RESEARCH ON EMOTION AND LEARNING: CONTRIBUTIONS FROM LATIN AMERICA

EDITED BY: Camilo Hurtado-Parrado, Carlos Gantiva, Leonardo A. Ortega, Alexander Gómez-A, Lucas Cuenya and Javier Leonardo Rico

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RESEARCH ON EMOTION AND LEARNING: CONTRIBUTIONS FROM LATIN AMERICA

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Latin America has increased its share of world scientific publications by nearly twofold during the last two decades (approximately from 2 to 4%). Despite this positive trend, the scholarly impact of scientific research produced in the region - measured in terms of citation rate - remains low. Two interrelated factors that contribute to this situation is that most research groups tend to work in isolation or in local sporadic collaboration, and results are often published in journals that are not indexed in major citation databases (e.g., SCOPUS, or Web of Science). Ultimately, part of Latin American high-quality research seems to remain hidden from the rest of the world.

Over the last decades, an important number of Latin American scientists have developed fruitful research agendas on questions on learning and emotion, focusing on basic and/or translational research with humans and other animal models, and implementing diverse methodologies. Notwithstanding the important contributions of these research programs, Latin American research on emotion and learning has followed the overall trend of other research fields throughout the region; namely, remaining partially hidden from the large scientific community of the world. This Research Topic aimed to engage researchers from Latin America to share their empirical and conceptual work on learning and emotion. Ultimately, this effort was expected to strengthen and integrate our regional community of experts, enhance global networking, and establish new challenges and developments for future investigation.

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EDITORIAL
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Editorial: Research on Emotion and Learning: Contributions from Latin America

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Editorial on the Research Topic

Research on Emotion and Learning: Contributions from Latin America

Contemporary research on emotions ("dispositions to action," Lang, 1995), primarily focuses on emotion regulation (McRae and Gross, 2020), emotional intelligence (Gong and Jiao, 2019), and the relationship between emotion and social cognition (Stevens and Jovanovic, 2019). Technological advances have allowed researchers to identify the neurobiological processes underlying the emotions, in particular, functional magnetic resonance imaging (fMRI, Weber-Goericke and Muehlhan, 2019), electrophysiological techniques (Gantiva et al., 2019), and virtual reality (Wechsler et al., 2019). Notwithstanding these major progresses, there is awareness of the need to continue developing methodologies that further integrate behavioral measures and increase ecological validity. For example, using dynamic stimuli (e.g., videos) and not just photographs, as well as combining recording techniques that allow to understand neuronal activity underlying emotion over the course of time (e.g., EEG in conjunction with fMRI, Pavlova, 2017).

The study of emotions is currently one of the more developed fields in behavioral sciences, which is showing a high potential for integration across disciplines and research areas, for example trough computational models and creation of virtual reality environments (de Gelder, 2017). Basic and applied efforts to understand the interplay of emotion and learning are two of those fruitful areas of interaction (Hascher, 2010; Tyng et al., 2017; Wortha et al., 2019). Mounting evidence indicates that emotion modulates encoding and helps retrieval of information efficiently. Also, now there is evidence that emotion strongly affects attentional and executive processes (e.g., modulating selectivity of attention and motivating to action), which in turn are intimately linked to learning processes (Tyng et al., 2017).

Aligned with this global trend in research on emotion and learning, an important number of Latin American scientists have developed fruitful research agendas over the last decades. Their efforts have focused on basic and/or translational research with humans and other animal models, and on implementing diverse methodologies (Ardila et al., 2005; Annicchiarico et al., 2013; Robayo-Castro et al., 2016; Forero et al., 2020). Notwithstanding these important contributions, and efforts to develop regional and international networking systems

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(e.g., Red Latinoamericana de Ciencias del Comportamiento—https://redlacc.org; Sociedade Brasileira de Neurociências e Comportamento—http://www.sbnec.org.br/site/ and Asociación Latinoamericana de Neurociencias Aplicadas—http://www.neurolatam.org/index.php/en/), Latin American research on emotion and learning has followed the overall trend of other research fields throughout the region; namely, remaining partially hidden from the large scientific community of the world.

Although Latin America has increased its share of world scientific publications by nearly 2-fold during the last two decades, moving from ∼2 to 4%, its output remains below the expected level based on its proportion of world population and Gross Domestic Product (GDP)—i.e., 5 to 6% (Van Noorden, 2014; Ciocca and Delgado, 2017). Moreover, recent analyses show that this growth has not been equally distributed across the region. While Brazil, Mexico, Argentina, Chile, and Colombia combined contribute to more than 90% of all scientific publications in the region, other neighboring countries remain behind the rest of the world (Huete-Perez, 2013). Ultimately, the scholarly impact of scientific research produced in the region remains below world average (e.g., citation rate remains below 1.0; Van Noorden, 2014). Two major interrelated factors that have contributed to this situation are that (a) most research groups tend to work in isolation or in local sporadic collaboration, and (b) results are often published in journals that are not indexed in key citation databases (e.g., SCOPUS or Web of Science; Van Noorden, 2014; Ciocca and Delgado, 2017).

In view of this situation, the present special issue entitled Research on Emotion and Learning: Contributions from Latin America aimed to engage researchers from the region to share empirical and conceptual work on emotion and learning in the journals Frontiers in Psychology and Frontiers in Education; two outlets that have high impact across different disciplines (emotion science, neuroscience, educational psychology, comparative psychology, health psychology, clinical psychology, and cognition). This effort was expected to result in strengthening and integrating our regional community of experts, enhancing global networking, and establishing new challenges and developments for future investigation.

The response to this initiative in the academic community was quite positive. Forty-six articles were submitted between November of 2017 and 2018, of which 33 were finally published. Most of these manuscripts were classified as *original research* (22); the remaining were distributed across *brief research reports* (5), *conceptual analyses* (2), *reviews* (2), *book reviews* (1) and *technology reports* (1).

An analysis of the published manuscripts keywords (**Figure 1**) indicates relationships to a wide range of topics. We observed differentiated emotion- and learning-related areas, covering basic and applied research with human and non-human species (e.g., rats, gerbils, birds, dogs) and expanding across various methodologies (e.g., psychophysiology, animal models, computer modeling). Worth noting is the fact that emotion-related topics were predominant, ranging from socially relevant areas, such as conflict and emotion regulation, to clinically and health-related areas, such as stress and psychopharmacology.

Figure 2 shows the affiliation's country of published manuscripts and collaboration networks. Brazil, Argentina, Chile, Mexico, and Colombia were the only countries with contributions from Latin America. Unfortunately, this replicates the unequal pattern of scientific publications output previously

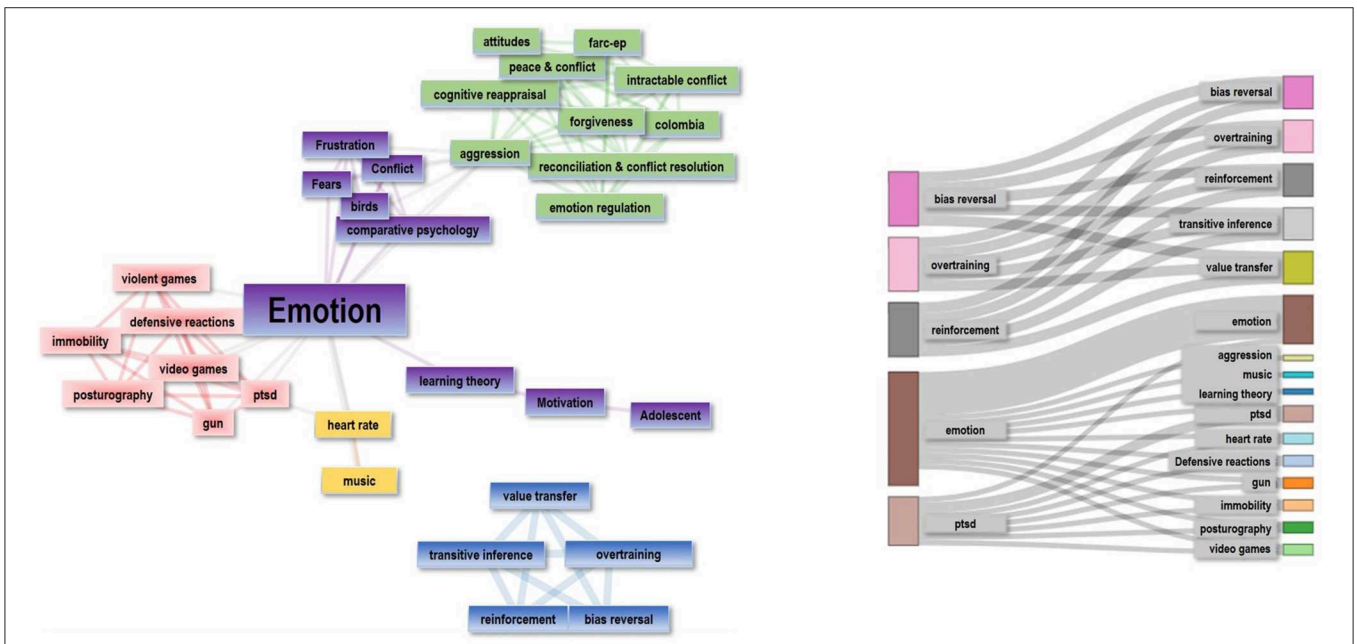

FIGURE 1 | Keywords network of the articles published in the Research Topic entitled Research on Emotion and Learning: Contributions from Latin America. Relevance of the keyword is represented with larger nodes and wider connecting lines. Created with Bibliometrix (Aria and Cuccurullo, 2017).

FIGURE 2 | Collaboration network based on affiliation country of published manuscripts. Higher number of articles from a given country are represented with darker blue color. Wider connecting red lines indicate more collaboration. Created with Bibliometrix (Aria and Cuccurullo, 2017).

identified in the region (Huete-Perez, 2013). In terms of collaboration, it practically only occurred between researchers with Latin American affiliations and institutions in other regions (e.g., United States, Canada, Spain, and Portugal). Collaboration between Latin American institutions was only observed in one instance (Colombia-Mexico). Both of these findings are aligned with previously reported patterns of collaboration (i.e., typically with non-Latin American institutions), which have been explained by the need of better visibility, research quality, impact factors, or international recognition (García et al., 2017).

Regarding financial support, 85.29% of the published studies in this special issue reported funding by different sources, of which 65.51% corresponded to Latin-American Governmental agencies. In terms of authorship indicators, a total of 140 authors participated in the Research Topic. Only four papers were authored by a single person, and the rest (29) included two or more authors, with a mean of 4 authors per article (an additional indicator of collaboration).

Concerning the number of citations, as of January 2020 the average per manuscript published in the special issue was 0.7, which impressively approached citation's average worldwide (1.0) in a short period of time (Van Noorden, 2014). The top-three cited papers in the Research Topic came from authors of Brazil, Chile, Spain, Belgium, and the Netherlands. The first two were related to positive psychology and well-being, while the third one was related to defensive emotional reactions to violent video games.

Finally, an additional indicator of visibility is the total number of views that the articles included in the special issue have reached. In that regard, for instance, as of January 2020, Torres-Berrio et al.'s was positioned at the first quartile of visibility with up to 6,600 views in <2 years since the publication of that study. Ten other papers were positioned in quartile two, 13 papers in quartile three, and 10 papers in quartile 4 (Frontiers Loop®-https://loop.frontiersin.org/).

CONCLUSIONS

When proposed, the aim of the present Research Topic was "to increase the visibility of Latin-American research on emotion and learning," something that has been achieved in a relatively brief period of time. Researchers from Latin-American countries like Brazil, Chile, Colombia, Argentina, and Mexico, mainly (but not uniquely) interested in emotions and learning, shared on a large scale contemporary and relevant research across a wide range of basic, applied, and translational fields. They also showed a very representative net of collaborations between countries known for a strong tradition of research in North America, Europe, and Asia.

Another important aspect evidenced in the present special issue relates to the limited investment in science in Latin America. Funding information on the published manuscripts indicates that near half of the reported studies were supported with governmental resources. This somewhat resembles the low percentage of spending in Research and Development (R&D) in Latin-American countries (with the exception of Brazil, most countries in the region allocate on average

<0.7% of the GDP—UNESCO Institute for Statistics—http://bit.ly/1USVQIP), as compared to that of developed countries in North America and Europe (beyond 2%—UNESCO Institute for Statistics—http://bit.ly/1USVQIP). This is a key point of interest for Latin-American researchers and their governmental agencies; namely, despite the low access to resources, researchers in the region are still capable of producing high-quality science. This is something that experts working on public policies need to "keep on the table" in order to continue advocating for increments in the resources that are greatly needed to support science in our countries.

Lastly, an aspect to highlight is the impact and visibility that most of the published articles are showing shortly after being released. This is aligned with previously reported growth in Latin-American science, as well as the recognition of its quality and applicability in other regions (Huete-Perez, 2013; Van Noorden, 2014; Ciocca and Delgado, 2017). Less than 1 year after the completion of the Research Topic, average citation per article is very close to reach worldwide standards (1.0 citation per article; Van Noorden, 2014).

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the project. CH-P and CG wrote the first draft of the manuscript. AG-A, LC, LO, and JR edited and added sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Association of Lower Spiritual Well-Being, Social Support, Self-Esteem, Subjective Well-Being, Optimism and Hope Scores With Mild Cognitive Impairment and Mild Dementia

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Introduction: Positive psychology (PP) constructs contribute significantly to a better quality of life for people with various diseases. There are still few studies that have evaluated the evolution of these aspects during the progression of dementia.

Objective: To compare the scores for self-esteem, life satisfaction, affect, spirituality, hope, optimism and perceived support network between elderly people with mild cognitive impairment (MCI), mild dementia and moderate dementia and control group.

Methods: Cross-sectional study. The sample consisted of 66 healthy controls, 15 elderly people with MCI, 25 with mild dementia and 22 with moderate dementia matched by age, gender, and schooling. The instruments used were: Spirituality Self Rating Scale (SSRS), Rosenberg Self-Esteem Scale, Medical Outcomes Study's Social Support Scale, Life Satisfaction Scale (LSS), Positive and Negative Affect Schedule (PANAS), Revised Life Orientation Test (LOT-R), and Adult Dispositional Hope Scale (ADHS).

Results: The scores for spiritual well-being, social support, self-esteem, life satisfaction, positive affect, optimism, negative affect, and hope differed significantly between the groups ($p < 0.05$). The individuals with MCI and mild dementia had lower spiritual well-being, social support, self-esteem, life satisfaction, positive affect, optimism and hope scores, and higher negative affect scores compared with the controls. The scores for PP constructs did not differ between the group of people with moderate dementia and the control group.

Conclusion: Dementia was found to impact several PP constructs in the early stages of the disease. For individuals with greater cognitive impairment, anosognosia appears to suppress the disease's impact on these constructs.

Keywords: aged, dementia, cognitive decline, well-being, positive psychology, optimism, hope, social support

INTRODUCTION

The maintenance of the well-being of elderly people with cognitive impairment is relevant to promote the independence and autonomy of these individuals. One of the demands on health professionals is to prevent or even delay the brain degeneration process, thus contributing to productive aging through the promotion of social engagement and significant interpersonal relationships (Agli et al., 2015; Casemiro et al., 2016). Elderly people exhibit different levels of cognitive impairment. A clinical picture of mild cognitive impairment (MCI) in one or more cognitive domains was recently described in which there was no significant impact on daily living activities. This diagnosis has been incorporated into important classification systems such as APA (American Psychiatric Association, 2014) and National Institute on Aging–Alzheimer's Association (NIA–AA) (Hyman et al., 2012), which have called it Mild Neurocognitive Disorder and MCI, respectively. This diagnosis is important in that signals a higher likelihood of progression to dementia (Roberts et al., 2014). On the other hand, dementia is a syndrome characterized by acquired cognitive and behavioral symptoms and a decline from previous functioning and performance levels, which interferes with one's ability to work and engage in usual activities (Hyman et al., 2012). The progressive loss of independence and functionality gives rise to the hypothesis that dementia has a major psychological impact to the subject (Ogawa et al., 2017).

Current studies on emotions have pointed out that individuals with dementia exhibit a lack of recognition, assessment, and even the ability to feel negative emotions (Balconi et al., 2015; Oliver et al., 2015; Bora et al., 2016) and preserve the ability to recognize positive emotions (Goodking et al., 2015; St Jacques et al., 2015). These altered perceptions may be related to the loss of their cognitive ability to understand their disease, known as anosognosia, which impairs their perceptions throughout the course of the dementia, but is associated with an improvement in their quality of life (Conde-Sala et al., 2013, 2014). In order to understand the individual attributes that improve one's quality of life, Positive psychology (PP) has been consolidated as a field of study interested in promoting well-being and developing individual strengths. PP proposes a change of focus from pathology and the remediation of suffering to positive individual potentials and characteristics (Seligmann and Lopes, 2011).

Positive psychology is the study of the conditions and processes that contribute to the flourishing and optimal functioning of people, groups, and institutions (Gable and Haidt, 2005). The following are the main PP constructs investigated in older people (Snyder and Lopez, 2009): Spirituality: a personal dimension of understanding questions about life, meaning and one's relationship to the sacred or transcendent (Gonçalves and Pillon, 2009); Social Support: resources made available by a group of people with whom an individual maintains contact and that correspond to certain functions, such as emotional, material, and affective support (Griep et al., 2005); Self-esteem: a visual aspect of self-conception that consists of a set of thoughts and feelings about oneself (Hutz and Zanon, 2011); Optimism: a stable personality trait that gives rise to positive expectations about future events (Scheier et al., 1994); Well-being: a three-part structure in which the first part refers to a cognitive judgment about the degree of satisfaction a person feels about his/her life and the latter two relate to affective components that specify how often a person experiences positive or negative affects (Diener et al., 2004); and Hope: thinking directed to objectives and composed of pathways and agency (Snyder and Lopez, 2009).

Regarding the PP construct approach in dementia, a systematic review that included 11 articles on religion and spirituality in patients with dementia concluded that intervening in these constructs appears to delay cognitive decline and helps them to cope with their disease (Agli et al., 2015). This finding was corroborated by a study by Wu and Koo (2016). These authors conducted a clinical trial with 103 patients with dementia and showed that spiritual interventions improved their hope, life satisfaction, and spiritual well-being. It has been suggested that the social support and sense of belonging that individuals share by being part of the same religious group are the factors that mediate the positive effect of spirituality on patients with dementia (Brewer et al., 2015). Furthermore, Kuiper et al. (2015) reviewed 19 longitudinal cohort studies investigating the association between social relationship and dementia. They concluded that individuals with poor social interaction had a faster decline into dementia. The effect of poor social interaction was similar to that of other well-established risk factors such as limited schooling, physical inactivity, and depression. This result was in line with the work done by Khondoker et al. (2017) in a cohort study that followed 10,055 participants over 10 years to investigate the relationship between dementia and social relationships. The authors concluded that dementia severity showed a significant and negative relationship with the number of bonds the subjects maintained as well as the quality of the social relationships they established. In addition, a systematic review evaluating the effectiveness of social support group interventions for individuals with dementia and MCI suggested that support groups can be psychologically beneficial to people with dementia by lessening their depression and increasing their quality of life (Leung et al., 2015).

Self-esteem in subjects with dementia has also been related to a better quality of life. Higher self-esteem scores predicted a better quality of life in a sample of 95 individuals with dementia and this association was partially mediated by depression and anxiety (Clare et al., 2013). Similar results were observed by Young et al. (2017), who evaluated 57 patient-caregiver dyads in a cross-sectional study and found that self-esteem predicted a higher quality of life for the individuals with dementia.

In addition to self-esteem, other positive variables investigated in patients with dementia are life satisfaction and daily experienced affection. Life satisfaction predicted dementia in a sample of 1.751 elderly people without cognitive impairment at baseline who were followed for 5 years (adjusted OR = 0.70, CI = 0.51–0.96; Peitsch et al., 2016). This dementia risk is also related to negative affects according to a study conducted by Korthauer et al. (2017). The authors assessed affects in 2,137 elderly women without depressive symptoms who were followed for 11 years and concluded that negative affects were associated with greater cognitive decline, even when adjusting for the

covariates of age, education, lifestyle, sociodemographic factors, global cognition, cardiovascular risk, and hormone therapy.

The incidence of cognitive impairment was also associated with optimism in a study by Gawronski et al. (2016). These authors evaluated 4,624 elderly people over a period of 4 years and concluded that high optimism was a protective factor for incident cognitive impairment, playing an important role in maintaining cognitive functioning. Likewise, higher levels of hopelessness in midlife were associated with cognitive impairment in a 21-year follow-up with a sample of 200 subjects (Hakanson et al., 2015). Similarly, a systematic review concluded that optimism and hope were beneficial to the treatment of individuals with chronic diseases (Schiavon et al., 2017).

The studies cited above demonstrate that there is great interest in the field of PP in older individuals with cognitive impairment, as they stress the role of many PP constructs as risk factors for this condition and/or the beneficial effects of interventions focusing on these PP constructs on some outcomes, such as quality of life in dementia. Nevertheless, few studies have investigated PP constructs at different levels of cognitive impairment. One investigation described a significant reduction in self-esteem as dementia progressed (Diesfeldt, 2007). Thus, the progression of PP aspects during the course of the disease is still unknown (Wolverson et al., 2009). In view of this, the present study aimed to compare self-esteem, life satisfaction, affect, spirituality, hope, optimism, and perceived support network scores among healthy elderly individuals and subjects with MCI and mild and moderate dementia.

Considering the literature described above, this study's first hypothesis was that there is a decreasing gradient in all PP constructs among healthy subjects and individuals with MCI and dementia–i.e., better cognitive performance is associated with higher scores for PP constructs. On the other hand, we predicted that individuals with MCI would present lower scores for PP constructs than those with dementia due the latter group's greater anosognosia–i.e., that the dementia group's greater cognitive impairment would reduce the individual's ability to perceive his/her daily limitations, thus affecting the PP constructs.

MATERIALS AND METHODS

This is a cross-sectional study. "Cases" were considered those individuals who had some type of cognitive change and "controls" were considered those who were healthy and undergoing a normal aging process. The sample characteristics and inclusion criteria are described below.

Participants

The sample size was previously calculated in the WinPepi program, version 11.43, based on the studies of Hernandez et al. (2009) and Moreno et al. (2010) resulting in a minimum number of at least 60 demented individuals and 62 healthy controls. The final sample was composed of 128 individuals with a minimum age of 60.

The clinical group (individuals with MCI and dementia) consisted of 62 outpatients who were being treated at a dementia clinic located in a southern Brazilian capital city. Their ages ranged from 60 to 89 (Mean = 72,52; Standard Deviation = 7,92). Dementia diagnoses were made by dementia experts according to the NIA–AA (Hyman et al., 2012), and Brazilian Academy of Neurology criteria (Frota et al., 2011; McKahan et al., 2011). Dementia severity was assessed by the Clinical Dementia Rating (CDR) (Morris, 1993; Chaves et al., 2007). Patients who scored 1 on CDR had mild dementia and who scored 2 presented moderate dementia. Individuals who had a CDR score of 3 (severe dementia) were excluded from the study, as the severity of their impairment would make it impossible for them to understand the questions posed by the instruments that evaluated PP aspects. MCI diagnosis was made by a comprehensive clinical evaluation, which included a clinical history and neuropsychological and functional assessments. Those patients who showed impairment in any cognitive domain and no functional impairment fulfilled the MCI criteria. A CDR score of 0.5 was also used to corroborate a MCI diagnosis. Patients with a current clinical diagnosis of major depression were also excluded. Doctors with clinical experience in evaluating depression and dementia carried out all of the assessments.

The control group consisted of 66 individuals aged 60–88 (Mean = 72,95; Standard Deviation = 7,63) who were selected from a group of elderly people who engaged in physical activities in an active aging group in a capital city in southern Brazil. The controls were chosen after clinical group was selected so that their age, gender, schooling, and social class would be similar to those of the individuals in the clinical group, aiming to match the groups. Depression and cognitive impairment were exclusion criteria for this group. The 15-item Geriatric Depression Scale (GDS) (Sheikh and Yesavage, 1986; Paradela et al., 2005) and the mini mental state examination (MMSE) (Folstein et al., 1975; Bertolucci et al., 1994) were applied to screen for these conditions. Subjects who tested positive for these conditions were not included. All of the subjects were able to perform their day-to-day activities independently.

Positive Psychology Instruments

The researchers were trained beforehand to apply the PP scales. The following instruments were used to evaluate the PP constructs.

- The Brazilian Portuguese Adaptation of the Spirituality Self Rating Scale (SSRS) (Galanter et al., 2007; Gonçalves and Pillon, 2009). This is a Likert scale composed of six items that assess an individual's spirituality–i.e., the importance of his/her spiritual dimension and how he/she applies it in his/her life. It is based on three factors: peace, meaning, and faith, and presented adequate internal consistency, with a Cronbach's alpha of 0.83 (Gonçalves and Pillon, 2009).
- The Brazilian Portuguese Adaptation of the Social Outcomes Study Scale (MOS) (Sherbourne and Stewart, 1991; Griep et al., 2005). This instrument covers five social support dimensions: material, affective, positive social interaction, emotional, and information. It's a Likert-type scale and presented a Cronbach's alpha equal to or greater than 0.8 (Griep et al., 2005).

- The Brazilian Version of the Rosenberg Self-Esteem Scale (Rosemberg, 1989; Hutz and Zanon, 2011). This unifactorial instrument consists of ten statements related to a set of feelings of self-esteem and self-acceptance and assesses global self-esteem. It presented a Cronbach's alpha of 0.90. The items are answered on a 4-point Likert scale, including the following responses: totally agree, agree, disagree, and totally disagree (Hutz and Zanon, 2011).
- The Brazilian Portuguese Version of the Life Satisfaction Scale (LSS) (Diener et al., 1985; Zanon et al., 2014). The LSS scale presented a Cronbach's alpha of 0.81 and is composed of 5 self-report items arranged on a 7-point Likert scale in which people agree or disagree with the statements to varying degrees (Zanon et al., 2014).
- The Brazilian Version of the Positive and Negative Affect Schedule (PANAS) (Watson and Clark, 1994; Zanon et al., 2013). The Brazilian version of the positive and negative affect schedule presented a Cronbach's alpha of 0.83 for positive affects and 0.77 for negative affects. It is a self-report scale composed of 10 items that evaluate positive affects and 10 items that evaluate negative affects. The items are adjectives with the answer keys for a Likert five-point scale that correspond to the frequency at which the person experiences the emotion described by the adjective (Zanon et al., 2013).
- The Revised Life Orientation Test (LOT-R) by Scheier et al. (1994) in Its Brazilian Version (Bastianello et al., 2014). Used to evaluate optimism, this scale is composed of a 10-item measure of optimism versus pessimism. Of the 10 items, 3 items measure optimism, 3 items measure pessimism, and 4 items serve as fillers. Respondents rate each item on a 5-point Likert scale with varying degrees of agreement or disagreement. The Cronbach's alpha of the LOT-R Scale is 0.68 (Bastianello et al., 2014).
- The Brazilian Version of the Adult Dispositional Hope Scale (ADHS) (Snyder et al., 1991; Pacico et al., 2013). The Brazilian version of the adult dispositional hope scale is used to evaluate hope and presented a Cronbach's alpha of 0.80. This instrument consists of 12 items. Four of these refer to the agency dimension, four refer to the pathway dimension, and four serve as distractors. The items are answered on a 5-point Likert type scale in which 1 is "totally false" and 5 is "totally true" (Pacico et al., 2013).

Social Class Assessment

Social class was established according to the Economic Classification of the Brazilian Economic Classification Criteria developed by Associação Brasileira de Empresas de Pesquisa [ABEP] (2016), taking into account the population's purchasing power. This instrument's final score results in the categories A, B, C, D, and E. Category A includes individuals with an average income of R$ 20,888.00 (Brazilian reals); Category B includes individuals with average incomes between R$ 4,852.00 and R$ 9,254.00; Category C includes individuals with average incomes between R$ 1,625.00 and R$ 2,705.00; and categories D and E include individuals with an average income of R$ 768.00. These amounts are expressed in Brazilian currency, as set forth in the National Household Sample Survey of 2014.

All of the instruments were used in their validated versions for application in Brazil and have good validity and reliability indicators.

Procedures

Data were initially collected from the individuals in the clinical group. Based on their characteristics (gender, age, schooling, and social class) they were paired with members of the control group when its participants were selected, aiming to match the groups. The elderly people who met the inclusion criteria were invited to participate in the study through an approach carried out in different places, according to the group to be studied. After the informed consent form was explained and signed in a silent and private room, the subjects were evaluated. The PP questionnaires were applied in random order to avoid response bias.

Ethical Issues

This study was carried out in accordance with the ethical recommendations for clinical research laid out in Resolution 446/12, with written informed consent from all subjects. It was previously approved by the UFCSPA Research Ethics Committee (Protocol No. 1,046,803). All subjects provided informed written consent in accordance with the Helsinki Declaration.

Statistical Analysis

The data were stored in an SPSS 22.0 database and analyzed using this statistical program. Variable distribution was assessed using the Shapiro–Wilk normality test and variance homogeneity was evaluated with the Levene test. The Student's *t*-test was used to compare age between the case and control groups; the Chi-square association test was used to compare the other demographic data between the groups. Since the normality and homogeneity tests did not present statistically significant values, parametric tests were applied. To compare the scales between the groups, we applied the one-way analysis of variance (ANOVA) complemented by Tukey's test. The significance level was set at 5% ($p < 0.05$).

RESULTS

In total, 128 subjects aged 60–89 (Mean = 72,74; Standard Deviation = 7,50) were included in the study. **Table 1** shows the comparison of the demographic data, presence of cognitive complaints and antidepressant use between the healthy individuals (controls) and those with MCI, mild dementia and moderate dementia (cases). The demographic data (age, schooling, and social class) did not differ between the groups. The MCI and dementia groups presented cognitive complaints. Such complaints became increasingly frequent the higher the level of cognitive impairment and dementia severity. Although major depression was one of the exclusion criteria, some individuals reported that they currently used antidepressants for minor anxiety or depression symptoms. Such use was significantly higher in the control group.

TABLE 1 | Comparison of demographic data and complaints of cognitive difficulties between the group of healthy individuals (controls) and the group of people mild cognitive impairment (MCI) and mild/moderate dementia (cases).

		Controls *N* = 66	MCI^{+} *N* = 15	Mild dementia *N* = 25	Moderate dementia *N* = 22	*P*
Age*		72.95 (7.63)	73.27 (8.78)	70.36 (7.39)	74.45 (7.67)	0.316
Gender**	*F*	54 (81.8)	11 (73.3)	17 (68.0)	16 (72.7)	0.512
	M	12 (18.1)	4 (26.6)	8 (32.0)	6 (27.2)	
Schooling**	None	0 (0)	0 (0)	2 (8)	2 (9.1)	0.199
	Primary school education	53 (80.3)	13 (86.7)	16 (64)	17 (77.3)	
	High school education	7 (10.6)	2 (13.3)	5 (20)	3 (13.6)	
	College and/or graduate school	(9.1)	0 (0)	2 (8)	0 (0)	
Social Class**	*A*	4 (6.1)	0 (0)	0 (0)	0 (0)	0.522
	B	20 (30.3)	3 (20)	5 (20)	5 (22.7)	
	C	39 (59.1)	12 (80)	19 (76)	17 (77.3)	
	D	3 (4.5)	0 (0)	1 (4)	0 (0)	
Complaint of cognitive difficulty**	Yes	24 (36.3)	9 (60.0)	20 (80.0)	20 (90.9)	<0.001
	No	42 (63.6)	6 (40.0)	5 (20.0)	2 (9.0)	
Antidepressant use**	Yes	7 (10.6)	3 (20.0)	3 (12.0)	2 (9.0)	0.748
	No	59 (89.3)	12 (80.0)	22 (88.0)	20 (90.9)	

*MCI^{+}, Mild Cognitive Impairment. *ANOVA – results presented as mean and standard deviation. **Chi-square – results presented as n (%).*

Table 2 presents a comparison of the scores on the spiritual well-being, social support, self-esteem, life satisfaction, positive affect, negative affect, optimism and hope scales among the healthy elderly people and those with MCI and mild and moderate dementia. There was a significant difference in all of the PP constructs between the groups.

The multiple comparisons using Tukey's test showed significantly lower scores for the majority of the PP constructs in individuals with MCI and mild dementia than in the healthy elderly individuals and those with moderate dementia. There was no difference in the total spiritual well-being score between the MCI and mild dementia groups, while individuals with MCI presented significantly lower scores for this construct than the healthy subjects and the individuals with moderate dementia. Multiple comparisons using Tukey's test also showed that the total social support scores were similar between the MCI and mild dementia groups and that both groups presented lower scores for this construct than the moderate dementia group and the healthy control group. Individuals with MCI showed lower scores in all of this construct's subgroups than those observed in the healthy group and the moderate dementia group, while the mild dementia group showed lower scores than the moderate dementia group only for the affective, information and positive social interaction sub-scales of social support. Scores for the self-esteem, life satisfaction, positive affect, and hope constructs did not differ between the MCI and mild dementia groups. These constructs were also similar between the moderate dementia group and the healthy individuals. On the other hand, the scores for these constructs were significantly lower in the MCI and mild dementia groups compared with the moderate dementia group and the healthy control group. The healthy controls showed the highest optimism scores among all the groups. The optimism scores did not differ between the mild dementia group and the MCI group and both presented lower levels of optimism than the healthy control group and the moderate dementia group. Negative affect scores were significantly higher in the MCI and mild dementia groups than in the healthy control group, while negative affect levels did not differ between these groups.

DISCUSSION

The present study evaluated self-perception in several PP constructs in elderly individuals with MCI and different degrees of dementia compared with that in healthy individuals. The study showed two main results: (a) Groups with MCI and mild stages of dementia had worse scores for all constructs, and (b) The scores of patients with moderate-stage dementia did not differ from those of the control group. Therefore, our first hypothesis that better cognitive performance would be associated with higher scores in the PP constructs was refuted. The second hypothesis that cognitive impairment would be associated with better scores in all of the PP constructs was validated.

It is worth discussing some points about the first result (worse scores in the early stages of dementia). It is known that the neuropsychological impacts of dementia include the deterioration of life quality, perception, mood, and behaviors that are associated with neurodegenerative diseases. These changes are mainly perceived at an early stage of the disease and are associated with a poor prognosis of progressive cognitive loss (Ismail et al., 2017). This may explain the first result described. The results of the present study are in partial agreement with those demonstrated by Diesfeldt (2007). This author verified a significant reduction in self-esteem with the progression of dementia. Our results were similar for milder degrees. However, the present study's assessment found no difference between

TABLE 2 | Comparison of mean and standard deviation in spiritual well-being, social support, self-esteem, life satisfaction, positive affect and negative affect, optimism and hope scores among healthy individuals with mild cognitive impairment and mild/moderate dementia.

	Control Group	Clinical group				
	N = 66	Mild cognitive impairment *N* = 15	Mild dementia *N* = 25	Moderate dementia *N* = 22	*P*	$F_{(dfB;dfW)}$
Spiritual well-being	39.06 (5.62)[c]	28.00 (6.92)[a]	31.28 (6.42)[a,b]	35.50 (7.89)[b,c]	<0.001	$17.46_{(3;124)}$
Peace	12.89 (2.12)[c]	8.6 (2.74)[a]	9.72 (2.33)[a,b]	11.41 (2.55)[b]	<0.001	$20.70_{(3;124)}$
Meaning	13.18 (1.92)[c]	8.73 (2.63)[a]	10.60 (2.02)[b]	11.95 (2.69)[b,c]	<0.001	$21.16_{(3;124)}$
Faith	13.13 (2.25)[b]	10.67 (3.88)[a]	10.96 (3.90)[a]	12.27 (3.16)[a,b]	0.003	$4.85_{(3;124)}$
Social support	94.74 (11.98)[b]	73.46 (27.02)[a]	74.39 (30.12)[a]	91.18 (18.68)[b]	<0.001	$9.35_{(3;124)}$
Material	95.30 (14.43)[b]	82.66 (22.58)[a]	88.20 (17.13)[a,b]	94.09 (15.93)[a,b]	0.029	$3.09_{(3;124)}$
Affective	96.63 (10.92)[b]	80 (27.25)[a]	82.12 (22.67)[a]	92.40 (19.00)[a,b]	<0.001	$6.43_{(3;124)}$
Emotional	94.16 (14.89)[b]	74.33 (28.77)[a]	76.8 (26.17)[a,b]	91.59 (19.11)[b]	<0.001	$7.19_{(3;124)}$
Information	93.78 (14.54)[b]	68.33 (30.09)[a]	73.2 (28.60)[a]	90.45 (21.03)[b]	<0.001	$9.86_{(3;124)}$
Positive social interaction	95.22 (13.34)[b]	63.05 (38.62)[a]	67.43 (33.81)[a]	90.00 (20.23)[b]	<0.001	$13.51_{(3;124)}$
Self-esteem	38.10 (2.68)[b]	28.53 (7.92)[a]	30.96 (6.99)[a]	35.95 (4.70)[b]	<0.001	$23.32_{(3;124)}$
Life Satisfaction	30.93 (3.73)[b]	25.53 (6.86)[a]	23.24 (5.81)[a]	29.27 (3.79)[b]	<0.001	$18.96_{(3;124)}$
Positive Affect	37.65 (4.88)[b]	28.27 (9.78)[a]	27.00 (7.63)[a]	33.41 (7.95)[b]	<0.001	$19.11_{(3;124)}$
Negative Affect	12.62 (4.60)[a]	25.27 (7.94)[b]	22.24 (7.71)[b]	14.41 (5.47)[a]	<0.001	$29.30_{(3;124)}$
Optimism	24.10 (4.58)[c]	13.87 (6.78)[a]	14.60 (3.96)[a]	20.32 (4.01)[b]	<0.001	$36.22_{(3;124)}$
Hope	36.93 (3.54)[b]	28.40 (7.97)[a]	28.32 (6.01)[a]	34.00 (5.10)[b]	<0.001	$24.34_{(3;124)}$

*[a,b,c] *Different letters indicate significant differences (Tukey test at 5% significance); dfB, degrees of freedom between groups; dfW, degrees of freedom within groups.*

the controls and the individuals with moderate dementia. This discrepancy may be due to differences in the methods each study used to assess dementia severity.

The reduction of the constructs studied in the earlier stages of dementia can also be understood as resulting from the impacts that the disease generates, posing itself as a threat to existence, the meaning of life and social context, since believing that the rest of one's life may be unpleasant overwhelms the individual, threatening his/her identity and lowering his/her self-esteem (Cheston et al., 2015). Regarding the wide range of emotions that elderly people with dementia can experience, positive and negative affects are present in varying degrees of intensity in day-to-day life, and it is the relationship between the frequencies with which they are experienced that leads to a greater or lesser feeling of well-being (Diener et al., 2004). In the literature, negative emotions are associated with subsequent dementia. However, since the disease has a long preclinical stage, is difficult to determine whether dementia causes such negative feelings or results from them (Hakanson et al., 2015). What a demented person feels and how he/she feels about the disease is something that has not yet been studied and needs more attention (Schiavon et al., 2017). It is important to point out that even patients with MCI (i.e., whose cognitive impairments do not cause disability) have scores equal to those with mild dementia (worse than the controls' scores). This could be attributed to their expectation of having an unfavorable progression toward an irreversible and incapacitating disease (Ogawa et al., 2017).

To deal with these disabilities, the literature suggests that dementia interventions in the various positive psychology constructs studied may help the elderly to cope with their disease. Recent studies have investigated the benefits of interventions in positive psychology for individuals with dementia (Coin et al., 2010; Agli et al., 2015; Hakanson et al., 2015; Kuiper et al., 2015; Wu and Koo, 2016; Khondoker et al., 2017). These studies suggest that spirituality in individuals with dementia tends to alleviate or stabilize cognitive disorders (Coin et al., 2010; Agli et al., 2015; Chen et al., 2017) and assists in the development of coping strategies to accept dementia, maintain their relationships, sustain hope, and find meaning in their lives (Jolley et al., 2010; Dalby et al., 2012; MacKinlay, 2012; Agli et al., 2015). Self-esteem interventions are indicated to reduce depressive symptoms and improve life quality (Lee and Park, 2007). Social support and positive relationships can play an important role in maintaining health and preventing dementia (Khondoker et al., 2017), as well as reducing depression (Logsdon et al., 2010; Leung et al., 2015). However, studies are still needed to assess self-esteem, life satisfaction, affect, spirituality, hope, optimism, and support networks in dementia and what effect the disease's progression has on these aspects.

Our investigation also found that the PP constructs did not differ between the patients with moderate dementia and the healthy controls–i.e., as their dementia severity worsened, the elderly patients' responses became more positive. This finding is similar to that of a study by Midorikawa et al. (2016) in which family members reported that individuals with mild and moderate dementia displayed more positive behaviors and emotions in their day-to-day lives. Considering the present study's results, it is hypothesized that this is due to the patient's perception. Among the constructs evaluated, only optimism continued to show a significant difference between the control group and the group with moderate dementia. A systematic review by Schiavon et al. (2017) emphasized the need for more studies on optimism and hope in people with chronic

diseases, highlighting the importance of these constructs in disease prognosis, life satisfaction, and life quality. Regarding emotions, impairment of left-hemisphere emotion regulators (including the left ventrolateral prefrontal cortex, orbitofrontal cortex, and anterior striatal insula) may impair one's ability to suppress positive emotions such as happiness, thus making individuals more inclined to positive affect (Conde-Sala et al., 2014; Sturm et al., 2015). In advanced stages, there is a functional reduction in these regions as a whole as well as a smaller volume of gray matter in regions of the prefrontal gyrus associated with the recognition of emotions (Stock et al., 2015). This reduction provides a hypothesis for this study's findings, which indicate that there is no difference in any of the constructs except optimism between the control group and the group with moderate dementia.

As the disease progresses, the perception of its initial impact may not be perceived as a threat, since the disease's own effects can lead to an inability to judge and understand the losses it causes, as well as to better responses in many domains, such as emotions and empathy (Fong et al., 2016). A patient's lack of concern about the losses caused by the disease is related to frontal and ventromedial cortical atrophy, which leads him/her to overestimate his/her individual and emotional abilities (Hornberger et al., 2014). Although these losses compromise an individual's ability to respond to his/her emotions, a qualitative study by Kaufmann and Engel (2016) found that, even with communication challenges, individuals with dementia have the ability to assess their own well-being and affect. The study also pointed to the need for specific methods to evaluate elderly dementia patients to see if they have an adequate understanding of the disease's impact on their lives. The present study found more frequent complaints of cognitive difficulty in the cases (79%) than in the controls (36.4%). This data suggests that, even if there is a difficulty in understanding the disease, the recognition of cognitive impairment is preserved. Even so, we cannot discard the hypothesis that the greater cognitive impairment of the moderate stage affects judgment and even the comprehension of the existing scales used to evaluate elderly people without dementia. This could also explain the lack of difference in the PP constructs between controls and patients with moderate dementia, in which increased cognitive deficit, anosognosia, and poor understanding of complex issues exert great influence (Poveda et al., 2017).

Greater impairment of an individual's ability to perceive his/her own limitations with the progression of dementia determines his/her awareness of the pathology's severity or even of being ill (known as anosognosia), which was mentioned earlier in this study (Conde-Sala et al., 2014; Martyr and Clare, 2017). Studies on anosognosia point to regions of the brain that may be related to this disorder (Perrotin et al., 2015; Arroyo-Anllo et al., 2017). Anosognosia, or the loss of the ability to perceive a disease, makes an individual incapable of responding clearly to his/her emotions or even of perceiving and/or feeling the difficulties experienced in his/her routine. This reduced perception is associated with a serious deficit in the processing and recognition of emotions, which also impairs an individual's perspective on his/her emotional responses (Poveda et al., 2017). In the literature, anosognosia is associated with the perception of better life quality in advanced stages of dementia (Conde-Sala et al., 2013) and may affect not only cognitive deficits and day-to-day functioning, but also affective symptoms ("affective anosognosia") described in previous studies (Conde-Sala et al., 2013; Munro et al., 2016). The present study's findings lead us to believe that anosognosia has a strong influence on an individual's perception in advanced degrees of dementia, as well as on PP construct scores, highlighting the need for further research in this field in order to better understand the relationships between dementia and emotions.

The scarcity of research on PP and the associations between its constructs and dementia is still evident in current literature (Machado et al., 2017). Considering the results found in the present study, the importance of performing specific evaluations to understand what happens in the various PP constructs during the course of dementia is reinforced.

Study Strengths

One of the present study's strengths is that it investigates several PP domains at different stages of dementia, including the earliest stages, such as MCI. It is original in that it provides a view of the differences that exist in PP constructs in terms of the degrees of dementia impairment and performs a broad analysis of the findings, aiming to improve the psychosocial conditions of elderly people.

Study Limitations

An important limitation of the study is that there is limited literature available in the field of PP, mainly due to the novelty of studies on PP in dementia. Important gaps still exist (Reppold et al., 2015; Freitas et al., 2016). This problem, especially when related to demented individuals and the different stages of dementia, occurs due to the difficulty of evaluating individuals with advanced dementia with existing instruments, since the responses obtained are usually non-specific, non-objective, and strongly influenced by the fluctuations in attention and difficulty in retaining information caused by cognitive impairment (Kaufmann and Engel, 2016).

For future studies, a longitudinal design is suggested with dementia patients in the assessment of the progression of losses in PP, since this may help in understanding the progression of the constructs studied. The impressions of caregivers would be an interesting topic for future studies. Potential causality, selection and measuring biases, and confounding effects are related to the study's design.

CONCLUSION

Mild cognitive impairment and mild dementia result in lower scores for the constructs of self-esteem, spirituality, social support, affect, life satisfaction, optimism, and hope. However, as dementia severity increases, the construct scores more closely resemble those of individuals in the control group, leading to the

hypothesis that factors such as anosognosia, a reduction in the suppression of positive emotions and a reduction in emotional self-perception and understanding of the disease gain influence with the progression of dementia.

AUTHOR CONTRIBUTIONS

SdS was the lead author in conceptualizing the study and writing the manuscript. GR, AdP, LF, and CR contributed to all stages of the research and then critically reviewed and revised the manuscript. CR was a doctoral advisor for this research. LF and AdP were co-advisors for this research. All authors were accountable for the final version of the manuscript.

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Pre-exposure Schedule Effects on Generalization of Taste Aversion and Palatability for Thirsty and Not Thirsty Rats

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The study reported four experiments aiming to test the effects of the pre-exposure schedule and water deprivation on the generalization of a conditioned taste aversion in rats, with a particular focus on testing whether or not the concurrent schedule might enhance generalization. In two experiments, non-water-deprived rats received concurrent, intermixed, or blocked exposure to a sweet-acid solution and a salty-acid solution before conditioning of one of these compounds and testing of both flavors. During pre-exposure, the rats consumed a greater amount of the sweet-acid solution than the salty-acid solution (Experiments 1 and 2), consumption of the former increasing during pre-exposure while consumption of the latter decreased (Experiment 1). Furthermore, consumption of the salty-acid solution was lower during concurrent than intermixed or blocked pre-exposure (Experiment 1 and 2) while consumption of the sweet-acid solution was greater during intermixed than concurrent or blocked pre-exposure (Experiment 1). It is discussed whether the pre-exposure schedule might modify stimulus perception beyond the mere enhancement of stimulus differentiation, by, for instance, affecting the palatability of gustatory stimuli. Evidence for enhanced generalization after concurrent pre-exposure was not found for either deprived (Experiments 1, 2, and 3) or non-deprived rats (Experiments 3 and 4), with deprivation leading to a general increase in consumption of both the conditioned and test flavors. This then raised the question of whether or not concurrent pre-exposure to flavors always increases generalization between them. The present study highlights the importance of this issue for various accounts of perceptual learning.

Keywords: perceptual learning, pavlovian conditioning, deprivation, discrimination, generalization, palatability, taste aversion, rats

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INTRODUCTION

A conditioned taste aversion established to one stimulus (e.g., AX) will, to some degree, generalize to another similar stimulus (e.g., BX), depending on the schedule with which the stimuli have been presented previously (e.g., Honey and Hall, 1989; Mackintosh et al., 1991). In particular, generalization between the stimuli appears to be lower after intermixed (i.e., AX, BX, AX, BX. . ., the stimuli being presented few hours apart) than blocked (i.e., AX, AX,. . ., BX, BX. . .) pre-exposure (e.g., Symonds and Hall, 1995, 1997; Mondragón and Hall, 2002). But shorter intervals

(a few minutes or seconds) between intermixed presentations of the stimuli (e.g., Bennett and Mackintosh, 1999; Wills and Mackintosh, 1999) or concurrent pre-exposure (e.g., Alonso and Hall, 1999; Rodríguez and Alonso, 2008), seems to increase generalization with respect to the standard intermixed or blocked schedules.

Within the field of perceptual learning, it is widely accepted that generalization decrements after stimulus pre-exposure must rely, at least in part, on changes in the extent to which the stimuli can be differentiated as a consequence of stimulus pre-exposure (see Hall, 1991; Mitchell and Hall, 2014, for a review). To the extent that less generalization would be expected between stimuli that are better differentiated from each other, then such an effect should be more marked when using an intermixed pre-exposure schedule, compared to a schedule in which the stimuli are pre-exposed in a series of blocks. According to such an analysis, it is expected that stimulus differentiation would be poorer after rapid-intermixed or concurrent pre-exposure relative to standard intermixed or blocked pre-exposure. A number of human perceptual learning experiments, however, have found quite the opposite result. In particular, several studies have established that people are more accurate in judging two similar visual stimuli as different, or assigning such stimuli to different artificial categories following concurrent exposure in comparison with either intermixed or blocked schedules (e.g., Mundy et al., 2007, 2009; Angulo and Alonso, 2012, 2013). It has therefore been concluded that at least for humans, the concurrent schedule increases stimulus differentiation to a greater extent than the intermixed or blocked schedules. This apparent discrepancy between the results of studies conducted with human and non-human animals has been explained in several ways.

For example, it has been argued that, even in the case in which concurrent pre-exposure is able to enhance stimulus differentiation for non-human animals in the way described for people, various sources of generalization might still be enhanced by this schedule. According to some authors, concurrent pre-exposure to the stimuli will strengthen the associative excitatory links between them (e.g., Honey et al., 1994; Alonso and Hall, 1999; Rodríguez et al., 2008) and, such links would be able to increase generalization between the stimuli, even in the case in which the subjects are able to discriminate between them. Indeed, it is expected that excitatory links would be established between concurrently presented stimuli only in the case in which such stimuli were discriminable. It has also been suggested that common elements of the stimuli (X, in the example outlined above) could be more strongly conditioned after concurrent than intermixed or blocked pre-exposure (e.g., Bennett and Mackintosh, 1999; Rodríguez and Alonso, 2008). Thus, irrespective of how well animals may differentiate between the stimuli after concurrent pre-exposure, the size of the generalized conditioned response (CR) will be stronger, simply because learning acquired by the common elements would be greater.

Other analyses have focused on the extent to which the instructions given to participants during the pre-exposure phase could play a role in determining the outcome of human perceptual learning studies. To date, human studies assessing the concurrent schedule (e.g., Mundy et al., 2007, 2009; Angulo and Alonso, 2012, 2013) have specifically asked participants to look for differences between the stimuli during pre-exposure, while for other animals, stimuli are assumed to be "merely" pre-exposed (e.g., Hall, 1991, 2001). It has been proposed that, in humans, instructions may activate top-down strategies for processing the stimuli that would not be operating in situations in which mere exposure is given to other animals (e.g., Mitchell and Hall, 2014). According to such analyses, the greatest benefit for humans (although not for other animals) of the concurrent schedule may lie in an interaction of some sort between the pre-exposure schedule and the instructions asking the participant to seek out stimulus differences (see Nelson, 2009; Mitchell and Hall, 2014, for a review of this discussion). A recent study conducted with humans found that, in the absence of instructions asking participants to specifically look for stimulus differences, people were less accurate in judging the pre-exposed stimuli as being same or different, following concurrent pre-exposure (Angulo et al., unpublished). It might therefore be tempting to conclude that the effects of the pre-exposure schedule could vary according to the specific demands of the instructions given during pre-exposure and, if this were indeed the case for humans, one might wonder whether the same may also be true for other animals. Rats and other non-human animals cannot be verbally instructed during pre-exposure. But it could still be the case that the animals' environment (external or internal) during pre-exposure may dictate the way in which the stimuli are processed, in the same way that verbal instructions are thought to influence stimulus processing in humans.

Experimental procedures employing taste aversion preparations with rats usually begin with a regime of water deprivation that is maintained throughout the experiment (e.g., Symonds and Hall, 1995, 1997; Honey and Hall, 1989; Mackintosh et al., 1991; Rodríguez and Alonso, 2008). Thus, the procedure induces a state of fluid deprivation under which the critical flavored solutions will be presented. In such a situation, rats receive exposure to stimuli with motivational value in a state of fluid depletion. It might therefore be a matter of debate as to what extent the rats are receiving mere exposure to neutral stimuli, as has traditionally been assumed. It might be possible to argue that the state of water deprivation — a part of the animal's internal environment — might provide some "like-instructions" for processing the stimuli. Accordingly, the aim of the present study was to examine the impact of giving the animals free access to water during the pre-exposure phase (i.e., not inducing a state of water deprivation). In particular, we wanted to explore whether this change in the deprivation state would allow us to observe differences in the extent to which the schedule of pre-exposure has an effect on stimulus generalization, using a standard taste aversion procedure. Because discrepancies between the studies conducted with human and non-human animals arise from the effects of the concurrent pre-exposure schedule, we focused particularly on the effects of this schedule.

EXPERIMENT 1

Using a conditioned taste aversion procedure, Experiment 1 tested the effect of concurrent, intermixed, and blocked pre-exposure to two similar flavored compound solutions, AX and BX, on subsequent generalization between them. Whilst this issue has previously been addressed (Rodríguez and Alonso, 2008; Rodríguez et al., 2008), the rats were fluid deprived during pre-exposure (but see Alonso and Hall, 1999 for concurrent and blocked pre-exposure effects when the flavored compounds were presented *ad libitum*). In this experiment, however, the deprivation regime began after pre-exposure. The experiment included eight groups of rats. Half of these groups received a single taste aversion conditioning trial with the compound AX (paired groups, - P) after pre-exposure, while the other half received presentation of the stimulus AX and the induced illness separately (unpaired groups, - U). Within the paired and unpaired groups, one received a concurrent pre-exposure schedule (e.g., AX–BX, AX–BX, AX–BX, etc., Groups CNC-P and CNC-U), another received the intermixed schedule (e.g., AX, BX, AX, BX, etc., Groups INT-P and INT-U) and the third the blocked schedule (e.g., AX, AX, etc., BX, BX, etc., Groups BLK-P and BLK-U). The remaining two groups received no pre-exposure to AX and BX before the induced illness (Groups CTRL-P and CTRL-U). Subsequently, all the rats received a single consumption test with the AX flavor, and another test with BX. Generalization between these stimuli was then assessed in two ways: by comparing AX and BX consumption in the paired groups; and by comparing consumption of BX in the paired groups with consumption of BX in the unpaired groups. Different consumption levels of AX and BX in the paired groups would indicate discrimination between the two stimuli. Since AX but not BX was aversively conditioned, lower consumption of AX than BX would be expected if rats were indeed able to differentiate between the stimuli. No differences between consumption of AX and BX in the paired groups would, however, be taken to indicate generalization between the stimuli. On the other hand, lower consumption of BX in the paired than in their respective unpaired control groups would also be indicative of generalization. Since no stimulus was conditioned in the unpaired groups, and thus there would be no aversion to be generalized from one stimulus to the other, consumption of BX and AX should be similar in this case, with consumption of BX being of the level that would be expected in the case of "zero generalization."

Experiment 1 also measured the free consumption of the two flavored solutions employed as stimuli, AX and BX, during concurrent, intermixed, and blocked pre-exposure. The reason for this is that it has recently been suggested (Mitchell and Hall, 2014), though not empirically tested, that rats might be able to differentiate *a priori* the stimuli usually employed in perceptual learning studies. For the current theoretical accounts of perceptual learning (e.g., McLaren and Mackintosh, 2000; Hall, 2003; Mitchell et al., 2008), it becomes crucial to test this possibility. Such accounts have been developed primarily within the framework of animal research and have provided evidence for mechanisms that are able to explain stimulus differentiation as a consequence of pre-exposure. But, if the stimuli are differentiated *a priori*, then differentiation must clearly not be a consequence of such proposed mechanisms. As in previous studies (e.g., Mackintosh et al., 1991; Symonds and Hall, 1995), the two flavored compound solutions used here as stimuli shared a common acid flavor (X), and were distinguished by the addition of other distinctive flavors (A and B), which are sugar and salt (counterbalanced in each group, see below). The sweet solution was expected to be more palatable than the salty solution. Thus, if rats were able to differentiate between the stimuli during pre-exposure, and had access to an unrestricted amount of the solution, they should display a preference for the sweet solution relative to the salty one. To the best of our knowledge, pre-exposure schedule effects on stimulus discrimination have not yet been tested in this simple and more ecological way.

Method

Subjects and Apparatus

Subjects were 64 experimentally naïve male Wistar rats with a mean weight of 350 g (range 346–355 g) at the beginning of the experiment. Rats were individually housed in cages with food and water *ad libitum* in a room with constant temperature (24°C) and humidity (50%). The room was artificially illuminated under a 12 h-dark/light cycle with the light period beginning at 8:00 a.m. All experimental sessions were conducted with the animals in their home cages.

The stimuli used were two compound solutions, AX and BX, consisting of diluted plain water presented at room temperature through 50-ml plastic tubes, fitted with a metal spout. Stimulus X was always a solution containing hydrochloric acid (HCl) at 1%, whilst Stimulus A and B were (counterbalanced) a solution of 1% salt and 10% sugar, respectively, for half the rats in each group, and the reverse for the remaining half. The unconditioned stimulus (US) was an intraperitoneal injection of 0.15 M lithium chloride (LiCl) administered at 10 ml/Kg of body weight.

Procedure

The Animal Welfare Ethics Committee of the Universidad del Pais Vasco (Experiments 1 and 2) and the Universidad Autónoma de Chile (Experiments 3 and 4) approved the procedures employed in the current experiments. The rats were randomly assigned to eight equal groups ($n = 8$): CNC-P, INT-P, BLK-P, CTRL-P, CNC-U, INT-U, BLK-U, and CTRL-U.

Pre-exposure

All the rats, except those belonging to Groups CTRL-P and CTRL-U, received four AX and four BX presentations during pre-exposure. This phase lasted for 4 days, with the stimuli being presented during two daily 30-min drinking sessions (at 11:00 a.m. and 5:00 p.m.). These drinking sessions began by removing the bottles containing *ad libitum* plain water, and replacing these with two tubes, each containing 50 ml of flavored solution, one located on the right-hand side and the other on the left-hand side of the rats' home-cages (30 cm apart). The sessions finished with the replacement of the two tubes with the bottle containing water *ad libitum*. For the CNC-P and CNC-U groups, in all the pre-exposure sessions one of the bottles contained the salty compound and the other the sweet compound. Half of the rats in

these two groups first received the salty flavor on the right and the sweet flavor on the left (with the remaining rats receiving the reverse arrangement), the position of the solution being switched from trial to trial. For the INT-P and INT-U groups, the two tubes always contained the same solution, but it was switched from trial to trial. Half of the rats received the salty flavor on all of the morning sessions and the sweet flavor on the afternoon sessions, while for the remaining rats this arrangement was reversed. The BLK-P and BLK-U groups also received the same solution in both tubes. However, in these groups, half of the rats received the salty flavor on the first 2 days (on both the morning and afternoon sessions) and the sweet solution on the latter 2 days, while the other half received the reverse arrangement. Finally, the CTRL-P and CTRL-U groups always received plain water in both tubes. For half the rats in each group the salty solution was flavor AX and the sweet solution the flavor BX, while the reverse was true for the remaining rats. One hour after the last pre-exposure drinking session, the bottles containing *ad libitum* water were removed from the home cages. On the following 4 days, all the rats only had access to fluids during the 30 min morning and afternoon drinking sessions, in a single bottle placed in the location otherwise occupied by the standard water bottle.

Conditioning

The day after the last pre-exposure trial, all the rats received a single intraperitoneal injection of LiCl, half of them in the morning session and the other half in the afternoon session. For the CNC-P, INT-P, BLK-P, CTRL-P, and CNC-P groups, the injection was immediately preceded by the presentation of 10 ml of the AX solution in a single bottle, with the rats receiving 10 ml of plain water on the other session. Half of the rats from Groups CNC-U, INT-U, BLK-U, and CTRL-U received 10 ml of the AX solution in the morning, and 10 ml of plain water followed by the injection in the afternoon, whilst the remaining rats received 10 ml of water followed by the injection in the morning and 10 ml of the AX solution in the afternoon. The day following conditioning, all the rats received free access to plain water in the morning and afternoon drinking sessions.

Test

Over the next 2 days, all the rats had access to free consumption of the AX or BX solutions in the morning drinking session and water in the afternoon. Half of the rats from each group received the AX solution on the first day and the BX solution on the second, with the remaining rats receiving BX first and then AX. For all the subjects, the fluid was presented in a single bottle.

The amount of fluid consumed during the drinking sessions was calculated by weighing the drinking tubes before and after consumption and converting the difference to ml. An analysis of variance (ANOVA) was then conducted on the consumption values, adopting a statistical significance criterion of $p < 0.05$. Subsequent pair-wise comparisons between groups were conducted with the Tukey's Honest Significant Difference (HSD) test.

Results

The mean consumption of the salty and sweet solutions during pre-exposure trials with the three schedules (Concurrent, Intermixed, and Blocked) is shown in **Figure 1**. Consumption of the sweet solution was always greater than consumption of the salty solution, with this consumption increasing in the former case and decreasing in the latter across the blocks of pre-exposure trials. Furthermore, consumption of the sweet solution was greater with the intermixed pre-exposure schedule than with the others, while consumption of the salty solution was lower with the concurrent schedule than the other schedules.

A 3 × 2 × 4 ANOVA with Schedule, Solution, and Trial as factors found a significant main effect of the three factors, Schedule, $F(2,45) = 10.99$, $p < 0.001$; Solution, $F(1,45) = 4.15$, $p < 0.001$; and Trial, $F(3,135) = 4.89$, $p = 0.003$. The Schedule × Solution, $F(2,45) = 3.62$, $p = 0.035$, and Solution × Trial, $F(3,135) = 13.29$, $p < 0.001$, interactions were also significant. A subsequent analysis of simple effects for these interactions confirmed the observations described above. Greater consumption of the sweet solution than the salty solution was found with all three pre-exposure schedules, $Fs(1,15) \geq 95.89$, $ps \leq 0.001$, with pre-exposure conditions differing in both cases, $Fs(2,45) = 10.25$, $p < 0.001$. Consumption of the sweet solution was greater with the intermixed than with the concurrent, $p = 0.003$, or the blocked schedule, $p = 0.007$, while consumption of the salty solution was lower with the concurrent than with the intermixed, $p < 0.001$, or blocked, $p = 0.005$, schedule. Consumption of the sweet solution was greater than the salty solution in all four blocks of pre-exposure trials, $Fs(1,47) \geq 44.47$, $p \leq 0.001$, with the former increasing across blocks of trials, $F(3,141) = 11.36$, $p < 0.010$, while the latter decreased, $F(3,141) = 3.95$, $p = 0.010$.

On the conditioning trial, consumption of AX was 8.04 (SEM ± 1.36), 9.66 (SEM ± 1.29), 9.86 (SEM ± 1.53), and 8.77 (SEM ± 1.43) for the Concurrent, Intermixed, Blocked, and Control conditions, respectively. An ANOVA conducted with these consumption data found no significant effect of Schedule, $F(3,60) = 0.35$, $p = 0.783$.

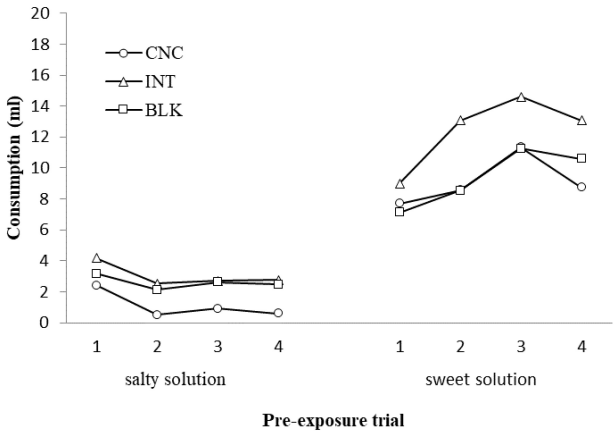

FIGURE 1 | Consumption (ml) of the salty and sweet solutions for the concurrent, intermixed, and blocked pre-exposure conditions across blocks of pre-exposure trials in Experiment 1.

The mean consumption of AX and BX for the paired and unpaired groups during testing can be seen in **Figure 2**. Irrespective of the pre-exposure schedule, unpaired groups consumed a similar amount of AX and BX, but consumption of AX was always lower than consumption of BX for the paired groups. In this latter case, consumption of AX was similar in all groups, while consumption of BX appeared to be greater for the CNC-P and BLK-P groups than for the INT-P and CTRL-P groups. Furthermore, consumption of AX was, in general, lower for the paired than for the unpaired groups. However, consumption of BX was observed to be somewhat lower for the INT-P and CTRL-P groups than for the INT-U and CTRL-U groups, respectively, and very similar for the CNC-P and CNC-U, and BLK-P and BLK-U groups. A 4 × 2 × 2 ANOVA conducted with Schedule, Stimulus, and Conditioning as factors supported all the above observations except for the latter. The main effect of Conditioning, $F(1,56) = 21.52$, $p < 0.002$, and Stimulus, $F(1,56) = 11.28$, $p = 0.001$, as well as the interaction Stimulus × Conditioning, $F(1,56) = 21.211$, $p < 0.001$, were significant (remaining Fs ≤ 0.89). A subsequent analysis of simple effects for the Stimulus × Conditioning interaction found lower consumption of AX than BX in the paired groups, $F(1,31) = 41.28$, $p < 0.001$, but not in the unpaired groups, $F(1,31) = 0.616$, $p = 0.439$. Furthermore, consumption of AX was lower in the paired than in the unpaired groups, $F(1,62) = 45.57$, $p < 0.001$, while consumption of BX did not differ reliably in the paired and unpaired groups, $F(1,62) = 0.470$, $p = 0.496$. Previous studies assessing the effect of the concurrent schedule on generalization have inferred its magnitude only from the consumption of BX (e.g., Alonso and Hall, 1999; Rodríguez and Alonso, 2008). Thus, although the Schedule × Stimulus interaction failed to reach significance, some planned comparisons were conducted in order to explore the differences in consumption observed for the paired groups. These analyses indicated that while the paired groups did not differ in their consumption of AX, $F(3,28) = 0.26$, $p = 0.84$, they differed in their consumption of BX, $F(3,28) = 4.24$, $p = 0.014$. In particular, consumption of BX was greater in Group BLK-P than in Group CTRL-P ($p = 0.026$).

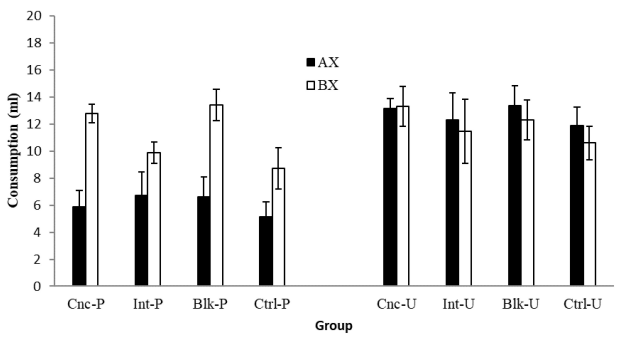

FIGURE 2 | Consumption (mean ± SEM) of the AX and BX solutions during the test trials for the 8 groups of Experiment 1.

Discussion

In Experiment 1, rats consumed a greater amount of the sweet-acid solution than the salty-acid solution from the first pre-exposure trial. This initial discriminative response to the solutions seems to indicate that the rats were able to differentiate between them *a priori*, the sweet solution being more palatable than the salty solution. The consumption of the salty solution decreased in general during pre-exposure while consumption of the sweet solution increased. Thus, at least when rats were not water-deprived during pre-exposure, consumption of the less palatable solution might decrease unconditionally, while consumption of the more palatable solution increases. Experiment 1 also found a clear effect of the schedule on the pattern of consumption elicited by the salty and sweet solutions during pre-exposure. Rats consumed less of the salty solution during concurrent than intermixed or blocked pre-exposure. However, the sweet solution was more readily consumed during intermixed than concurrent or blocked pre-exposure. If we accept that the stimuli were readily discriminated *a priori*, it is possible that the schedule affected consumption of the most and least palatable stimuli not by increasing stimulus differentiation, but rather through some other, as yet unspecified, effect of the schedule. This issue will be taken up again in the General Discussion.

On the test, all the rats from the paired groups drank less of the aversively conditioned flavor AX than the BX flavor. Again this discriminative response would be possible only if the stimuli were differentiated. Rats from the unpaired groups did not differ in their consumption of AX and BX. Given that no stimulus was conditioned in these groups, there would be no reasons to expect any difference in consumption of AX and BX even when they are discriminable (note that half of the rats that received the AX compounds were given the sweet solution and the other half received the salty solution). Consumption of AX was lower in the paired than in the unpaired groups, indicating that an aversion to AX was successfully established in these cases. And importantly, there were no significant differences between the paired and unpaired groups in terms of their consumption of BX. Since consumption of BX in the unpaired groups would be free from any generalization of conditioned aversion, this last result may indicate that the paired groups did not generalize the conditioned aversion from AX to the other stimulus, BX. In brief, none of the results obtained on the test appear to indicate generalization between AX and BX in the paired groups, and we can thus rule out the possibility that generalization was differentially affected by the pre-exposure schedules. Nevertheless, in the paired groups, consumption of BX was observed to be greater after blocked pre-exposure to the stimuli relative to the control group that did not receive prior pre-exposure to the stimuli. Taking the consumption of BX alone, it might be possible to conclude that the blocked but not the intermixed and concurrent pre-exposure schedules reduced generalization between the stimuli (relative to the control group not receiving pre-exposure to the stimuli). Such a conclusion would be incompatible with the results produced by previous studies (Alonso and Hall, 1999; Rodríguez and Alonso, 2008; Rodríguez et al., 2008), in which rats drank less BX after

concurrent than intermixed or blocked pre-exposure, concluding that generalization was greater in the former than in the latter cases. However, the results of Experiment 1 appear to suggest that consumption of BX might not be a net indicator of generalization between the stimuli. Given that there is no sign of generalization between the stimuli, and taking into account that pre-exposure schedules might directly affect consumption, it seems likely that differences in consumption of BX between the groups could reflect some effect of the schedules on consumption rather than on stimulus generalization.

Presumably, the discrepancy between our findings and those of previous studies regarding the consumption of BX might be related to the state of fluid deprivation during pre-exposure. However, it should be noted that this and the previous studies also differed in terms of the amount of solution that was made available during pre-exposure. In the experiments conducted previously, rats received only a small and fixed amount of the solutions during pre-exposure (10 ml), and these were usually fully consumed given that the rats were water deprived (but see Alonso and Hall, 1999, Experiment 1a). But in Experiment 1 of the present study, rats had free access to the flavored solutions. Therefore, the main aim of Experiment 2 was to test whether the principal findings of Experiment 1 could be replicated if rats received a fixed amount of the solutions during pre-exposure, as in previous experiments reported in the literature.

EXPERIMENT 2

The methods used in Experiment 1 and those employed in previous studies (e.g., Rodríguez and Alonso, 2008; Rodríguez et al., 2008, but see Alonso and Hall, 1999), differed in two important ways. Firstly, in Experiment 1 (but not in previous studies), the rats were not in a state of water deprivation during pre-exposure (but see Alonso and Hall, 1999). And secondly, in Experiment 1 (but not in the previous studies), rats were allowed free access to the solutions during pre-exposure instead of a limited, fixed amount of the flavor. In Experiment 2, rats in the paired groups also received a limited amount of the solution during pre-exposure in order to see if such a variable could play a role in our failure to observe the expected effect of enhanced generalization after concurrent pre-exposure.

Method

Subjects and Apparatus

Subjects were 32 experimentally naïve male Wistar rats with a mean *ad libitum* weight of 398 g (range 391–405 g) at the beginning of the experiment. The stimuli, apparatus, and all other details not specified here were identical to those described for Experiment 1.

Procedure

Rats were randomly assigned to four equal groups ($n = 8$), CNC-P, INT-P, BLK-P, and CTRL-P, which, as in Experiment 1, differed only in terms of the pre-exposure schedule received (Concurrent, Intermixed, Blocked, or No exposure, respectively). However, unlike in the previous experiment, in this case the rats received only 10 ml of solution on each pre-exposure trial (5 ml in each bottle). All other procedural details not specified here were identical to those described for Experiment 1.

Results

Consumption of the salty and sweet solutions during pre-exposure with the Concurrent, Intermixed, and Blocked schedules can be seen in **Figure 3**. Again, consumption of the salty solution was lower than the sweet solution and in this case, consumption of both solutions was observed to be lower with the concurrent than with the intermixed or blocked schedules. In general, consumption of the salty solution also decreased somewhat across blocks of trials (with all schedules), while consumption of the sweet solution was observed to increase in general (with all schedules). A 3 × 2 × 4 ANOVA conducted with Schedule, Solution, and Trial as the factors found significant main effects of Schedule, $F(2,21) = 47.66$, $p < 0.001$, and Solution, $F(1,21) = 262.440$, $p < 0.001$. Of the interactions, Schedule × Solution was found to be significant, $F(2,21) = 36.94$, $p < 0.001$, remaining, $Fs \leq 2.70$. Subsequent analyses of simple effects for the interaction found that for all schedules, consumption of the sweet solution was always greater than consumption of the salty solution, $Fs(1, 7) \geq 22.50$, $ps \leq 0.002$. Groups differed in their consumption of both the salty, $F(2,21) = 7.39$, $p = 0.004$, and the sweet solution, $F(2,21) = 70.77$, $p < 0.001$, with consumption always being lower for subjects that received the concurrent schedule compared with the intermixed or blocked schedules, $ps \leq 0.032$.

On the conditioning trial, consumption of AX was 6.37 (SEM ± 1.39), 5.48 (SEM ± 1.15), 5.70 (SEM ± 1.32), and 5.57 (SEM ± 1.0) for the CNC-P, INT-P, BLK-P, and CTRL-P groups, respectively. An ANOVA conducted on these consumption scores revealed that differences between the groups were not significant, $F(3,28) = 1.61$, $p = 0.208$.

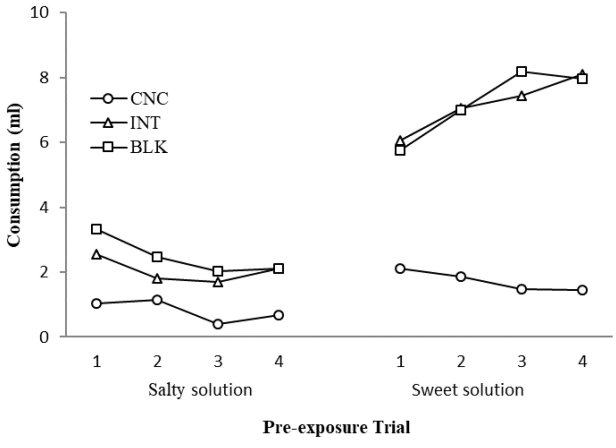

FIGURE 3 | Consumption (ml) of the salty and sweet solutions for the concurrent, intermixed, and blocked pre-exposure conditions across blocks of pre-exposure trials in Experiment 2.

Finally, consumption of AX and BX on test for all four groups can be seen in **Figure 4**. In all four groups, consumption of AX was lower than consumption of BX, and in this case, consumption of both AX and BX was observed to be lower in Group CTRL than in the others. A 4 × 2 ANOVA conducted with Schedule and Stimulus as factors found only the main effect of Stimulus to be significant, $F(1,28) = 81.46$, $p < 0.001$, although the effect of Schedule also approached significance, $F(3,28) = 2.81$, $p = 0.057$. The interaction Schedule × Stimuli was far from reliable, $F(3,28) = 0.687$, $p = 0.567$ and, if anything, a subsequent pair-wise comparison between groups revealed that only the CTRL and BLK groups differed marginally ($p = 0.077$) in terms of their general consumption, as in Experiment 1.

Discussion

As observed in Experiment 1, consumption of the sweet solution was greater than consumption of the salty solution from the outset of pre-exposure, with consumption of the salty solution being lower during concurrent than intermixed or blocked pre-exposure. In this case, consumption of the sweet solution was also lower during concurrent than intermixed or blocked pre-exposure. This result, however, might readily be explained in terms of a procedural artifact. In order to provide the same overall amount of flavored solution during pre-exposure for all the groups, rats given concurrent pre-exposure to the stimuli received only 5 ml of each solution on each trial while rats receiving intermixed or blocked pre-exposure received 10 ml (5 ml in each tube). Thus, the rats receiving the concurrent schedule were never able to drink more than 5 ml of the sweet solution during pre-exposure. The consumption of the sweet solution in the groups receiving the intermixed and blocked schedules was similar and asymptotic (note that the last ml of fluid in the tube is usually inaccessible). It thus seems likely that the greater consumption of the sweet solution observed in the intermixed group relative to the other groups in Experiment 1 was not observed here because an insufficient amount of solution was available to reveal this difference.

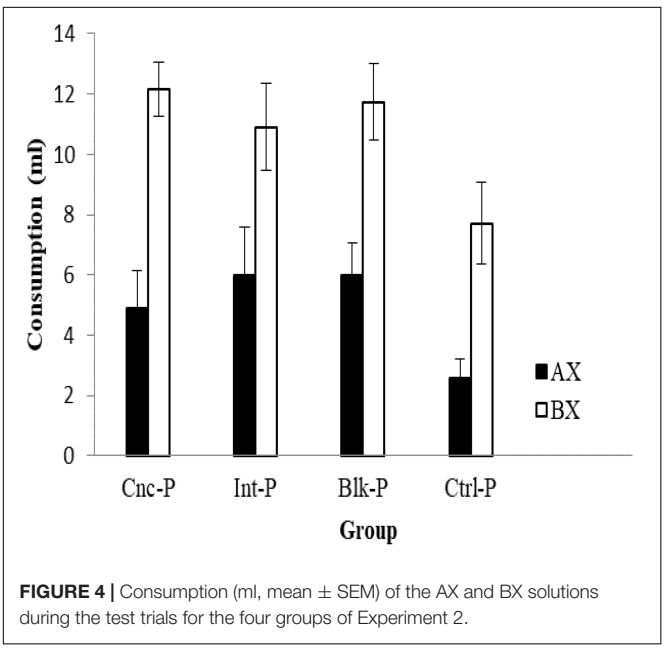

FIGURE 4 | Consumption (ml, mean ± SEM) of the AX and BX solutions during the test trials for the four groups of Experiment 2.

It should perhaps be noted that, regarding the ability to discriminate the stimuli, lower consumption of the stimuli during concurrent pre-exposure relative to the intermixed or blocked schedules would lead, if anything, to poorer stimulus discrimination in the former than in the latter cases. In spite of this, however, on the subsequent test, nothing in our results indicated poorer discrimination or greater generalization between the stimuli after concurrent exposure in comparison with intermixed or blocked pre-exposure. All rats consumed less of the conditioned stimulus (CS) flavor (AX) than BX, indicating good discrimination between the stimuli in all cases. According to some theoretical analyses, generalization between the stimuli should be increased by the concurrent schedule because this arrangement should boost the establishment of excitatory links between the stimuli and strengthen conditioning of their common elements. If this were the case, consumption of BX should have been lower after concurrent than intermixed or blocked pre-exposure. But in the present experiment, consumption of both AX and BX was similar after concurrent and intermixed or blocked pre-exposures. Again, this experiment also provides no evidence that stimulus generalization differs according to the pre-exposure schedule and, if anything, the blocked schedule may have increased the general consumption of the solutions relative to the control group. It might be worth noting, however, that contrary to our expectations, consumption of BX appeared to be somewhat higher following concurrent exposure compared with the intermixed schedule, although this difference was not significant (see also Experiment 1).

In brief, the results of the two experiments presented above appear to be incompatible with the idea that concurrent pre-exposure to the stimuli increases generalization between them, or at least, not in all cases. Such a result would be largely unexpected, given that two previous studies (Rodríguez and Alonso, 2008; Rodríguez et al., 2008) found greater generalization of a conditioned taste aversion after concurrent exposure in comparison with intermixed pre-exposure. Further, according to some authors, associative mechanisms would be operating to increase generalization after concurrent pre-exposure. In particular, the latter arrangement should facilitate the establishment of excitatory links between the pre-exposed stimuli. Furthermore, according to previous evidence, the common elements of the stimuli might have acquired a stronger aversion during conditioning following concurrent pre-exposure due to an attenuation of latent inhibition (i.e., Bennett and Mackintosh, 1999; Rodríguez and Alonso, 2008). Given that the main procedural difference between those experiments reporting increased generalization after concurrent pre-exposure and those reported here was the state of fluid deprivation, it is reasonable to suppose that this factor could be responsible for the discrepancy between the results of these studies. To be more specific, it is well-established that contextual changes after pre-exposure usually attenuate latent inhibition (i.e., Hall and Honey, 1989) and, it would be expected that the change in the state of deprivation between the pre-exposure and

conditioning phases in our experiments might be able to change the internal context of the animals. If this were the case, such a contextual change might serve to attenuate latent inhibition of AX for subjects in all the pre-exposure conditions. It might then be possible to argue that the differences in latent inhibition expected for the common elements of the stimuli according to the pre-exposure schedule might also be attenuated, thus reducing the likelihood of detecting subsequent differences in the generalization of the conditioned taste aversion. This possibility is supported by the fact that in the previous experiments the subjects who received pre-exposure to the stimuli did not differ from the non-preexposed control group in terms of test consumption of AX, as would be expected on the basis of a very robust effect such as latent inhibition. Following this logic, the aim of Experiment 3 was to explore the possible role played by the deprivation state of the animals in observing an effect of intermixed and concurrent pre-exposure on the generalization of a taste aversion. In order to meet the ethical requirement of reducing the number of animals used, in the following experiment only were maintained such pre-exposure conditions critical for the theoretical discussion, that is, those subjects in the intermixed and concurrent pre-exposure conditions.

EXPERIMENT 3

The chief aim of Experiment 3 was to test the effect of water deprivation during intermixed and concurrent pre-exposure on the subsequent generalization of a conditioned taste aversion, and in particular whether or not the enhanced generalization observed after concurrent pre-exposure might occur only in deprived rats. This latter result would be expected if such an increase in generalization were due to an attenuation of latent inhibition, where the change in the deprivation state might disrupt latent inhibition for both pre-exposure conditions. In order to test this possibility, a conditioned taste aversion was established to the compound AX, as in the previous experiments, followed by a consumption test in which animals are presented with this flavor and a second, similar flavor, BX. In a previous phase, the rats received either concurrent (CNC conditions) or intermixed (INT conditions) pre-exposure to the stimuli, and were either water deprived or not (D and ND conditions respectively) during pre-exposure.

Method

Subjects and Apparatus

Subjects were 32 naïve male Sprague Dawley rats with a mean *ad libitum* weight of 299 g (range 286–312 g) at the beginning of the experiment. The stimuli, apparatus, and other details not specified were identical to those described for Experiment 2.

Procedure

The rats were randomly assigned to four equal groups ($n = 8$): CNC-D, INT-D, CNC-ND, and INT-ND. For the deprived conditions, 4 days before the pre-exposure phase, access to water was limited to two daily 30 min sessions. This drinking schedule was also maintained during pre-exposure. However, for the non-deprived rats, fluid was always available until the conditioning phase. In this case, during the pre-exposure phase the tubes containing the flavored compounds replaced the bottles containing plain water, with the latter being returned after pre-exposure. Given that the aim of this experiment was to specifically test the effect of deprivation, the level of fluid available in each trial for both the intermixed and concurrent pre-exposure conditions was 10 ml (5 ml in each tube), being the fluid the same for the intermixed conditions and different for the concurrent conditions.

Results

The consumption of the salty and sweet solutions for the four groups can be seen in the upper part of **Table 1**. In general, and as expected, the consumption of both solutions was greater for those subjects in the deprived conditions than in the non-deprived conditions. Furthermore, for the non-deprived conditions the consumption of the sweet solution appeared to be greater than the salty solution, as observed in previous experiments; however, for the deprived conditions this difference was less apparent. Furthermore, as found in Experiment 1, in the non-deprived conditions the consumption of the sweet solution appeared to increase with the intermixed schedule whilst consumption of the salty solution seemed to decrease with the concurrent schedule. A 2 × 2 × 2 × 4 ANOVA with Deprivation (Deprived vs. Non-Deprived), Schedule, Flavor, and

TABLE 1 | Consumption (ml) of the salty and sweet solutions during the pre-exposure trials for all the groups of Experiments 3 and 4.

Group	Salty 1	Salty 2	Salty 3	Salty 4	Sweet 1	Sweet 2	Sweet 3	Sweet 4
Experiment 3								
CNC-D	10.00	7.84	9.42	4.47	9.79	6.34	10.00	7.64
INT-D	7.75	8.53	9.63	9.82	9.14	9.99	9.99	10.00
CNC-ND	3.10	2.24	2.92	2.05	5.00	5.00	5.01	5.00
INT-ND	1.36	1.62	1.18	4.35	2.61	4.97	4.81	8.42
Experiment 4								
CNC-1B	4.49	4.69	5.00	5.00	4.32	4.72	5.00	4.90
CONC-2B	4.44	5.00	5.00	5.00	4.26	4.89	5.00	4.77
INT-1B	8.27	10.00	10.00	10.00	8.06	8.61	9.83	9.68
INT-2B	7.18	10.00	10.00	9.64	7.59	9.71	9.42	9.80

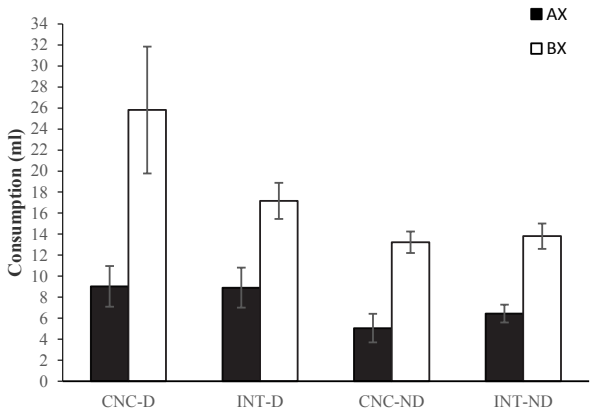

FIGURE 5 | Consumption (ml, mean ± SEM) of the AX and BX solutions during the test trials for the four groups of Experiment 3.

Trial as the factors found significant main effects of Deprivation, $F(1,28) = 148.26$, $p \leq 0.001$, and Flavor, $F(1,28) = 22,87$, $p \leq 0.001$. Furthermore, the interactions Deprivation × Schedule, $F(1,28) = 4.67$, $p = 0.039$, Flavor × Deprivation, $F(1,28) = 8.23$, $p = 0.008$, Deprivation × Trial, $F(3,84) = 6.91$, $p \leq 0.001$, and Schedule × Trial, $F(3,84) = 19.29$, $p \leq 0.001$, were also significant (remaining, $Fs \leq 1.95$). The subsequent analysis of simple effects found that rats in the deprived conditions consumed more than those in the non-deprived conditions, both with the Concurrent, $F(1,15) = 36.78$, $p \leq 0.001$, and Intermixed $F(1,15) = 161,44$, $p \leq 0.001$, schedules, but the effect of schedule was significant only for the deprived rats, $F(1,15) = 9.38$, $p = 0.008$. Furthermore, rats in the deprived conditions drank more of both the salty, $F(1,31) = 169.61$, $p \leq 0.001$, and sweet solutions, $F(1,31) = 32.82$, $p \leq 0.001$. However, the consumption of the sweet solution was greater than the salty solution only for the non-deprived rats, $F(1,15) = 40.58$, $p \leq 0.001$. In addition, the effect of Deprivation was significant on the four pre-exposure trials, $Fs \geq 15.24$, with the effect of trial only being significant for the deprived rats, $F(3,45) = 3.24$, $p = 0.031$. Finally, the general effect of Schedule was significant for the first, $F(1,31) = 4.46$, $p = 0.043$, and latter pre-exposure trials, $F(1,31) = 21.145$, $p \leq 0.001$, with a significant effect of trial for the Deprived, $F(3,45) = 3.24$, $p = 0.031$, but not for the Non-Deprived rats, $F(3,45) = 2.10$, $p = 0.112$.

On the conditioning trial, consumption of AX was 8.88 (SEM ± 0.24), 8.67 (SEM ± 0.194), 9.34 (SEM ± 0.18), and 8.86 (SEM ± 0.17) for the CNC-D, CNC-ND, INT-D, and INT-ND groups, respectively. An ANOVA conducted on these consumption scores revealed no significant differences between the groups, $F(3,28) = 1.97$, $p = 0.141$.

Figure 5 shows the consumption of the compound AX and BX on the test for the four experimental conditions. In general, consumption of both AX and BX was greater for rats in the deprived conditions compared with the non-deprived conditions, with consumption of BX being generally higher than that of AX. Consumption of BX appeared to be particularly high for the CNC-D groups in comparison with the other groups.

A 2 × 2 × 2 ANOVA conducted on these data with Deprivation (Deprived vs. Non-Deprived), Schedule, and Stimulus found significant main effects of Deprivation, $F(1,28) = 10.46$, $p = 0.003$, and Stimulus, $F(1,28) = 29.34$, $p < 0.001$ (remaining, $Fs \leq 2.38$). Planned comparisons to examine possible differences in the consumption of AX and BX according to the schedule found an effect of the schedule for the consumption of BX, $F(3,28) = 3.20$, $p = 0.038$, but not for AX, $F(3,28) = 1.52$, $p = 0.229$, with consumption of BX being higher for subjects given the concurrent schedule than those given the intermixed schedule.

Discussion

As observed in Experiments 1 and 2, Experiment 3 found that when rats were not deprived during pre-exposure, they displayed a preference for the sweet solution over the salty solution during pre-exposure, which clearly indicates that they can discriminate the compounds *a priori*. The three experiments appeared to be consistent in this regard. Unsurprisingly, deprived rats tend to consume all the fluid available and thus the preference would not be detected when a deprivation regime is introduced in the taste aversion preparation. The fact that the deprived rats drank more than the non-deprived rats during pre-exposure requires no special comment and, since the opportunity for consumption in this experiment was also limited, the present experiment presented no opportunity to replicate the principal findings of Experiment 1. Thus, no further comments regarding the pre-exposure phase are necessary.

In addition, Experiment 3 found a general effect of deprivation on the test that was reflected in the greater consumption of both AX and BX for the deprived rats. Although all the rats were deprived from the conditioning phase, the most parsimonious explanation for this finding was that even in this case the general state of deprivation was greater for the rats that were maintained in a state of deprivation for a longer time period. Notwithstanding, maybe one might also consider the possibility that the incentive value of the flavors might to differ according to the previous status of deprivation, being the hedonic impact higher for the deprived than non-deprived rats. If this was the case, one might also to expect a greater consumption for the deprived than non-deprived rats both during conditioning and test. But because during conditioning only a limited amount of fluid was available, the effect would have been observed only during the test. In any case, these findings are also entirely consistent with the notion that the change in the internal context for the non-deprived rats might attenuate the latent inhibition suffered by AX, increasing the conditioning of this compound and hence the generalization of the aversion to BX. Given that this experiment does not allow us to distinguish between these alternatives, on the basis of parsimony it seems reasonable to accept the first explanation. But whatever the explanation, the results of this experiment were largely unexpected. In general, but particularly for the deprived rats, the consumption of BX was higher for those rats in the concurrent pre-exposure condition than those given the intermixed schedule. This result is precisely the opposite of that expected according to the literature and the associative

mechanisms described previously. Thus, how might this result be interpreted?

Before entering into further theoretical speculation, a final experiment was conducted in order to examine the effect of the concurrent and intermixed pre-exposure schedule effects in deprived rats with a conditioned taste aversion preparation more similar to that employed by Rodríguez et al. (2008). The rationale for this is that first, in the previous experiments we only employed a single conditioning trial while Rodriguez et al. used two. If the effect of the concurrent schedule reported by these authors relies on the attenuation of latent inhibition of the common elements produced by this schedule, then it is possible that the reduction in the general level of conditioning hindered the possibility of finding the effect. Secondly — and also related to the potential attenuation of latent inhibition for the common elements with the concurrent schedule — another procedural detail that might serve to obscure the effect is the change in the number of bottles presented during the pre-exposure and conditioning phases. In our experiments, but not in those reported previously, during conditioning AX was presented only in one tube while during pre-exposure the compounds were always presented in two tubes. Thus, a change in context might not only be provided by the deprivation state, but also by a change in the number of bottles presented between pre-exposure and conditioning; whilst the former represents a change in the internal context of the animal, and the latter an external change, both of these modifications could potentially disrupt latent inhibition as suggested previously.

EXPERIMENT 4

Experiment 4 aimed to test whether or not the enhanced generalization expected after concurrent pre-exposure relative to the intermixed schedule might be observed in a preparation more similar to that employed by Rodriguez et al. In particular, we wanted to verify whether a contextual change such as the one that might be provided by changing the number of bottles between pre-exposure and conditioning could mask the increment in generalization expected for the concurrent schedule, and thus supply an explanation for our apparent failure to find this effect.

Experiment 4 therefore included two conditions for which the compound AX was presented in two bottles during conditioning, in the same way as during pre-exposure. The other two groups of the experiment received identical treatment to Groups CNC-ND and INT-ND in the previous experiments except that in this case, all rats received two conditioning trials and the amount of fluid available in each tube was 5 ml. Whilst it is true that changing two factors from Experiment 3 makes it difficult to identify which of these is important, it should be noted that the general aim was to more closely follow the procedure that has been shown to generate the concurrent/intermixed effect described previously.

Method

Subjects and Apparatus

Subjects were 32 naïve male Sprague Dawley rats with a mean *ad libitum* weight of 284 g (range: 222–331 g) at the beginning of the experiment. Any other details of the apparatus, stimuli, and procedures not specified here were identical to those of Experiment 2.

Procedure

Rats were randomly assigned to four equal groups ($n = 8$): CNC-1B, INT-1B, CNC-2B, and INT-2B. During pre-exposure, all the rats received 5 ml of the compounds in each bottle, these being the same for the intermixed condition and different for the concurrent. For half the participants in the intermixed and concurrent pre-exposure conditions the compound AX was presented in one bottle for conditioning, as in the previous experiments (Conditions-1B) while for the remaining half the compound AX was presented in two bottles (as in the experiments reported in other studies). Furthermore, in this experiment all the rats received two conditioning trials rather than one. All the rats then received a consumption test with the compound AX followed by a single generalization test with the BX flavor.

Results

The consumption of both the salty and sweet solutions during pre-exposure is displayed in **Table 1**. Given the fact that the available amount of each flavor for the subjects in the INT condition was twice that given to those in the concurrent condition, the overall amount consumed was greater in the former than in the latter case. The consumption of the sweet solution appeared to be somewhat higher than the salty solution but in general, from the second trial, the rats consumed most of the fluid available. A $2 \times 2 \times 4$ ANOVA conducted on these data with Schedule, Solution, and Trial as the factors found a significant main effect of Schedule, $F(1,30) = 1.975, 67$, $p \leq 0.001$, Solution, $F(1,30) = 9.17$, $p \leq 0.005$, and Trial, $F(3,30) = 44.67$, $p \leq 0.001$. There was also a significant interaction between Schedule and Trial, $F(3,30) = 12.92$, $p \leq 0.001$ (remaining, Fs ≤ 1.61). The subsequent analysis of this interaction revealed a significant effect of Schedule for the four pre-exposure trials, Fs ≥ 152.710, and an effect of Trial for both Schedules, Fs ≥ 27.34.

Figure 6 shows the mean consumption of compound AX on the two conditioning trials and the test for the four experimental conditions. It appears that consumption of AX was similar for the four conditions, and it declined across the trials in all cases. A $2 \times 2 \times 3$ ANOVA conducted on these data with Schedule, Number of bottles, and Trial as the factors found a significant main effect of Trial, $F(1,28) = 41.249$, $p \leq 0.001$ (Remaining Fs ≤ 1.16).

Finally, **Figure 7** shows the mean consumption of the BX compound for the four experimental groups. It is clear that consumption of this compound was similar for all groups. A 2×2 ANOVA with Schedule and Bottle as the factors failed to find any significant main effects of these factors or any interaction between them, Fs ≤ 1.11.

Discussion

The final experiment failed to find an effect of either the schedule or the change in the number of bottles presented during the pre-exposure and conditioning phases on conditioning or

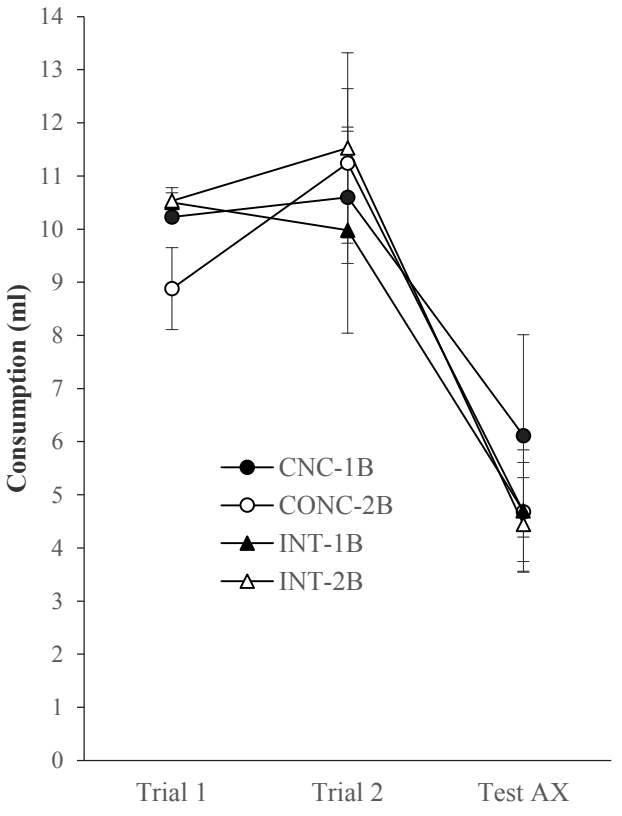

FIGURE 6 | Consumption (ml, mean ± SEM) of the AX solution during the two conditioning trials and the test for the four groups of Experiment 4.

generalization. Thus, it might be thought that this supposed contextual change was either ineffective or was of insufficient strength to be detected with our experimental procedure.

Again, this experiment also found no evidence of an enhancement in generalization after concurrent pre-exposure compared with the intermixed schedule. It is possible that our failure to find an effect that has previously been reported in the literature could still lie in parametric factors not considered in the present study. However, if the incremental effect of concurrent pre-exposure on generalization can only be obtained under a very specific set of conditions such as those reported in previous experiments, then perhaps the generality of the effect should be questioned. This is particularly important when we consider that it is precisely in this efect where we find a mismatch between the results from human and non-human animal experiments, and that there are only very few studies addressing this issue using animal experiments of the sort carried out in the present paper.

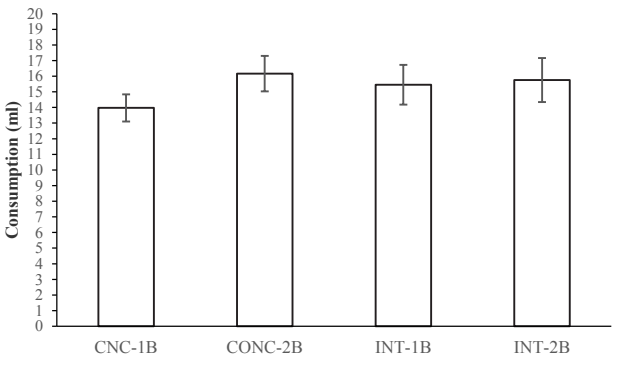

FIGURE 7 | Consumption (ml, mean ± SEM) of the BX solution during the test for the four groups of Experiment 4.

GENERAL DISCUSSION

The present study was conducted with the principal aim of examining whether the state of water deprivation exerts an effect on stimulus generalization in rats and whether such an effect might modulate the effects of pre-exposure schedules that have been established in the literature. Recently, several studies have reported important interactions between the pre-exposure schedules and the instructions given to people during pre-exposure. Thus, we may question whether these general effects of pre-exposure can be regarded as unequivocal. If this is true for humans, then perhaps other factors might be modulating the pre-exposure effects for other animals with other experimental paradigms, and these factors could be responsible certain discrepancies between the results of studies conducted with human and non-human animals. The most controversial findings in this regard are those related to the concurrent pre-exposure schedule, and therefore the present series of experiments set out to specifically examine the effect of this pre-exposure schedule on stimulus generalization.

Before entering into a discussion of the results that are relevant to the principal aims of this study, it is worth mentioning some rather unexpected but very interesting findings regarding the effects of the pre-exposure schedule on consumption in thirsty rats. Experiments 1 and 2 found greater consumption of the sweet-acid solution than the salty-acid solution from the first pre-exposure trial. This result indicates that both solutions were initially differentiated, given that the sweet solution is more palatable than the salty solution. In Experiment 1, consumption of the salty solution was reduced in general during pre-exposure, while consumption of the sweet solution increased. Because the rats were not thirsty during pre-exposure, these results might easily be explained in terms of the palatability of the solutions. Rats would progressively avoid consuming the less palatable solution, while increasing their consumption of the more palatable flavor. But rather more difficult to explain is the observation that consumption of the least and most palatable solutions differentially changed according to the pre-exposure schedule. Experiments 1 and 2 found that consumption of the less palatable solution was lower with the concurrent schedule than the other schedules. This result might perhaps be regarded as an effect of sensory contrast, consumption of the more palatable solution serving to devalue the less palatable flavor. Sensory contrast would be hindered, however, during intermixed pre-exposure. Thus, the greater consumption of the

sweet solution during intermixed than concurrent or blocked pre-exposure could not be explained in the same way. Given that the rats were able to differentiate the stimuli *a priori*, it seems unlikely that these pre-exposure schedule effects on consumption could arise from increments in stimulus differentiation. It is possible, however, that the different pre-exposure schedules might change the perception of the stimuli in a way that is distinct from increasing their discriminability. For example, it is possible that some perceptual learning mechanism that is modulated by the pre-exposure schedules could change the palatability of the stimulus. This suggestion, however, is merely speculative, and it seems reasonable to avoid any further development of this hypothesis until the appropriate experiments have been conducted to address the issue. The results for pre-exposure obtained in Experiments 3 and 4 were constrained by the limited amount of flavors available during pre-exposure and the state of water deprivation, as usually occurs in standard conditioned taste aversion procedures. These findings therefore are unable to shed further light on this issue. Nonetheless, the results from the first experiments might be regarded as a first step toward an interesting and more ecological way to assess the pre-exposure schedule effects in non-human animals, which might not be limited to effects on generalization.

Irrespective of the pre-exposure schedule received beforehand, and even when rats had no previous experience with the stimuli (CTRL groups, Experiments 1 and 2), test consumption of the conditioned flavor AX was lower than consumption of the other, similar, non-conditioned flavor BX. Thus, these latter results appear to confirm that rats were able to differentiate the stimuli even without pre-exposure. As acknowledged previously, generalization between the stimuli might be increased or reduced by different means and, in this regard, similar responses to different stimuli would not necessarily imply that the stimuli were not discriminable. However, a clear discriminative response to the stimuli on test, as found in the experiments reported here, would clearly only be possible if the stimuli had been discriminated. Furthermore, Experiment 1 found that the paired and unpaired groups did not differ in their consumption of BX. For the unpaired groups, consumption of BX would be free from generalization because AX was never conditioned. Thus it might be suggested that similar consumption of BX in the paired groups indicates that in these cases, there was no generalization of the aversion from AX to BX. This finding precludes the possibility that generalization between the stimuli would always increase after the concurrent schedule, as would be expected if excitatory links between the stimuli had been established, or their common elements had been strongly conditioned (e.g., Rodríguez and Alonso, 2008; Rodríguez et al., 2008).

Regarding the specific effect of the pre-exposure schedule on test, Experiment 1 found greater consumption of BX on the generalization test after concurrent or blocked pre-exposures to AX and BX, and in comparison with the control condition that had received no pre-exposure to any of the stimuli. If only this latter result were taken into consideration, one might conclude that concurrent and blocked pre-exposure (although perhaps not the intermixed schedule), may reduce generalization between the stimuli for no-thirsty rats. Nonetheless, the absence of any evidence of generalization between the stimuli (see Experiment 1) raises doubts about this conclusion. In the light of the findings obtained during pre-exposure, the results, on the whole, appear to indicate that the pre-exposure schedule also affected consumption of the solutions on test over and above their potential effects on generalization. Although only speculative at present, pre-exposure schedule effects could potentially serve to modulate stimulus palatability, or interact with conditioning, thus explaining such differences in the consumption on test. Again, further research is needed to directly address this possibility. Nonetheless, one of the merits of the present study is that it includes for the first time the unpaired control groups needed to address this hypothesis. Without these control groups, it is not possible to establish whether differences in consumption of the test stimulus might be related to generalization or some other factor that could potentially affect consumption of the test flavor.

Experiments 3 and 4 consistently failed to find any evidence for the expected increase in generalization after concurrent pre-exposure relative to intermixed exposure when the rats were deprived during pre-exposure, as is a routine feature of this type of experimental procedure. Experiment 3 showed a general effect of the deprivation state on increasing the consumption of the conditioned flavor, AX and the test flavor, BX. As discussed above, this finding might be interpreted in several ways. It is possible that for the non-deprived rats the possible change in the internal context that might be caused by the introduction of deprivation between the pre-exposure and conditioning phases might disrupt latent inhibition of AX. In this case, the aversion acquired by AX — and hence generalization to BX —would be stronger for rats in the non-deprived conditions. However, in the absence of independent evidence for this possibility, the more parsimonious explanation is that consumption was generally higher for the deprived rats because they were subjected to a limited drinking schedule for a longer period of time. Surprisingly, in Experiment 3 consumption of BX was higher after concurrent than intermixed pre-exposure for deprived rats. Given the absence of any differences in consumption of AX between the groups in the intermixed and concurrent pre-exposure conditions, this result might be taken to indicate that, for the deprived rats, given the similar levels of conditioning to AX, the generalization of the aversion to BX was less after concurrent pre-exposure. Clearly, this result is exactly the opposite of that reported by Rodríguez et al. (2008), and was largely unexpected, although it is perhaps worth noting that in our previous experiments such a tendency was also consistently observed. Finally, Experiment 4 failed to find evidence for an effect of a contextual change produced by changing the number of bottles presented at test and it appears that the rate of conditioning to AX and generalization to BX was similar after concurrent and intermixed pre-exposure. It is important to recognize that since the experiments differed in terms of several parameters, it is difficult to know exactly why Experiment 3 found an effect of the schedule but that the final experiment did

not. But what it is very clear here is that in none of the four experiments, conducted with different parameters, did we find the effect previously reported: an enhancement in generalization after concurrent pre-exposure relative to the intermixed schedule.

At this point, it is important to note that only two previous studies have directly compared the effectiveness of the concurrent and intermixed pre-exposure conditions regarding the generalization of a conditioned taste aversion. One study compared the concurrent schedule with the blocked schedule (Alonso and Hall, 1999), whilst another study compared intermixed schedules with varying intervals between presentations, and whilst this interval was close to 0 in some cases (see Bennett and Mackintosh, 1999), it did not constitute the type of concurrent pre-exposure as that provided here. Other studies have also tested the effect of concurrent pre-exposure to visual stimuli with other preparations (Gibson and Walk, 1956; Wills and Mackintosh, 1999). But only the studies reported by Rodríguez and Alonso (2008) and Rodríguez et al. (2008) demonstrated greater generalization of a conditioned taste aversion after concurrent exposure in comparison with the intermixed schedule. One potentially important aspect of those experiments might be that the rats did not receive concurrent pre-exposure to two similar compounds but rather presentation of one compound and an element of such a compound (i.e., AX, X). This could be an important issue, given that evidence for stronger conditioning of the common elements of the stimuli after concurrent pre-exposure has been found only when the common elements were conditioned alone, and not when such elements were conditioned in compound with the distinctive element (see, for example, Bennett and Mackintosh, 1999). Moreover, it is important to consider that evidence for excitatory links after concurrent pre-exposure has also been found with simple flavors presented as compounds (e.g., Rescorla and Cunningham, 1978) but not when compounds of flavors were presented simultaneously (e.g., Alonso and Hall, 1999).

The results of the present study do not allow us to identify which factor might be critical in determining the results reported by Rodriguez et al. and why their findings were not replicated here. But what seems clear is that, if the incremental effect of the concurrent schedule on generalization can be obtained only in specific circumstances such as those reported by Rodriguez et al., the generality of the effect should possibly be questioned, particularly if we consider that relatively few studies have focused on the effect discussed here. This issue of generality is of critical importance for the field of perceptual learning. In particular, the findings regarding the concurrent schedule effect are precisely those in which the results of research with human and non-human animals appear to disagree. Moreover, the effects of the concurrent schedule are of particular importance for both the associative and non-associative accounts of perceptual learning.

AUTHOR CONTRIBUTIONS

RA completed the experiments, analyzed the data, and wrote the paper.

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Psychometric Properties of the 20-Item Toronto Alexithymia Scale in the Chilean Population

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Alexithymia can be defined as inability to identify and describe emotions in the self. Has shown to be related to several psychological and pathological processes that can result in unsatisfactory interpersonal relationships and decreased social adjustment. Advances in research of alexithymia require the development and validation of assessment instruments, and its application to different population. With this aim, we studied the psychometric properties of the Twenty-Item Toronto Alexithymia Scale (TAS-20) in Chilean population using various modeling procedures (e.g., CFA, ESEM) in different structures (i.e., Correlated, Unidimensional, Hierarchical or Wording factors). Among the 10 models tested, the four-dimensional structure offered the best fit but with item-loading problems in the last factor (Pragmatic Thinking). We suggest that the studied version of the scale needs improvement (theoretical and empirical) to ensure optimal indices of validation for Chilean population.

Keywords: alexithymia, emotion, TAS-20, psychometric properties, ESEM, wording factor

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INTRODUCTION

The concept of Alexithymia (Sifneos, 1973) can be defined as cognitive and an affective deficit in the way that some individuals recognize and communicate emotional states (Taylor, 1984). This concept has proved to be related to several psychological and pathological processes such as: interoceptive awareness (Herbert et al., 2011), traumatic memories (Nandrino et al., 2006), suicide risk (De Berardis et al., 2017), depression (Arancibia and Behar, 2015; Melin et al., 2017), eating disorders (Behar et al., 2014), somatoform and conversion disorders (Arancibia et al., 2016), chronic pain (Saariaho et al., 2017) and also psychosomatic illness (Kano and Fukudo, 2013; Porcelli et al., 2017), among others. Resulting in unsatisfactory interpersonal relationships and decreased social adjustment (Taylor and Bagby, 2012). Alexithymia has also been associated with different kinds of addictive disorders, such as pathological gambling (Lumley and Roby, 1995; Maniaci et al., 2017), Internet addiction (Dalbudak et al., 2013; Scimeca et al., 2014), maladaptive sexual behavior (Scimeca et al., 2013), and abnormal illness behavior (Scimeca et al., 2016).

Taylor (1984), basing on a literature review of alexithymia, proposes a scale to measure this construct (Toronto Alexithymia Scale-TAS-26), based on five dimensions: (1) difficulty identifying feelings and distinguishing between feelings and bodily sensations of emotional arousal; (2) difficulty describing feelings to others; (3) externally oriented thinking (or lack of introspection); (4) social conformism and (5) lack of daydreaming and other imaginative activity (Taylor et al., 1985).

Later, Bagby et al. (1994) proposes a revised brief version of this self-report Likert scale: the TAS-20. This scale conserved only the first three traits as factors. In some way, the last two traits remained present in factors 2 and 3 as a more general operatory thinking component oriented to the preference for the external details of everyday life instead of thought content related to inner experience.

TAS-20 has been validated in clinical and non-clinical population, including mental and chronic physical illness. As point out by Taylor et al. (2003), the English version of TAS-20 has been translated to many different languages. In the last years, there have been validation reports in Arabic population (El Abiddine et al., 2017), Chinese population (Zhu et al., 2007), Croatian (Kocijan et al., 2015), Portuguese (Brazil) (Yoshida, 2007), Greek (Tsaousis et al., 2010), Dutch (Adolescents) (Meganck et al., 2012), and Turkish population (Gülec et al., 2009; Bolat et al., 2017). In Latin-American countries there has been reported a Peruvian (Loiselle and Cossette, 2001) and two Mexican versions (Pérez-Rincón et al., 1997; Moral, 2010).

In the psychometric field, the TAS-20 has demonstrated good internal consistency and test–retest reliability. The validation studies yielded to a three-factor structure congruent with the theoretical construct of alexithymia and this structure remains relatively stable in several cultures and languages (Taylor et al., 2003). In addition, it has been shown invariance of the three factors between men and women (Parker et al., 2003). Despite, is also possible to find research reports with results that show good fit indicators for four or more factors models (Tsaousis et al., 2010; Meganck et al., 2012).

Evidence has been found about the possible role of culture in the factorial structure. Culhane et al. (2009), presented evidence of invariance comparing US-Anglo and US-Hispanic student samples. On the opposite, Peruvian (Loiselle and Cossette, 2001) and Mexican (Pérez-Rincón et al., 1997; Moral, 2010) studies showed poorer fit indices. These different findings open the question about the possible role of culture in the factorial structure. The Peruvian sample contrasted a three-factor model using Confirmatory Factor Analysis. These authors reported lack of fit, particularly in the third factor, and they mention as possible reasons that this problematic factor includes 4 negative keyed items (4 of the 5 total, and 4 of 8 of the third factor), which they think it could mean a greater difficulty in answering these items and a low reliance on introspection when describing affective states. With this respect, Fernández-Jiménez et al. (2013) says that these Spanish adaptations of the scale have certain limitations: (a) the Mexican and Peruvian versions present some local particularities in language use, when compared with the Spanish spoken in Spain; (b) Latin-American versions, and the version developed in Spain, contain some items whose back-translation does not adequately reflect the meanings of the original English version of the items; (c) the indices to assess the fit of the proposed models do not meet the standards that are currently recommended (CFI $\geq$ 0.95, TLI $\geq$ 0.95, and RMSEA $<$ 0.06; Hu and Bentler, 1999; Schreiber, 2017). Moreover, the size of the sample with which the psychometric properties of Spanish version were supported was tight for some of the tested models according to Wolf et al. (2013) indications.

In Chile, only one undergraduate thesis was found to evaluate the reliability and validity of the TAS-20 in 236 university students in the city of Chillán (Sáez and Tiznado, 2012). However, only a principal component analysis was applied in this study.

Advances in research of alexithymia require the development and validation of assessment instruments, and its application to different population. With this aim, we studied the psychometric properties of the Twenty-Item Toronto Alexithymia Scale (TAS-20), which now is, the most widely used instrument to measure Alexithymia. We have applied this scale to Chilean university students and we performed analysis using different model testing procedures.

MATERIALS AND METHODS

Participants

A total of 516 students voluntarily participated in this study. Most were female (n = 340, 65.8%, *Mean Age* = 22; *SD* = 5.1), and 176 were males (34.2%, *Mean Age* = 22; *SD* = 3.7). 54.7% (n = 282) of the students were from the cities of La Serena and Coquimbo, 8.9% (n = 46) from Iquique, 9.1% (n = 47) from Antofagasta, 8.1% (n = 42) from Santiago, 9.5% (n = 49) from Temuco and 9.7% (n = 50) from Punta Arenas, throughout Chile. They all spoke Spanish as their mother tongue.

Measures

The English version of the TAS 20 (see **Table 1**; Meganck et al., 2008) was translated and adapted to the Spanish language following the international guidelines (Hambleton, 1994; International Test Commission, 2010).

This Spanish version of TAS-20 includes 20 self-report questions distributed into three subscales: (1) difficulty identifying feelings and distinguishing between feelings and bodily sensations in emotional activation, (2) difficulty in the verbal expression of emotions, and (3) externally oriented thinking. The answers values fluctuate between 1 and 5 points (1 is the lack of it and 5 is most present), and items 4, 5, 10, 18, and 19 must be inverted before adding up scores. Total score interval is 20–100, while a person is considered alexithymic with a score $\geq$ 61.

Procedure

The students voluntarily completed the scales after reading and written informed consents. All procedures in this study followed (a) the principles of Helsinki Declaration (World Medical Association, 2013), (b) the APA ethical standards (Including 2010 and 2016 Amendments), and (c) the guidelines of the National Commission for Scientific and Technological Research of Chile (CONICYT). There were no missing data in this study.

Tested Models

The six basic models tested by Meganck et al. (2008, 2012) were compared using Confirmatory Factor analysis (see **Table 2**). The first Model (a) is proposed as a unidimensional structure where all items reflect alexithymia. Model (b) is a two-factor structure with DIF and DDF items forming one factor and EOT items

TABLE 1 | Items of the TAS-20 Spanish version.

Number	Dificulty identifying feelings (Dificultad identificando sentimientos)
1	A menudo estoy confuso con las emociones que estoy sintiendo
3	Tengo sensaciones físicas que ni incluso los doctores entienden
6	Cuando estoy mal no se si estoy triste, asustado o enfadado
7	A menudo estoy confundido con las sensaciones de mi cuerpo
9	Tengo sentimientos que casi no puedo identificar
13	No sé qué pasa dentro de mi
14	A menudo no se por qué estoy enfadado
Number	**Dificulty describing feelings (Dificultad describiendo sentimientos)**
2	Me es difícil encontrar las palabras correctas para mis sentimientos
4	*Soy capaz de expresar mis sentimientos fácilmente*
11	Me es difícil expresar lo que siento acerca de las personas
12	La gente me dice que exprese más mis sentimientos
17	Me es difícil revelar mis sentimientos más profundos incluso a mis amigos más cercanos
Number	**Externally oriented thinking (Pensamiento orientado al exterior)**
5	*Prefiero analizar los problemas mejor que sólo describirlos*
8	Prefiero dejar que las cosas sucedan solas, mejor que preguntarme por qué suceden de ese modo
10	*Estar en contacto con las emociones es esencial*
15	Prefiero hablar con la gente de sus actividades diarias mejor que de sus sentimientos
16	Prefiero ver espectáculos simples, pero entretenidos, que dramas psicológicos
18	*Puedo sentirme cercano a alguien, incluso en momentos de silencio*
19	*Es útil examinar mis sentimientos para resolver problemas personales*
20	Buscar significados ocultos a películas o juegos disminuye el placer de disfrutarlos

Translated and adapted from Meganck et al. (2008); italic items = negatively keyed.

forming the second factor (Haviland and Reise, 1996; Loas et al., 1996; Erni et al., 1997). Model (c) (Kooiman et al., 2002) proposed the same structure of model (b) but with only 16 items (items 16, 17, 18, and 20 were erased). The fourth model (d) is composed by three factors: DIF, DDF, and EOT (Bagby et al., 1994) and the fifth model (e) is a three-factor solution (Ritz and Kannapin, 2000); DIF and DDF items as one factor and EOT split into two factors (PR and IM). Finally, the sixth model (f) is a four-factor solution that considers the dimensions DIF and DDF plus de sub-dimensions PR and IM that were split from EOT (Müller et al., 2003).

Further models were also tested in this study for those models described above that provided adequate fit to data: hierarchical (Hi), wording factor (Wf), and exploratory structural exploratory models (ESEMs). When the relationship between first-order factors is high, hierarchical models of indirect effects could be proposed. The Hi second-order structure was tested with the general alexithymia concept as a higher level.

TABLE 2 | Basic models of the TAS-20 (proposed in previous literature).

		Model					
Dimensions	**Items**	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**
ALEX	1-20	F1					
DIF	1, 3, 6, 7, 9, 13, 14		F1	F1	F1	F1	F1
DDF	2, *4*, 11, 12, ~~17~~				F2		F2
EOT	*5*, 8, *10*, 15, ~~16~~, ~~*18*~~, *19*, ~~20~~		F2	F2	F3		
IM	*10*, 15, 16, *18*, *19*					F2	F3
PR	*5*, 8, 20					F3	F4
	Dimensional structure	Un	Cf	Cf	Cf	Cf	Cf

Un, unidimensional; Cf, correlated factors; ALEX, alexithymia; DIF, difficulty identifying feelings; DDF, difficulty describing feelings; EOT, externally oriented thinking; IM, lack of importance emotions; PR, pragmatic thinking; italic items, negatively keyed; cross items, not proposed in model (c).

The choice of one model or another is a theoretical, applied, and parsimony-based decision. The Wf was tested as an orthogonal method factor (bifactor) on which the negatively keyed items are located (4, 5, 10, 18, and 19). Finally, ESEM approach integrates the flexibility of EFA and the advantages of CFA (Asparouhov and Muthén, 2009; Garrido et al., in press). Even if these models are not contemplated in the classical frameworks (models 1–6), the exploratory approach could end up suggesting a more efficient latent structure than those that have been derived from previous studies (e.g., less or specific dimensions in a bifactor structure; Arias et al., 2016).

Data Analysis

We firstly explored the reliability and adequacy of factor analysis indices for each TAS-20 scale. These statistics were: the explained proportion of variance (PEV), Barlett's test and KMO index for the adequacy of the analysis, the number of advised dimensions in each scale with the parallel analysis technique (Garrido et al., 2013), Cronbach's α and McDonald's ω as an estimate of the reliability. For this purpose, we employed an unweighted

TABLE 3 | Descriptive statistics and Pearson correlations for the TAS-20 scales.

	ALEX	DIF	DDF	EOT	IM	PR
ALEX						
DIF	0.797*					
DDF	0.794*	0.585*				
EOT	0.684*	0.209*	0.301*			
IM	0.637*	0.171*	0.307*	0.934*		
PR	0.514*	0.201*	0.175*	0.747*	0.461*	
n ítems	20	7	5	8	5	3
M	53.11	16.37	15.25	21.49	13.66	7.83
SD	14.14	6.84	5.24	6.67	5.00	2.68
Skewness	0.27	0.77	0.29	0.32	0.34	0.30
Kurtosis	−0.23	0.17	−0.35	−0.64	−0.78	−0.39

*ALEX, alexithymia; DIF, difficulty identifying feelings; DDF, difficulty describing feelings; EOT, externally oriented thinking; IM, lack of importance emotions; PR, pragmatic thinking. *$p < 0.01$.*

least-squares (ULS) estimator based on polichoric correlations because of the ordinal nature of data. The use of robust estimators as ULS are recommended because they may produce more accurate parameter estimates than Maximum Likelihood (Forero et al., 2009; Schmitt, 2011; Li, 2014). If necessary an oblique rotation was used because of possible secondary and related dimensions (Promin; salient larger values > 0.30; Lorenzo-Seva, 1999).

In addition to the descriptive and complementary factorial indices of the scales, different latent structures were tested (CFA and ESEM). These models were executed using the same factorial considerations as the previous (polichoric correlations and ULSMV estimator available in Mplus version 7.4). Cut-off point recommendations of Schreiber (2017) were followed for goodness of fit indices criteria: CFI $\geq$ 0.95, TLI $\geq$ 0.95, and RMSEA < 0.06.

RESULTS

Table 3 shows descriptive statistics computed with SPSS 22. The average values of the scales showed a similar central tendency to those reported by previous studies for students (Taylor et al., 2003; Meganck et al., 2012). However, the variance of the items was slightly higher in the sample of Chilean students. The most closely related scales were DIF and DDF on the one hand, and IM and PR on the other.

DIF and DDF proposed scales (**Table 4**) had good exploratory values to be considered as possible single dimensions (one advised dimension per scale, adequate proportions of explained variance, Bartlett test $p < 0.01$ and KMO $\geq$ 0.8; Lorenzo-Seva and Ferrando, 2013). EOT, IM, and PR scales did not have adequate exploratory values to be considered as possible single dimensions. It should be noted its lack of reliability (α or

TABLE 4 | Reliability and adequacy factor analysis indices for the TAS-20 proposed scales.

	ALEX	DIF	DDF	EOT	IM	PR
n items	20	7	5	8	5	3
AND	4	1	1	2	2	2
PEV	0.59	0.54	0.51	0.36	0.47	0.40
Bartlett (*df*)	3792.6 (190)*	1362.1 (21)*	487.3 (10)*	1252.4 (28)*	790.5 (10)*	13.2 (3)*
KMO	0.869	0.898	0.767	0.786	0.835	0.503
Cronbach's α	0.829	0.855	0.730	0.663	0.650	0.102
McDonald's ω	0.838	0.861	0.764	0.690	0.677	0.311

*ALEX, alexithymia; DIF, difficulty identifying feelings; DDF, difficulty describing feelings; EOT, externally oriented thinking; IM, lack of importance emotions; PR, pragmatic thinking; AND, advised number of dimensions by Horn's parallel analysis; PEV, proportion of explained variance; Barlett, Barlett's test; KMO, Kaiser-Meyer-Olkin Index. *$p < 0.001$.*

TABLE 5 | Fit indices for the basic models of the TAS-20.

	Model				Fit índices					
		s	Dimensions	i	χ^2	*df*	χ^2/df	CFI	TLI	RMSEA
1 (a)	CFA	Un	ALEX	20	1955,11	170	11.50	0.612	0.566	0.143
2 (b)	CFA	Cf	DIF/DDF-EOT	20	1000,04	169	5.92	0.819	0.797	0.098
3 (*c*)	CFA	Cf	DIF/DDF-EOT	16	786,10	103	7.63	0.908	0.893	0.113
4 (d)	CFA	Cf	DIF-DDF-EOT	20	918,69	167	5.50	0.837	0.814	0.093
5 (e)	CFA	Cf	DIF/DDF-PR-IM	20	997,93	167	5.98	0.819	0.794	0.098
6 (*f*)	CFA	Cf	DIF-DDF-PR-IM	20	904,80	164	5.52	0.938	0.813	0.094

s, structure; Un, unidimensional; Cf, correlated factors; i, number of items; CFI, Comparative Fit Index; TLI, Tucker-Lewis Index; RMSEA, root-mean-square error of approximation.

TABLE 6 | Fit indices for the alternative structures of the TAS-20.

	Model				Fit índices					
		s	Dimensions	i	χ^2	*df*	χ^2/df	CFI	TLI	RMSEA
7 (f)	CFA	Hi	DIF-DDF-PR-IM	20	1259,64	166	7,59	0.762	0.728	0.113
8 (f)	CFA	Wf	DIF-DDF-PR-IM	20	785,05	160	4,91	0.870	0.846	0.085
9 (f)	ESEM	Cf	DIF-DDF-IM-PR	20	273,65	116	2,36	0.966	0.944	0.051
10 (f)	ESEM	Wf	DIF-DDF-IM-PR	20	238,45	112	2,13	0.972	0.953	0.047

s, structure; CFA, confirmatory factor analysis; ESEMs, exploratory structural equation models; Hi, hierarchical alexithymia 2nd order factor; Wf, wording factor on which the negatively keyed items load (Items 4, 5, 10, 18, and 19); Cf, correlates factors; i, number of items; CFI, Comparative Fit Index; TLI, Tucker-Lewis Index; RMSEA, root-mean-square error of approximation.

$\omega < 0.70$). Note that these factors reflect a small number of items (especially PR).

Reliability indices of the TAS-20 scales were similar to those reported by Bagby et al. (1994) and Meganck et al. (2012). Both DIF and DDF tend to show good magnitudes of reliability, whereas EOT (and its sub-factors IM and PR), as previous studies, showed inadequate reliability indices (<0.80).

All confirmatory modeling analyzes on the basic models reported poor or not adequate fit indices (**Table 5**). Only models (c) and (f) reached an acceptable CFI value (>0.90). Models (d) and (f) referred the lowest RMSEA. In relation to the other basic models and considering the set of indicators (χ^2, RMSEA, CFI, and TLI), the best structure for these datasets was four correlated factors (f).

We also tested two bifactor models with the purpose of exploring other dimensional structures (correlated and uncorrelated specific factors together with a general factor of alexithymia). These tested solutions were uninterpretable, that is, poor fit indices (i.e., RMSEA > 0.10) and mostly low or negative factor loadings.

CFA analysis considering Hi or Wf structures in model (f) did not meet acceptable fit indices. As expected, the alternative ESEM approach of the TAS-20 was supported by good fit indices ($\chi^2/df < 0.3$, RMSEA < 0.06, CFI and TLI > 0.90). Although not shown in **Table 6**, the rest of the basic models (a–e) were also tested with an ESEM approach. None of these models reported better ESEM fit indices than the (f).

Table 7 shows the estimates of model 10 (f) (with the best fit-indices among the tested). In the table we can see clearly how the estimates loaded mainly in their corresponding factors (≥0.30) except for the fourth factor. PR was defined mainly by one loading (20) and two cross-loadings (15 and 16). Item 8 did not load substantially on any factor of the extracted ones.

TABLE 7 | ESEM estimates for (f) + Wording factor.

		Model 10 (f) + Wf			
D	**i**	**F1**	**F2**	**F3**	**F4**
DIF	01	**0.65**	0.19	−0.08	−0.07
	03	**0.55**	−0.01	−0.01	0.04
	06	**0.76**	0.04	−0.03	0.03
	07	**0.85**	−0.04	−0.06	0.05
	09	**0.84**	0.02	−0.02	0.02
	13	**0.65**	0.19	0.08	0.01
	14	**0.54**	0.17	0.09	0.03
DDF	02	**0.48**	**0.32**	0.03	−0.08
	04*	0.02	**0.62**	−0.29	0.00
	11	0.24	**0.51**	0.12	−0.07
	12	0.01	**0.66**	0.08	−0.09
	17	−0.03	**0.63**	0.29	0.08
IM	10*	−0.03	−0.02	**0.60**	−0.15
	15	−0.11	0.35	**0.39**	**0.38**
	16	0.03	−0.01	**0.31**	**0.59**
	18*	0.03	−0.01	**0.48**	−0.25
	19*	−0.01	0.03	**0.64**	−0.24
PR	05*	0.20	−0.23	**0.43**	0.26
	08	0.20	0.00	0.17	−0.26
	20	0.19	−0.16	0.07	**0.44**
	F2	0.53			
	F3	0.01	0.17		
	F4	0.27	0.10	−0.05	

*D, dimension; i, item number; *negatively keyed items. Loadings greater than 0.30 are in bold. Correlations between factors are presented in the lower part of the table.*

DISCUSSION

A Chilean version of TAS-20 has been studied and the results show evidence of its reliability and construct validity, detecting some problems that must be addressed in future studies (e.g., items 5, 8, and 15 could be complicated to translate into Spanish because it requires the interpretation of "mejor que" [best than] in the sense of "en vez de" [instead of]; or item 10 in our culture the meaning of "estar en contacto" could be interpreted as a more concrete physical touch and therefore its comprehension requires the capacity to understand a metaphor, which is not of a common sense use).

Among the 10 tested models, including a unidimensional factor or the combination of different factors, we only found relatively good fit estimates for a model of four factors. These results do not follow the direction of some previous studies in English speaking population (that support a three-factor structure). They rather show consistency with the results observed in Latino-American population reported by Loiselle and Cossette (2001) in a Peruvian sample and Moral (2010) in a Mexican sample. Compared to these studies there is also a difference to be considered, that is, the fourth factor shows weaker indices of fit in our study. As a possible explanation of differences, we adhere to Taylor et al. (2003) hypothesis that points out that differences between Latino-American and Anglo-American population could be due to translation problems or related to cultural aspects of alexithymia in Latino-American population. The third factor structure not only has been problematic in Latinamerica. Moreover, this can be enhanced by (a) respondent's low reading comprehension skills, particularly affecting negatively keyed items and those that include more abstract ideas, (b) a culture that is not used to make verbal language distinctions between "internal and external" world, and (c) the presence of patriarchal cultural beliefs that contradict the idea of affectivity as a positive domain. These beliefs usually see emotions as dangerous and to be controlled (Arón, 2001; Blanco and De la Corte, 2003).

On the other hand, it could be as in previous studies, that the psychometric properties of some factors were not adequate (Zhu et al., 2007). For this reason, it is understandable that structures modeled with CFA forcing IM and PR dimensions regularly show inadequate adjustment. As an alternative analysis, (f) ESEM model showed the best-fit indices in comparison with previous

studies. In this sense the work of Craparo et al. (2015) already showed an oblique solution that substantially improves the fit of a TAS-20 structure.

The improvements that we recommend include the revision of dimensions IM and PR items, particularly item number 8, for its lack of load on the extracted factors. It may be necessary to reduce the scale. Gori et al. (2012) measured alexithymia and reported good reliability and validity indices with only five items (the PTI-Alexithymia Scale; "PTI-AS"). The PTI-AS highly correlates with TAS-20.

A limitation of this study is that the sample includes only university students, so it is not generalizable to the entire population and it is not directly comparable with studies performed with samples of young adolescents or clinical population. Given the use of a non-probabilistic and homogeneous sample for this study, more empirical support of the proposed factorial solution of this study with Chilean samples is necessary (e.g., young adolescents or clinical population).

CONCLUSION

We found evidence that reliability and construct validity of TAS-20 are not optimal for Chilean student population. Factor analysis shows a structure of four-factor model being the best fit, but with problems in the fourth factor. Therefore, we suggest that the studied version of the scale needs improvement to ensure optimal indices of validation for Chilean population.

AUTHOR CONTRIBUTIONS

MG-A led the project and made part of the Introduction, Method, and Discussion. AM-M made part of the Introduction and the Discussion and led Method and Results. SG made part of the Introduction, part of the Method, and part of the Discussion. AU made part of the Introduction and the Discussion and led part of the Method.

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BRIEF RESEARCH REPORT
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"Keep That in Mind!" The Role of Positive Affect in Working Memory for Maintaining Goal-Relevant Information

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Some studies have demonstrated a beneficial role of Positive Affect on working memory (WM) by either applying protocols of mood induction or assessing naturally occurring state Positive Affect. However, there are no studies directly linking Positive Affect as a stable personality-like trait with WM. We aimed to address this potential relationship using the Positive and Negative Affect Schedule scale and contra-lateral delay activity (CDA) as measures of trait Positive Affect and WM Capacity, respectively. We also sought to investigate the impact of a neutral or unpleasant emotional state on this relationship. Participants performed a change detection task, while a neutral or an unpleasant emotional state was induced. Our results showed a positive robust correlation between trait Positive Affect and WM Capacity for both neutral and unpleasant emotional states, as revealed by the neuroelectrophysiological gold-standard measure of WM, namely, CDA. These data suggest a tangible role of trait Positive Affect in the cognitive ability of maintaining goal-relevant information in WM, such that even a highly disruptive state is not sufficient to corrupt this relationship.

Keywords: Positive Affect, PANAS, emotion, working memory, event-related potential (ERP), contralateral delay activity (CDA), individual variability

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Figueira JSB, Pacheco LB, Lobo I, Volchan E, Pereira MG, de Oliveira L and David IA (2018) "Keep That in Mind!" The Role of Positive Affect in Working Memory for Maintaining Goal-Relevant Information. Front. Psychol. 9:1228. doi: 10.3389/fpsyg.2018.01228

INTRODUCTION

Positive emotions can lead to feeling more active and enthusiastic, and they have been associated with psychological health (Vazquez, 2017) and well-being (Burton and King, 2009). The amount and the tendency to experience positive emotions are together known as Positive Affect, which can be described from the perspective of either a transient mood (state) or a stable personality (trait) (Watson et al., 1988). When referring to trait, Positive Affect is associated with more frequent and intense episodes of pleasant state (Watson and Tellegen, 1985; Watson, 2002). It has been suggested that Positive Affect influences cognitive processes by increasing cognitive flexibility and/or by fostering goal-pursuit motivation (Gray, 2001; Isen and Reeve, 2005; Nadler et al., 2010).

Working memory (WM) is a cognitive system with limited capacity responsible for keeping goal-relevant information in focus (Goldman-Rakic, 1996; Cowan, 2001; Baddeley, 2012). WM allows the manipulation, use, and recall of relevant information and, if necessary, enables behavior

changes to better cope with the challenge of achieving a goal (D'Ardenne et al., 2012; Stout et al., 2013). Some studies have investigated whether a direct effect of state Positive Affect on WM exists (Carpenter et al., 2013; Yang et al., 2013; Brose et al., 2014; Storbeck and Maswood, 2016). For example, both Yang et al. (2013) and Storbeck and Maswood (2016) observed better performance on a WM task by individuals in whom a positive mood had been induced. These studies focused on the affect state by applying different protocols of mood induction (Yang et al., 2013; Storbeck and Maswood, 2016) or assessing naturally occurring state Positive Affect (Brose et al., 2014). Affect trait and state may influence cognition in a different and interactive way, and considering affect trait is important (Dunn et al., 2010; Harlé and Sanfey, 2010; Hur et al., 2015; Riepl et al., 2016). Furthermore, no neuroelectrophysiological studies have addressed this association.

The contralateral delay activity (CDA) is a robust neuroelectrophysiological biomarker for WM Capacity (Vogel and Machizawa, 2004; Luria et al., 2016). An advantage of CDA in relation to other behavioral/neural measures of WM capacity is that it allows us to isolate in time the sustained activity exclusively related to the maintenance of items in WM (Fukuda et al., 2010). This event-related potential (ERP) is a great representative of the limited capacity of WM, as the amplitude of CDA increases along with the number of items to be maintained in WM but reaches a limit at approximately four items (Vogel and Machizawa, 2004; Vogel et al., 2005).

Previously, using measures of CDA, we showed that an unpleasant emotional state diminishes the WM capacity limit, demonstrating the disruptive effect of unpleasant emotional stimuli on WM (Figueira et al., 2017). Indeed, unpleasant stimuli and states can blunt goal-directed behavior and impact our decisions (Vuilleumier and Schwartz, 2001; Erthal et al., 2005; Dolcos and McCarthy, 2006; Pereira et al., 2006, 2010; Fernandes et al., 2013; Stout et al., 2013). In this vein, Sanchez et al. (2015) observed that neural reactivity for distractive unpleasant images is attenuated by trait Positive Affect, suggesting a potential crossplay between trait Positive Affect and the processing of unpleasant stimuli. In fact, Hur et al. (2015) supports the idea that trait Positive Affect may play a protective role as a compensatory mechanism in cognitive control when the individual is experiencing an unpleasant state.

To our knowledge, there is a gap in the literature regarding the influence of trait Positive Affect on WM using a neuroelectrophysiological approach. The current study aimed to fill this gap using the CDA, a gold-standard neuroelectrophysiological index of WM, to elucidate the putative interplay between trait Positive Affect and WM Capacity. Additionally, we investigated whether the induction of an unpleasant emotional state would disrupt this relationship.

MATERIALS AND METHODS

Participants

The sample consisted of 33 participants (undergraduate students) from Figueira et al. (2017) and three added participants, for a total of 36 participants. The data sets of seven participants had to be excluded due to excessive behavioral errors (2), the production of extremely noisy data (2) and extensive eye movements (3). The remaining 29 participants (19 women) had a mean age of 21.67 years (SD = 4.69). All the participants were right-handed (Oldfield, 1971), reported normal color vision and normal or corrected-to-normal visual acuity. They also reported no psychiatric or neurological problems and were not using any central nervous system drugs.

Stimuli and Procedure

The emotional state was created by the presentation of 120 pictures (20° × 16°) that were equally distributed in two categories: neutral (intact bodies) and unpleasant (mutilated bodies). They were presented in a blocked fashion to ensure the induction of a sustained modulatory effect by emotional picture viewing, as tested in Figueira et al. (2017). The neutral and unpleasant picture categories differed in both valence and arousal (see Supplementary Material, item 1).

After picture offset, the participants performed a change detection task (Vogel and Machizawa, 2004) that consisted of an arrow cue pointing to the to-be-attended hemifield and two sequential arrays (the memory array and the test array) of 2 or 4 colored squares in both hemifields (**Figure 1A**). The participants were instructed to covertly shift their attention toward the cued hemifield and press one of two buttons to indicate whether one of the squares changed color in the test array in relation to the previous memory array. The memory and test arrays differed in 50% of the trials. The uncued hemifield remained unchanged for all trials. For more information regarding the experimental paradigm, see Figueira et al. (2017).

Trait Positive Affect Evaluation

Individual trait Positive Affect was assessed with the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) at the beginning of the experimental session. This scale comprises two subscales consisting of 10 adjectives each: Positive Affect and Negative Affect. Because the aim of this study was to evaluate trait Positive Affect, only the Positive Affect dimension score was considered. To reflect TRAIT-LIKE Positive Affect stability, we instructed the participants to report the amount of positive emotions they experienced in general (Watson et al., 1988).

Electroencephalogram (EEG) Recording

The EEG data were recorded and analyzed using our standard procedures (Figueira et al., 2017). As the CDA reflects visual WM processing (Luria et al., 2016), we computed CDA waveforms from the parietal-occipital P3/P4 and O1/O2 electrode pairs (Vogel and Machizawa, 2004) within an epoch of 1200 ms, starting 200 ms prior to the onset of the arrow cue and ending 1000 ms later. The mean peak amplitude was obtained over a 500–1000 ms time window during the retention interval (RI), in which the contralateral negativity of CDA is observed (**Figure 1B**).

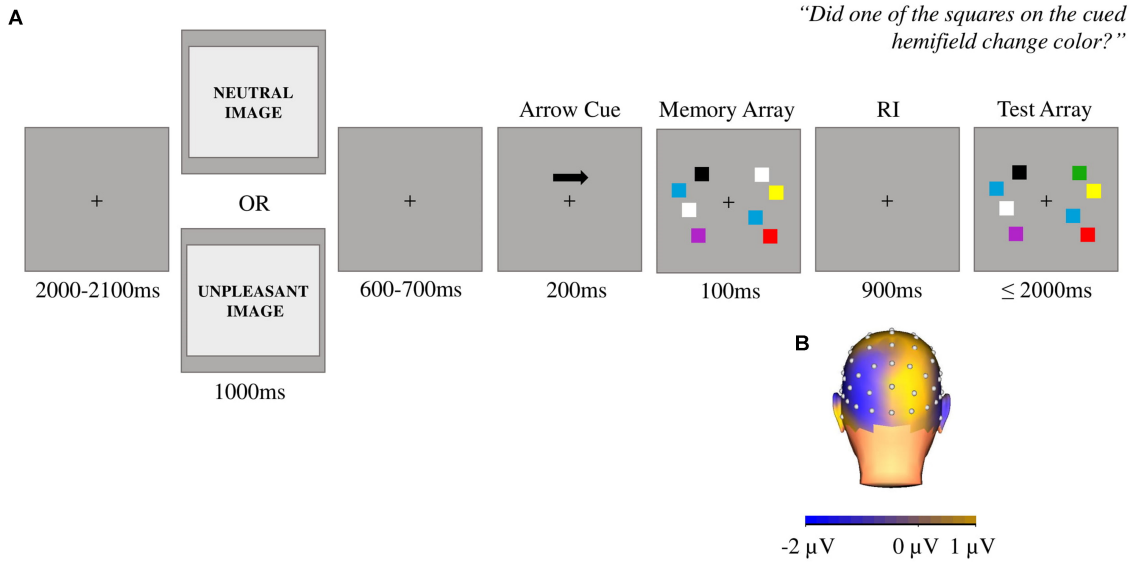

FIGURE 1 | (A) Example of the sequential order of events in a trial. Each trial began with a fixation cross that remained on the screen throughout the entire trial. After 2000 to 2100 ms from the fixation cross onset, a neutral or an unpleasant picture was presented for 1000 ms in a blocked fashion. The change detection task started at 600 to 700 ms following the offset of the picture with an arrow cue for 200 ms. The arrow cue was replaced with a 100 ms memory array. The arrow pointed to the left on half of the trials. Then, there was a 900 ms retention interval (RI), followed by a test array. Participants were instructed to respond as quickly as possible and to try not to commit errors. **(B)** Topographical map showing the expected contralateral negativity during the RI arising from the left parietal-occipital electrodes when the cued hemifield was on the right.

Statistical Analysis

To ensure that the results reported in Figueira et al. (2017) were not affected by the added participants, we replicated the following analysis: the mean peak amplitude obtained over the CDA time window was submitted to a repeated-measures ANOVA with the within-subject factors *site* (P3/P4 vs. O1/O2), *number of squares* (2 vs. 4) and *emotional state* (neutral vs. unpleasant). The Newman–Keuls procedure was used to test for *post hoc* differences when applicable.

To investigate the relationship between trait Positive Affect and WM Capacity, we conducted a robust Spearman correlation analysis (Rousselet and Pernet, 2012) between the PANAS Positive Affect score and the increase in CDA amplitude between the 2- and 4-squares task conditions during the unpleasant and neutral emotional states. The 95% confidence interval (CI) was computed using the MATLAB toolbox to perform robust correlation analysis available at http://sourceforge.net/projects/robustcorrtool/ (Pernet et al., 2013), based on bootstrapping procedures. Correlations were considered significant when CI did not encompass zero. Because CDA has a negative amplitude, for better visualization purposes, we multiplied the values by (−1). Thus, larger values represent a higher WM Capacity because they represent the increase in CDA amplitude between the 2- and 4-squares conditions.

RESULTS

The repeated-measures ANOVA revealed a significant main effect of *number of squares*, $F(1,28) = 7.05$; $p < 0.05$; however, the main effects of *site* and *emotional state* did not approach significance, $F(1,28) = 1.79$; $p = 0.21$ and $F(1,28) = 0.38$; $p = 0.54$, respectively. The interaction effect between the *number of squares* and *emotional state* was significant, $F(1,28) = 5.66$; $p < 0.05$. As demonstrated by *post hoc* analysis, the expected increment in CDA amplitude from 2 to 4 squares was revealed in the neutral emotional state, $p < 0.05$. On the other hand, the unpleasant emotional state affected the increment in CDA amplitude from 2 to 4 squares, $p = 0.49$. CDA's amplitudes for neutral and unpleasant emotional states did not differ during the 2-squares condition, $p = 0.25$. During the 4-squares condition, the CDA amplitude was greater during the neutral emotional state than during the unpleasant emotional state, $p < 0.05$. The error results and CDA grand average waveforms can be found in the Supplementary Material, items 2 and 3.

The mean Positive Affect score was $M = 33.65$ ($SD = 4.64$), whereas the mean WM Capacity, indexed by the increase in CDA amplitude from the 2- to 4-squares conditions (Vogel and Machizawa, 2004; Figueira et al., 2017), was $M = 0.81$ μV ($SD = 1.04$) for the neutral emotional state and $M = 0.14$ μV ($SD = 1.37$) for the unpleasant emotional state. The increase in CDA amplitude from 2 to 4 squares showed a significant positive robust correlation with Positive Affect score for the neutral emotional state ($\rho = 0.41$; $p < 0.05$, CI = [−0.706304 to 0.00408713]) and for the unpleasant emotional state ($\rho = 0.49$; $p < 0.05$, CI = [−0.764525 to 0.131]) (**Figure 2**). Hence, as the Positive Affect scores increase, the difference between CDA amplitude during the 2- and 4-squares conditions also increases, independently of whether the present emotional state is neutral or unpleasant.

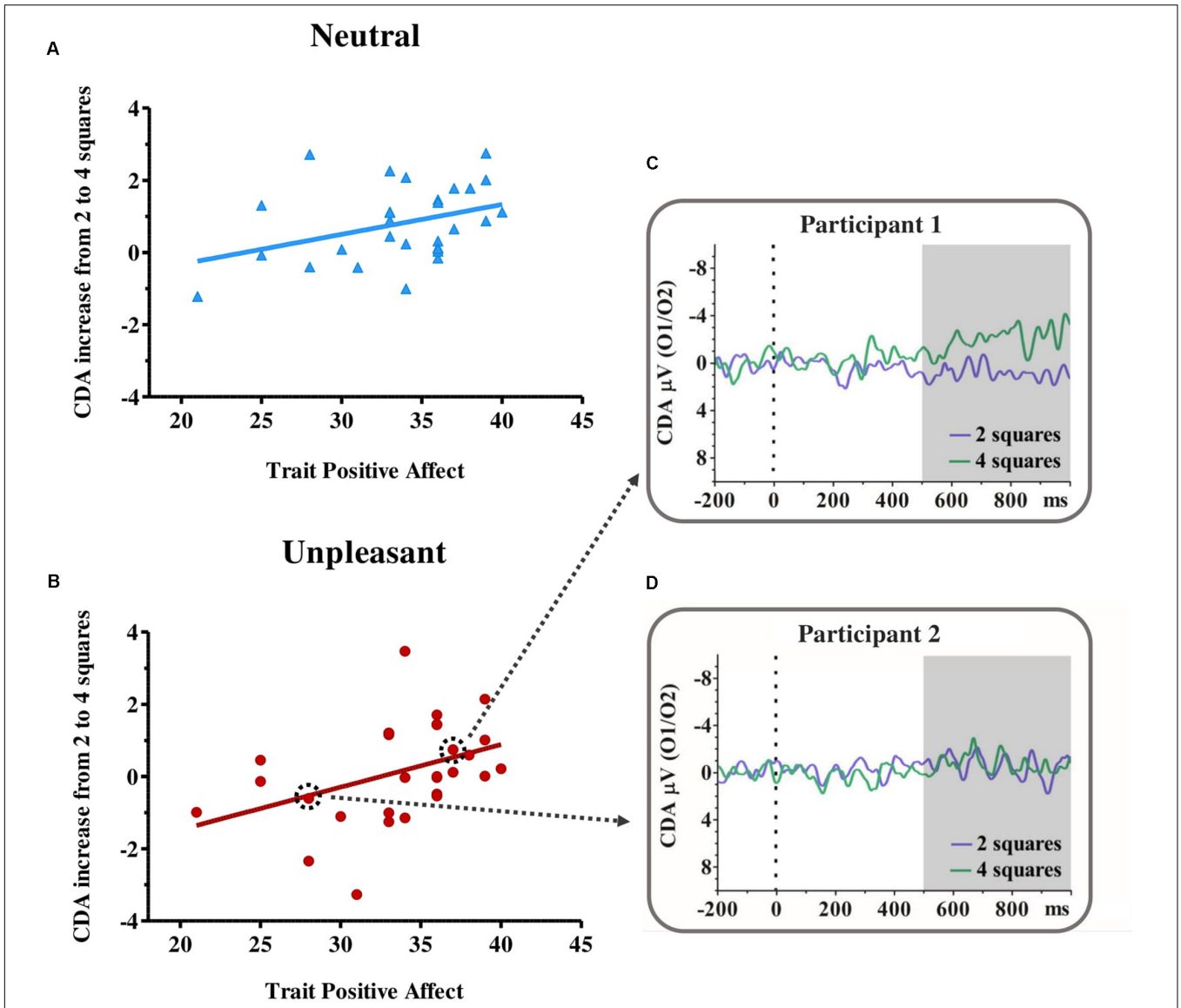

FIGURE 2 | Correlation between participants' working memory capacity [CDA mean amplitude (μV) increase from 2 to 4 colored squares] and trait Positive Affect during the neutral **(A)** and unpleasant emotional state **(B)**. On the right, two examples of the CDA waveform for the 2- (purple) and 4- (green) squares condition during the unpleasant emotional state are shown, obtained from an individual who had a higher score on trait Positive Affect [Participant 1 **(C)**] and an individual who had a low score on trait Positive Affect [Participant 2 **(D)**]. Note that in the correlations, there are overlapping points, 4 in the neutral state and 5 in the unpleasant state.

DISCUSSION

In this work, we provide strong evidence for the beneficial effect of Positive Affect on WM Capacity. To our knowledge, this is the first study to provide evidence of a constructive relationship between Positive Affect and WM Capacity using a gold-standard neuroelectrophysiological marker. Our data indicate that trait Positive Affect increases with WM Capacity, even in the face of an unpleasant state.

Most research on the relationship between Positive Affect and cognition has focused on state Positive Affect. For example, in the systematic review performed by Vanlessen et al. (2016), the authors proposed that the transient experience of positive emotions grants a flexible management of attentional resources that are built on cognitive demand. However, the mechanism through which trait Positive Affect influences cognitive processes, such as WM, remains unclear. Therefore, our study addresses this topic and demonstrates a positive relationship between trait Positive Affect and WM Capacity, suggesting that trait Positive Affect enhances the cognitive ability of holding information in memory.

The literature greatly supports the idea that unpleasant emotional states or stimuli are highly disruptive to cognitive resources (Vuilleumier and Schwartz, 2001; Erthal et al., 2005; Dolcos and McCarthy, 2006; Pereira et al., 2006, 2010; Fernandes et al., 2013; Stout et al., 2013). Indeed, Figueira et al. (2017) have

demonstrated that WM Capacity is diminished by unpleasant states, leading to a failure that may disrupt the ability to perform many activities on a daily basis. Surprisingly, our results demonstrate a positive correlation between trait Positive Affect and WM Capacity that is preserved even during a concurrent disruptive unpleasant state. This result may also be explained by a down-regulation of unpleasant emotions through emotion regulation, as the latter is positively related to both Positive Affect (Fredrickson, 2001) and WM (Xiu et al., 2016). Considering the absence of a pleasant-state condition in our study, we are unable to uncover the cognitive relationship between trait Positive Affect and emotional state as a whole. Addressing this issue in the future would be interesting.

Our study adds new data showing that WM Capacity is case sensitive to trait Positive Affect. Our results provide a new avenue toward understanding the interplay between Positive Affect and WM. In view of our results, we hypothesize that Positive Affect enhances WM Capacity through two distinct mechanisms: by assuming a mediator role by maintaining a goal throughout a task and by shielding task disruption from unpleasant distractors. Further studies should address how trait Positive Affect can enable better coping strategies in the face of unpleasant emotional states or stimuli.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Federal Fluminense University, University Hospital Ethics Committee (HU). The protocol was approved by the University Hospital Ethics Committee (HU, CAAE: 53505615.0.0000.5243). All the subjects provided written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

ID, MP, LdO, and JF developed the study concept and the study design. JF and IL performed the data collection. JF and LP performed the data analysis and interpretation under the supervision of ID. JF and LP drafted the manuscript. ID, MP, LdO, and EV substantially contributed to the interpretation of the data and provided important critical revisions. All the authors approved the final version of the manuscript. They also agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.01228/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Maternal Odor Exposure Modulates Acceptance of a Bitter Taste in Newborn and Infant Rats

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The acceptance of bitter, aversive, substances during early life is enhanced by stimulation with familiar, pre-exposed odors. Newborn rats exhibited heightened grasp responses toward an artificial nipple dispensing quinine, and drank more of this bitter solution, if concurrently stimulated with a lemon odor they had been exposed to shortly after birth. It yet unknown, however, if odors made familiar via normative developmental milestones also acquire modulatory influence upon seeking and intake of basic tastants. The current study assessed the influence of exposure to maternal odor on intake and grasp responses toward a surrogate nipple providing quinine, in 3-day (Experiment 1) or 12-day (Experiment 2) old, Wistar rat pups. The results revealed enhanced seeking and intake of the bitter solution, but not of water, in animals tested in the presence of the mother (and hence exposed to its odor cues), at both ages, compared to counterparts given either no explicit odor stimulation or stimulation to the odor of an unrelated dam. These results, obtained with a biologically relevant odor, are consistent with those previously found with a neutral, arbitrary odor. It seems that during the early stages of development, familiar odors regulate the acceptance of non-palatable, otherwise rejected, flavors.

Keywords: odor, taste, ontogeny, bitter, mother, rats

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INTRODUCTION

Various neuroendocrine mechanisms are in place to keep the caregiver in close proximity during the early development of altricial species, which in turn increases access to food, protection from predators, and warmth (Upton and Sullivan, 2010). Attachment behavior is regulated by sensorial stimuli such as sight and olfaction (Morrow-Tesch and McGlone, 1990; Janzen et al., 1999; González-Mariscal and Poindron, 2002). Birds learn to identify the caretaker through visual stimuli, briefly after birth. However, in mammals, olfaction has a more predominant role (Nowak et al., 2011).

In humans, olfactory stimuli are essential in the acquisition of early learning and induce approach toward the maternal breast, thus helping deploy intake behaviors (Mennella et al., 2016). During the last trimester of gestation fetuses ingest the amniotic fluid (Lipchock et al., 2011), detecting odors and tastes carrying individualized sensory information from the mother. Re-exposure to these stimuli, for instance during breastfeeding, impacts diet choice during postnatal life (Mennella et al., 2001; Lipchock et al., 2011). Taste is essential to ingestive behavior

and is a product of the gustatory and olfactory systems working together (Auvray and Spence, 2008).

Infants from a variety of species usually reject bitter food. There seems to be, however, a sensitive period in which early experiences with bitter tastes predispose them to accept this, otherwise unpalatable, taste (Beauchamp and Mennella, 2009). Human babies accept bitter tastes during the first 4 months, but reject them afterwards (Mennella et al., 1996; Beauchamp and Mennella, 2011). This sensitive period would allow culture-dependent shifts in innate reactivity to food and flavors, which are essential for establishing long-term healthy eating habits (Trabulsi and Mennella, 2012).

The newborn rat is an appropriate animal model for the study of gustatory learning. Rat pups develop the sense of sight and hearing by the third week of life (Sullivan and Holman, 2010), therefore they depend on their mother's odor to monitor the localization of the nest and the caregiver (Hofer, 2005). The absence of that odor triggers locomotion and distress calls (i.e., ultrasonic vocalizations) that increase the likelihood of reuniting with the dam. Thus, olfactory learning can help discriminate changes in odors resulting from the fluctuating dietary patterns from the mother (Landers and Sullivan, 2012). The evolutionary relevance of olfactory learning apparently favored the emergence of a sensitive period (i.e., first 10 postnatal days, PND) in which familiar odors are preferred, even after their association with a moderate aversive situation (Sullivan et al., 2000). Even neutral, arbitrary, odors can significantly control the pup's behavior if made familiar by odor pre-exposure. For instance, exposure to a lemon odor, made familiar by pre-exposure, increased responsiveness toward a surrogate nipple in neonatal rats, compared to rats that did not have experience with this odor (Miller and Spear, 2008, 2009, 2010).

Neonate rats discriminate between different tastants, as shown by differential suckling behavior toward saline and quinine, in the first hours of life (Nizhnikov et al., 2002). Yet there are few studies assessing the interaction of taste and smell at this early age. Kamenetzky et al. (2014) gave rats a brief exposure to a lemon odor (or no exposure, control group), shortly after birth, and then tested intake from and seeking of a lemon-odor scented, surrogate, nipple dispensing 0.1% quinine. Pups that had been pre-exposed to the lemon odor exhibited, when compared to controls, significantly greater seeking of the nipple and quinine intake than controls. This effect did not occur when the nipple provided saccharin. This intriguing experiment suggests that familiar odors can enhance the acceptance of aversive solutions.

The present study assessed seeking and drinking of a bitter solution in 3-day old or 12-day old rats, in the presence of a scent extracted from their own mother or from another, unrelated, mother. We aimed at assessing the generality of the pre-exposure effect described in our previous work. We used, instead of an arbitrary (e.g., lemon) odor, a biologically relevant odor, made familiar through the natural history of interactions and shared milieu of the dam/pup dyad. Our hypothesis was that the explicit stimulation with the maternal odor, yet not the odor of an unrelated dam, would increase quinine seeking and intake.

MATERIALS AND METHODS

Subjects

Ninety-six, 3 or 12 day-old male and female pups, rats were employed. These animals were derived from 13 dams, mated at the vivarium of Instituto de Investigaciones Médicas Alfredo Lanari (IDIM-CONICET, Argentina). Thirty-four and sixty-two animals were employed in Experiments 1 and 2, respectively. Pups were born by natural delivery, the day of parturition was considered PND 0, and litters had *ad libitum* access to water and food (Cooperación Co., Buenos Aires, Argentina). The vivarium had a 12 h/12 h light/dark cycle, with lights on at 7 am, and controlled temperature (22°C) and humidity. Rats used in these experiments were maintained and treated in accordance with the guidelines for animal care and use established by the National Institutes of Health (National Research Council, 1996). The protocol was approved by the Animal Care and Use Committee of IDIM-CONICET of Instituto de Investigaciones Médicas Alfredo Lanari (Approval number 0602-17).

Experimental Designs

Experiment 1 measured seeking and intake of quinine in 3-day old rats via a two-group design (Experimental and Control, 18 and 16 animals in each group, respectively). Experiment 2 assessed intake of quinine or water in 12-day old rats. In Experiment 2, the pups were distributed in four groups, as a function of the solution delivered via the nipple (water or quinine), and depending on whether the test was conducted in the presence of the pup's dam or in the presence of an unrelated dam. Each group was composed by 15–16 animals.

Surrogate Nipple

The surrogate nipple used in Experiment 1 was made from rubber latex (AMACO rubber latex, Indianapolis, IN, United States) and molded into a conical form to measure 12 mm long with a rounded tip measuring 1 mm in diameter and the base measuring 2.5 mm in diameter. The base of the surrogate nipple was attached to the end of an angled dental probe to facilitate presentation by the experimenter (Petrov et al., 1997). Polyethylene tubing (Clay Adams, Sparks, MD, United States) extended throughout the length of the nipple and attached to a syringe containing the solutions. The syringe had a hole on the wall that generated a hydraulic system. When the oral cavity of the pup contacted with the latex and the tip was pressed by the mouth, a negative pressure was generated that allowed voluntarily intake. The subject was clamped in a semi-supine posture into a "vest" made from ultra-thin, elastic rubber. This light restraint prevented righting attempts but did not otherwise produce discomfort nor hinder movement. The pups were kept, before the test, in a controlled heat chamber (Simen, Buenos Aires, Argentina) maintained at 35°C (PND3) or 28°C (PND12).

Procedure

On the morning of PND 3 (Experiment 1) or PDN12 (Experiment 2), the dam was separated from their pups and anesthetized with a combination of ketamine (40 mg/kg) and

xylazine (5 mg/kg). The pups were placed into the heating chamber for 15 min. Each pup was gently stimulated in the urogenital region with cotton to induce urination or defecation and subsequently weighted in a precision scale.

Experiment 1 followed an adapted version of the protocol described in Kamenetzky et al. (2014). Briefly, each pup was equipped with a vest and attached to a tempered mirror. The test involved a 6-min stimulation with a 0.1% quinine solution (i.e., 100 mg quinine in 100 ml of distilled water), dispended through the artificial nipple. The tip of the nipple was gently applied on the perioral area, in the presence (Experimental Group) or absence (Control Group) of the mother. The anesthetized mother was placed 2 cm away from the nose of the pup.

In Experiment 2 the pups, 12-day old rats, were separated from the dam and intraorally cannulated, as described in Pautassi et al. (2008). The intraoral devices (PE 10 polyethylene tubing, 5 cm length) were made using a heat source to flatten one of the ends. A dental needle, attached to the non-flanged end, was used to place the cannula in the middle portion of the mucosa, with the flattened end inside. This procedure requires ±8 s per subject and does not induce major stress (Spear et al., 1989). After the cannulation, the pups were group-housed into a chamber kept warm (i.e., 28°C) until the time of the test.

Animals were then weighted, stimulated in the urogenital region and placed into the heated chamber for 10 min. The anesthetized mother (either the pup's dam or an unrelated dam) was also placed into the chamber, in close proximity to the pups. The inclusion of pups exposed to an anesthetized nursing mother controlled the effects of factors other than the exposure to the mom's odor (e.g., heat source, social contact). In order to assess the specificity of the type of taste associated to the mother's odor, another condition included the delivery of water.

To prevent pups from suckling from the nipples, the dams were wrapped in a Clingfilm envelope, which had an opening in the back to allow dissemination of the mother's odor but prevented the pups from coming in contact with the nipples. Animals were weighted and placed into the heated chamber for 10 min. Each intraoral cannula was attached to a PE50 length, which in turn was connected to an infusion pump (APEMA, Buenos Aires, Argentina), equipped with four Prexajet 5-ml syringes. The pump delivered water or quinine at a continuous rate, and the volume was adjusted to deliver 2.5% of the pup's body weight. This procedure combines forced administration of the solution with voluntary intake, because the rats can actively reject the solution infused by emitting aversive taste responses (e.g., chin rubbing, passive drips, etc.). Body weights were registered at the end of each test, and the heated chamber was cleaned with a damp cloth.

The percentage of body weight gained [(post-weight–pre-weight)/pre-weight] × 100] was calculated in both Experiments, whereas total time of grasp, number of grasp, latency to grasp and mean grasp duration toward the artificial nipple were recorded in Experiment 1 only. The behavioral coding was analyzed by two independent observers. The inter-rater reliability was >85% for all the behaviors, and was calculated was follows [Agreements/(Agreements + Disagreements)]*100; for the frequency, and (Shorter amount of time/Longer amount of time)*100, for duration.

Statistical Analysis

The body weight gained and the behavioral responses emitted during Experiment 1 were analyzed via Analysis of Variance (ANOVA). In Experiment 2 we used two-way ANOVA guided by our priory hypotheses, we used planned comparisons to analyze intake scores, separately for water and quinine, between pups tested in the presence of the pup's dam or in the presence of an unrelated dam. Preliminary analysis indicated that, across variables, sex did not exert significant main effects nor significantly interacted with the remaining variables. Therefore, data were collapsed across sex. The alpha level was set at 5%.

RESULTS

Figure 1 shows percentage of body weight gained during the test, total time of grasp, number of grasps, mean grasp duration, and latency to grasp, in Experiment 1. It seems that the presence of the maternal odor significantly reduced the latency to grasp the nipple, increased the time attached to it and heightened quinine intake. These impressions were confirmed by the statistical analyses. Subjects evaluated in the presence of the mother exhibited, when compared to controls assessed in isolation, significant increases in body weight gained [$F(1,32) = 4.98$, $p < 0.03$] and in total time of grasp [$F(1,32) = 6.58$, $p < 0.01$]; and a significant decrease in the latency to grasp the nipple [$F(1,32) = 4.53$, $p < 0.04$]. Likewise, the ANOVA for number of grasp responses neared significance [$F(1,32) = 3.78$, $p > 0.06$]. Mean grasp duration was similar across groups [$F(1,32) = 2.97$, $p > 0.09$].

The data gathered in Experiment 2 has been depicted in **Figure 2**. The analysis of ANOVA did not show interactions between the different groups. Therefore, guided by our priory hypotheses, we used planned comparisons to analyze intake scores. The animals stimulated with quinine in the presence of the odor of an unrelated dam, but not those tested in the presence of their own dam, rejected, for the most part, the quinine solution. Planned comparisons indicated that quinine intake in the group tested in the presence of their own dam was significantly higher compared to the rest of the groups, $F_{3,61} = 4.83$, $p < 0.005$. Pups stimulated with the odor of their own mother drank as much quinine as water ($p > 0.05$).

DISCUSSION

Olfactory cues produced by the dam regulate the attachment of pups (Sullivan and Holman, 2010). It is possible that maternal odors regulate other behavioral domains, yet the information on this topic has been scarce. The present study stimulated pups with a bitter solution, delivered through an artificial nipple at PND 3 (Experiment 1), or through intraoral administration at PND 12 (Experiment 2). The odor of the familiar significantly enhanced, when compared to control groups that were evaluated

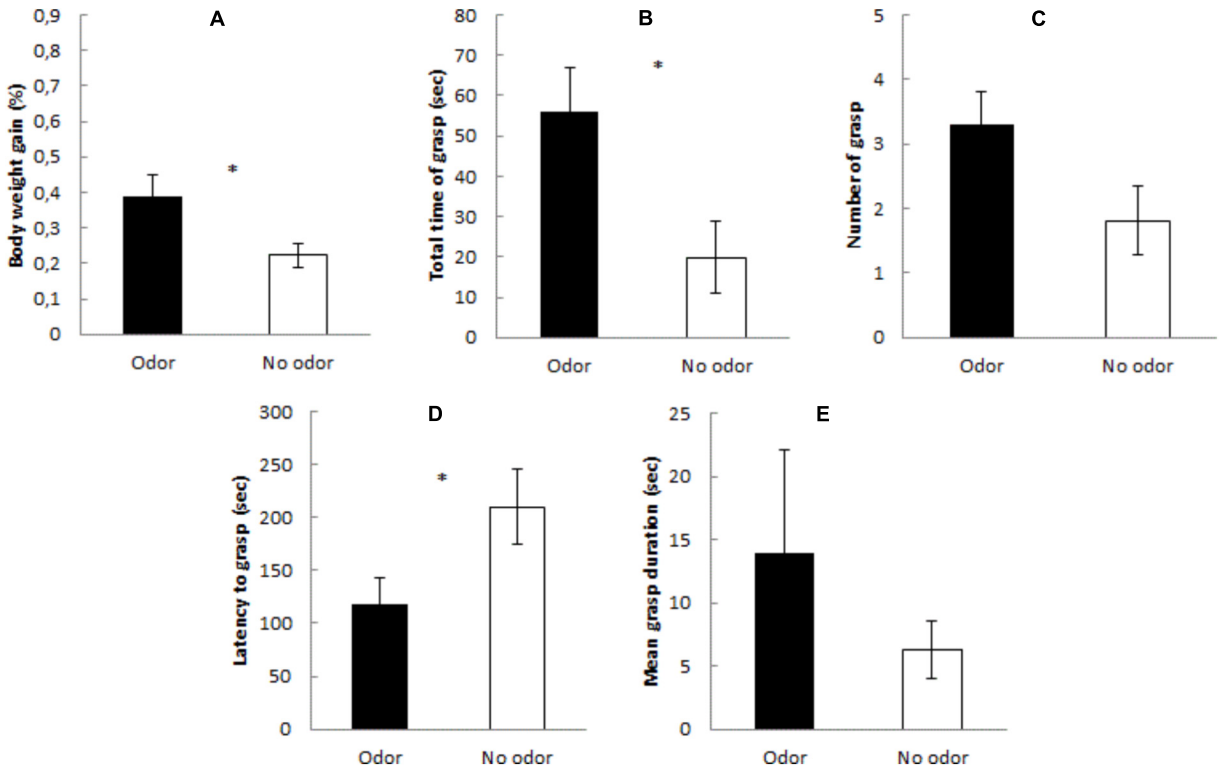

FIGURE 1 | Mean percentage (±SE) of **(A)** body weight gain, **(B)** total time of grasp, **(C)** number of grasp, **(D)** latency to grasp, and **(E)** mean grasp duration during the 6-min presentation of the artificial nipple containing quinine in the presence of the mother (black bars – Odor condition) or in the absence of the mother (white bars – No odor condition). *Indicates $p < 0.05$.

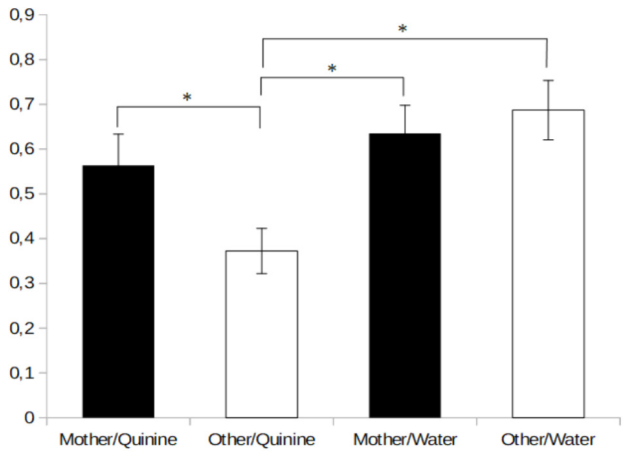

FIGURE 2 | Mean percentage (±SE) of body weight gain during the 6-min administration of quinine or water in the presence of the mother's odor or in the presence of another lactating mother. *Indicates $p < 0.05$.

in isolation (Experiment 1) or in the presence of non-familiar dam (Experiment 2), seeking and intake of this bitter, normally aversive, solution.

These results suggest that exposure to the stimuli present in the nest are sufficient to overcome the innate disposition to reject aversive solutions, which was likely inherited due to the association of bitter tastes with poisons and malaise. Experiment 1, however, has the caveat of testing controls in isolation. It can be proposed that greater quinine intake, as found in the "dam group," is merely a side-effect of the additional warmth or social contact provided by the mother. Experiment 2, however, tested all pups in close proximity with an adult female, yet those tested in the presence of an unrelated dam rejected quinine (i.e., the level of quinine intake was lower than the level of water intake in peers tested in similar conditions) and drank significantly less of this bitter solution quinine than pups tested in the presence of their own dam. Therefore, it seems that only the odor of a familiar adult female (i.e., the dam) heightens quinine intake. The mother's odor does not increase water consumption, further pinpointing the specificity of the phenomenon under analysis. The pups stimulated with water drank as much of that fluid regardless the presence of their own mother or the presence of another, unrelated mother. The data is consistent with, and generalize, previous results in which exposure to a lemon odor increased responses to an artificial nipple dispensing a 0.1% solution of quinine, but only when the lemon odor had been pre-exposed immediately after birth. A possible limitation of the present study is, however, that the experiments were conducted at different ages. On the positive side, this suggests that, in the rat, the ability of maternal odor to inhibit the deployment of aversive responses seems to last until the end of the second week of life.

These results relate to previous evidence suggesting that the presence of the mother decreases the offspring response to aversive stimulation. More in detail, during a sensitive period that lasts up to PND 10, approximately, the pups learn conditioned odor preferences even after pairing odors with an electric shock of moderate intensity (Sullivan et al., 2000). Furthermore, maternal odor exposure seems to decrease stress responses in rats' 2-weeks old or older, a phenomenon known as *social buffering* (Gunnar and Sullivan, 2017).

Also related to the present results is that, due to nutritional reasons, some newborns are fed with a protein-hydrolysate formula that has a strong bitter taste (Beauchamp and Mennella, 2011). This formula is rejected by babies older than 4–5 months, yet it seems to be accepted by younger babies, who display normative weight gain curves after chronic feeding with the formula (Mennella et al., 2011). Also, babies that had been exposed to garlic (Mennella and Beauchamp, 1993) or alcohol (Mennella and Beauchamp, 1991), due to prior maternal consumption, also seem to increase acceptance of milk contaminated with these flavors. Unpublished data from our laboratory also showed that infant rats stimulated with an alcohol taste in the presence of maternal odor increased consumption responses toward alcohol. Altogether, these data and studies suggest that during the early stages of development there is a sensitive period for flavor learning, in which the presence of the maternal odor may increase responses to bitter compounds, otherwise rejected, or can endow neutral, arbitrary learned odors with the ability to increase acceptance of these compounds.

Human infants learn about flavor stimuli through the amniotic fluid and later through breast milk and through formula. This learning can affect the development of food choices, thus exerting long-term effects upon health (Trabulsi and Mennella, 2012). The present report represents progress toward the development of an animal model for analyzing the influence of factors that increase the intake of bitter-tasting foods.

AUTHOR CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, and analysis of the data and review of the manuscript. MI, AS, and GK conducted the experiments and analyzed the data. MI, AS, RP, and GK wrote the manuscript.

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Transitive Inference Remains Despite Overtraining on Premise Pair C+D−

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Transitive inference (TI) has been studied in humans and several animals such as rats, pigeons and fishes. Using different methods for training premises it has been shown that a non-trained relation between stimuli can be stablished, so that if A > B > C > D > E, then B > D. Despite the widely reported cases of TI, the specific mechanisms underlying this phenomenon remain under discussion. In the present experiment pigeons were trained in a TI procedure with four premises. After being exposed to all premises, the pigeons showed a consistent preference for B over D during the test. After overtraining C+D− alone, B was still preferred over D. However, the expected pattern of training performance (referred to as serial position effect) was distorted, whereas TI remained unaltered. The results are discussed regarding value transfer and reinforcement contingencies as possible mechanisms. We conclude that reinforcement contingencies can affect training performance without altering TI.

Keywords: transitive inference, value transfer, reinforcement, overtraining, bias reversal

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INTRODUCTION

In the context of animal behavior, transitive inference (TI) refers to the establishment of a relation between pairs of stimuli which have not been previously trained. In the general procedure, the subject is exposed to pairs of stimuli named: A+B−, B+C−, C+D−, D+E− in a training phase, where letters represent stimuli, +means that the stimulus is followed by the delivery of a reinforcer, and − means that the stimulus was not followed by a reinforcer. After reaching over chance level of correct responses (usually more than 80% or 85%) on each pair of stimuli, the subject is exposed to non-adjacent pairs (BD, AC, AD, CE, BE, and AE) under extinction or non-differential reinforcement, which is called the test phase. The BD pair is regarded as the crucial pair, so that when the subject prefers B over D, TI is assumed. BD is regarded as the crucial pair of the so-called anchor effect. Because B and D were equally reinforced and non-reinforced, it is assumed that for solving BD TI is required.

Performance during training phase usually follows a U-shaped pattern with better performance in more extreme premise pairs (e.g., AB and DE), an effect referred to as the *serial position effect* (SPE). During the test phase, performance follows a similar pattern in which accuracy is better for more extreme premise pairs (e.g., AE) than for more central premise pairs (e.g., BD). Additionally, latencies tend to be greater for more central premises than for more extreme pairs. This pattern of performance and latencies is referred as *symbolic distance effect* (SDE). Both effects are usually reported in TI procedures (Vasconcelos, 2008).

The phenomenon of TI has been reported in different species, such as rodents (Roberts and Phelps, 1994; Takahashi and Ushitani, 2008), birds (von Fersen et al., 1991; Weiß et al., 2010; Lazareva and Wasserman, 2012; Mikolasch et al., 2013), fishes (Grosenick et al., 2007), chimpanzees

(Gillan, 1981), and humans (Lazareva and Wasserman, 2010). TI has also been simulated with neural networks (Frank et al., 2003).

Different approaches have been addressed in order to explain the preference for B over D when non-adjacent pairs of stimuli are presented across test phase. According to biological approaches TI could be adaptive under conditions of social complexity where ranking possible adversaries is necessary during mating competition. Several studies with mongoose lemurs (*Eulemur mongoz*) (Maclean et al., 2008), pinyon jays (*Gymnorhinus cyanocephalus*) and scrub-jays (*Aphelocoma Californica*) (Bond et al., 2003) support this explanation, where social complexity predicts the ability to establish TI. Other studies with fishes (*A. burtoni*) (Grosenick et al., 2007) and chickens (*Gallus gallus domesticus*) (Daisley et al., 2010) indirectly support the effect of social complexity on TI.

From a reinforcement-based approach, the preference for B over D during a test cannot be predicted from the direct values based on reinforcement strength acquired during training, since both stimuli were equally reinforced and not reinforced. Therefore, in order to explain TI, it is necessary to assume that the value acquired by a particular stimulus does not depend exclusively on the number of reinforcers directly received, but it could also depend on the value transferred from other stimuli presented during each trial. This is captured by the value transfer hypothesis (Zentall and Sherburne, 1994). According to this hypothesis, B would receive more indirect value from A than the indirect value received by D from C. Because B is paired with A, which is always reinforced (A+B−), whereas D is paired with a partially reinforced C (B+C−, C+D−) and a never reinforced stimulus (D+E−). Therefore, this difference will favor more associative strength for B compared with D. Since value transfer has been shown in simultaneous discriminations (see Zentall and Sherburne, 1994) and in TI procedures (Steirn et al., 1995), the aforementioned inequity in reinforcement has been proposed as an explanation for TI. Several models based on reinforcement and value transfer hypothesis have been developed in order to explain TI (see, for example, Wynne, 1995; Siemann and Delius, 1998).

Finally, cognitive approaches appeal to mechanisms such as verbal and spatial representations, memory and logical rules. Based on this approach, TI is allowed by a ranking of the stimuli in which the most reinforced stimuli are earlier in the inferred sequence while less reinforced stimuli are later. Thus, the subject's performance requires the storage of the serial position of each stimulus, and deficits in stablishing TI can be predicted by learning impairments or alterations in the serial position of the stimuli. Studies in animals have shown that circular arrangements of the stimuli impair TI in rats (Roberts and Phelps, 1994), which supports the idea of serial representation. Other studies have shown that hippocampal lesions may impair TI in rodents (Dusek and Eichenbaum, 1997; Heckers et al., 2004), as well as in other species such as pigeons (Strasser et al., 2004; Lazareva et al., 2015), which supports the involvement of memory in TI.

The present study is focused on the relationship between reinforcement contingencies and the formation of TI. More specifically, our aim is to explore the effect of extended training of all premise pairs and overtraining in a single premise pair. Previous studies have analyzed the effect of overtraining on TI. For example, Lazareva et al. (2004) and Lazareva and Wasserman (2006, 2012) explored the effect of overtraining the pair D+E−, as a way to increase associative strength for D, and the preference for D over B on the later test performance. In our study, we overtrained the pair C+D− (usually the most difficult discrimination to learn). Assuming value transfer, the overtraining of premise C+D− should have an indirect effect over the performance in premise B+C−. The latter premise should become more difficult to solve, because C gets more associative value through overtraining and, therefore, competes with premise B+ to receive the response. If only the direct associative strength is responsible for TI, then the effect of overtraining premise C+D− should be only a better discrimination in this pair without affecting the pigeon's performance in the B+C− pair. Subsequent performance during the test would be disrupted.

MATERIALS AND METHODS

Participants

Ten pigeons (*Columba livia*) maintained at approximately 85% of their free-feeding body weight by food deprivation, with permanent access to water, served as subjects. All the subjects had previous experience in an autoshaping procedure; a blue light operated as the cue. After this training, they were exposed to different ratio schedules; a white light operated as the cue. All pigeons were housed in metal cages, two pigeons were individually housed (25 cm × 25 cm × 30 cm) and eight were paired housed (40 cm × 40 cm × 45 cm). Pigeons were exposed to a 12:12 day–night cycle during the experiment, with lights on from 07:00 to 19:00 h.

The experimental procedure was approved by the local Ethical Committee of the Center for Studies and Investigations in Behavior, by the University of Guadalajara committee for animal experiments, and met governmental guidelines.

Apparatus

Two acoustically isolated operant chambers (MED ENV-007, 25.4 cm wide × 21 cm high × 31.8 cm long). The frontal panel of the cages was flat and composed of three subpanels. The middle panel had a food hopper (ENV-123AM). Over the food hopper a 2.5 cm diameter response white-lighted cue was installed (ENV-123AM), 20.5 cm above the floor grid bars. On each side subpanel, at the same height as the white-lighted cue, it was installed a 2.5 cm diameter key that could be illuminated in different colors (ENV-131M). A house light (ENV-215M) was installed in the rear panel. Each experimental cage was placed inside a sound isolated chamber (ENV-018V) equipped with a fan (VF80A11- AC 115 v). MED-PC IV software was used for programming and recording data.

Procedure

Pigeons were trained in five simple overlapped item pairs A+B−, B+C−, C+D−, D+E− where the positive stimuli were always reinforced and the negative stimuli were never reinforced. Stimuli A, B, C, D, and E were red, green, blue, yellow, and white cues,

respectively, for all pigeons. The position of the stimuli in each trial was randomized.

The general procedure was based on the work of Lazareva and Wasserman (2010), with the main differences being the presence of correction trials, the manipulation of response rates, and the presence of correct responses criterion from one phase to other (see **Table 1**). We omitted those features trying to isolate the effect of extended exposure and pavlovian contingencies from the effect of response cost. All sessions ended after reaching the number of trials programmed for the session, or after a duration of 1 h (see **Table 1**), whichever came first.

We used an ABCB design, where A means training, C represents overtraining and B means test (see **Table 1**).

Following Lazareva and Wasserman (2010), training (A) was divided in four phases. In phase 1 the pair A+B− was trained for 2 sessions up to 200 trials per session. Therefore, 2 more sessions (phase 2) were programmed to train the pairs A+B− and B+C−. Once again, the maximum number of trials by session was 200. Then, the pair C+D− was trained alone (phase 3) for 2 more up to 200-trials sessions each one. Finally, the four pairs were trained together (phase 4) in 9 more up to 200-trials sessions until reaching 80% of correct responses on average. In phases 2 and 4 the order of the pairs training was randomized. Therefore, at the end of training (A) each pigeon was exposed to up to 3,360 trials, distributed as follows: pair A+B− up to 1,140 trials, pair B+C− up to 740 trials, pair C+D− up to 940 trials, and finally pair D+E− up to 540 trials.

Overtraining consisted in exposition to 200 programmed trials of pair C+D− alone. Thereafter, 400 programmed trials of all premises. Therefore, overtraining consisted in a maximum amount of 600 trials for each pigeon. Considering training and overtraining, each pigeon was exposed to the following number of pairs presentations: A+B− up to 1,240 trials, pair B+C− up to 840 trials, pair C+D− up to 1240 trials, and finally pair D+E− up to 640 trials.

The day after finishing training and overtraining phases one test session was programmed. In each test session, up to 240 trials of non-adjacent pairs BD, AC, AD, CE, BE, and AE were presented in random order under non-differential reinforcement.

A trial started with the house light and white center key illuminated. After the white key was pecked, it turned off and the side keys were illuminated. The first response in one of the two side keys turned them off, and a reinforcer was delivered or not depending on the chosen stimulus.

During training and overtraining, correct choices (i.e., reinforced stimuli in the pair presented: +) were always followed by 4 s of food access to the food hopper that operated as the reinforcer. After a correct choice, an inter-trial interval (ITI) of 10 s was inserted. After an incorrect choice (i.e., non-reinforced stimuli in the pair presented: −), no reinforcer was delivered and the ITI lasted 14 s. During the ITIs the house light stayed off.

During tests, a non-differential reinforcement schedule operated. Thus, both premises on each pair were partially reinforced with an equal probability of 0.5.

Results

To explore the effect of training and overtraining, we considered the average percentage of correct choices and latencies of response on each premise for the last two sessions of training (i.e., Phase 4) and overtraining (i.e., Phase 6). For test phases we considered only the last two sessions of Test 1 and the single session of Test 2. Because we found in a few rare trials very extreme latencies, especially during test phases, we did not consider for analysis trials with latencies higher than 2 s. For all phases, latencies showed a positive asymmetrical distribution. Thus, we estimated the average of median latencies for each session considered. All statistical analyses were performed with SPSS v20.

The total number of programmed presentations of each premise was not the same (see **Table 1**). **Table 2** shows the number of trails completed for all subjects and sessions for each premise, as well as the average percentage of correct responses considering only the two last sessions of training and overtraining.

Figure 1A shows the average percentage of correct responses in the last two sessions of training. Each dot represents the average percentage of correct responses in the pair where the stimulus was always reinforced. We

TABLE 1 | Experimental design.

	Training (A)				Test 1 (B)	Overtraining (C)		Test 2 (B)
	Phase 1	Phase 2	Phase 3	Phase 4		Phase 5	Phase 6	
Stimuli					B D			B D
				A+ B−	A C		A+ B−	A C
	A+ B−	A+ B−	C+ D−	B+ C−	A D	C+ D−	B+ C−	A D
		B+ C−		C+ D−	C E		C+ D−	C E
				D+ E−	B E		D+ E−	B E
					A E			A E
Sessions	2	2	2	9	4	1	2	1
Trials by session	200	200	200	240	200	200	200	240

Training was divided in four phases and overtraining in two phases. Pairs were sequentially trained or overtrained following the Lazareva and Wasserman (2010) sequence. After training and overtraining, the test session of the non-adjacent pairs was also included. The number of sessions of each phase is described, as well as the maximum number of trials presented by each session. The number of pairs trained or tested by session was equally distributed.

TABLE 2 | Number of effective presentations of each pair regarding all subjects and sessions across training and overtraining.

Premises	# of presentations	
	Training	Overtraining
A+B−	8347 (78.72 ± 3.86)	9331 (86.68 ± 2.86)
B+C−	5902 (87.21 ± 2.05)	6888 (47.03 ± 5.57)
C+D−	7448 (40.28 ± 5.44)	10400 (78.56 ± 3.46)
D+E−	4360 (91.08 ± 1.74)	5363 (84.26 ± 3.83)

In parenthesis appears the average percentage of correct responses considering the last two sessions of training and overtraining phases.

found a function that resembles a SPE after training, with high percentages of correct responses in more extreme premises.

Our data show that, as has been previously reported, the worst performance is in premise C+D− (39.87%), and high accuracies are in the most extreme premises, D+E− (91.36%) and A+B− (78.76%). A within-subjects ANOVA with Greenhouse-Geisser correction showed differences in accuracy between premises [$F(2.03,18.27) = 49.89$; $p < 0.01$]. The Bonferroni *post hoc* test showed that accuracy in premise C+D− differed from the other three premises ($p < 0.01$), and the accuracy in premise A+B− was statistically higher than in premise C+D− ($p < 0.05$) and premise D+E− ($p < 0.05$). B+C− did not differ from D+E− ($p > 0.05$). Additionally, a one-sample *t*-test revealed that accuracy in premise C+D− did not differ from chance [$t(9) = -1.97$; $p = 0.08$]. Latencies increased as a function of serial position (see **Figure 1B**), meaning that they were shorter for the A+B− pair than for the D+E− pair. Significant differences were found between premises [$F(2.16,19.4) = 15.73$; $p < 0.01$] using a within-subjects ANOVA with Greenhouse-Geisser correction. Latencies in premise A+B− were shorter than in premises C+D− and D+E−, but they were apparently different from the B+C− latency. Premise A+B− is the only pair in which one stimulus (A+) is always reinforced across the training. In contrast, latency in premise D+E−, being the only pair in which one stimulus (E−) is never reinforced, was the highest, but it did not differ of latencies corresponding to premises B+C− and C+D−.

As an attempt to correct deficits in premise C+D− after training phase, we increased training in pair C+D− during overtraining phase (see **Table 2**), so that at the end this pair was the premise most trained across sessions. As far as we know, this manipulation has not been done before. After overtraining this premise (see **Figure 2A**), the average accuracy in C+D− increased to 78.74% (*SEM* = 3.32), whereas performance on B+C− significantly decreased to 46.9% (*SEM* = 5.71). A within-subject ANOVA with Greenhouse-Geisser correction showed differences in accuracy between premises [$F(2.29,20.06) = 25.44$; $p < 0.01$]. The Bonferroni *post hoc* test showed that only the accuracy in premise B+C− differed from the other three premises ($p < 0.05$). No further differences were observed between premises. The accuracy in premise B+C− did not differ from chance [$t(9) = -0.57$; $p = 0.58$]. Latencies for each premise (see **Figure 2B**) stayed as in previous training. Once again, latency increased as a function of serial position.

A change in performance from Test 1 to Test 2 would be expected after overtraining the premise C+D−, regarding exclusively the frequency of reinforcement for each premise (see **Table 2**). Nevertheless, because performance in both Tests was equal, we will describe both tests at the same time.

Our data were not consistent with any hypothesis of TI based on the frequency of reinforcement of each premise. There was no effect of overtraining premise C+D− on test performance. We observed an increase in accuracy as a function of symbolic distance between stimuli.

Figure 3 shows the pigeons' performance and latencies of response on Test 1 and Test 2 when stimuli were simultaneously presented in non-adjacent pairs. The averaged pigeon performance (see **Figure 3**) is consistent with the SDE. Therefore, the worst performance is in pair BD and the best is in pair AE. During Test 1 the averaged accuracy in the most central pair, BD, differed significantly from the most distal pair, AE, and pair BE [$F(2.28,20.5) = 0.67$; $p < 0.01$]. The same pattern was observed in Test 2. Pigeons also showed a slight and non-significant decrease in latencies as symbolic distance increased. Latency in pair CE was significantly higher than in the other pairs. Exactly the same pattern was observed in Test 2, with a slightly better performance in accuracy and less variable latencies. Latencies for incorrect responses were higher than for correct responses. Considering all the premise pairs, latencies slightly decreased as the distance between non-adjacent premises on each pair increased.

Therefore, TI was established in the absence of correction trials and despite deficits in performance during premise C+D. This pattern of performance during the test remained even when overtraining was administered. The preference for B stimulus in pair BD, was higher and differed from chance for Test 1 [$t(9) = 4.83$; $p < 0.01$] and Test 2 [$t(9) = 4.48$; $p < 0.01$].

DISCUSSION

Our data show that using the employed training, pigeons were able to establish TI in the absence of correction trials and even when performance deficits in C+D− were found and persisted across the Training Phase. Overtraining the premise pair C+D− did not affect TI but distorted SPE, so that three pairs (i.e., A+B−, C+D−, D+E−) reached the highest accuracy and only one pair (B+C−) dropped near to chance. However, this extra training in C+D− did not affect TI. The performance in the worst solved premise pair before overtraining (i.e., C+D−) and after overtraining (i.e., B+C−) did not differ from chance.

Latencies increased after training related to SPE. Therefore, for premise A the latency was shorter than for premise B, and so on. Exactly the same was observed after overtraining. In both tests (after training and overtraining), latencies slightly decreased as the symbolic distance between non-adjacent premise pairs increased. The higher latency was observed in the pair CE.

Interestingly, latencies were always shorter for correct responses, no matter what the non-adjacent premise pair presented, than for incorrect responses, except for the most inner pair BD.

Because we did not counterbalance the stimuli's color between pigeons, it is possible to argue that this could affect the choices. Besides, counterbalancing colors is a common practice in TI procedures. However, some previous studies have shown that reversing the order of colors and pre-exposition to the same colors during training do not affect TI performance. For example, Strasser et al. (2004) used figures as stimuli but without counterbalancing and they found TI. Additionally, Steirn et al. (1995) conducted two experiments with relevance to this question. During the first one, they assigned one group to a set of premises A+B−. . . D+E− (group A+) and the other to the reverse A−B+. . . D−E+ (group A−). All pigeons had previous exposition to the same stimuli employed during training. They only found more variability in the amount of sessions to reach the criterion (90% of correct responses during two consecutive sessions) for group A− with no statistical differences during test pair BD. Moreover, in their second experiment, they compared pigeons with previous experience with the stimuli and naïve pigeons, finding no differences to reach the criterion, although there was a lower performance in naïve pigeons during the first sessions of each phase. The performance during the test did not differ between groups. Therefore, it seems that previous experience with the stimuli and the counterbalance of stimuli can slightly affect performance, however, TI seems to be unaffected.

Because overtraining premise pair C+D− seems to not have an effect over TI, but it decreased the discrimination of pair B+C− during overtraining, we will discuss first the effects of training and overtraining over premise pairs discrimination and later, we will discuss its effect on TI. Finally, we will focus on latencies of response during training (and overtraining) and tests.

Training and Overtraining Effects on Premise Pairs Discrimination

Vasconcelos (2008) describes the U-shape as the idealized curve performance for the five-term series. However, the shape of the curve can take several forms depending on many features during training. There are many ways for training the premise pairs and consequently different outcomes in acquisition. Studies differ in the amount of training trials. For example, von Fersen et al. (1991) trained the complete set of stimuli across all sessions reaching 27,000 stimulus pair presentations, and they found the expected U-shape in performance with naïve pigeons as subjects. Steirn et al. (1995) reported above chance performance and TI with only 2,112 training trials. Siemann et al. (1996) reported 2,700 trials to reach above chance performance and TI, and their performance curve resembles an exponential ascending function. Therefore, there is not a specific criterion that determines the number of trials or sessions necessary to establish above chance performance and TI, and the idealized U-shape is not always found.

After our training procedure, the expected U-shape was not exactly found, although performance above chance was reached for all premise pairs except C+D−. This finding does not resemble precisely what other studies have found. For example, in von Fersen et al. (1991) the pair C+D− is the worst solved but it is still above chance. In Siemann et al. (1996), the pigeons' performance is noticeable better, since they found an ascending function from A+ to D+ (contrary to the expected U-shape), but the training began with central premise pairs (B+C− and C+D−) and then proceeded with extreme pairs, first pair A+B− followed by pair D+E−. They used a different number of presentations of each pair and imposed a criterion of 80% of correct responses before starting the test phase to avoid the emergence of an end-anchor effect. Other studies have also found this ascending function in performance. For example, Lazareva and Wasserman (2006) -with pigeons- and Lazareva and Wasserman (2010) -with humans- also reported ascending training performances from A+B− to D+E− (from 80 to 90%) using the same pattern of premise training as we did. In this respect, it is important to point out that in both studies manipulations in response rates and correction trials were employed, which could have improved accuracy for all premise pairs, whereas in our experiment the absence of those

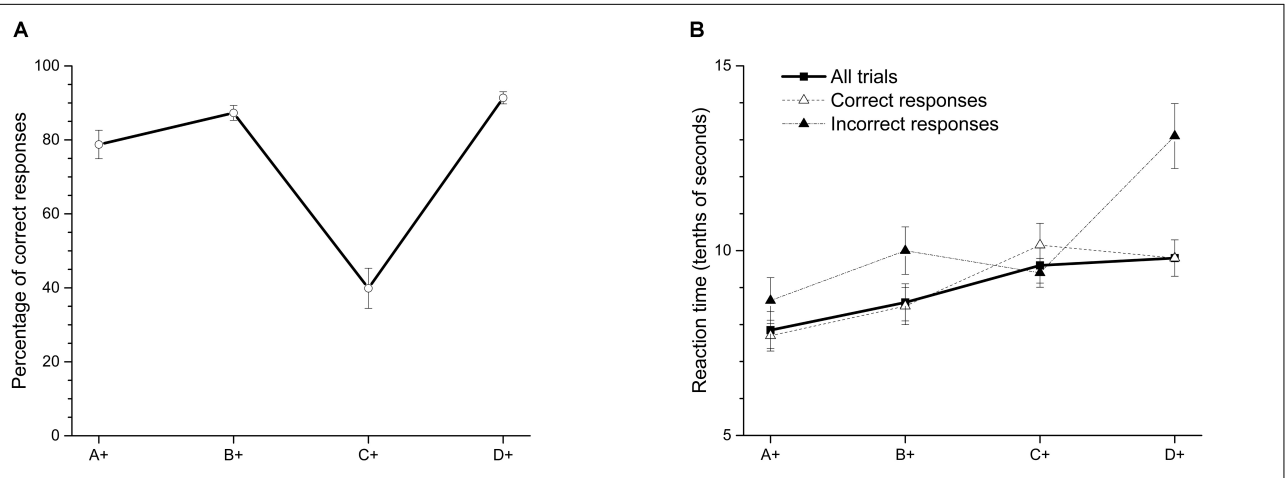

FIGURE 1 | (A) Average percentage (±SEM) of correct responses and **(B)** reaction time (tenths of second) on each stimulus in the last two sessions of training.

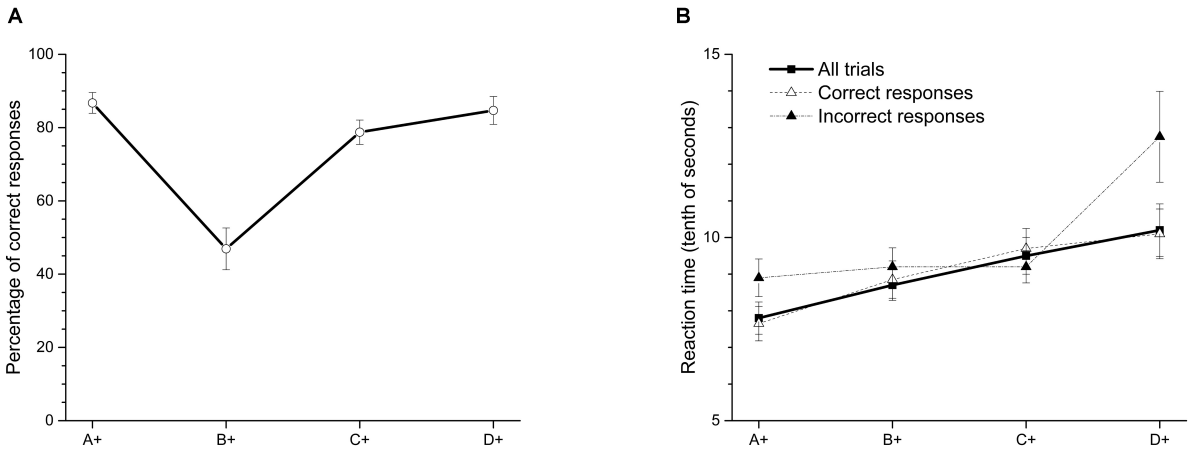

FIGURE 2 | (A) Average percentage (±SEM) of correct responses and **(B)** reaction time (tenths of second) on each stimulus in the last two sessions of overtraining premise C+D−.

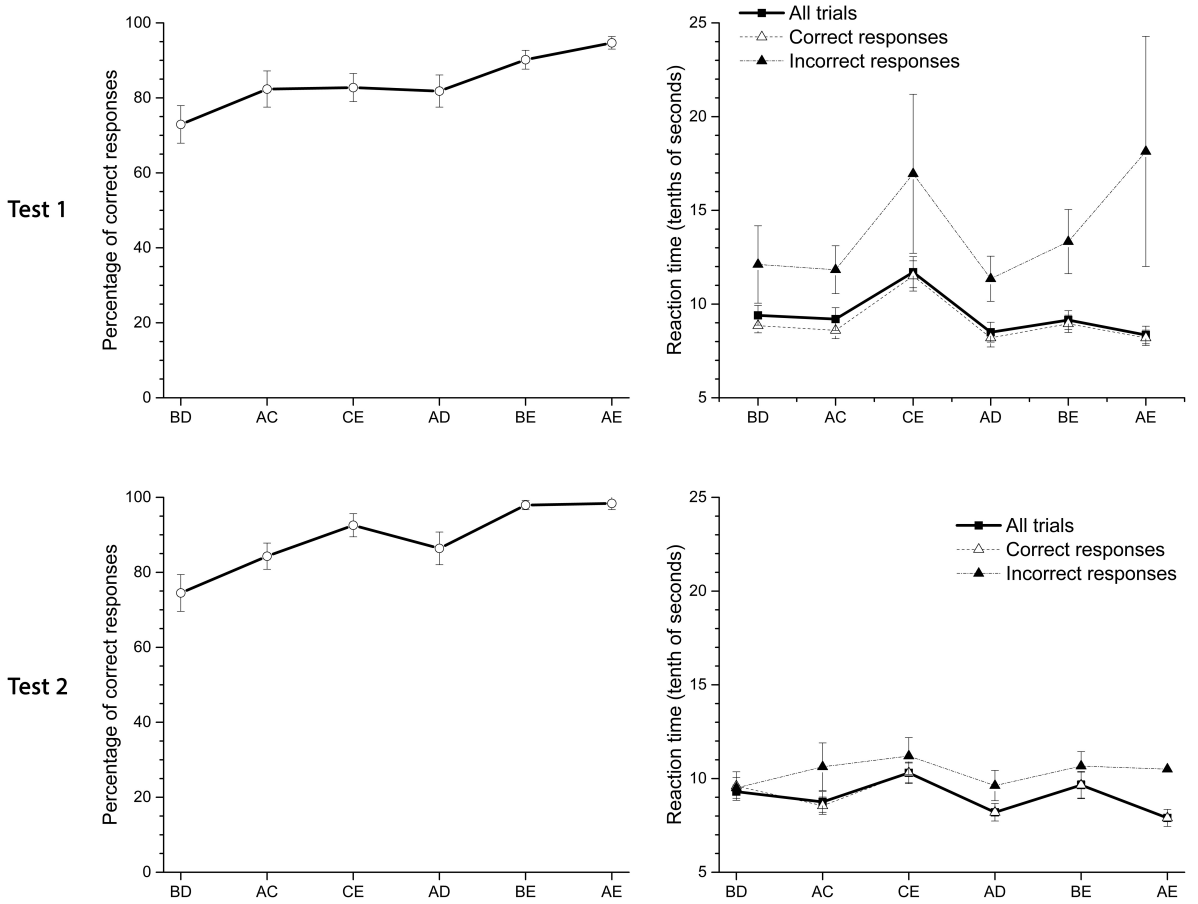

FIGURE 3 | Average percentage (±SEM) of correct responses and reaction time (tenths of second) in Test 1 and Test 2. From left to right, pairs of stimuli are ordered by their symbolic distance.

manipulations could have provoked deficits in performance. Support for this hypothesis comes from serial learning studies. For example, Straub and Terrace (1981) employing pigeons as subjects, trained a sequence of stimuli A→B→C→D where "→" denotes the consequent stimulus and the pecking of the last stimulus in the chain delivered the reinforcer. With gradual

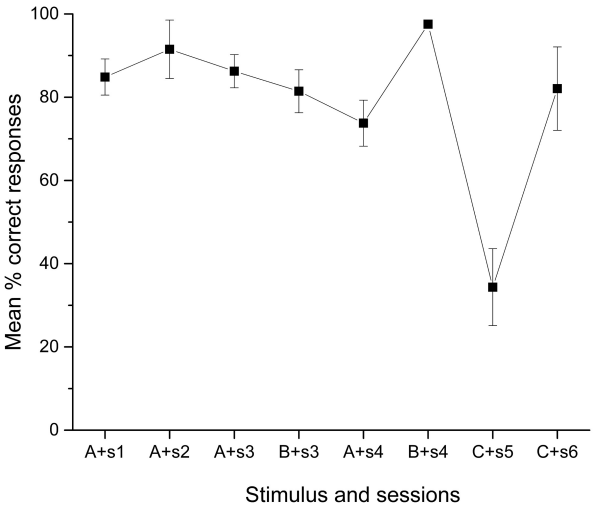

FIGURE 4 | Mean percentage (SEM±) of correct responses for each stimulus until Phase 3 (sessions 1 to 6).

training (A→B, A→B→C and A→B→C→D) and different levels of correct responses required (25, 50, and 70%), the performance showed different shapes, having "C" the worst performance with the complete chain trained at the lowest correct responses requirement. They found a performance curve that closely resembles the one we found, which suggests that in absence of correction trials and with lower correct responses requirement, the learning of relations between stimuli is impaired by the addition of a third stimulus. Nevertheless, the fact that C+D− was constantly the worst solved across four sessions of training all premises, cannot be explained by this assumption.

It is also worth mentioning that other studies do not report the U-shape during training although TI is still reported (see Strasser et al., 2004 with pigeons, and Acuna et al., 2002 with humans). Therefore, the observed differences in performance depending on training administration, and especially the absence of the U-shape, deserve further verification.

There is evidence in humans in which learning a second list of verbal stimuli decreases the remembered items for the first list, an effect referred to as retroactive inhibition (Barnes and Underwood, 1959). If this phenomenon affected extensively trained stimuli, the observed effect of overtraining C+D− could be explained. In fact, looking at the performance during the first training session in the pair C+D−, the percentage of correct responses was below chance but without significance [34.36%; $t(9) = -1.691$, $p = 0.13$], which did not happen with any other pair, but significantly improved over chance [82.06%; $t(9) = 3.201$, $p = 0.01$] by the second training session (see **Figure 4**).

However, the performance in C+D−, never over chance, remained stable across the entire Phase 4 (data not shown), and improves during overtraining. Therefore, it is unclear why the discrimination C+D− is correctly solved when presented alone but poorly solved when presented along with the other premise pairs. Additionally, the improvement in C+D− diminishes from the first to the second session after overtraining (see **Figure 5**), so that the function acquires a U-shape with B+C− being the worst pair solved and D+E− the best solved.

More direct evidence related to overtraining comes from studies in TI where bias reversal is employed. For example, in Lazareva et al. (2004), after training all premise pairs, their subjects -hooded crows- were exposed to D+E− presentations until reaching a reinforcement ratio in D greater than in B. Despite of this manipulation, B was preferred over D (especially in the ordered feedback group). Lazareva and Wasserman (2006), using pigeons, employed the same bias reversal and found a slight improvement in general performance during training, but the preference of B over D remained the same during the test. The same effect of bias reversal has been found in Lazareva and Wasserman (2012) using pigeons. Nevertheless, in a more recent study, Lazareva et al. (2015) have shown that bias reversal is reported (changing BD preference) in some pigeons but not in others, which suggests that in some pigeons, performance depends on reinforcement history, whereas in others, performance depends on the implicit order of the stimuli.

In the present experiment, overtraining C+D− unexpectedly decreased performance in B+C− discrimination. However, that decrement did not change BD preference in any test. It is worth mentioning that in all the previously mentioned studies, bias reversal on D+E− slightly improved general performance during training, whereas in our procedure overtraining C+D− distorted general performance. This fact suggests that methodological differences with others experiments, such as using correction trials or the response requirement, have noticeable effects on the expected U-shape during training.

Tests Performance

In our results, despite the already mentioned deficits during performance, particularly for C+D−, and despite the changes provoked by the extra training in C+D− (which lowered B+C− near to chance), the preference for B over D and the complete pattern of performance during Tests 1 and 2 remained unaffected. This finding turns out to be unexpected based on the mere calculations of reinforcement ratios, and it suggests that learning during acquisition and performance during test could be affected by independent mechanisms. It would then follow that impairments during training do not always translate into impairments during test. Siemann et al. (1996) pointed out a similar argument, stating that with sufficient training reinforcement histories have minor consequences in TI. Steirn et al. (1995) reported a negative correlation between reinforced responses during B+C− and D+E− and the preference of B over D. Moreover, they found a preference of B over D even when the premise pairs' training (A+B−, C−E+, C+D−, A+E−) did not support it by reinforcement history. This trend has also been shown in monkeys, where TI was established despite predictions from reinforcement ratios (Boysen et al., 1993). More recently, Daniels et al. (2014) did not find a significant correlation between the proportion of relative reinforcement and TI performance in pigeons.

There are also indirect evidence supporting this independency between training and test performance. For example, Strasser et al. (2004) did not find differences during training and

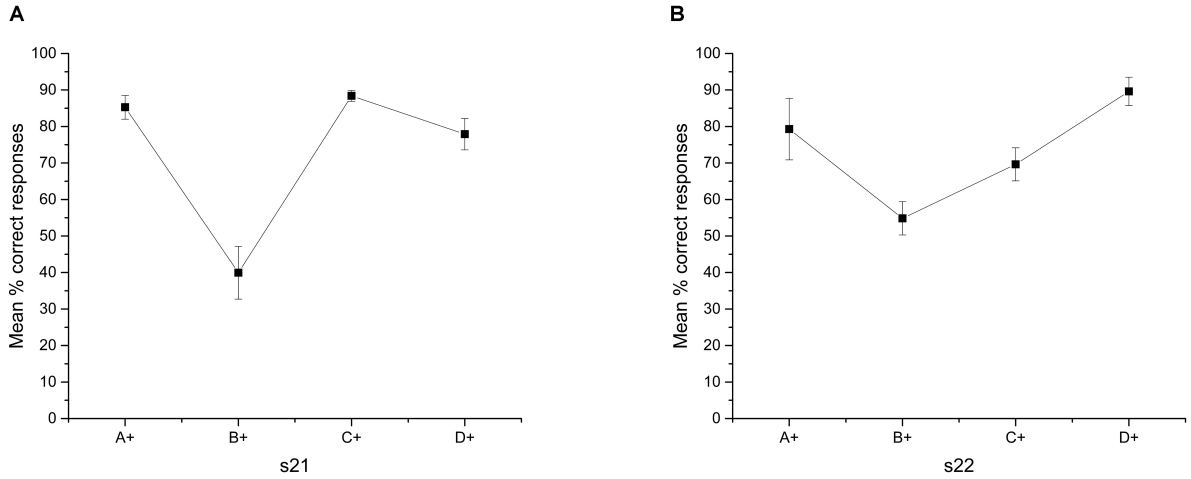

FIGURE 5 | Mean percentage (SEM±) of correct responses for each stimulus during overtraining phase. **(A)** Session 21 and **(B)** Session 22.

TI between hippocampal-lesioned and intact pigeons, which would imply that impaired spatial representation (caused by hippocampal lesions) does not affect neither acquisition nor TI. Similar findings can also be found in reinforcement models. For example, Lazareva et al. (2004) using crows (*Corvus cornix* L.) as subjects reported TI, but when they applied two different models of associative strength these models successfully fit the data from training but failed to fit BD performance. In another study from Lazareva and Wasserman (2006), both associative models failed to predict BD performance in pigeons after bias reversal. A similar inconsistency can also be found in humans (Lazareva and Wasserman, 2010) and pigeons (see Lazareva and Wasserman, 2012), where associative models produced better prediction for training than for testing.

Looking at the total reinforcement ratios after the training of all premise pairs and the over-trained premise pair C+D− (see **Table 2**), it seems that reinforcement ratios cannot account for the persistence of TI in our results, since B did not have a greater value than D. Performance during training and its effect on the tests seem unable to be predicted by inequalities of reinforcement.

On the other hand, cognitive approaches would be also insufficient, since the extended exposure to all premise pairs (Phase 4 of training) did not improve C+D− performance (data not shown). Additionally, overtraining the pair C+D− impaired B+C− performance without affecting TI. Studies in humans have shown that extended training can improve working memory even in long-term periods and with transference to different tasks (see Klingberg, 2010 for a review). However, the effect of extended training in TI procedures remain elusive. Studies with neural networks have found that overtraining specific premise pairs (for example, pair E+F− in a seven-term series) in impaired networks improves performance during BD pair (Frank et al., 2003) but without reporting effects during training, which turns out to be inconsistent with our findings.

Considering that the direct values of reinforcement cannot explain the observed pattern, we explore the possible effect of value transfer. In order to calculate values in indirect associative strength, we took the formula of value transfer theory (VTT) from von Fersen et al. (1991): $V_i = R_i + a^* V_{i+1}$, where V_i represents indirect value of a particular stimulus when it is paired with a reinforced stimulus, R_i is the value from the stimulus directly reinforced, a is a weighting parameter (ranging from 0.1 to 0.5), and V_{i+1} is the value of the rewarded stimulus when presented with stimulus i.

Taking the overall performance, we used the reinforcement ratios [rewarded trails/(rewarded + non-rewarded trials)] and updated the values from session 1 to session 15 based on parameter a. Then, the last two training sessions before overtraining were averaged and contrasted with the obtained data. Taking those values, our data do not support value transfer, since running the equation the best fitting obtained with least squares method was with a near to zero (data not showed), which would imply no value transfer. Despite of several models which assume value transfer (Wynne, 1995; Siemann and Delius, 1998), other studies have also suggested TI in absence of value transfer (Weaver et al., 1997). Regarding the above mentioned, although value transfer remains as a possible intervening mechanism in TI procedures our data neither support an explanation based on value transfer.

Latencies

Latencies during performance remained as an ascending pattern from the most central to the most extreme premises, which turns out to be contrary to the widely reported SDE. This finding is also inconsistent with the decrement in latencies predicted by cognitive approaches as an effect of practice (Logan, 1992). In a previous study we found a monotonic decrement in latencies as a function of extended exposure to two alternatives predicted by a power law (Camarena-Pérez and García-Leal, 2017). This effect has been also reported in perceptual discrimination procedures in monkeys, where more alternatives provoke less steep decrease in latencies (Albantakis and Deco, 2009). Whether a comparison process between alternatives were operating, larger latencies would be expected in pair BD and shorter latencies in the pair AE,

contrary to our findings. On the other hand, if latencies decrease as a function of practice, we would have found steeper or flat functions instead of ascending latency function.

How latencies change in TI procedures seems to be more widely reported in humans (Woocher et al., 1978; Acuna et al., 2002; Munelly et al., 2010) than in non-human animals. D'Amato and Colombo (1990) reported increasing latencies in monkeys (*Cebus paella*) during testing when they grouped pairs containing A, B, C, and D as the first stimulus in the pair. The same trend is shown in D'Amato and Colombo (1988). These findings are regarded by them as SDE.

The valuation process (if any) in TI procedures remains unclear, and along with the fact that not all TI studies reported data about latencies, the question as to what should be the observed trend in latencies remains unanswered. Previous studies about the effects of practice in learning curves have suggested a hyperbolic improvement until reaching an asymptotic performance across sessions, as an accumulation of correct responses (Thurstone, 1919; Mazur and Hastie, 1978). For the present experiment, that hyperbolic improvement is clearly absent, which in turn suggests that learning of TI cannot be explained by mere accumulation of correct responses.

CONCLUSION

TI can be established even with deficits in performance during acquisition and overtraining in one premise. Nevertheless, it remains unclear why the overtraining of one single premise did not affect TI and impaired performance of the immediate previous premise. Our findings suggest that the specific way in which premises are trained could have effects on the expected functions of performance without affecting TI, and that even when subjects can discriminate a single premise alone, this discrimination becomes impaired when all premises are presented together. Studies focusing on the performance premise by premise and session by session, along with a more detailed analysis of latencies, could be helpful in determining the specific mechanisms involved in the establishment of TI.

AUTHOR CONTRIBUTIONS

All authors jointly designed the experiment and discussed the results. HC collected the data. HC and OG-L jointly wrote the manuscript.

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CORRECTION
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Corrigendum: Transitive Inference Remains Despite Overtraining on Premise Pair C+D–

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Keywords: transitive inference, value transfer, reinforcement, overtraining, bias reversal

A Corrigendum on

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In the original article, there was an error. The letter "C" was incorrectly provided and should instead be "B."

A correction has been made to the **Introduction**, paragraph seven:

"The present study is focused on the relationship between reinforcement contingencies and the formation of TI. More specifically, our aim is to explore the effect of extended training of all premise pairs and overtraining in a single premise pair. Previous studies have analyzed the effect of overtraining on TI. For example, Lazareva et al. (2004) and Lazareva and Wasserman (2006, 2012) explored the effect of overtraining the pair D+E−, as a way to increase associative strength for D, and the preference for D over B on the later test performance. In our study, we overtrained the pair C+D− (usually the most difficult discrimination to learn). Assuming value transfer, the overtraining of premise C+D− should have an indirect effect over the performance in premise B+C−. The latter premise should become more difficult to solve, because C gets more associative value through overtraining and, therefore, competes with premise B+ to receive the response. If only the direct associative strength is responsible for TI, then the effect of overtraining premise C+D− should be only a better discrimination in this pair without affecting the pigeon's performance in the B+C− pair. Subsequent performance during the test would be disrupted."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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ORIGINAL RESEARCH
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Effect of Intranasal Oxytocin Administration on Human-Directed Social Behaviors in Shelter and Pet Dogs

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A wide variety of evidence has demonstrated that oxytocin is involved in socio-cognitive skills in domestic dogs (*Canis familiaris*). The purpose was to evaluate the effect of oxytocin administration on socio-cognitive abilities in two populations of dogs with different levels of daily human contact: shelter and pet dogs. Additionally, the effect of different doses of oxytocin (i.e., 16 and 24 IU) was assessed. To this end, dogs were tested on two tasks: a sociability test to assess their social responses and a communicative task focused on the learning of gazing responses. Results showed that pet dogs performed better than shelter dogs on the sociability and the gazing tests showing the relevance of dogs' previous experience and learning when interacting with people. The administration of 16 IU as well as 24 IU of oxytocin improved the performance on the communicative learning task, producing an increment in gaze duration during extinction. This difference was observed in both pet and shelter dogs. Therefore, oxytocin seems to participate in the persistence of this communicative response. However, the treatment did not modify the behaviors during the sociability test. Furthermore, oxytocin appears to be beneficial to increase the communicative abilities of shelter dogs.

Keywords: oxytocin, gaze, sociability, pet dogs, shelter dogs

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INTRODUCTION

An increased interest in the comparison of human-dog interactions on physiological levels has been seen in recent years. Accordingly, investigation of the effects of oxytocin (OT) on companion dogs' socio-cognitive skills and the related increase of affiliative behaviors has become more relevant.

Oxytocin is a neuropeptide and hormone synthesized in the supraoptic and paraventricular nuclei in the hypothalamus and it is related to a wide range of affiliative and socio-cognitive behaviors in a variety of species (Lee et al., 2009). For instance, OT is involved in cooperative behavior in suricates (*Suricata suricatta*) (Madden and Clutton-Brock, 2010), social behaviors in newborn monkeys (Simpson et al., 2014), social grooming in bats (*Desmodus rotundus*) and chimpanzees (Crockford et al., 2013; Carter and Wilkinson, 2015) and a longer gaze duration

toward the eye region among macaques (Dal Monte et al., 2014) and humans (Guastella et al., 2008).

With regard to the human-dog bond, different genetic markers of OT have been associated with human-directed social behaviors in dogs such as microsatellites markers close to the OT receptor gene (OXTR) (Oliva et al., 2016) and OXTR polymorphisms (Kis et al., 2014a; Kubinyi et al., 2017; Oláh et al., 2017; Persson et al., 2017; Turcsán et al., 2017; Konno et al., 2018; Kovács et al., 2018). Furthermore, in relation to epigenetic mechanisms, similar social behaviors were associated with DNA methylations in the promoter region of OXTR in dogs (Cimarelli et al., 2017).

Positive interactions have been shown to increase endogenous OT levels in both species. For example, an increase in OT was observed in both owners and their dogs after approximately 30 min of interaction which included gently stroking and scratching, playing and talking in a positive tone (Odendaal and Meintjes, 2003; Miller et al., 2009). Similar but shorter interactions (i.e., 3–4 min) also increased OT levels in male Labrador dogs (Handlin et al., 2011) and female Beagle dogs (Rehn et al., 2014). Rehn et al. (2014) found that the combination of physical and verbal contact elevated OT levels more than verbal contact only. Thus, both physical and verbal interactions appear to be important for the release of OT during human-dog exchanges. In addition, assistance dogs that experienced a wide variety of interactions with people had higher endogenous OT levels compared to pet dogs (MacLean et al., 2017). Moreover, Nagasawa et al. (2009, 2015) found that the OT levels of both the dogs and their owners, were increased when the dogs gazed more at the owner compared to those that gazed for a shorter time. The authors concluded that the dog's gazing would likely induce an activation of the OT neuroendocrine system in both of them.

Similar results were found with the administration of exogenous OT in dogs. For example, the application of intranasal OT promoted affiliative behaviors in dogs toward humans as well as toward conspecifics, compared to controls receiving saline (Romero et al., 2014). It also encouraged dogs' social play with conspecifics (Romero et al., 2015). Additionally, OT improved the performance on a communicative task where dogs had to follow pointing cues to find hidden food (Oliva et al., 2015; Macchitella et al., 2017). In line with this, Nagasawa et al. (2015) demonstrated that the intranasal administration of OT produced an increase in dogs' gaze duration toward their owners after mutual interaction, although this increment was only seen in female dogs. Furthermore, recent studies found differential breed effects, such as Border Collies looking more toward a human than Siberian Huskies after OT administration, in an unreachable food situation (Kovács et al., 2016b). Moreover, OT administration increased gazing toward people in ancient Japanese dog breeds, which have lower baseline gazing levels than European breeds (Nagasawa et al., 2017). Other changes observed include cognitive bias effects, such as an increase in a positive bias to assess ambivalent stimuli (Kis et al., 2015), looking at projected images on a screen (Kovács et al., 2016a), and gazing at their owners for a longer period when approached in a threatening manner (Hernádi et al., 2015).

The first purpose of our study is to evaluate the effect of the intranasal administration of OT (16 IU) on dogs' social behaviors during the interaction with a stranger in a sociability test. Secondly, considering that gazing appears to be important for the release of OT during human-dog interactions, we aim to assess the effect of OT on learning a communicative task consisting of gazing at the human face to ask for food. The learning process comprises three phases – an initial phase where the gaze behavior is reinforced with food; an extinction phase where no food is given even if the dog performs the gazing response; and finally, a re-acquisition phase where dogs receive the reinforcement again. We hypothesize that an OT increase will favor the development of social and communicative behaviors in dogs toward humans, given the role of this hormone on social relationships. Furthermore, we propose that these effects could be different according to the dogs' previous levels of social contact with humans. To this end, in Study 1 we compare the performance of pet and shelter dogs on these tasks. Previous studies have pointed out differences between these populations both in sociability and gazing tasks (Barrera et al., 2010, 2011) and in other communicative tasks (Udell et al., 2008). The poorer performance of shelter dogs on social tasks suggests that such skills could be affected by reward history during ontogeny. As far as we know, there are no studies exploring the effect of OT on shelter dogs, despite the relevance this could have on rehabilitating behavioral problems (Romero et al., 2015; Thielke and Udell, 2015). These results would expand on the knowledge regarding the importance of OT in the human-dog bond, as well as in how it might shape learning and how previous experiences may modulate its effects.

Furthermore, on study 2, we carry out the same tasks in pet dogs after administering a higher dose of OT (24 IU). In previous studies there is no consensus on the OT dose that should be applied, as it ranges from 12 to 40 IU. Therefore, in the current study we set out to investigate whether a higher OT dose for the same tasks would produce an increase on the observed effects. Researching the effects of different OT doses is important given that intranasal OT has been proposed in the applied area as treatment of dog behavioral problems and to improve dog training.

ETHICAL APPROVAL

These studies complied with the current Argentine law of animal protection (Law 14.346) and were developed with the approval of the CICUAL (Institutional Commission for the Care and Use of Laboratory Animals) from the Medical Research Institute IDIM UBA-CONICET (Res. Nro. 084-18). All owners and shelter staff signed an informed written consent for the participation of their dogs.

STUDY 1

Materials and Methods

Subjects

We assessed 45 dogs, 21 pet dogs (PD), 11 females and 10 males, and 24 shelter dogs (SHD), 10 females and 14 males.

Six additional dogs (1 PD and 5 SHD) were excluded from the sample as they showed fear responses to the situation and/or did not reach the criterion for the communicative learning task described below. All of them were adults (2–10 years old) and mongrels with no clear resemblance to any particular breed. Shelter and pet dogs were randomly divided in two groups according to the treatment: OT or Placebo. Therefore, there were 4 groups: pet dogs with OT (PO, $N = 10$), pet dogs with placebo (PP, $N = 11$), shelter dogs with OT (SHO, $N = 12$) and shelter dogs with placebo (SHP, $N = 12$).

All shelter dogs had lived in the shelter for at least 2 years before the testing and their background history was not available. Seventeen SHDs came from a shelter sponsored by the "Asociación Dignidad Animal" (Association of Animal Dignity) in Santa Fe, Argentina; while the remaining seven SHDs were from another shelter belonging to the "Sociedad Protectora de Animales de Santa Fe" (Society for the Protection of Animals of Santa Fe), Argentina. Both shelters offered similar accommodations for the dogs, with large open separate areas (10 m long × 8–10 m wide), holding between 5 and 15 dogs per sector. Their contact with shelter staff was limited to feeding and cleaning activities. They were all in good health and none of them had previously participated in a study.

The selection criteria used for PD was to choose dogs that had spent most of their lives in a household and had daily interaction with their owners inside the house. A total of 4 pet dogs had already been assessed in other tasks (self/inhibitory control).

Dogs had free access to water and the last meal before the communicative learning task had been received between 4 and 6 h earlier.

Administration

The dogs received intranasal OT (16 IU) Syntocinon-Spray (Novartis) or saline solution as placebo, 40 min before the sociability test and the communicative learning task. This time frame was selected as it is the time it takes for OT to reach the brain (MacDonald et al., 2011; Quintana et al., 2015).

Immediately after application, the animals remained in their homes or enclosures for 30 min while performing their usual activities and not interacting with the humans. They were then taken to the evaluation room for 10 min so that they became familiar with the surroundings, accompanied by an unfamiliar experimenter that was indifferent to the dog. After the 10 min of habituation, this experimenter left the room and the testing began immediately after.

Procedure

The procedure comprised one test (sociability test) and one task (communicative learning task). They were always carried out in this order and on the same day. The interval between them was 2 min. The fixed order of procedures was to ensure the assessment of the animals' initial reaction to the presence of a stranger in the sociability test. In addition, the sociability test acted as a familiarization to the Experimenter (E). Both tests were performed by the same female E who was unknown to the dog and blind to the dogs' treatment (OT-placebo). The tests were scheduled in a quiet familiar room in the dogs' usual environment (home or shelter).

Sociability test

Apparatus. The test was carried out in a closed room where there was a chair placed against a wall. Tape marks on the floor 1 m away from the chair were used to later determine the distance kept by the dogs. Only the E and the dog were present during testing. The evaluation was videotaped by a camera (Sony DCR 199 SX-85) located on a tripod.

Procedure. The sociability test was the same as Jakovcevic et al. (2012). It was divided into two 2 min phases: (a) Passive phase: The E entered the room and sat on the chair reading a book. If the dog made physical contact with her, E petted its head, neck, or back twice and then withdrew her hand. During this phase the E did not make visual contact with the dog. (b) Active phase: The E stood up, called the dog by its name and made visual contact with it. If the dog approached, E interacted by petting and talking to it. If the dog did not approach, E called it three times. If the dog approached and then went away, E called it up to three times. During this second phase, E stayed in the same place to avoid possible fear reactions in the dog.

The following variables were registered for both phases (passive and active):

Time (s) the subject remained near the E (<1 m distance).

Time spent in physical contact (s) between the E and the subject.

Communicative learning task

The task was the same as in Barrera et al. (2010) and Jakovcevic et al. (2010).

Apparatus. A container with the reward was placed on a high shelf, so that it was visible to the dogs but out of their reach. The E stood beside the container. All trials were video-taped by a Sony DCR 199 SX-85 camera. The person taping the trial ignored the dog and was located behind the E, so as to be able to film the dog's gaze and head position. The task was performed in the same area as the sociability test. Each session involved the dog, the E, and the assistant operating the camera. The reward were pieces of cooked liver.

Procedure. The procedure consisted of three phases:

Acquisition (ACQ). The phase began after a warm up in which the E called the dog by its name, actively sought physical contact and gave it three pieces of liver. Acquisition trials started with E standing beside the food container and once again calling the dog by its name and giving it a piece of liver. Gazing at E's face for at least 1s was reinforced at every occurrence. Usually, dogs moved their gaze from E's face to her hand as soon as E reached for the food, and a new reinforcer was delivered when the dog turned its gaze back to E's face for 1 s. A selection criterion where dogs had to respond to their names and gaze at E at least four times during the last acquisition trial was established. Dogs received three 2 min trials of differential reinforcement of gazing at the E. The inter-trial interval (ITI) lasted 2 min.

Extinction (EXT). The interval between acquisition and extinction phases lasted 2 min. Three 2 min extinction trials were performed. The ITI lasted 2 min. During this phase the dogs never received food, so the gazing response was no longer reinforced. The E and the food remained in the same place as in previous trials.

Reacquisition (RA). Two minutes after extinction, the dogs received one trial of reacquisition which was identical to the acquisition trials. This phase discarded potential satiety or fatigue effects.

During acquisition, extinction and reacquisition trials, the E remained in the same position gazing at the dog's face.

The dependent variable in all trials was the cumulative gaze duration (s) toward the E's face. To measure this, a stopwatch was activated each time the dogs directed its head/gaze to the E's face and stopped when the direction of the head/gaze changed/they looked away.

Data Analyses

For both tasks, an experimenter blind to the dogs' treatment (OT-placebo) measured 100% of the video-taped material. Additionally, in order to assess interobserver reliability, a second observer analyzed 40% of the material. We calculated Pearson's coefficients of correlation for all the measures and they showed high reliability for both tasks (Sociability test: $r > 0.99$, $p < 0.0001$, $n = 18$; Communicative learning task: $r > 0.92$, $p < 0.0001$, $n = 18$).

The sociability test was analyzed using generalized linear mixed modeling on the time spent near and in contact. The distribution of the variables (seconds) was specified as normal and related to the fixed factors through the identity link function. Phase (passive and active), treatment (OT, placebo), housing (SHD, PD) and sex (male, female) were included as fixed factors into the model. Additionally, six two-way interactions were specified: three interactions resulting from crossing the within-subject factor (i.e., phase) with each of the between-subjects factors (i.e., treatment, housing and sex), and three interactions resulting from pair-crossing the between-subjects factors. The random effect structure for near and contact was by-participants intercepts.

For gazing, analyses, statistical software and fixed and random factors' specifications were the same as in the sociability test. It is important to note, however, that the factor phase was composed of three levels (acquisition, extinction, and reacquisition) in this case, rather than two, as in the previous task. The random effects' structure included intercepts to account for variability across participants and across trials, since data collection took place in seven different trials.

All tests were two-tailed, $\alpha = 0.05$ and were carried out in SPSS 22.0.

Results

Sociability Test

The mean duration (s) and standard deviations of time spent near and in contact are reported in **Table 1**. The analyses yielded a similar pattern for both variables: a significant main effect of housing: near $F(1,77) = 5.05$, $p = 0.027$; contact $F(1,77) = 6.99$, $p = 0.01$, and a significant main effect of phase: near $F(1,77) = 50.91$, $p < 0.0001$; contact: $F(1,77) = 65.74$, $p < 0.0001$, were found. As shown in **Table 1**, the average duration of social behaviors in the PD group and the active phase condition were significantly longer than those from the SHD group and the passive phase condition, respectively. The rest of the factors did not yield significant effects, $p > 0.05$.

TABLE 1 | Near and contact to the E on sociability test of study 1.

	Passive phase		Active phase	
	Near	Contact	Near	Contact
SHO	**14.48** ± 24.82	**4.55** ± 8.78	**78.16** ± 50.96	**67.76** ± 52.94
PO	**59.36** ± 43.76	**43.01** ± 46.93	**111.45** ± 9.53	**111.36** ± 9.65
SHP	**29.85** ± 37.84	**16.63** ± 31.68	**74.99** ± 47.86	**72.85** ± 46.83
PP	**28.02** ± 36.32	**16.46** ± 36.31	**84.53** ± 54.50	**84.53** ± 54.50

Both behaviors are calculated in ratios of the cumulative durations in seconds on each phases (passive and active). Cells indicate the mean (in bold) and the standard deviation of the time ratio that SHO (shelter dogs with OT); PO, pet dogs with OT; SHP, shelter dogs with placebo; and PP, pet dogs with placebo; spent near and contact to the E on each phases.

Communicative Learning Task

The mean duration (s) and standard deviations of gazing are reported in **Figure 1** and **Table 2**. The model yielded a significant main effect of housing, $F(1,297) = 5.78$, $p < 0.017$, so that the average gaze duration from the PD group was significantly longer than that from the SHD group. The model also yielded a significant main effect of phase, $F(2,297) = 8.52$, $p < 0.0001$. The *post-hoc* Bonferroni comparisons indicated that the average gaze duration in extinction was significantly longer than in the acquisition phase, $t(297) = 3.94$, $p < 0.0001$, and reacquisition, $t(297) = 2.53$, $p = 0.002$. As expected, gaze durations from phase 1 and phase 3 did not differ significantly, $t(297) = 0.25$, $p = 0.8$. Finally, the model also revealed a significant treatment × phase interaction, $F(2,297) = 7.65$, $p < 0.001$, so that the group that received OT showed longer gaze times than the group that received placebo, but only in phase 2, $t(297) = 3.63$, $p < 0.0001$. Gaze duration did not differ in phase 1 and phase 3 as a function of treatment, $p > 0.05$. The other comparisons were non-significant, $p > 0.05$.

These results indicate that PD performed better than SHD on both the sociability and the communicative learning task. This could be related to the dogs' prior history as well as SHD low daily contact with humans, which limits their possibilities to learn communicative responses.

Secondly, in both PD and SHD dogs, the administration of OT increased the duration of gazing to the human face to ask for food during the extinction phase, when this response was no longer successful. This suggests that OT modulates the persistence of this learned communicative response. Conversely, no OT effects were observed during the acquisition and reacquisition phases. Probably, the delivery of food masks any potential OT effect. Additionally, no differences between these dog populations were observed during the sociability test. One possibility is that the

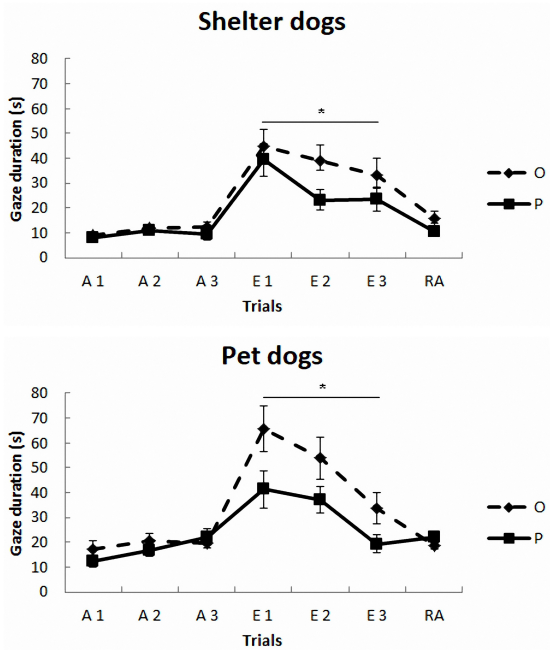

FIGURE 1 | Communicative learning task of the Study 1. Gaze duration (s) in acquisition (ACQ), extinction (EXT), and reacquisition (RA) trials for the groups SHO (shelter dogs with OT), PO (pet dogs with OT), SHP (shelter dogs with placebo) and PP (pet dogs with placebo) (means ± SEM). Dogs were required to gaze at the experimenter for 1 s to receive food in the acquisition and reacquisition phases. During extinction no food was delivered. *$p < 0.05$.

dose used in this study was insufficient to produce differences in this test so in Study 2 we set out to evaluate the effects of a higher dose of OT.

STUDY 2

The aim of this study was to replicate the findings of Study 1, contributing to a larger sample size and using a higher OT dose. Given that OT affected both pet and shelter dogs similarly on the previous study, in this case only pet dogs were evaluated.

Materials and Methods

Subjects

We assessed 41 dogs, 22 females and 19 males. Four additional dogs were excluded from the sample as they showed fear responses to the situation and/or did not reach the criterion for the communicative learning task. All of them were adults of 1–10 years of age (mean age: 4.54 years, SD: 2.55 years old) and mongrels with no clear resemblance to any particular breed. The dogs were randomly assigned into two groups: OT or Placebo. Therefore, there were 2 groups: pet dogs with OT (O, $N = 20$) and pet dogs with placebo (P, $N = 21$).

Procedure

The procedure was exactly the same as in Study 1, except that the OT administration was of 24 IU.

Data Analyses

Regarding inter-observer reliability, the procedure was the same than in Study 1, Pearson's coefficients of correlation for all the measures were run and they showed high reliability for both tasks (Sociability test: $r > 0.98$, $p < 0.0001$, $n = 17$; Communicative learning task: $r > 0.93$, $p < 0.0001$, $n = 17$).

Data were examined using generalized linear mixed models. For the sociability test, two identical generalized linear mixed models were specified, one for each outcome variable (near and contact). The models included phase (passive, active), treatment (OT, placebo), and sex (male, female) as fixed factors. The models also included all possible interactions (i.e., three two-way interactions and a three-way interaction) as additional fixed factors and by-participants random intercepts ($\alpha = 0.05$).

For the communicative learning task, gazing was entered as the outcome variable. Phase (acquisition, extinction and reacquisition), treatment (OT, placebo), and sex (male, female) were entered as fixed factors into the model. The fixed effects' structure also included three two-way interactions, resulting from pair-crossing the three fixed factors, and a three-way interaction including all factors ($\alpha = 0.05$). The random effects' structure included intercepts to account for variability across participants and across trials, since data collection took place in seven different trials. The Satterthwhaite approximation method was used to estimate the degrees of freedom due to different cluster sizes in the between and within-subjects factors. When necessary, additional analyses were conducted using *post-hoc* paired comparisons (Adjusted Sequential Bonferroni).

Result and Discussion

Sociability Test

In the sociability test (see **Table 3**), the analyses yielded significant effects of phase on both outcomes (near $F(1,74) = 30.06$, $p < .0001$; contact $F(1,74) = 75.96$, $p < 0.0001$). As shown in **Table 2**, the average duration of the behaviors in the active phase

TABLE 2 | Mean (in bold) and SD of the gaze duration (s) across trials for each group of Study 1.

Group	Acq 1	Acq 2	Acq 3	Ext 1	Ext 2	Ext 3	Reacq
SHO	**8.92** ± 5.78	**11.72** ± 5.79	**12.67** ± 5.92	**45.03** ± 22.95	**39.24** ± 21.41	**33.10** ± 23.62	**15.68** ± 9.85
SHP	**7.94** ± 6.11	**11.00** ± 6.69	**9.41** ± 7.12	**39.33** ± 22.52	**23.26** ± 14.75	**23.43** ± 15.56	**10.73** ± 6.87
PO	**17.28** ± 10.09	**20.79** ± 9.03	**19.82** ± 6.10	**65.64** ± 29.52	**54.05** ± 26.76	**33.71** ± 20.46	**18.94** ± 5.52
PP	**12.53** ± 8.24	**16.89** ± 8.71	**22.27** ± 10.54	**41.34** ± 24.74	**37.34** ± 17.64	**19.46** ± 12.23	**22.03** ± 7.63

Acq, acquisition; Ext, extinction; Reacq, reaquisition; SHO, shelter dogs with OT; SHP, shelter dogs with placebo; PO, pet dogs with OT; and PP, pet dogs with placebo.

TABLE 3 | Near and contact to the E on sociability test of study 2.

	Passive phase		Active phase	
	Near	Contact	Near	Contact
O	**41.76** ± 43.82	**19.98** ± 29.10	**87.09** ± 44.76	**86.20** ± 44.82
P	**54.78** ± 48.33	**26.59** ± 36.54	**83.84** ± 48.75	**83.72** ± 48.97

Cumulative durations in seconds of each phase (passive and active). Cells indicate the mean (in bold) and the standard deviation of the time that O (oxytocin) and P (placebo) spent near and in contact to the E during each phase.

was significantly longer than those from the passive phase. The rest of the fixed factors did not yield significant effects, $p > 0.05$.

Communicative Learning Task

Regarding the gazing task (see **Figure 2** and **Table 4**), the mixed model revealed a significant effect of phase on gazing, $F(2,4) = 31.37$, $p = 0.004$. The paired comparisons indicated that gaze duration was longer in extinction phase than in acquisition ($p = 0.005$) and reacquisition phases ($p = 0.013$), which did not differ between them ($p = 0.97$). The model also yielded a marginally significant effect of treatment, $F(1,42) = 3.79$, $p = 0.058$, and a significant treatment by phase interaction, $F(2,234) = 6.21$, $p = 0.002$, so that the OT treatment led to longer gaze times than the placebo treatment, but only in extinction (Sequential Bonferroni, $p < 0.0001$). No significant effects were found as a function of the sex of the participants $F(1,1016) = 3.14$, $p = 0.08$, and the rest of the interactions ($p > 0.05$).

The results of Study 2 are consistent with those of Study 1. The administration of OT had no effects on the sociability test. However, it increased the duration of gazing toward the human face during extinction. Therefore, a higher dose of OT did not increase the previously observed effects during these tasks.

GENERAL DISCUSSION

The results of our studies demonstrate that pet dogs exhibit more approach and physical contact behaviors toward a stranger than shelter dogs. This is in disagreement with our previous study in which shelter dogs spent more time near a stranger than pet dogs (Barrera et al., 2010). Conversely, Shin and Shin (2017) did not find any difference in the sociability of shelter and companion dogs. It is possible that the characteristics of the shelters were different and this would affect in a different way the behavior of the dogs. It is necessary to evaluate shelter dogs with several standardized tests in order to clarify their relative levels of sociability compared to pet dogs. Moreover, the responses were higher during the active phase compared to the passive one. This is probably related to the active E's attitude during the second phase that promotes the appearance of more social behaviors in dogs.

Furthermore, we found that the intranasal administration of OT (16 IU or 24 UI) does not change the reactions to a stranger in the sociability test. These findings are contrary to previous studies in dogs that show that OT modifies affiliative behaviors, particularly approach and physical contact (Mitsui et al., 2011; Romero et al., 2014). This difference may be due to the fact that the standardized experimental situation, especially in the passive phase, failed to promote the appearance of more social responses. Additionally, the presence of a stranger may have triggered less social responses than when the owner is

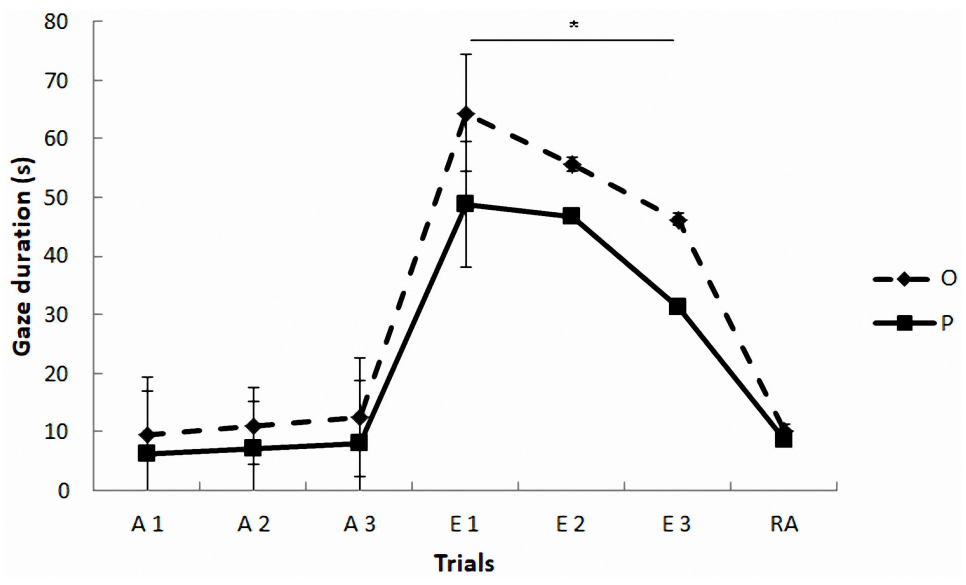

FIGURE 2 | Communicative learning task of the Study 2. Gaze duration (s) in acquisition (ACQ), extinction (EXT), and reacquisition (RA) trials for the groups O (OT) and P (placebo) (means ± SEM). Dogs were required to gaze at the experimenter for 1 s to receive food in the acquisition and reacquisition phases. During extinction no food was delivered. *$p < 0.05$.

TABLE 4 | Mean (in bold) and SD of the gaze duration (s) across trials for each group of Study 2.

Group	Acq 1	Acq 2	Acq 3	Ext 1	Ext 2	Ext 3	Reacq
O	**9.62** ± 5.31	**10.94** ± 4.92	**12.52** ± 4.59	**64.37** ± 20.09	**55.56** ± 27.60	**46.24** ± 27.67	**10.16** ± 4.31
P	**6.38** ± 5.14	**7.09** ± 5.89	**8.17** ± 4.74	**48.87** ± 22.38	**46.59** ± 17.96	**31.38** ± 19.32	**8.55** ± 3.75

Acq, acquisition; Ext, extinction; Reacq, reaquisition; O, oxytocin; P, placebo.

present, even after OT administration. There is evidence that OT may have differential effects in the case of a familiar person compared to a stranger (see De Dreu, 2012; Persson et al., 2017). Finally, the test used here was probably not sensitive enough to detect differences between groups, especially during the active phase in which the experimenter promotes interaction and thus facilitates the appearance of social responses. This could have equated the performance of dogs from both groups.

However, our results are in line with recent studies that did not find OT to improve proximity seeking and contact toward people (Nagasawa et al., 2017; Thielke et al., 2017). It has been suggested that OT could increase social responses according to the context and its perception as positive or threatening (Bartz et al., 2011; Turcsán et al., 2017). As stated by Buttner (2016), the effect of OT is complex and interacts with multiple modulating factors, such as the context, the sex of the dogs and the stress levels.

On the other hand, pet dogs gazed more at the human face to ask for food than shelter dogs. This replicates our previous findings (Barrera et al., 2011) and may be related to the fact that shelter dogs have a deficit in some communicative responses associated with a long learning history with low human contact. These findings are consistent with the Two Stage hypothesis (Udell et al., 2010) that states that dogs' communicative abilities are not only the product of domestication, but of learning and experiences during ontogeny.

Regarding the effect of treatment, no significant difference was found during acquisition. The presence of food probably made both groups react similarly, by making them reach a ceiling level in their response. Moreover, it could be argued that the experimenter's hand reaching toward the food became a conditioned reinforced by its repeated pairing with it, signaling the availability of reinforcement shortly. Because of this, dogs rapidly started gazing toward the hand before receiving the food and this may have contributed to the appearance of a ceiling effect on the gazing behavior toward the experimenter's face.

Conversely, we have demonstrated that OT increases the duration of the gaze at the human face during the extinction phase, when the animals are no longer reinforced to gaze at the person. This effect was observed with the administration of both 16 and 24 IU of OT. Oxytocin probably increases not only the duration of the gaze *per se* but also its persistence when this response is unsuccessful, so that the most significant differences are seen during the extinction phase when dogs receive no food.

Contrary to our predictions, the OT effect was similar on pet and shelter dogs. This suggests that the increase of gazing as a requesting behavior, appears besides the level of daily social contact of the dogs.

The differences observed during extinction, may also be due to the effect of OT on decreasing stress and anxiety (e.g., Kis et al., 2014b). For instance, OT reduces the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Uvnas-Moberg and Petersson, 2005). In addition, increased levels of OT were associated with stressful events (Engert et al., 2016; Li et al., 2016). Given that the extinction phase produces emotions similar to stress (Konorski, 1959), the anxiolytic effect of OT could facilitate the persistence of the gazing response.

It has been suggested that this stress reducing effect of OT is related to a drop in attention and vigilance toward socially threatening stimuli (Kis et al., 2017; Somppi et al., 2017; Turcsán et al., 2017). For instance, after OT treatment, dogs gazed less toward angry faces in comparison to happy or neutral ones (Kis et al., 2017; Somppi et al., 2017). Additionally, human gaze may be interpreted as threatening or positive according to the context (Tops et al., 2018). Taking this into account, one possible explanation for these results could have been that dogs interpreted the human gaze as threatening and the administration of OT helped reduce this effect. However, the context of this study was appetitive as dogs receive food for gazing at the human face. Therefore, OT effects appear to be related to affiliative mechanisms and not to a decrease of negative stimuli during the test.

In relation to this, one important issue to consider is that shelter dogs lived at least 2 years in a potentially stressful environment. Therefore, the interaction between OT and stress could influence the performance of dogs from this group. Further research involving physiological assessments of the basal levels of stress (i.e., heart rate, cortisol levels) in shelter and pet dogs is needed to confirm this hypothesis.

Finally, it should be noted that the sex of dogs did not affect any of the responses assessed, although in some previous studies OT effects were only noted in female dogs (Nagasawa et al., 2015; Oliva et al., 2015).

One limitation of this study is the little information available about the shelter dogs' history. For instance, it is impossible to know whether they ever lived in a family, if they had past traumatic experiences with people or if they were surrendered because of behavioral problems. Another limitation is that, when generalizing these results, it must be considered that only mongrel dogs were evaluated in these studies. Therefore, it is not possible to conclude whether the observed differences would appear in dogs of particular breeds, especially given that the effects of OT have been shown to be differential across breeds (e.g., Kis et al., 2014a; Kovács et al., 2016b; Nagasawa et al., 2017).

Finally, it must be taken into account that in this study only two OT doses were evaluated, while in the literature even higher doses (i.e., 40 IU) are used. It would be interesting to compare the effects of more OT doses in further studies.

In sum, our findings demonstrate that OT is related to the persistence of gazing at the human face when food is inaccessible in pet dogs. We have demonstrated for the first time that shelter dogs also increase their gaze at the human face to ask for food after OT application. This finding is particularly relevant for two reasons. First, prior studies indicated that shelter dogs gaze less than pet dogs. This suggest a communicative response deficit in shelter dogs that may be reversed with the administration of OT. Second, gazing is a key response in the human-dog bond (e.g., Miklósi et al., 2003), so it is critical that shelter dogs acquire this response to encourage the possibility of successful adoption and reintegration to family life. In the future, it would be interesting to assess the effect of OT on other communicative tasks in shelter dogs. Moreover, we found that two different doses of OT produce similar effects on gazing to the human face as a communicative response to request food.

The present results show that it is possible to analyze the neurophysiological mechanisms and, particularly, the role of OT in the human-dog bond, by using a minimally invasive method like nasal administration. This helps to examine the relationship between OT and a wide range of socio-cognitive responses in different dog populations as well as provides valuable information on the potential therapeutic value of OT.

AUTHOR CONTRIBUTIONS

GB, VD, CC, and MB designed the studies and participated in the data collection. GB, VD, and CC measured the videos. MB and GB analyzed the data. All authors contributed in the writing of manuscript, collaborated to its revision for important intellectual content, read and approved the submitted version.

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BOOK REVIEW
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Book Review: Scientific Method: How Science Works, Fails to Work and Pretends to Work

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A Book Review on

Scientific Method: How Science Works, Fails to Work and Pretends to Work

John Staddon, (Routledge; Taylor & Francis Group, London), 2017, 158 pages,
ISBN: 978-1138295353.

This book aims to disinfect egregious practices in social science by illustrating errors commonly made by many social scientists, including some with Nobel prizes. Professor John Staddon (JS) is one of those rare individuals held in equally high regard for his seminal works in experimental and theoretical Psychology (Staddon, 2001), and readers stand to greatly benefit from the litany of suggestions borne of JS's experience. One notable highlight is the author's long-standing criticism of static (time-independent) theorizing in Psychology, which generally requires some "executive control" system to initiate operations (cf., Bandura's self-system—p. 63). The author suggests that psychological scientists incorporate *time* as a constitutive element within their explanatory models, fortifying his thesis through examples of complex behavioral systems, such as a child's expectation of punishment following a display of aggression (p. 66), without reference to some intervening all-knowing homunculus. Professor JS is among the forerunners of the silent behavioristic renaissance (Staddon, 2014) and readers may be surprised to know how this once maligned science of "muscle twitches" and "glandular squirts" (Bower, 2014) evolved into a compelling alternative to buttress against the excessive "surplus meaning" underlying information processing approaches in Psychology (Amsel, 1992).

The book consists of eight brief and informative chapters, with Chapters 1 through 3 showcasing to the reader methodological oversights common to scientific conduct. The remaining chapters focus on problems more specific to a given area in social science. Chapter 1 eloquently posits the processes of induction (approximating some rule behind a phenomenon's occurrence) and deduction (generating causal predictions, or hypotheses, from the earlier approximated rule) as equally necessary for scientific understanding, countering claims by those who imply the utility of one process over another (e.g., Hayes et al., 2001, p. 144). This opening salvo will re-orient social scientists who have been led astray through misinformation about what constitutes scientific understanding.

Chapters 2 and 3 go on to detail specifics regarding experimental methodology and the disadvantages from relying exclusively on null hypothesis testing, which can include (for example) not considering how differences in *a priori* information affects the probability distributions of the variables under investigation, and consequently, using the same null hypothesis and alpha criteria across all research questions. Indeed, the $p < 0.05$ significance threshold employed across much of social science was originally meant to discriminate across crop yields (pp. 33–34), not for disentangling behaviors of complex systems! Finally, it is worth restating that rejecting a null statement about the relationship between groups of subjects tells us nothing about any *specific* relationship between the groups compared. Alongside recommending more rigorous

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statistics, including the reporting of effect sizes, more conservative significance thresholds ($p < 0.001$), and the use of Bayesian priors, the author reminds us of the merits underlying the neglected, but still powerful, single-subject approach in experimental psychology. First championed by B. F. Skinner, single-subject approaches can verify cause-and-effect relations across individuals with a resolution group comparison cannot match. Keeping in mind that many psychologically interesting topics are defined, and must correspondingly be explained, at the group level (e.g., gender identity, or political affiliation), JS's exposition remains invaluable as it showcases to readers how sound psychological analyses need not be restricted to large-scale group comparisons and classical frequentist approaches.

Most methodological errors, at least in social science, should be detectable by the reader if the lessons in the first three chapters were carefully attended to, but JS's suggestions go beyond mere technical refinements. Over the remainder of the text, JS focuses on the thornier issue of confused terminologies in social science. An excellent example is provided in the discussion on "heredity" and "intelligence" in Chapter 4, where JS points out that most individuals outside the intelligence research community assume researchers talk about *genetic* heritability when what researchers typically imply is *statistical* heritability. Regarding the former, JS argues we know little about how parental genes cause a child's brain, let alone her behavior, whereas statistical heritability describes how transformed parental variables (e.g., mean IQ of parents) relate to transformed offspring variables (e.g., mean IQ of children), telling us nothing about the relative contribution of each parent's "intelligence" genes (whatever these may be) to the child's IQ. As for "intelligence," it is tautologically defined as being "that" which intelligence tests measure (p. 58), providing little scope for external validation of the construct. The author suggests we replace "intelligence" with "adaptability" as a more quantifiable and better defined proxy of human achievement (p. 61), converging with mainstream approaches declaring the many benefits of psychological flexibility in learning contexts (Asikainen et al., 2018). The chapter provides excellent talking points for the always-popular coffee table topics of heredity and intelligence.

The remainder of the book focuses on Economics and related areas, which are shown to be also rife with fuzzily defined concepts (how do we measure "affluence"?—p. 73; when is a market "efficient"?—p. 116) mired under a guise of mathematical formalism. Of note is a fascinating critique of the highly-cited Prospect Theory (PT), which led its authors to a Nobel Prize in Economics. JS is unimpressed, chastising PT's authors for re-framing operations under labels like "combination, segregation, isolation, coalescing" without actually explaining what these processes involved. Furthermore, the author illustrates how nearly a third of all subjects tested under PT produced results directly contradicting PT (p. 107), highlighting how even eminent Nobel prize winners are not impervious to scientific error.

The book concludes with a discussion on why outcome-focused (functional) and causal (mechanistic) models are equally essential for scientific understanding, for while a good functional model can produce predictable outcomes, the lack of any constraining causal analyses can obviate the very error-detection mechanisms that shields a scientist from erroneous inferences and inept theorizing (e.g., Heebner, 1891; for more recent examples, see Burgos, 2003).

The author paints a worrying picture of academia, where motions of "doing science" and the production of publications are incentivized over actual scientific understanding. Fortunately, JS is more then up to the task of providing a much-needed counter-balance, producing a text that should be required reading in any introductory class on research methods. *SciM* is a welcome addition to the literature on scientific research methods and is highly recommended for all students of behavior.

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The author confirms being the sole contributor of this work and has approved it for publication.

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TECHNOLOGY REPORT
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The Adventures of Amaru: Integrating Learning Tasks Into a Digital Game for Teaching Children in Early Phases of Literacy

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In low-income countries, the history of academic failure is a liability for children acquiring literacy skills. It is thus important to develop strategies that motivate and focus these students on specific strategies to learn to read. Digital games can be useful in motivating students and assisting teachers in the teaching-learning process, but there are few interactive tools that effectively integrate tasks of direct instruction and good gameplay. This technical report describes an interactive digital game to engage students in the initial phase of reading skills acquisition, whose design incorporates evidence-based procedures. The game, called "The Adventures of Amaru," aims to promote word coding-decoding skills and vocabulary growth through teaching trials. We discuss the adaptation of reading teaching curricula, their limitations and future implications of the use of this game by children from a low-income background.

Keywords: digital game, teaching, reading, matching-to-sample, narrative and gameplay

INTRODUCTION

Adopting new technologies of gaming as part of the teaching-learning process can promote engagement in academic activities with attractive experiences that meet educational needs and lead to better outcomes for the students (Katsikitis et al., 2014; Abdul Jabbar and Felicia, 2015; Ke, 2016). This can be particularly significant for children of developing countries such as Brazil, where the average performance in reading of students is meaningfully below the Organization for Economic Cooperation and Development (OECD) average: with 407 points, compared to the average of 493 points on the PISA (Program for International Student Assessment) standardized test. An aggravating factor is that the share of low performers in reading in Brazil has remained the same since 2009 (OECD, 2015). Besides that, according to the Brazilian Institute of Geography and Statistics (IBGE), in 2011 more than 4 million children aged between 5 and 7 years were illiterate, and many of the students in the subsequent grades did not have basic reading skills (IBGE, 2011). The experience of academic failure has negative impacts on reading performance and can cause demotivation, school dropout, and reading non-comprehension (Pajares, 2003; Neild et al., 2008; López et al., 2017).

One workaround indicated by researchers is the positive influence of direct instruction procedures to foster the learning of basic reading skills, such as the teaching program called Learning to Read and Write in Small Steps (ALEPP – acronym in Portuguese) (De Souza et al., 2009; Rose et al., 2012). The ALEPP curricula are aimed at identifying and remediating the basic reading skills of students with a history of academic failure in regular classes. This teaching program uses the consolidated discrete-trial procedure Match-to-Sample (MTS) to promote learning of the initial basic reading skills and vocabulary formation (Perfetti and Hogaboam, 1975). When integrated into a computerized learning path manager, this program allows for the monitoring of progress through educational units (set of activities to be taught) which, in turn, allows for analysis of the learning needs of each student (Orlando, 2009). However, ALEPP presents the teaching tasks (the same used in basic cognitive and behavioral process research) in a classic MTS layout and structure because of the growing and consistent learning curve, which often leads students to boredom and considerable abandon rates, as discussed in a series of doctoral and masters theses that tested this procedure in Brazil.[1]

In this context, researchers (Battaiola et al., 2008; McGonigal, 2011; Kim et al., 2015; Cezarotto and Battaiola, 2016) also assert that the narrative of a game and its characteristics can be determinant in maintaining the player's engagement. Generally, learning is sustained by motivational applications and by conventional methods (Marques et al., 2013; Mourgues et al., 2016), but the students prefer educational methods based on games, rather than other applications that do not have game characteristics (Sarmanho, 2012; Hwang and Wang, 2016).

Some studies (Smith et al., 2013; Ronimus et al., 2014; Hwang and Wang, 2016) have had positive results in improving teaching using motivational aspects. Smith et al. (2013) investigated the learning and engagement of Chinese university students in teaching English vocabulary; there were indications of learning in computer games with better knowledge gains than traditional methods. Hwang and Wang (2016) developed an educational game with the goal of observing learning and engagement in language teaching; the study confirmed that games could engage students in the search for better answers. In the research developed by Ronimus et al. (2014), the objective was to help children with dyslexia identify the sounds of letters and words; the effects of reward, challenges, and engagement of children in educational games have showed that digital games could be good tools for aiding literacy.

In the domain of stimuli association using the MTS procedure for improving reading, the game used by Marques et al. (2013) and Siqueira et al. (2012) has a story wherein the player walks through the stages in a style similar to the classic RPGs (Role-Playing Games), such as Zelda^{TM}, Chrono Trigger^{TM} and Phantasy Star^{TM}. Marques et al. (2013) used the digital game with a playful plot and a progressive transition of learning tasks based on the student's performance. The learning trials were presented as a solution to puzzles and the discovery of paths. The authors indicated that the use of interactive multimedia such as games and web applications stimulated the student's interests. In this protocol, the player must accept some challenges in the course of the game to complete a set of items. However, at the time of learning words, the player is presented in the style of classic MTS trials with little interactivity. This can lead the player to "escape" these challenges by looking for other challenges inherent in the game itself. There are tools that have several forms of interactivity and gameplay, but the mini-games do not interconnect in a narrative or story that could aid in the engagement of the player for a longer time in the teaching, as does the game used by researchers Richardson and Ronimus (Richardson and Lyytinen, 2014; Ronimus and Richardson, 2014).

The objective of this technology report is to present a digital game called "The Adventures of Amaru" (in Portuguese – "As Aventuras de Amaru"), that addresses these issues by means of an innovative game-based design, and assists written word recognition using image-text-speech relations. The MTS procedure was embedded in a personalized learning path and game-like activities in the hope of increasing engagement and learning. The game uses features that can be attractive to children, such as story and interesting characters, rewards after the completion of challenges and fewer repetitive mini-games (Ronimus et al., 2014). Additionally, the integration and inclusion of trials and stimuli are intended to be relatively simple for a teacher trained in the behavioral analysis process.

MATERIALS AND METHODS

The digital game used in the research was built using the Unity 3D engine (Unity, 2018) and C# programming language, with the mechanics of platform games (e.g., Super Mario Bros^{TM}, Sonic^{TM}, and Alex Kid^{TM}). The game presents MTS teaching trials integrated to a story that revolves around the character named Amaru, along with his robot friend named Urama. The 3D objects were modeled in the Blender tool (Blender, 2018), with main characters and the 2D objects edited in the GIMP (GNU Image Manipulation Program) tool (Peck, 2006; GIMP, 2013). The graphic arts of the characters were inspired by virtual pets generally found in cartoons (e.g., Pokemon^{TM}, Digimon^{TM}, and Medabot^{TM}), to further appeal to children. Moreover, the sound, audio, and image aspects of this game were built by a team specialized in multimedia. The game narrative was created with gamification aspects, as shown in the story clipping in **Figure 1**.

In the context of the game narrative, the teaching trials are intended to teach the character to communicate in the local language with the two children presented at the beginning of the story, hence Amaru's need to learn to read the objects in the new planet to fix his spaceship, in this case, words written in Brazilian Portuguese.

A test conducted with an early prototype of the game proved to be valuable, allowing us to improve its utility by better

[1]The ALEPP curriculum has been developed by a group of researchers motivated to handle the performance literacy gap of brazilian children. These efforts details can be found on the SubProject 7A (Reading, writing, and mathematics in the classroom) from the "National Institute of Science and Technology on Behavior, Cognition, and Teaching/" Final Report (http://www.inctecce.com.br/images/annual-report/web_inct-ecce-7_high-2.pdf).

FIGURE 1 | After a meteor shower, Amaru was forced to land on earth, where he meets two kids that soon befriend him. Amaru has a faithful squire who accompanies him, the robot Urama.

understanding the features of the game, fixing bugs and making software improvements, such as the insertion of new mini-games and types of tasks (de Souza et al., 2017).

The basic skills that the ALEPP promotes are words and syllables written to spoken bidirectional relations through MTS trials, similar to decoding skill as (context-free) word recognition or phonics. The MTS is structured as a discrete trial task with one stimulus, functioning as a model and two or more comparisons stimuli, from which the subject can choose, promoting a conditionally discriminative behavior (Sidman and Tailby, 1982). Then, the subject can identify which is the correct option to choose (i.e., the written word "*cake*") in the presence of a certain model (i.e., the spoken word "*cake*").

Additionally, the task ordering and conditionalities (alternative options that are displayed) ensure the evaluation of a basic level of comprehension by two MTS tasks: (1) figure-word recognition test, when the spoken word is the model and different figures are the comparisons; (2) emergent behavior, based on the assessment of non-direct relations between written words and figures that illustrate it. The emergent behavior means that to demonstrate mastery of this skill, the student needs first to relate the spoken word with the figure, and, afterward, relate the written word with its spoken counterpart. A form of conceptual learning occurs if the relation between the written word and the figure emerges, according to the equivalence paradigm (Sidman et al., 1989; Sidman, 2000).

The order of tasks implemented in the game promotes equivalence relations among words via procedures of modalities association, such as written, spoken, figures (representative) and syllables (as word components). These word modality associations are always based on MTS and CRMTS (Constructed Response – MTS); the latter for the training of composition of words by the correct composition of letters and syllables. The behavioral teaching procedures programmed in the ALEPP curriculum focus on word decoding and in reinforcing the Brazilian Portuguese vocabulary. **Table 1** presents examples of tasks that promote the word modality relationships used in the game.

THE PROPOSED TEACHING DIGITAL GAME

The digital teaching game produced in this work is named "The Adventures of Amaru" and can be used for both training and teaching words, as well as for evaluation (similar to a test). For evaluation purposes, however, there is no correct or error feedback, and the player only has one chance to succeed in each trial.

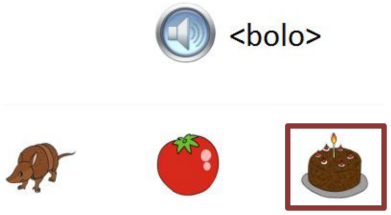
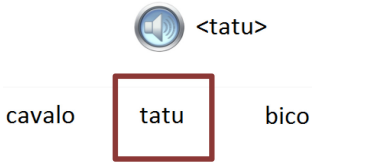
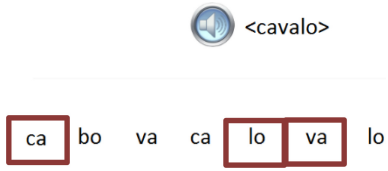
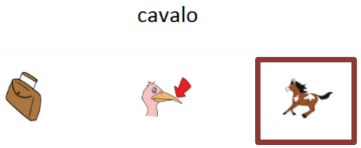
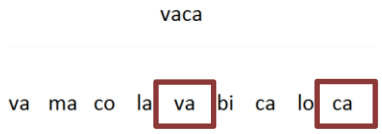
To aid in the insertion of trials by the teacher, an auxiliary tool was developed that allows the inclusion of a CSV (Comma-Separated Values) file, where each row is a trial and each column is an attribute of this trial, as shown in **Figure 2**. The first column indicates the task type, or the word modality relation being taught. The game makes a uniform division of trials per stage: for example, with a set of 45 trials inserted in the game, each stage will have 9 trials, following the order stipulated in the CSV file. The images, sounds, and texts are stored in folders with their respective settings, which allows the easy inclusion and removal of the assets used to compose a trial.

The main goal of the player, playing as Amaru, is to rebuild the spaceship, and to do so he/she needs to collect items such as gears, bolts, and nuts in game stages. Each item has a specific score and, based on the items collected at the end of the journey, the player gains a ship, as shown in **Figure 3**. The rewards after performing an activity and collecting items are also gamification aspects of the MTS process. In the game, these items are found and the character is successful on the trial. For each wrong answer on a trial, the item to be collected undergoes depreciation, that is, if the player were to make only correct choices, the item will have a higher value, making a better-looking ship available at the end of the game. In training mode, the player has three chances to answer correctly on each trial. If the player chooses incorrectly three times on a given trial, the player advances to the next attempt, but does not gain any item to improve his ship. A tutorial at the beginning of the gameplay shows the commands, the player's mission and the ships he/she can win based on the items collected, as shown in **Figure 4**.

A stage in the game is basically composed of modified MTS trials that the player executes controlling an avatar (i.e., Amaru), with keyboard, mouse, joystick or computer touch-screen. The novel fun game features may only have a short-term positive impact (Ronimus et al., 2014). Soon after each trial, an obstacle arises that has the collectible item as a reward. This cycle repeats itself until the trials of that stage are finished. The game has five stages, set in the following environments: forest, countryside, city, industry, and beach. At the end of each stage, there is a screen transition that shows a mini-map and the progress of the score (**Figure 5**). This status display is a gamification feature that generates a sense of advancement and ultimate goal to be fulfilled.

One of the characteristics of classic MTS trials in computing environments is that the person must select (i.e., clicking the mouse button) the option which he/she considers correct. In this

TABLE 1 | Types of equivalence relations of the MTS and CR-MTS procedure used in this proposal.

Trial illustration	Trial description	Trial illustration	Trial description
	Identification of the type of relation (nomenclature): AB. Based on a sound instruction, the student must choose the picture of a "bolo" (cake).		Identification of the type of relation (nomenclature): BB. Based on the picture of a "mala" (suitcase), the student must choose the picture of a "mala" (suitcase).
	Identification of the type of relation (nomenclature): AC. Based on a sound instruction, the student must choose the word "tatu" (armadillo)		Identification of the type of relation (nomenclature): CC. Based on the word "bico" (beak), the student must choose the word "bico" (beak).
	Identification of the type of relation (nomenclature): BC. Based on the picture of an "apito" (whistle), the student must choose the word "apito" (whistle).		Identification of the type of relation (nomenclature): AE. Based on a sound instruction of word "cavalo" (horse) the student must choose the syllables in the correct order: ca - va - lo.
	Identification of the type of relation (nomenclature): CB. Based on a written instruction, he student must choose the picture of a "cavalo" (horse).		Identification of the type of relation (nomenclature): BE. Based on the picture of a "tomate" (tomato), the student must choose the syllables in the correct order: to - ma – te.
			Identification of the type of relation (nomenclature): CE. Based on the word "vaca" (cow), the student must choose the syllables in the correct order: va - ca.

	TrialType	Choices	CorrectAnswer	MiniGame	TrainingOrTesting
2	BB	tatu luta lata	tatu	Cube	training
3	AB	lata fita tubo	lata	Platform	training
4	BC	mala mato luta	mala	Platform	training
5	CB	tatu lata mato	tatu	Shot	training
6	CE	ta-tu ma-pa lu-ta	tatu	Cube	training
7	CB	mala tatu mapa	mala	Platform	training
8	CC	tatu tubo lata	tatu	Cube	testing
9	BC	mala mapa mato	mala	Cube	testing
10	BE	ma-la ma-to ma-pa	mala	Platform	training
11	AC	lata luta fita	lata	Platform	training
12	AC	lata luta fita	lata	Cube	testing
13	AE	la-ta fi-ta lu-ta	lata	Shot	training

FIGURE 2 | CSV file configuration of a set of trials inserted for in-game presentation.

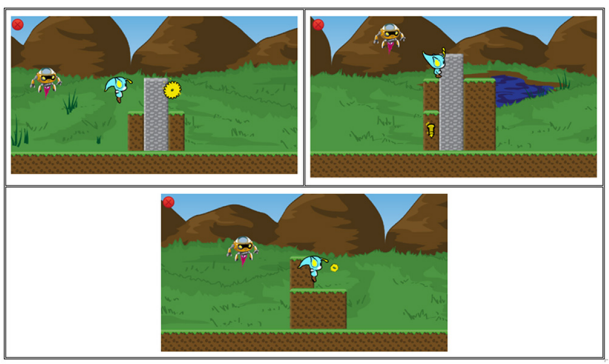


FIGURE 3 | Game items and obstacles between trials.

FIGURE 4 | Game tutorial screens.

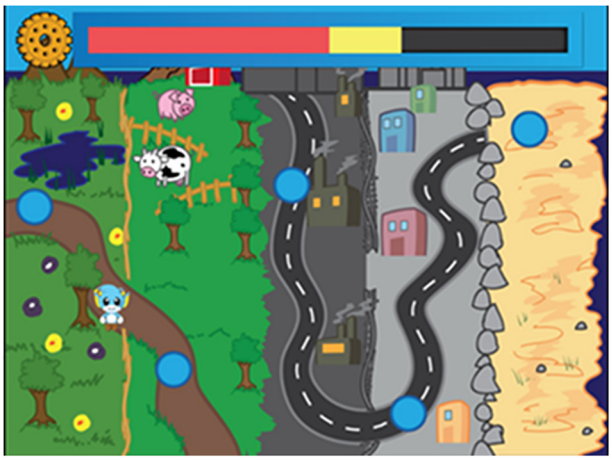


FIGURE 5 | Mini-map between stages of the game, similar to a previous prototype (de Souza et al., 2017) (Reproduced with the consent of the IEEE copyright).

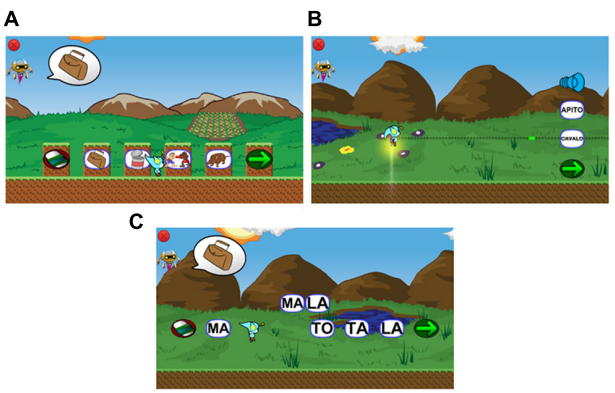


FIGURE 6 | Trials presented in the game. **(A)** platform mini-game, **(B)** shooting mini-game and **(C)** cubes mini-game.

game, different means are implemented for selecting the options (**Figure 6**). In the first kind of player-task interaction, the avatar must jump and touch one of the floating options above the screen. In the second kind, the player must jump on a platform to choose the option. In the third, the avatar can shoot with a projectile to hit the target options. The position of the options is shuffled trial-by-trial to avoid repeating positions of the correct choice and to stimulate the screening behavior of the students. These strategies can make the game more dynamic and less repetitive. Despite the response type variations on the stages, at the behavior control level, all the game mechanics used to promote the same learning relations are found in regular MTS.

The character Urama is a helper avatar that has the role of presenting the reference model (the word in the format of speech, text or figure). The game provides positive feedback when the option is correct and a 'try again' feedback when it is incorrect. In order for Amaru to complete the task and move on to the next trial, the player must select the green arrow, **Figure 7A**, that

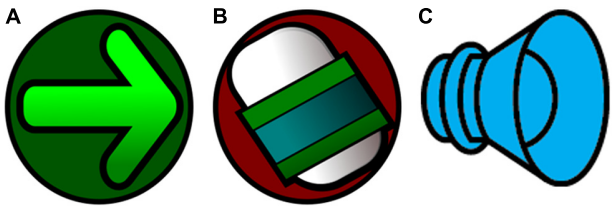


FIGURE 7 | (A) image of the "arrow" that must be activated to confirm the option selected in the trial, **(B)** image of "eraser" to correct the option and redo the trial, **(C)** image of a "speaker" to hear the trial model/command again.

indicates continuation of the gameplay, or he/she can redo the task by selecting the icon of an eraser, **Figure 7B**. In the case of trials that have sound (speech model), the player can select the option to listen again to the sound of the word dictated, **Figure 7C**.

At the end of the stages, the game generates a log file in tabulated format. The log arranges the trials configuration and the user responses (see in **Figure 8**), allowing further analysis by the teacher regarding the student's progress.

Figure 9 illustrates the use of a teaching activity in an environment with teachers and students, using the game as an educational tool. At an early stage, the teacher inserts the trials in the game to be presented to the student. Students are then given guidance on how to use the software. In order to evaluate learning, a pre-test is applied. Trials are presented to the student individually, and if the student meets a condition, he/she may be presented with further trials that teach other words. For example, if the student achieves more than 70% of the correct trials, he/she advances to a new teaching stage. In the end, a post-test can be applied and compared to the pre-test; in this way, the teacher can identify improvements in the student's learning.

DISCUSSION AND FUTURE WORKS

According to the Brazilian National Literacy Assessment, in 2016 more than 50% of children aged between five and eight

	User	TrialType	Choices	CorrectAnswer	GivenAnswer	NumberOfAttempt	Latency(s)	Result	MiniGame	DateTime
2	student1	AB	tatu luta lata	tatu	tatu	1	6,25	CORRECT	Cube	2016-12-12T08:59:47.6648-03:00
3	student1	AB	lata fita tubo	lata	lata	1	16,98	CORRECT	Platform	2016-12-12T09:00:12.1438-03:00
4	student1	AB	mala mato luta	mala	mala	1	21,21	CORRECT	Platform	2016-12-12T09:00:41.4848-03:00
5	student1	CB	tatu lata mato	tatu	tatu	1	3,88	CORRECT	Shot	2016-12-12T09:06:15.3248-03:00
6	student1	CB	tatu mapa luta	tatu	luta	1	7,89	INCORRECT	Cube	2016-12-12T09:06:46.6738-03:00
7	student1	CB	tatu mapa luta	tatu	mapa	2	10,48	INCORRECT	Cube	2016-12-12T09:06:57.1508-03:00
8	student1	CB	mala tatu mapa	mala	mala	1	19,05	CORRECT	Platform	2016-12-12T09:11:43.7788-03:00
9	student1	CB	tatu tubo lata	tatu	tatu	1	7,98	CORRECT	Cube	2016-12-12T09:11:55.6088-03:00
10	student1	BC	mala mapa mato	mala	mapa	2	14,32	INCORRECT	Cube	2016-12-12T09:17:32.2308-03:00
11	student1	BC	mala mapa mato	mala	mato	3	4,14	INCORRECT	Cube	2016-12-12T09:17:36.3708-03:00
12	student1	BC	mala mato mapa	mala	mala	1	26,59	CORRECT	Platform	2016-12-12T09:18:08.5698-03:00
13	student1	BC	lata luta fita	lata	luta	1	34,95	INCORRECT	Platform	2016-12-12T09:19:10.4668-03:00
14	student1	BC	lata luta fita	lata	lata	2	14,28	CORRECT	Platform	2016-12-12T09:19:24.7508-03:00
15	student1	AC	lata luta fita	lata	lata	1	23,28	CORRECT	Cube	2016-12-12T09:19:52.8008-03:00
16	student1	AC	lata fita luta	lata	lata	1	10,89	CORRECT	Shot	2016-12-12T09:20:14.5728-03:00
17	student1	AC	lata fita luta	lata	luta	1	7,55	INCORRECT	Platform	2016-12-12T09:20:54.5138-03:00

FIGURE 8 | Log file generated at the end of the game.

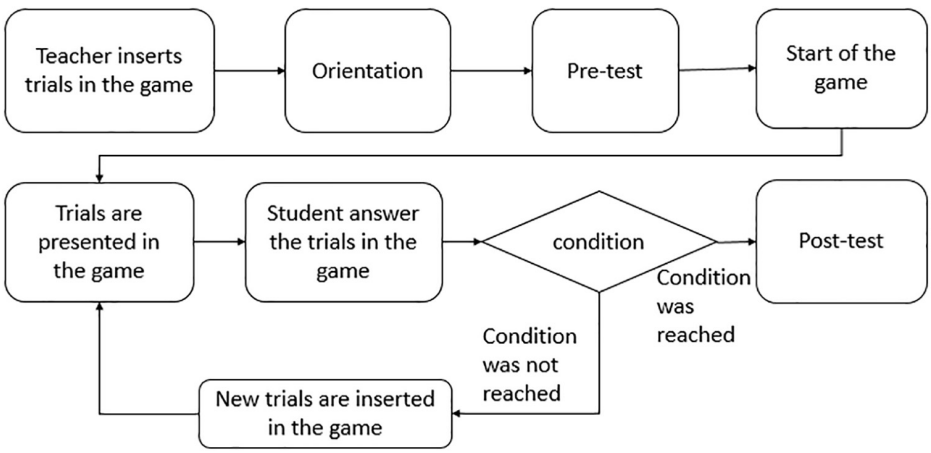


FIGURE 9 | Flowchart with an example of teaching activity using the game.

had insufficient knowledge according to Bermúdez (2017). The northern region of Brazil was the most worrisome, with approximately 70% of school-age children presenting some difficulty in reading and writing (Bermúdez, 2017). "The Adventures of Amaru" is a game that uses evidence-based teaching procedures to help promote basic reading skills. This kind of technology can aid schools in low-income areas that do not have internet or updated computers. For example, schools in cities such as Belém, Brazil, have computers but do not have stable internet connections.

Using play tools to teach children to read increases the possibility of engaging them and even their learning. In the literature that investigates the use of games as a teaching tool, reviews show not only an increase in academic performance, but also improved motivation of the participants (Clark et al., 2016; Ke, 2016). The main application of a playful technology intended to teach reading is geared to the school environment, but not exclusively. Domestic use of the game can also be useful, as it exposes the child to a pleasurable activity that also teaches. The main advantage of educational games using artificial intelligence is the capacity of identifying student performance and, based on it, programming the ensuing tasks. This saves time for the educator, who can then serve as a mentor, and exposes the students to the training while maintaining motivation through engagement and introducing new challenges through novel tasks. Students who are in the initial stage of literacy can use this technology as a complementary tool for learning idioms. The teacher can use it both as a class activity and as homework. In a playful way, technology can teach, using the combination of images and sounds and through a paradigm that tries to understand human behavior and, as well, employ an approach that matches how effective teaching actually occurs.

The difference between "The Adventures of Amaru" and other computerized games designed for teaching lies in the approach of integrating the teaching procedure into a narrative and merging it with the gameplay. The items and stimuli are integrated with the story, making sense within the narrative, thus aiming to discourage the player from choosing to engage in the non-educational aspects of the game over the educational aspects. In contrast, other digital games (i.e., Tetris^{TM}, Snake^{TM}, and Pong^{TM}) use gameplay disconnected from the story, and with extra classic mini-games as rewards to sustain the motivation.

"The Adventures of Amaru" presents the MTS trials as a series of continuing challenges, implemented as "mini-games" planned to entertain and teach at the same time. Additionally, the game's architecture enables the creation and inclusion of new mini-games in future versions of the software, allowing new kinds of playful interactions. An additional advantage is that the game can be used at school or home, likely increasing its probability of use.

One strength of the game design is that it has the flexibility to be used with other languages and even different domain areas (e.g., math operations). Students with learning disabilities could also be benefited, since the game implements automated, instruction-free procedures, in a ludic context. For this reason, the game will be available and freely accessible to interested researchers accessing the web page, with the game installation step by step, the supplementary material, access to explanations videos and the current state of this research by the link http://linc.ufpa.br/amaru-mts/.

The project is heading toward the use of artificial intelligence techniques to produce new trials personalized for students. The authors intend to use assistive tools for the automatic generation of individualized reading tasks estimated by the performance of non-literate students.

Analysis of the learning of children in low-income areas can also be accomplished; it is possible that new data related to these experiments will be released at the end of this year. It is expected that, with further studies in this area, "The Adventures of Amaru" will be a valid, meaningful, and useful tool for the teaching-learning process.

This technology is a low-cost approach. Despite this, a major limitation is a need for financial resources to obtain a minimal structure, such as a computer, which not all children and schools are able to access, especially in low-income areas. In addition, the content that is presented in the game in its current version is preliminary and restricted to initial stages of literacy. There is a moderate amount of words or combinations of them. Further research can show and test the complexification the words to be taught.

AUTHOR CONTRIBUTIONS

GdS, MT, LM, PG, and DM made substantial contributions to the development of the concepts. GdS, MT, LM, PG, DM, and ÁS contributed to the drafting and approval of this manuscript and agreed to be held accountable for all aspects of this work. GdS and YB provided the computer programming of this work. GdS originated the project and coordinated the paper team.

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REVIEW
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Interaction Between Stress and Addiction: Contributions From Latin-American Neuroscience

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Drug addiction is a chronic neuropsychiatric disorder that escalates from an initial exposure to drugs of abuse, such as cocaine, cannabis, or heroin, to compulsive drug-seeking and intake, reduced ability to inhibit craving-induced behaviors, and repeated cycles of abstinence and relapse. It is well-known that chronic changes in the brain's reward system play an important role in the neurobiology of addiction. Notably, environmental factors such as acute or chronic stress affect this system, and increase the risk for drug consumption and relapse. Indeed, the HPA axis, the autonomic nervous system, and the extended amygdala, among other brain stress systems, interact with the brain's reward circuit involved in addictive behaviors. There has been a growing interest in studying the molecular, cellular, and behavioral mechanisms of stress and addiction in Latin-America over the last decade. Nonetheless, these contributions may not be as strongly acknowledged by the broad scientific audience as studies coming from developed countries. In this review, we compile for the first time a series of studies conducted by Latin American-based neuroscientists, who have devoted their careers to studying the interaction between stress and addiction, from a neurobiological and clinical perspective. Specific contributions about this interaction include the study of CRF receptors in the lateral septum, investigations on the neural mechanisms of cross-sensitization for psychostimulants and ethanol, the identification of the Wnt/β-catenin pathway as a critical neural substrate for stress and addiction, and the emergence of the cannabinoid system as a promising therapeutic target. We highlight animal and human studies, including for instance, reports coming from Latin American laboratories on single nucleotide polymorphisms in stress-related genes and potential biomarkers of vulnerability to addiction, that aim to bridge the knowledge from basic science to clinical research.

Keywords: addiction, stress, Latin America, biomarkers, mesocorticolimbic pathway, CRF

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INTRODUCTION

Drug addiction is a chronic neuropsychiatric disorder characterized by the compulsive intake of drugs of abuse and the loss of control over this consumption, in spite of the devastating consequences it carries for the individual (American Psychiatric Association, 2013). According to the World Health Organization, drug addiction affects between 3.4 and 6.6% of the population

worldwide and represents a major contributor to the global burden of disease and disability (World Drug Report, 2017). It is estimated that 0.4% of the annual deaths are due to drug abuse, with cannabis, cocaine, opioids, and amphetamine as the most frequently consumed illegal drugs worldwide (World Drug Report, 2017). Furthermore, in Latin America, the geographical region that comprises a part of North America (Mexico), Central America, South America, and the Caribbean, there is a strong association between drug use and having a history of violence, sexual abuse, and other stressful and traumatic events (Montoya et al., 2003; Alegría et al., 2004; Vera et al., 2005; Alvarez et al., 2007; Tucci et al., 2010; Frade et al., 2013; SAMHSA, 2016).

Addiction is cyclical: Individuals who suffer from this disorder transition from periods of abstinence to a return to drug use, complicating therapeutic efforts. The *addiction cycle* — the leading theory in the field — posits that chronic drug users go through three types of phases, one characterized by the preoccupation with consumption, with constant obsessing and craving, followed by another stage dominated by binging and intoxication that leads to a period of withdrawal and negative affect (Koob and Le Moal, 2008). This last stage, also referred to as the "dark side of addiction" and characterized by negative emotional states and stress, eventually transitions back to the preoccupation and craving stage, and the cycle begins again. Major efforts have been made to understand which factors contribute to the development of addiction and drug abuse and also to elucidate the mechanism behind the phases of the addiction cycle. Human imaging and preclinical studies indicate that addiction involves maladaptive changes in the brain's reward system that result from complex gene–environment interactions (Sweitzer et al., 2012; Walker and Nestler, 2018). Importantly, environmental factors such as early exposure to drugs or chronic stress facilitate the acquisition and maintenance of drug-related behaviors in vulnerable individuals, and are strong predictors of addiction relapse (Saal et al., 2003).

Addiction does not only comprise the positively reinforcing effects of drug consumption. Excessive activation of the neural reward system leads to its dysfunction and to hyperactivation of the brain's stress response (anti-reward), resulting in an increase in reward thresholds (reward deficit and stress surfeit). Stress therefore plays a very prominent role in all stages of the addiction cycle but particularly in the negative affect phase, also dubbed *the dark side of addiction* (Koob and Le Moal, 2005). However, there is still a gap in the literature regarding the precise molecular and neurophysiological mechanisms that are at play in the interaction between stress and addiction, and how these relate to the behavior exhibited by individuals with this disorder. In order to fill this gap, different laboratories in Latin America have been advancing research that focuses on every level of this relationship, from the molecular pathways of stress and their deleterious effects on the reward system, to the genetic and epigenetic factors that may facilitate these effects. The works of Latin American laboratories seem to not be as strongly acknowledged by the broad scientific audience as studies coming from developed countries (Meneghini et al., 2008). For this reason, here we wish to showcase these Latin American scientific contributions.

This review is the first attempt at compiling a series of studies conducted by Latin American-based neuroscientists, that have devoted their careers to studying the interaction between stress and addiction, from a neurobiological and clinical perspective. For this purpose, we included manuscripts that have been peer-reviewed and published in indexed journals, in which the leading or corresponding authors are affiliated to a university or institution located in Latin America. In the first section, we introduce the neurocircuitry associated with addiction, with a special focus on the mesocorticolimbic system, and we present the most commonly used animal models of drug addiction. In the second section, we briefly describe the role of stress as a main risk factor for drug addiction. In the third section, we report the different contributions made by Latin American researchers who study the molecular and cellular role of stress in drug abuse and addiction using rodent behavioral models. In the fourth section, we emphasize promising preclinical findings coming from Latin America, that could guide novel therapeutic advances for drug addiction. Further, we cover a number of human studies conducted in Latin American population that relate SNPs in stress-related genes to drug addiction vulnerability. Finally, we provide a concluding perspective and discuss opportunities for new research on which Latin American neuroscience could take the lead.

DRUG ADDICTION: ESTABLISHED MECHANISMS AND ANIMAL MODELS

The brain's reward system comprises several regions that are activated in response to rewarding stimuli — such as food, water, or sex — and constitutes an important regulator of complex cognitive processes, like motivation, expectations, and emotions (Koob and Le Moal, 2005; Volkow et al., 2017). This system involves primarily the mesolimbic and the mesocortical DA pathways as well as the medial forebrain bundle, and the extended amygdala (Koob and Le Moal, 2005; Lüscher and Malenka, 2011). The mesolimbic pathway includes DA projections that originate in the VTA and innervate primarily the MSNs of the NAc. The mesocortical pathway, on the other hand, comprises the projections of VTA DA neurons to the PFC (**Figure 1A**) (Lüscher and Malenka, 2011; Lammel et al., 2014). Release of DA by VTA

Abbreviations: 2-AG, 2-arachidonoylglycerol; ACTH, adrenocorticotropic hormone; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain derived neurotrophic factor; BNST, bed nucleus of the stria terminalis; CB, cannabinoid receptors; CB1, cannabinoid type 1 receptor; CB2, cannabinoid type 2 receptor; CBD, cannabidiol; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPP, conditioned place preference; CRF, corticotropin releasing factor; CRF-BP, corticotropin releasing factor binding protein; CRFR1, corticotropin releasing factor receptor 1; CRFR2, corticotropin releasing factor receptor 2; CRH, corticotropin releasing hormone; *CRHR1*, corticotropin releasing hormone genes; DA, dopamine; DEX, dexamethasone; EE, environmental enrichment; GDNF, glial cell derived neurotrophic factor; GPCRs, G protein-coupled receptors; GRs, glucocorticoid receptors; GWAS, genome-wide association study; HPA, hypothalamic–pituitary–adrenal; IL-4, interleukin-4; IL-6, interleukin-6; LHA, lateral hypothalamic area; LS, lateral septum; MSNs, medium spiny neurons; MRs, mineralocorticoid receptors; mRNA, messenger RNA; NAc, nucleus accumbens; NE, norepinephrine; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; PND, postnatal day; PVN, paraventricular nucleus; SNP, single nucleotide polymorphism; TNF-α, tumor necrosis factor α; UCN I, urocortin I; UCN II, urocortin II; UCN III, urocortin III; VTA, ventral tegmental area.

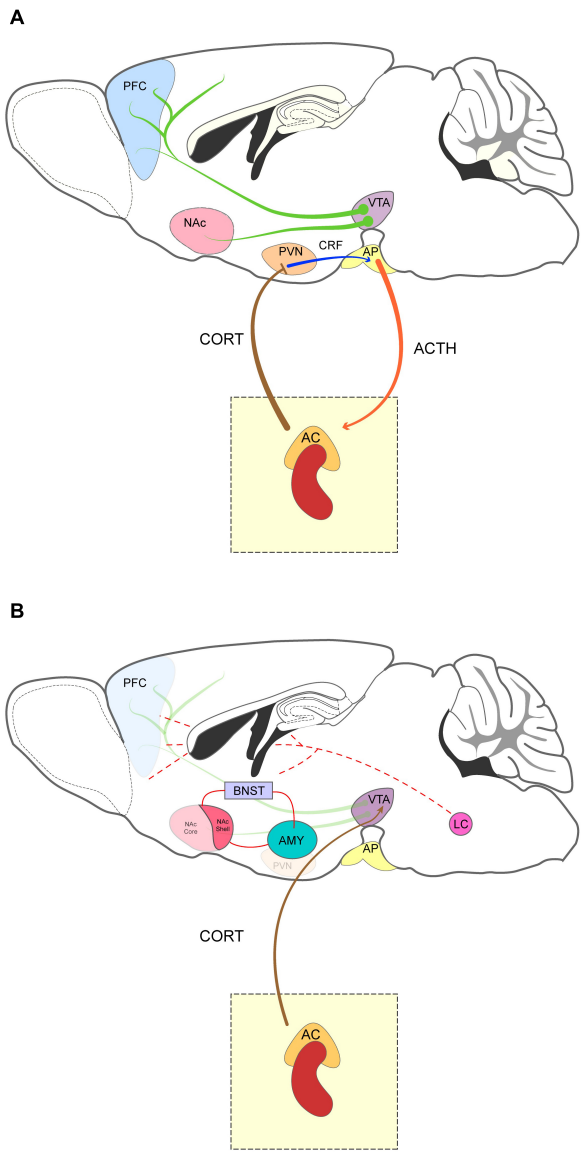

FIGURE 1 | Sagittal representation of the mesocorticolimbic system and the brain stress-related areas. **(A)** The mesocorticolimbic system is composed of dopamine projection neurons (green arrow) that innervate the nucleus accumbens (NAc) and prefrontal cortex (PFC). Drugs of abuse such as cocaine or amphetamine increase the activity of VTA neurons and lead to dopamine release in the NAc and PFC. The HPA axis comprises the paraventricular nucleus of the hypothalamus (PVN), the anterior pituitary gland (AP) and the adrenal cortex (AC). Stressful stimuli activate the PVN, which releases CRF (blue arrow) in the AP and stimulates adrenocorticotropic hormone (ACTH) (orange arrow) secretion into the bloodstream. In turn, ACTH reaches the AC to induce release of corticoids (CORT, brown arrow). Activation of the HPA is terminated by a negative feedback mechanism induced by corticoids in the PVN and AC. Acute and chronic exposure to stress lead to vulnerability to drug addiction by strengthening plasticity across the mesocorticolimbic pathway. Indeed, stress induces DA release in the PFC and NAc and "*sensitizing*" VTA neurons (Saal et al., 2003). **(B)** Relevant stress-related areas and their interaction with the brain's reward system. Corticosteroids from the adrenal gland stimulate the VTA and the extended amygdala [bed nucleus of the stria terminalis (BNST), amygdala (AMY), and the shell of the NAc]. The arousal system and the noradrenergic projections from the locus coeruleus (LC) is also represented (dashed lines).

neurons is further regulated by the PFC and the NAc. Pyramidal neurons in the PFC and NAc MSNs innervate VTA GABAergic interneurons which, in turn, exert an inhibitory control over DA neurons of the VTA (Carr and Sesack, 2000). For a more comprehensive review, see Lüscher and Malenka (2011).

Acute intake of drugs of abuse leads to rapid activation of DA neurons in the VTA and results in DA release in target areas. Imaging studies report that healthy individuals exhibit increased DA efflux in the NAc after acute alcohol and amphetamine consumption (Leyton et al., 2002; Boileau et al., 2003). These findings are consistent with preclinical evidence from rodents exposed to acute administration of cocaine, heroine, or endocannabinoids (Kalivas and Duffy, 1990; Weiss et al., 1992). In addition, repeated exposure to drugs leads to long-lasting maladaptive changes in the mesocorticolimbic system, rendering individuals more vulnerable for the development of addiction and relapse (Saal et al., 2003; Hyman et al., 2006). For example, cocaine users display increased DA release in the PFC and striatum in response to craving-inducing drug associated cues, suggesting that complex context-drug associations activate the mesocorticolimbic system during craving (Volkow et al., 2009). Furthermore, increases in the expression of monoamine transporters have also been observed in chronic cocaine and opioid users (Malison et al., 1998; Kish et al., 2001; Crits-Christoph et al., 2008; Shi et al., 2008).

The use of animal models has become a tremendous tool for studying drug-associated behaviors and deciphering the molecular mechanisms underlying the long-term effects of exposure to drugs. Different paradigms such as behavioral drug sensitization, CPP, and self-administration have been developed to study drug-reward, craving, and relapse in rodents. **Behavioral drug sensitization** was introduced to measure the enhanced behavioral and motivational effects induced by repeated administration of stimulant drugs (Vezina and Leyton, 2009). This paradigm is divided in two phases: development and expression. During the development of sensitization, repeated injections of psychostimulants such as cocaine or amphetamine are given intermittently to rodents over a period of several days, while a separate group of animals are injected with the drug's vehicle (e.g., saline). Expression of drug sensitization is assessed during a drug challenge administered following a period of drug abstinence, and is associated with an enhanced locomotor activity observed in animals previously exposed to the psychostimulant, in comparison to rodents treated with vehicle (Vezina and Leyton, 2009). CPP is a form of appetitive Pavlovian conditioning used to determine the association strength between drugs of abuse and a specific context. The basic procedure consists of two learning stages and one test: first, the drug of interest (e.g., cocaine or heroin) is administered in a particular environment (e.g., a chamber with white walls). In the second stage, a different environment (e.g., a chamber with black walls) is paired with the absence of the drug (e.g., vehicle administration). During the test, the time spent in each of the environments in the absence of the drug is recorded. Rodents that exhibit heightened place preference spend significantly more time in the drug-paired compartment and are therefore responding to the rewarding effects of the drug (Prus et al., 2009). Finally,

self-administration is a behavioral procedure used to assess *voluntary* drug-intake in rodents. This paradigm is based on operant learning in which drug-naïve rodents are trained to perform a specific behavior (e.g., lever pressing or nose-poking) that is contingent on the availability of the drug, thus making the drug a reinforcer of that behavior (Panlilio and Goldberg, 2007). Drug self-administration is particularly useful to studying processes such as drug motivation, extinction, and reinstatement (relapse) following an abstinence period (Panlilio and Goldberg, 2007). It is important to mention that certain situations — such as stress or drug exposure — can enhance the subsequent behavioral or neural responsiveness to a different addictive drug (Berridge, 2004). This phenomenon, known as **cross-sensitization**, can be observed in the three behavioral paradigms described above. In subsequent sections, we will discuss the remarkable work of Latin American neuroscientists who make use of these behavioral models to understand the neurobiological mechanisms of addiction.

STRESS AS A RISK FACTOR FOR DRUG ADDICTION

Stress is a neurophysiological and behavioral response that allows organisms to respond to environmental challenges perceived as threatening (Koolhaas et al., 2011). This process is directly controlled by the HPA axis and the autonomic nervous system, top-down systems that prime an organism for *fight-or-flight* or freeze responses, and contribute to restoring homeostasis (Koolhaas et al., 2011). Upon exposure to a stressor, the PVN of the hypothalamus secretes corticotropin-releasing factor (CRF), into the anterior pituitary gland, where it stimulates the release of ACTH into the bloodstream. As a result, ACTH reaches the adrenal cortex where it binds to its receptors to induce secretion of corticosteroids. Once in the bloodstream, corticosteroids are transported to different target organs where they bind to MR and GR receptors. Corticosteroids also induce a mechanism of negative feedback, suppressing the release of CRF and ACTH and ceasing the stress response (**Figure 1A**) (Carrasco and Van de Kar, 2003; McEwen, 2007).

Epidemiological studies have identified stress as one of the main risk factors for the development of drug addiction, and as a strong predictor of high craving and relapse to drug use (Sinha, 2008; Mantsch et al., 2016). Both cocaine and opioid-dependent individuals report having experienced stressful life events prior to drug seeking or relapse (Khantzian, 1985; Sinha, 2008). They exhibit increased levels of stress-related hormones, including CRF and ACTH at baseline or after pharmacological and psychological challenges (Schluger et al., 2001; Contoreggi et al., 2003). Preclinical models of addiction have demonstrated that exposure to stress can enhance the rewarding effects of drugs and the acquisition of drug-seeking behaviors. In this perspective, stress facilitates the formation of associations between contextual information and drugs (Mantsch et al., 2016).

In addition to the HPA axis, other relevant brain structures mediate the stress response and are involved in the pathophysiology of drug dependence, including the central and medial nucleus of the amygdala, the BNST, and the medial NAc (together known as the extended amygdala) (**Figure 1B**) (Alheid and Heimer, 1988; Koob, 2009). These areas are key substrates for the feedforward interaction between CRF and NE. CRF activates NE release in the locus coeruleus (Valentino et al., 1991) which in turn increases CRF in the extended amygdala. These two factors jointly participate in the progression of drug consumption from a positively reinforcing experience to a relief-seeking behavior from the negatively reinforcing effects of withdrawal (Heilig and Koob, 2007; Koob, 2009). NE induces CRF release and this cross-talk between both systems participates in the negative emotional states and drug-seeking behavior associated with drug withdrawal (Koob, 2009). NE is involved in the arousal-promoting effects of certain drugs like psychostimulants, but it has also been implicated in the increase in drug-cue salience and the attentional bias that are known to contribute to relapse (Chaijale et al., 2015; España et al., 2016). The interaction between PVN-CRF and midbrain DA systems in the VTA also contributes to the stress response associated to addictive behavior (Kelly and Fudge, 2018). Recent evidence indicates that projections from the BNST to the VTA requires the signaling of CRF receptor type 1 (CRFR1) and receptor type 2 (CRFR2) to modulate binge-like ethanol drinking (Rinker et al., 2017).

Several laboratories located in Latin America have contributed to the understanding of how acute or chronic stress, associated with exposure to addictive drugs, lead to plastic changes in the brain's reward system and increase vulnerability to addiction and relapse. In the following section, we describe this very diverse work, which spans the molecular and cellular levels, focusing on different pathways affected by stress mediators, and also explores the behavioral and clinical consequences of stress on addicted populations.

CONTRIBUTIONS FROM LATIN AMERICA

Stress and Psychostimulants: Amphetamine and Cocaine

For more than two decades, the laboratory of Dr. Liliana Cancela, at the *National University of Córdoba*, Argentina, has worked on characterizing the molecular mechanisms behind the increased vulnerability to developing substance use disorders observed in previously stressed subjects. Specifically, using rats as an animal model, Dr. Cancela's group has focused on the effects of acute and chronic restraint-induced stress on the subsequent response to drugs of abuse, including amphetamine, cocaine, and morphine. We describe here her work on the psychostimulants amphetamine and cocaine.

Amphetamine

In a series of studies published between 1997 and 2007, Dr. Cancela's group evaluated the effects of acute and chronic restraint stress on the rewarding and stimulating properties of amphetamine (Kalivas and Duffy, 1990; Díaz-Otañez et al., 1997; Pacchioni et al., 2002, 2007). Using behavioral drug

sensitization, Dr. Cancela's group revealed that both acute and chronic restraint stress lead to increased locomotor activity (sensitization) after a subsequent challenge with different doses of amphetamine. The authors showed that systemic administration of antagonists against D1, D2, or opioids receptors performed before acute restraint exposure, suppressed the subsequent stress-induced sensitization to amphetamine. These findings reveal an opioid-DA interaction underlying stress-induced behavioral sensitization (Díaz-Otañez et al., 1997). Moreover, in 1999 Cancela's group reported that exposure to acute, but not chronic stress, led to an increase in the time the animals spent in the drug-paired compartment during a CPP paradigm, a measurement of the rewarding properties of amphetamine. Interestingly, while stress-induced increase in the rewarding properties of amphetamine depends on the activations of the D1 and/or D2 DA receptors, this effect is independent from the opioid system (Capriles and Cancela, 1999) contrasting with their previous finding on stress-induced behavioral sensitization (Díaz-Otañez et al., 1997). Together, these two publications demonstrate that stress alters opioid-DA signaling, modifying the subsequent locomotor and rewarding properties of amphetamine.

Dr. Cancela's group extended their findings by studying the interaction between DA and glutamate signaling. To this end, the authors chose *in vivo* brain microdialysis, a technique that allows to sample extracellular fluids by means of an implanted hollow fiber constructed from a dialysis membrane (Westerink, 1995), to determine neurotransmitter levels in discrete brain areas. They found that acute stress-induced behavioral sensitization to amphetamine, previously reported (Díaz-Otañez et al., 1997), was related to a DA overflow in all the striatal nuclei. More specifically, there was a significant increase in DA release after an amphetamine challenge in the NAc core, NAc shell, and dorsal striatum, 24 h after a single stress exposure in comparison to non-stressed animals (Pacchioni et al., 2002, 2007). Remarkably, 8 days after restraint-induced stress, the enhanced DA release was maintained only in the NAc core (Pacchioni et al., 2007). Regarding glutamate transmission, they showed that stress-induced behavioral sensitization to amphetamine is prevented by a systemic administration of an NMDA receptor antagonist before stress exposure (Pacchioni et al., 2002, 2007). Moreover, this systemic NMDA blockade also prevented the increase in DA release (Pacchioni et al., 2002, 2007). Altogether, the evidence presented in these reports revealed that stress-induced behavioral sensitization to amphetamine is mediated by a sensitized response to the psychostimulant, at the level of dopaminergic neurotransmission in the different nuclei of the striatum, as well as by NMDA-glutamatergic receptor activation.

Cocaine Sensitization

Using the restraint-induced stress model, Dr. Cancela's laboratory also sought to determine the molecular mechanism mediating the cross-sensitization induced by stress on the locomotor effects of a cocaine injection in adult rats. In a study published in 2012, the group evaluated the effects of repeated stress on actin cytoskeleton remodeling in the NAc and PFC, and assessed their role in the expression of cross-sensitization to cocaine-induced locomotor activity (Esparza et al., 2012). To this end, the authors exposed adult rats to 2 h restraint stress, for seven consecutive days. Three weeks after the completion of the restraint protocol, they challenged these animals with a cocaine injection. The results showed that repeated stress exposure was associated with changes in proteins that regulate the actin cytoskeleton in the NAc and PFC. Remarkably, a microinfusion of Latrunculin A, an inhibitor of actin polymerization, performed before the cocaine challenge into the NAc but not the PFC, was sufficient to inhibit the expression of cocaine cross-sensitization. Similarly, changes in postsynaptic morphology after acute exposure to cocaine were found exclusively in the NAc. AMPA receptor (AMPAR) surface expression in the NAc increased after cocaine exposure in previously stresses animals, a process that was also blocked by latrunculin A. Glutamate inhibition by the antagonist CNQX prevented the stress-induced sensitized response. Taken together, these findings suggest that restraint-induced stress modifies the surface expression of the AMPAR triggered by a cocaine challenge, by altering the actin dynamics in the NAc, giving rise to the behavioral cross-sensitization phenomenon.

In their next paper, published in 2016, Dr. Cancela's group revisited the effects of acute stress in the response to psychostimulants later in life, using a longer period — 3 weeks — between the acute stress experience and the exposure to the drug (Garcia-Keller et al., 2013). With this work, the authors were able to dissect for the first time the role of the subdivision of the NAc (*core* and *shell*) in the behavioral cross-sensitization to cocaine. By mean of microdialysis probes located in the different subregions of the NAc, Garcia-Keller et al. (2013) demonstrated that acute restraint-induced stress leads to an enhanced behavioral response to a cocaine challenge due to an exacerbated release of DA and glutamate in the *core*, but not in the *shell,* of the NAc. More specifically, the expression of behavioral sensitization to cocaine after stress depended on the activation of the AMPAR in the NAc *core*. Indeed, the stimulation of AMPAR in the *core* of the NAc of pre-stressed animals was sufficient to trigger an enhanced behavioral response in comparison to non-stressed animals. As pointed out by the authors, all these changes induced by stress resembled the enduring plastic modifications observed after repeated exposure to cocaine, and support the idea that stress leads to a facilitation of the processes involved in the development of cocaine addiction in animal models.

Finally, in another study the authors explored the relationship between acute stress and the rewarding properties of cocaine (De Giovanni et al., 2016). More specifically, they explored the reinstatement of an extinguished cocaine-induced CPP by exposing the animals to acute restraint-induced stress. The authors found that glutamate NMDA receptors were involved in the development and the expression of cocaine-CPP reinstatement after restraint-induced stress. Interestingly, microinfusions of the NMDA antagonist MK 801 in the *core* of the NAc but not the *shell* prevented this reinstatement, suggesting the core of the NAc is key for the behavioral expression of the rewarding effects of cocaine.

With all these studies, using a combination of behavioral and biochemical techniques, Cancela's group has contributed

specific evidence of the molecular mechanisms involved in the synergistic effect of stress on psychostimulant drug addiction and relapse. While not explored yet by Dr. Cancela's laboratory, one interesting possibility is that all these synergistic effects between stress and psychostimulants, are mediated by a neurotransmitter-release-modulation due to stress-induced alterations in CRF levels (Koob, 2015; Kelly and Fudge, 2018).

Corticotropin-Releasing Factor (CRF) and Cocaine Addiction

Corticotropin-releasing factor, also known as corticotropin-releasing hormone (CRH), is a major neuropeptide that regulates the endocrine and behavioral response to stress (Bale and Vale, 2004). CRF is member of a larger family of peptides that also include CRF binding protein (CRF-BP), UCN I, UCN II, and UCN III, as well as two GPCRs, CRFR1 and CRFR2. Cell bodies that express CRF are located in brain areas involved in the stress response, such as the PVN, the extended central amygdala, the basal forebrain, and the brainstem (Swanson et al., 1983; Kelly and Fudge, 2018). CRFR1 is highly expressed in the cerebral cortex, cerebellum, medial septum, and anterior pituitary (Radulovic et al., 1998), whereas CRFR2 is detected in the LS, ventromedial hypothalamus, and choroid plexus (Chen et al., 2005). During the stress response, CRF binds preferentially to CRFR1 in the anterior pituitary to activate the HPA axis (Bale and Vale, 2004).

Preclinical models have demonstrated an important role for CRF in the susceptibility to drug addiction (Corominas et al., 2010; Koob and Zorrilla, 2010). Indeed, CRF administration increases cocaine-induced locomotor activity (Sarnyai et al., 1992), enhances amphetamine-induced stereotypic behaviors (Cole and Koob, 1989), and promotes relapse to cocaine seeking in rodents (Erb et al., 1998). In contrast, CRFR1 antagonists prevent stress-induced CPP to cocaine (Lu et al., 2001) and sensitization to amphetamine (Cole et al., 1990). These behavioral alterations are associated with changes in the expression of *Crfr1* and *Crf* mRNA in areas such as the NAc or amygdala (Hansson et al., 2007; Sommer et al., 2008). Notably, humans with SNPs in the *CRH-BP* and *CRHR1* genes who have experienced adverse life events exhibit higher risk to alcohol consumption in comparison to non-carriers (Blomeyer et al., 2008; Enoch et al., 2008). In addition, SNPs in the *CRH-BP* gene have been related to cocaine and heroin abuse (Levran et al., 2014), further supporting the role of the CRF family in addiction.

The laboratory of Dr. Katia Gysling at the Department of Cellular and Molecular Biology of the *Pontifical Catholic University of Chile,* has extensively studied the mechanisms by which drugs of abuse induce plastic changes in the rodent brain, as well as the role of stress in promoting vulnerability to addiction-like behaviors. Specifically, her studies have been focused on understanding how CRF modulates the connection between the LS and the VTA in response to drugs of abuse (Sotomayor et al., 2005; Sotomayor-Zárate et al., 2010, 2013; Renard et al., 2014). The LS is a brain nucleus located in the subcortical forebrain. The LS is highly innervated by VTA DA neurons and sends GABAergic projections to the NAc, lateral hypothalamus, and VTA, thus contributing significantly to reward and motivation processes (Renard et al., 2014).

Using pharmacological manipulations and microdialysis, the Gysling laboratory has demonstrated that drugs of abuse such as amphetamine, morphine, or cocaine increase DA extracellular levels in both the VTA and LS (Sotomayor et al., 2005; Sotomayor-Zárate et al., 2010, 2013; Renard et al., 2014). Remarkably, this effect can be modulated either by stress exposure or by direct activation of CRFR1 in the LS (Sotomayor-Zárate et al., 2013, 2015). To illustrate this connection, Sotomayor-Zárate et al. (2013, 2015) from Gysling's group, reported that activation of CRFR1 increased DA release in the LS of saline-treated rats. However, repeated treatment with cocaine prevented CRFR1-induced DA levels in the LS at short and long-terms after drug withdrawal, indicating that cocaine exposure results in long-lasting alterations of the DA release to the LS (Sotomayor-Zárate et al., 2013). These findings suggested that CRF-CRFR1 signaling initiated by stress impairs LS function and, in turn, leads to altered VTA activity. In this regard, Sotomayor-Zárate et al. (2013), later reported that rats injected with cocaine displayed elevated DA levels in the VTA as compared to saline-treated rats. Interestingly, repeated exposure to stress by immobilization, or local infusion of CRF, blunted cocaine-induced DA levels. Furthermore, blocking CRFR1 increased DA levels in the VTA of rats previously exposed to repeated stress by immobilization (Sotomayor-Zárate et al., 2015). More recently, Vega-Quiroga et al. (2018), showed that stimulation of the LS increased DA levels in the VTA by innervating GABAergic interneurons and decreasing GABA-induced inhibition of DA neurons (Vega-Quiroga et al., 2018). Together, these results demonstrate that stress determines DA levels in the LS and VTA in response to cocaine by regulating CRF binding on CRFR1.

Another important contribution from Dr. Gysling's group was to provide the first report that CRF-BP and the α isoform of CRFR2 are co-expressed in VTA synaptosomes innervating the LHA. Importantly, these VTA innervations are glutamatergic and GABAergic. Similar to the LS, the LHA regulates motivation as well as feeding and drinking behaviors (Stuber and Wise, 2016). Using a protein–protein interaction model, Dr. Carlos F. Lagos in collaboration with Dr. Gysling, predicted the interaction between CRF-BP and the α and β isoforms of the CRF2R, and revealed that CRF-BP exhibited higher affinity for the CRF2αR isoform (Slater et al., 2018). Indeed, CRF-BP functions as an escort protein that allows the trafficking of CRF2αR from the intracellular space to the cell surface (Slater et al., 2016). Previous evidence demonstrated that reinstatement of cocaine self-administration induced by foot-shock stress was blocked by the infusion of CRF2R antagonists in the VTA (Wang et al., 2007). This effect is mediated by CRF-BP, which leads to DA and glutamate release and potentiates NMDA-dependent synaptic plasticity in the VTA (Ungless et al., 2003; Wang et al., 2007). Taken together, these findings suggest that alterations of the CRF-BP-CRF2R signaling in the VTA-LHA pathway can also contribute to the enhanced effects of stress on cocaine addiction and stress-induced relapse, and introduces the CRF2αR isoform as a potential therapeutic target.

Future studies involving pharmacological manipulations in the VTA and microdialysis in the LHA in rodents exposed to psychostimulants would be required to causally evaluate this hypothesis.

Molecular Studies: The Role of Wnt/β-Catenin Pathway

Wnt factors are a family of secreted signaling molecules that have been associated with different processes ranging from cell fate determination during embryonic development, cellular polarity, cell proliferation, cell cycle arrest, differentiation, apoptosis, and tissue homeostasis (Oliva et al., 2013, 2018). During development of the central nervous system the Wnt signaling pathway is implicated in a wide spectrum of physiological processes, including neuronal connectivity and synapse formation. Wnt proteins and components of the Wnt pathway are expressed in the brain since early development to adult life, however, little is known about its role in mature synapses. Here, we review evidence indicating that Wnt proteins participate in the remodeling of pre- and post-synaptic regions, thus modulating synaptic function. We include the most recent data in the literature showing that Wnts are constantly released in the brain to maintain the basal neural activity. Also, we review the evidences that involve components of the Wnt pathway in the development of neurological and mental disorders, including a special emphasis on *in vivo* studies that relate behavioral abnormalities to deficiencies in Wnt signaling. Finally, we include the evidence that supports a neuroprotective role of Wnt proteins in Alzheimer's disease. We postulate that deregulation in Wnt signaling might have a fundamental role in the origin of certain neurological diseases, by altering the synaptic function at stages where the phenotype is not yet established but when the cognitive decline starts (Oliva et al., 2013). Dysregulation of the Wnt pathways is related to multiples diseases including degenerative disorders, cancer, and psychiatric conditions (Ciani and Salinas, 2005; Oliva et al., 2018). Once secreted to the extracellular space, Wnt factors interact with specific receptors to activate one of three 'cascades': (1) the canonical Wnt or Wnt/β-catenin pathway, which acts through the transcriptional activity of β-catenin, plus two β-catenin-independent pathways: (2) the non-canonical planar cell polarity pathway, and (3) the non-canonical Wnt/calcium pathway, each one with specific physiological functions (Oliva et al., 2018).

The laboratory of Dr. Alejandra Pacchioni, located in the *National University of Rosario*, Argentina, has studied the relationship between the canonical Wnt pathway and the effects of cocaine exposure in rats. Dr. Pacchioni's group revealed that the canonical Wnt pathway is crucial for the cocaine-induced neuroadaptations in the PFC and the NAc that underlie the development and expression of behavioral sensitization (Cuesta et al., 2017a,b). Furthermore, the authors revealed that pharmacological modulation of the canonical Wnt pathway impacts and modifies the long-lasting effects on cocaine-induced neuroplasticity (Cuesta et al., 2017a).

Environmental factors, such as stimulant drugs, disrupt the patterns of neural networks that are still maturing during adolescence by altering crucial developmental signaling proteins (Cuesta et al., 2018). In this regard, more recently, Dr. Pacchioni's group became interested in evaluating how stress-induced by social isolation early in life confer a vulnerability to develop addiction-like behaviors during adulthood. Using behavioral sensitization and CPP, Cuesta et al. (2018) first revealed that rats exposed to only 5 days of social isolation during adolescence (from PND 30 to 35) increased cocaine-induced CPP and drug sensitization when tested during adulthood, indicating that social isolation early in life enhances the rewarding and stimulating properties of cocaine. Importantly, the authors also examined whether stress-induced by social isolation in adolescence altered the activity of the canonical Wnt pathway, before and after cocaine exposure. They found that social isolation in adolescence is sufficient to modify the levels of β-catenin and glycogen synthase kinase 3β (GSK3β), two main effectors of the Wnt/β-catenin pathway, in the PFC. This change was observed even 10 days after the completion of the social isolation protocol. During adulthood, animals previously exposed to stress in adolescence exhibited a higher behavioral response during the expression of cocaine sensitization, an effect associated to elevated activity of the Wnt/β-catenin pathway in the NAc. Taken together, the studies of Dr. Alejandra Pacchioni's laboratory describe a novel function of the canonical Wnt pathway as a mediator of the effects of drugs of abuse like cocaine. Her research posits the Wnt/β-catenin pathway as one of the molecular pathways affected by stress that would be involved in increasing later vulnerability to addiction and relapse (Cuesta and Pacchioni, 2017).

Alcohol Intake, Sensitization, and Stress

There is a strong association between stress and alcohol consumption. Alcohol use is elevated in individuals with high levels of anxiety (i.e., social phobia), whereas some people consume alcohol to reduce anxiety and stress (Kushner et al., 1990; Terra et al., 2006). In this context, stress has a main role in increasing the vulnerability for alcohol abuse and in triggering relapse. A considerable number of research groups in South America has studied the association between stress and alcohol intake using a preclinical approach. For example, Dr. Roseli Boerngen-Lacerda at *Universidade Federal do Paraná* (Brazil) demonstrated that ethanol intake induced an anxiolytic-like effect in mice with high-anxiety (HA) but not in medium-anxiety (MA) mice. However, HA and MA mice displayed a similar preference for ethanol intake when subjected to three different concentrations of ethanol, suggesting that anxiety-related traits were not a determining factor for the acquisition of ethanol intake (Correia et al., 2009). Another study by the same lab revealed that CRF1 receptor signaling in the amygdala is involved in ethanol consumption in "heavy drinking" mice, pointing to a relationship between this stress-mediated mechanisms and alcohol use severity (Correia et al., 2015). Ferraz and Boerngen-Lacerda (2008) reported that increased serotonergic neurotransmission reduced ethanol sensitization induced by chronic-handling stress. They suggested that the protective effects of mianserin (an tetracyclic antidepressant)

against stress is possibly mediated by 5-HT2 receptors (Ferraz and Boerngen-Lacerda, 2008).

The group of Dr. Rosana Camarini at Departamento de Farmacologia, *Universidade de São Paulo* (Brazil), has contributed significantly to the understanding of the role of stress in ethanol-induced sensitization. In a recent study, her team showed that cross-sensitization between chronic unpredictable stress and ethanol is mediated by the nitric oxide system (Santos-Rocha et al., 2018). Additional contributions from the Camarini lab and collaborators, include the role of Homer2 (an scaffolding protein system associated with glutamate receptors) in stress-alcohol locomotor cross-sensitization (Quadir et al., 2016), and the main role of housing conditions and other environmental changes that affect stress response and ethanol-induced reward (Araujo et al., 2005; Faria et al., 2008; Rueda et al., 2012; Novaes et al., 2017; Pautassi et al., 2017). Finally, we highlight the studies of Dr. Roberto Frussa-Filho from *Universidade Federal de São Paulo* about environmental modifications (Araujo et al., 2005; Fukushiro et al., 2010) and sleep deprivation (Araujo et al., 2006) in alcohol-induced behavioral effects.

Early-Life Stress and Ethanol Consumption: Sex Differences

Dr. Ricardo Pautassi, from the *Instituto de Investigación Médica Mercedes y Martín Ferreyra* (Argentina), has extensively studied how exposure to stress early in life affects ethanol intake. To this end, Pautassi's team has used a stress model of early maternal separation in rats, from PND 1 to PND 14, and evaluated ethanol consumption immediately after treatment (PND 15) or later in life (adult) (Pautassi et al., 2012; Fernández et al., 2014). His groups reported that early maternal separation led to altered ethanol-induced motivational learning, without affecting ethanol metabolism or ethanol intake (Pautassi et al., 2012). In addition, rats exposed to maternal separation exhibited significantly greater reactivity to the motor stimulating effects of 1.25 g/kg ethanol in comparison to control animals, yet greater motor suppression after 2.5 g/kg ethanol (Fernández et al., 2014). These results revealed that the effects of early life stress on ethanol responses emerged quickly during development. Intriguingly, none of these effects seems to be associated to the kappa opioid receptor system (Pautassi et al., 2012; Fernández et al., 2014), that is thought to mediate some of the aversive consequences of stress (Torres-Berrio and Nava-Mesa, 2018).

In a subsequent set of papers, Dr. Pautassi's group compared the effects of acute or repeated restraint-induced stress during adolescence and adulthood on ethanol consumption in male and female rats (Acevedo et al., 2013, 2016; Fernández et al., 2016; Wille-Bille et al., 2017). The authors found no effects of acute restraint stress on spontaneous or ethanol-induced locomotion at either age, whereas adult rats showed an enhanced and long-lasting sedative effect due to ethanol exposure as compared to adolescent rats (Acevedo et al., 2013). Intriguingly, 5 days of restraint-induced stress exacerbated free choice ethanol drinking in adolescent, but not adult rats. Furthermore, adult rats had a greater level of ethanol sedation in comparison adolescents rats, but only the adolescent animals exhibited ethanol-induced motor stimulation (Acevedo et al., 2013; Fernández et al., 2016), suggesting that stress modulates the effects of ethanol in an age-dependent manner. Interestingly, Pautassi's group further reported that female rats that show high baseline anxiety levels displayed higher ethanol intake as compared to female rats with average levels of anxiety. In addition, average-anxiety animals exhibited a significant increase in ethanol intake across sessions after three sessions of restraint stress. Intriguingly, they also found that high-anxiety female rats exposed to stress not only consumed less ethanol than their unstressed counterparts, but also failed to exhibit a significant increase in ethanol consumption across intake sessions (Acevedo et al., 2016), suggesting that individual differences at baseline anxiety levels might account for the effects of stress on ethanol intake in female rats. Finally, in more recent work, the authors exposed adolescent and adult (PND 70) female rats to restrain-induced stress and evaluated ethanol intake. Using similar paradigms for stress and ethanol exposure than in previous papers, they found that restraint stress significantly increased alcohol intake and preference in female adolescent rats. This effect was prevented by administering a single systemic dose of the long-lasting kappa opioid receptor antagonist, norbinaltrophimine, before the first session of restraint. In contrast, stress exposure led to a decrease in alcohol intake and preference in female adult rats (Wille-Bille et al., 2017). Taken together, Dr. Pautassi's work represents a very innovative contribution to the field, that focuses on how sex differences, developmental stages, and individual factors interact with the effects of stress on alcohol use.

Cannabinoid System, Stress, and Addiction

Cannabinoids are part of a neuromodulatory system comprising the endocannabinoids anandamide and 2-AG, and the CB1 and CB2 receptors. CB1 receptors are enriched in the brain, including areas such as the hippocampus, basal ganglia, and cerebellum, whereas CB2 receptors are preferentially expressed in cells of the immune system (Mechoulam and Parker, 2013), and sparse localization in VTA dopaminergic neurons and perivascular microglial cells (Núñez et al., 2004; Liu et al., 2017; Zhang et al., 2017). The role of the endocannabinoid system in addictive behavior has been thoroughly reported in preclinical studies (Bilbao, 2013; Gamaleddin et al., 2015; Chen et al., 2017). For example, CB1 receptors modulate reward-related behaviors by affecting VTA and NAc functions (Yazdi et al., 2015), and their expression is upregulated by repeated cocaine administration in the PFC (Blanco et al., 2014). Accordingly, GWAS studies have indicated that the CNR2 gene, which encodes the CB2 receptor, is associated with substance abuse disorders (Liu et al., 2017, p. 2). Moreover, the cannabinoid system modulates the effects of stress on drug-related behaviors such as cocaine acquisition (Miller et al., 2008), alcohol seeking (Onaivi et al., 2008) stress-induced reinstatement to cocaine

(Vaughn et al., 2012) and drug relapse (De Vries et al., 2001).

Different laboratories in Brazil have focused on the functional interaction between the glucocorticoid and endocannabinoid systems during learning paradigms, including fear conditioning and drug-induced CPP (Bitencourt et al., 2014; De Carvalho et al., 2014). For example, the team of Dr. Reinaldo Takahashi at the *Universidade Federal de Santa Catarina*, demonstrated that an injection of the CB1 receptor antagonist Rimonabant, impaired reconsolidation of morphine-CPP while the inhibition of anandamide metabolism with URB597 enhanced CPP expression (De Carvalho et al., 2014). In a similar line, studies conducted by the group of Dr. Francisco Guimarães at the Department of Pharmacology of the *University of São Paulo*, have focused on the multifunctional phytocannabinoid, CBD. This molecule weakly antagonizes the CB1 receptor, while serving as an inverse agonist of the CB2 receptor and an inhibitor of Anandamide uptake. In this context, Fogaça et al. (2018), from the Guimarães' group, reported that CBD induced anxiolytic responses in stressed animals (chronic unpredictable stress), an effect that was blocked by CB1 and CB2 antagonists. Similarly, in a recent study from de Carvalho and Takahashi (2017) rats that acquired morphine or cocaine CPP were reactivated with a single re-exposure to the reinforced compartment. A subset of these animals were treated shortly after reactivation with an injection of CBD and exhibited decreased expression of CPP 1, 7, and 14 days after, suggesting CBD can impair CPP reconsolidation. In another experiment, the authors reactivated CPP and injected CBD in a subset of rats as described before, but then proceeded to complete an 8-day 16 session extinction protocol. After extinction, rats were reinstated with a single dose of morphine and with restraint-induced stress, but only the CBD-treated group showed reduced CPP. Similarly, CBD after reactivation reduced CPP after naltrexone-induced withdrawal. These results indicate that CBD can reduce morphine and cocaine CPP, and this effect seems to be persistent even after a stress-induced reinstatement test (de Carvalho and Takahashi, 2017). These and other studies by Dr. Takahashi and Dr. Guimarães have pointed to the cannabinoid system as an interesting target for the treatment of anxiety-related disorders, substance abuse disorders, and stress-induced relapse (Lee et al., 2017; Stern et al., 2018). In addition, these studies provided neurobiological plausibility in a recent prospective study in a Canadian sample of illicit drug users. In this longitudinal cohort study, intentional use of cannabis was associated with reduced frequency of crack use (Socías et al., 2017). However, Dr. Jimena Frontera and Dr. Alicia Brusco, at the *Universidad de Buenos Aires* in Argentina, have studied the role of the endocannabinoid system in facilitating the reinforcing effects of different drugs of abuse. In an interesting experiment, repetitive exposure to the cannabinoid agonist WIN 55,212-2 (WIN) during early adolescence in mice led to an increase in anxiety-like behaviors, as well as to an enhanced preference for ethanol intake in adulthood (Frontera et al., 2018). This result suggests that the activation of CB1/CB2 receptors could induce anxiety and stress-induced drug seeking behavior in specific periods of vulnerability. The aforementioned studies indicate that endocannabinoid system is an interesting common pathway between emotional states, stress, and addiction.

LATIN AMERICAN NON-CLINICAL STUDIES AND TRANSLATIONAL EFFORTS

Latin American researchers have also made significant strides in translating the findings of animal studies on the neurobiological link between stress and addiction to human behavioral and clinical studies. We will end this review by highlighting these efforts and discussing how they may guide future research.

Studies on Stress and Genetic Vulnerability to Addiction

Understanding how genetic makeup may influence the potential vulnerability or protection for addiction is a topic of great interest to psychiatry. A more specific question is how genes related to the stress response play a role in determining the risk for addiction, and how these genotypes interact with stressful environments. Many regions of Latin America suffer from under-development, scarcity, and high levels of violence, which could represent significant environmental stressors. The laboratory of Dr. Claiton H. D. Bau, at the *Universidade Federal do Rio Grande do Sul* in Porto Alegre, Brazil, has been a steady source of studies on the interaction between stress and different relevant genes to the risk of addiction. Rovaris et al. (2015), from Bau's team, set out to explore the connection between early life stress, genetic variants of MR and GR receptors, and risk of crack/cocaine addiction, in a genetic association study (Rovaris et al., 2015). They reported an interesting gene–environment interaction between high levels of childhood physical neglect and the rs5522-Val allele of the MR gene (*NR3C2*), which very significantly modulated cocaine addiction severity. Furthermore, the study showed that the gene–gene interaction between *NR3C2* and the GR gene (*NR3C1*) significantly modulated the severity of withdrawal symptoms over time. This finding is in concordance with the reported effect of this and other interactions of the *NR3C1* gene on nicotine addiction severity found in several of their studies (Elkind-Hirsch et al., 1991; Rovaris et al., 2013). These results suggest that stress-response related genotypes, together with a concomitant history of early life stress, determine addiction vulnerability.

In a separate study, also in women addicted to crack cocaine, Bau's group found that C allele carriers for the *NR3C1* gene rs41423247 SNP had significantly higher depressive symptoms during abstinence (Rovaris et al., 2016). Given the relationship between negative affect and return to drug use, this indicates that this GR genotype could potentially increase the risk for relapse (Heilig et al., 2010; Carelli and West, 2014; Koob et al., 2014). Furthering the case for stress and gene interactions in worsening outcomes and severity of addiction, the group of Dr. Bau reported that variable number tandem repeats of the monoamine oxidase A (MAO-A) promoter — a gene region heavily regulated by glucocorticoids — were related to earlier onset, concomitant drug

abuse, and antisocial symptomatology in alcohol use disorder (Contini et al., 2006).

The epidemiological relationship between polysubstance use, defined as the consumption of more than one drug within a specified period of time, and several psychosocial factors (i.e., alcohol use, anxiety symptoms, and family functioning) in a Latin American sample was recently described by the group of Dr. Diego Forero at the *Universidad Antonio Nariño*, in Bogotá, Colombia (Pereira-Morales et al., 2017). In the same laboratory, methylenetetrahydrofolate reductase (MTHFR) methylation levels were found to be associated with perceived stress scores (Cohen's Perceived Stress Scale), supporting vast evidence of epigenetic changes in stress and psychiatric disorders (Jiménez et al., 2018). In fact, the *MTHFR* C677T polymorphism has been associated with depression in the context of traumatic stress in early life (Lok et al., 2013), again pointing to genetic and epigenetic determinants of stress-induced negative affect. Another genetic study on Colombian population indicated an association between a polymorphism of the *SLC6A3* gene, which encodes the DA transporter or DAT, and addiction to heroin or cocaine (Isaza et al., 2013). Similarly, the same polymorphism was found to be correlated with cocaine dependence in a Brazilian sample (n = 699) (Guindalini et al., 2006). These results are in line with the DA dysfunction hypothesis of the pathophysiology of addictive behaviors through the reward system. Interestingly, several types of chronic psychosocial stressors reduce the number of binding sites of DAT in the basal ganglia in animal models (Isovich et al., 2000).

Biomarkers of Stress-Induced Vulnerability to Addiction

Non-invasive biomarkers are biological characteristics that provide information about normal or pathological processes, and about traits or states associated with a disease (Strimbu and Tavel, 2010). They are crucial diagnostic tools for the identification of individuals who are at higher risk of developing a disorder (*predictors*) or who will greatly benefit from early therapeutic interventions (*mediators*). Biomarkers can range from blood pressure or pulse measurements to complex biochemical analyses of blood and other peripheral fluids, and should be measured in a reliable and reproducible manner (Strimbu and Tavel, 2010). However, one of the current challenges in neuropsychiatry is to find reliable biomarkers of vulnerability to addiction (Kwako et al., 2018).

The laboratory of Dr. Rodrigo Grassi-Oliveira, at the *Pontifical Catholic University of Rio Grande do Sul*, Brazil, has extensively studied the role of early life stress in cocaine addiction and how peripheral levels of stress hormones, transcription factors, and cytokines can serve as potential biomarkers of addiction (Coelho et al., 2014). For example, Grassi-Oliveira et al. (2012) measured the levels of cortisol in hair of women admitted to hospitalization seeking detoxification from crack and cocaine. The authors also recorded the number of stressful events 3 and 6 months prior to hospitalization. According to their results, women with high drug dependence exhibited a positive correlation between hair cortisol concentration and the number of stressful events 3 months before treatment admission, suggesting that hair cortisol provides information about stress response in drug abusers (Grassi-Oliveira et al., 2012). More recently, Levandowski et al. (2016) from Grassi-Oliveira's group, reported that variations in the levels of cytokines could correlate with addiction treatment and early life trauma. Specifically, the authors reported that the levels of the cytokine TNF-α were significantly lower in cocaine-dependent women at baseline as compared to reference values obtained from healthy women. Interestingly, addicted women who suffered from childhood trauma exhibited higher levels of TNF-α in comparison to those who did not. In addition, while the levels of IL-4 and IL-6 levels approached the reference values after 3 weeks of detoxification in women without trauma, those levels were still elevated in women with a history childhood trauma. Most interestingly, the imbalance between cytokines correlated with severity of withdrawal symptoms 3 weeks after treatment, demonstrating the role of cytokines as indicators of clinical states (Levandowski et al., 2016). Finally, Viola et al. (2014), demonstrated that peripheral levels of growth factors such as GDNF or BDNF were significantly elevated in crack cocaine users during early abstinence. Interestingly, GDNF levels remained elevated after 3 weeks of detoxification in women with a history of childhood sexual abuse (Viola et al., 2014).

CRF Family as a Potential Pharmacological Target

Animal and human studies on the neurobiology of stress and the pathophysiology of addictive behaviors make CRF an interesting therapeutic candidate (Koob, 2016). Furthermore, the association between SNPs in genes of the CRF family and addiction uncovers its potential role as a predictor biomarker of vulnerability (Enoch et al., 2008; Rovaris et al., 2017). To illustrate this, Rovaris et al. (2017), from the group of Dr. Bau, evaluated whether three different SNPs in the *CRFR1* gene (rs12944712, rs110402, and rs878886) would predict plasma levels of BDNF in addicted individuals. As previously shown, alterations in the levels of circulating BDNF are observed in addiction (Viola et al., 2014, 2015). According to their results, SNPs in the *CRFR1* gene determined circulating levels of BDNF in control individuals, however, this relationship was lost in individuals suffering from cocaine or crack addiction (Rovaris et al., 2017). These results suggest that genetic variants in families of the CRF family and vulnerability to addiction could be readily detected in peripheral fluids.

Clinical trials conducted by the National Institute on Alcohol Abuse and Alcoholism at NIH, Bethesda, MD, United States, have shown that the use of CRFR1 antagonists does not provide therapeutic effects in individuals suffering from alcohol dependence (Shaham and de Wit, 2016; Spierling and Zorrilla, 2017). For example, Kwako et al. (2015) evaluated the ability of pexacerfont, a CRFR1 antagonist, to reduce stress-induced alcohol craving in alcohol-dependent patients during early abstinence. They further assessed HPA activation following a challenge with the synthetic glucocorticoid DEX and with CRF

(dex-CRF challenge). The results show that Pexacerfont failed to reduce alcohol craving and suppress HPA axis activation after the dex-CRF challenge in alcohol-dependent individuals (Kwako et al., 2015). In a similar line, Schwandt et al. (2016) administered the CRFR1 antagonist, verucerfont, to anxious, alcohol-dependent women. Again, verucerfont failed to reduce alcohol craving but induced changes in the HPA activation following the dex-CRF challenge (Schwandt et al., 2016). Collectively, these results highlight the urgent need to develop other antagonists of the CRF family to test their potential efficacy in treating alcoholism and drug dependencies, including cocaine or amphetamine. To this point, the research of Dr. Gysling and Dr. Lagos has presented a novel and interesting interaction between the CRF2αR isoform and the CRF-BP, opening the possibility for developing pharmaceuticals that target CRF2αR (Slater et al., 2016, 2018). Their results could guide future human studies that evaluate the potential association between SNPs in CRF2αR and vulnerability to addiction.

Studies on Addiction, Stress, and Memory Processes

Chronic exposure to drugs of abuse leads to structural changes in key brain areas that support cognitive processes, executive function, and memory. Cocaine users for example, exhibit an accelerated decrease in global gray matter compared to normal aging individuals. This gray matter decline seems to be especially exacerbated in prefrontal and temporal cortices, an observation that suggests chronic cocaine users undergo a "fast-track" brain aging process (Ersche et al., 2013). Dr. Rodrigo Grassi-Oliveira's laboratory at the *Universidad Católica do Rio Grande do Sul*, tested this proposed phenomenon behaviorally, in a sample of female crack cocaine users compared to healthy aged-matched controls and to healthy older controls (over 60 years of age) who were asked to perform an N-back working memory task. Consistent with the "fast-track" theory, crack cocaine users performed worse than same age controls but no different than older non-users (Sanvicente-Vieira et al., 2016). Moreover, the same laboratory reported that memory deficits in drug users are in fact worsened by early life stress. The authors specifically interrogated a group of crack-cocaine inpatients for any history of childhood maltreatment, that is, any failure of their caregivers to meet their physical, emotional, educational, or healthcare needs during childhood. They found that patients with a positive history of childhood neglect had a significantly lower recall performance in an episodic memory task (Tractenberg et al., 2015). Grassi-Oliveira's work points to the long-term effects of early life stress in disrupting memory function in individuals with addiction. Interestingly, while these patients may suffer from poor working and episodic memory, drug-related contextual associations seem to be enhanced (Nestler, 2013; Kutlu and Gould, 2016). Indeed, environmental cues are thought to be strong drivers of the reinstatement of learned associations between drug consumption and the context in which it takes place. Stress also plays an important role in these associations. On the one hand, exposure to stress facilitates encoding and retention of rigid and inflexible forms of memory that require associations with an immediate stimulus (Torres-Berrio and Nava-Mesa, 2018), and on the other, stressful environments can reinstate these drug-related memories, facilitating relapse after periods of abstinence. Extinction has been proposed as an intervention that can disrupt this process. It is an active process by which a new inhibitory association is learned, which masks the originally learned association but does not erase it (Bouton, 2004). Dr. Javier Nieto, at the *Universidad Autonoma de Mexico*, has made several contributions to the understanding of how extinction can be enhanced for therapeutic purposes through experiments performed in rodents. His studies suggest that adding a sensory cue at the time of extinction ("cue-exposure") could reduce spontaneous recovery and reinstatement of the association (Bernal-Gamboa et al., 2017), and that increasing the interval of time in between extinction sessions could also boost its efficacy (Bernal-Gamboa et al., 2018). Clinicians are starting to revisit these findings to incorporate them into relapse-prevention therapies (Havermans and Jansen, 2003).

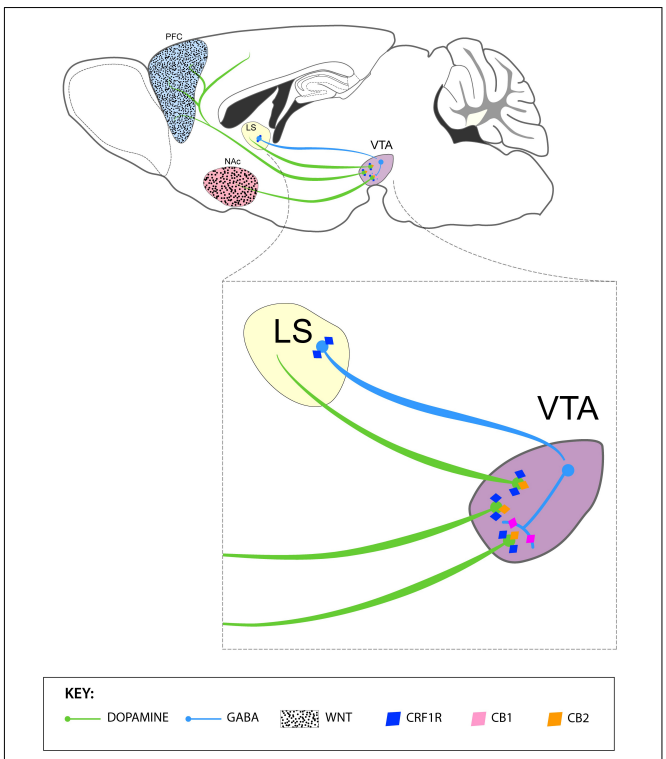

FIGURE 2 | Summary of the contributions by Latin American-based neuroscientist in the neurobiology of stress and addiction. The upper panel depicts VTA DA projections to the PFC and NAc and the modulatory role of GABAergic neurons of the lateral septum (LS) and VTA in the function of DA neurons. Activation of GABAergic neurons of the LS inactivates VTA GABA interneurons and, in turn, disinhibits DA neurons in the VTA. The PFC and NAc express WNT proteins, which play a crucial in the development and expression of cocaine sensitization in rats (black dots). The lower panel represents the expression of *(i)* CRF1 receptors by GABAergic neurons of the LS and DA neurons of the VTA, *(ii)* CB1 receptors GABAergic interneurons of the VTA and *(iii)* CB2 receptors in DA neurons of the VTA.

Therapeutic Potential of Exercise for Addiction

Animal studies have shown that exercise may reduce stress and addictive symptoms in several models. For instance, forced exercise attenuates nicotine withdrawal syndrome-induced anxiety, depression, and cognition impairment in rats (Motaghinejad et al., 2016). The same effect was also reported in a model of amphetamine relapse (Segat et al., 2014). Similarly, modifications in animals' housing conditions that facilitate voluntary exercise (e.g., running wheels, ladders, tubes, etc.) seem to have beneficial effects that reduce stress levels (Novaes et al., 2017). These and other EE interventions, such as enhancements in the animals' sensory, motor, and social stimulation have been studied in the context of substance dependence. A study from Dr. Rosana Camarini at Departamento de Farmacologia, Universidade de São Paulo, (Brazil), showed that EE reduced consumption after acute restraint stress, in rats that had been previously sensitized to ethanol (Marianno et al., 2017). However, the effect of EE on ethanol consumption has shown divergent results. For instance, the same group reported that EE enhanced ethanol's rewarding effects via an oxytocinergic-dependent mechanism (Rae et al., 2018). The authors suggested this could be explained because chronic oxytocin release induced by EE may have anxiolytic effects that could reduce the aversive effect of initial alcohol intake.

Cabral et al. (2017) at the *Federal University of Rio Grande do Norte* Brazil, found that individuals with substance use disorder had lower baseline metabolic activity in the PFC. Remarkably, one session of aerobic exercise was sufficient to improve PFC oxygenation and inhibitory cognitive control as compared to controls (Grandjean da Costa et al., 2017). They also reported a case of an alcohol use disorder patient in treatment at a psychiatric hospital, who after undergoing a continuous 3-month running program, improved not only in physical performance, but exhibited clear cognitive and autonomic benefits as well (Cabral et al., 2017). Fontes' group's work suggests that the addition of exercise to other therapeutic interventions could be effective in minimizing the biological influence of stress on addictive behaviors.

CONCLUSION

The vast majority of studies on addiction have been conducted by laboratories located in North America (United States and Canada) and Europe. Indeed, the work of world-leading researchers has inspired a new generation of neuroscientists interested in understanding drug-induced brain alterations. Remarkably, some of the advances in the field of addiction have also been developed by researchers located in Latin America. They have carried forward work on the topic of stress and addiction at every level, from the genetic and epigenetic, to the molecular and cellular, to the behavioral and clinical. Their studies have contributed to our understanding of the influence of stress on addiction vulnerability, of its role in the pathophysiology of relapse, and of opportunities for pharmacological, behavioral, and lifestyle interventions that can ameliorate outcomes for addiction, by counteracting the deleterious effects of stress. Their findings present new potential avenues for research for which Latin America could be at the forefront.

Based on results largely coming from Latin American laboratories, we highlight the association between SNPs in stress-related genes and addiction. Interestingly, the presence of those SNPs may interact with blood levels of stress hormones, transcription factors, and cytokines, suggesting a promising pathway toward identifying reliable biomarkers of vulnerability to drug abuse, and relapse in Latin American population. Preclinical evidence also points at CRF receptors in LS and the VTA, specifically the CRF2αR isoform, as a potential therapeutic target for drug addiction. We also emphasize their contributions in the study of neuronal plastic mechanisms of cross-sensitization of psychostimulants and ethanol, as well as the identification of the Wnt/β-catenin pathway as a critical neural substrate for the interaction between stress and addictive behavior. Finally, Latin American studies indicate that the cannabinoid system is a promising target for the treatment of anxiety-related disorders and stress-induced relapse. A summary of these non-clinical contributions is represented in the **Figure 2**.

Taking into account the high prevalence of stress in Latin American population, as well as their higher rates of addiction and comorbidity with stress-related disorders, the study of the mechanisms behind vulnerability and resilience in this specific population can be a very interesting model, that may have impact abroad. Likewise, the development of new specific drugs that target the molecular pathways and receptors described in this review, is very promising, especially for addressing therapeutically the perverse relationship between stress and substance use disorders.

AUTHOR CONTRIBUTIONS

AT-B and MN-M designed and supervised the project. AT-B, SC, SL-G, and MN-M wrote the manuscript. All authors discussed the literature and commented and edited the manuscript.

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Teachers' Interpersonal Style in Physical Education: Exploring Patterns of Students' Self-Determined Motivation and Enjoyment of Physical Activity in a Longitudinal Study

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This longitudinal study explored patterns of basic psychological needs and self-determined motivation, as well as its association with the teaching style and the physical activity enjoyment in a group of students. The sample consisted of 200 secondary education students (105 girls and 95 boys) aged 11 to 13 years (M = 12.65, SD = 0.79) at the start of the study. Students were assessed twice in a 22 month-period. Descriptive analyses were conducted between major variables at both time points, and to explore the number and nature of clusters, we relied on latent profile analysis. The statistical analyses showed three different patterns: downward, stable and upward, with different outcomes and determinants. The downward pattern was associated with more negative enjoyment and a controlling style, while the upward pattern with more positive enjoyment and autonomy support. This study, which was based on a person-centered approach, provided a bigger picture of the interplay between autonomy, competence, relatedness, self-determined motivation, autonomy/control support, and physical activity enjoyment.

Keywords: motivation, self-determination, physical activity, enjoyment, adolescents

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INTRODUCTION

The importance of regular exercise is indisputable in maintaining quality of life and health. Aspects related to physical exercise are particularly investigated in childhood and adolescence, since during this period a significant relationship is established between acquired behaviors and the resulting actions in adulthood (Hallal et al., 2012). Thus, a gradual increase in physical activity would be beneficial; however, a decline in these activities has been observed from adolescence onward, with high rates of physical inactivity in young people (Sousa and Hallal, 2015).

The factors that interfere in exercise adherence and a healthy lifestyle are related to motivational aspects. In this respect, the self-determination theory (SDT; Deci and Ryan, 1985, 2000), and the hierarchical model of intrinsic and extrinsic motivation (HMIEM) (Vallerand, 1997, 2001, 2007) offer an explanation of factors that promote or inhibit a healthy lifestyle, such as enjoyment during physical activity classes.

Basic psychological needs (autonomy, competence, and relatedness) and motivation are factors that have been extensively studied (Tessier et al., 2010; Cheon et al., 2014; Sparks et al., 2015). Cross-sectionals and longitudinal studies have focused on the linear relationships between autonomy, competence, relatedness or self-determined motivation and positive outcomes during physical activity classes (e.g., enjoyment, engagement or satisfaction). Similar relationships have been observed between contextual factors (e.g., teachers' control or autonomy support) and autonomy, competence, relatedness or self-determined motivation (Lim and Wang, 2009; Cairney et al., 2012; Cheon et al., 2012).

Unfortunately, these studies only provide information on the relationship between the determinant and the outcome, such as high values on autonomy correspond to high values on enjoyment. These types of studies rely on a variable-centered approach. This approach provides a small picture of the interplay among the studied variables, however, a person-centered approach allows the study of different variable configurations whilst providing a bigger picture (Kimiecik and Horn, 2017; León and Liew, 2017). For instance, in longitudinal research we could forecast groups of students based on autonomy, competence, relatedness and self-determined motivation changes, and then test how these groups differ on the outcomes (e.g., enjoyment) and the determinants (e.g., autonomy support).

The present study addresses this gap in the literature by examining students' patterns of autonomy, competence, relatedness and self-determined motivation changes, and how such patterns predict enjoyment/disenjoyment, and how these patterns relate to autonomy support.

Self-Determination Theory and Basic Education

The SDT, proposed by Deci and Ryan (1985, 2017), aims to explain human behavior, based on different motivational styles, influences of the context, and interpersonal perceptions. Three basic psychological needs are related to motivation: autonomy, which is related to the level of independence and control of the choices made by an individual; competence, which refers to a person's ability to perform a task; and relatedness, which is linked to the perception of a sense of connection with other people (Deci and Ryan, 2012).

Motivation varies from the most self-determined form, called intrinsic motivation, when the choice is personal, characterizing total autonomy in terms of the activity, which generates interest, enjoyment and satisfaction inherent to the activity (Deci and Ryan, 2000) to the least self-determined levels, which are an extrinsic motivation and amotivation (Deci and Ryan, 2000).

Satisfaction of basic psychological needs and self-determined motivation are associated with enjoyment and effort in performing activities and other positives outcomes (Deci and Ryan, 2017). For instance, Brunet et al. (2016) observed in adolescents that autonomy, competence and relatedness predicted moderate-to-vigorous-intensity physical activity 4 months later. Similarly, Richards et al. (2017) observed that self-determined motivation predicted physical activity.

Unfortunately, these longitudinal studies relied on a variable-centered approach, and thus, it is not possible to see a big picture of the interplay among the studied variables. Nevertheless, there are cross-sectional studies that relied on a person-centered approach to increase adolescents' motivation. Most of these studies analyzed the coexistence of motivational regulations (Ullrich-French and Cox, 2009; Wang et al., 2016), but little information is available about the interplay between autonomy, competence, relatedness and self-determined motivation. To the best of our knowledge, only Kimiecik and Horn (2017) explored different clusters based on adolescents' needs, observing three clusters based on students' autonomy, competence, relatedness, subjective well-being and self-awareness, and perceived choice as indicators of self-determination. In this cross-sectional study, they observed three different clusters: low, moderate and high. Interestingly, students in the high cluster reported the highest levels of moderate-to-vigorous physical activity and the most healthy eating patterns.

Interpersonal Teacher Style in Physical Education

In the educational and behavioral context, the SDT was broadened by Vallerand (1997, 2001, 2007) through the hierarchical model of intrinsic and extrinsic motivation (HMIEM). This model analyzes motivational changes (intrinsic, extrinsic and amotivation) that may occur over time, depending on three levels of social factors. The global level refers to overall motivation, which is related to family and cultural aspects developed in the first socialization processes. The contextual level refers to a specific context, such as physical activities. Finally, the situational level is influenced by the global level, and as a function of the learning styles displayed in the latter, perceptions and future styles at the contextual level may vary, in a specific situation, a particular physical exercise, for example.

During physical education classes, one of the contextual factors that can influence motivation is the teacher's interpersonal style. Teachers offer support along a continuum, which ranges from extreme control to total support for autonomy (Tessier et al., 2010). According to Reeve (2009), supporting autonomy consists of nurturing the student's inner motivational resources, providing basic explanations, using non-controlling language, and showing patience in allowing them the necessary time to learn at their own pace. As such, students become more involved in decision making and use an inquisitive methodology, attributing more importance to the process and encouraging effort and personal improvement (Moreno-Murcia et al., 2014).

In contrast to an interpersonal style that supports autonomy in the classroom, teachers with a controlling style ensure that activities are performed exactly according to their way of thinking, feeling and behaving. When teachers use controlling support, they make students abandon their own inner motivational resources in order to undertake activities and seek to solve problems based on the teachers' needs (Moreno-Murcia et al., 2012).

The motor and affective experiences perceived by students during class are influenced by the teachers' interaction and how they present class content (Jang et al., 2012; Ntoumanis et al., 2018). The teaching strategies used can affect how competent students feel to execute activities and pursue results, leading to a positive or negative change in behavior in relation to the proposed objective (Sparks et al., 2015).

Investigations on the SDT indicate that a teaching style aimed at supporting autonomy results in improved student motivation. Wang et al. (2016) observed that students in clusters characterized by the highest levels of autonomous regulation were associated with teachers with a more autonomy-supportive style.

Physical Activity Enjoyment

A determining factor in physical education classes and physical activity in general is the feeling of joy. Studies show that self-determined motivation to engage in physical activity is influenced by perceived enjoyment and challenges that activities can generate in children and adolescents (Motl et al., 2001; Cairney et al., 2012). The pleasure experienced during physical education classes was also identified as a predictor of future physical activity in both children and adolescents (Sallis et al., 2000).

The SDT highlights the positive effect of enjoyment and the feeling of pleasure as a crucial point for physical education self-determined motivation. Perceptions of competence, autonomy, success and good relationships with others increase pleasure during physical activity and reduce negative perceptions such as boredom (Baron and Downey, 2007). Taken together, these findings suggest that autonomy, competence and relatedness may be important factors that affect the pleasure experienced during physical activity.

The Present Study

Understanding different patterns of self-determined motivation in physical education classes may help teachers improve the quality of interactions with students, favoring and increasing positive experiences during classes (Moreno-Murcia and Sánchez-Latorre, 2016). Different researchers studied the relationship between motivational variables within the SDT, unfortunately there is lack of research focusing on patterns of longitudinal changes. Therefore, in this longitudinal study we aim to explore patterns of change of motivational variables (autonomy, competence, relatedness and self-determined motivation), and how these patterns differ on enjoyment and on teacher style (autonomy/control support).

We hypothesize the existence of different clusters and that the students in motivational clusters with more self-determination will show more pleasure in practicing physical activity and more autonomy support from their teachers. Similarly, we expect that motivational clusters with less self-determination will show less pleasure in practicing the activity and will receive more controlled classes from their teachers.

MATERIALS AND METHODS

Participants

The sample consisted of 200 schoolchildren, 105 girls, and 95 boys, in the final grades of elementary schooling at four public schools located in the urban zone of three municipalities in Midwest Santa Catarina state, Brazil. The students were 11–13 years old (M = 12.65, SD = 0.79) at the beginning of the study. For the data analysis, the same individuals were considered for collection 1 when they were still in the sixth, seventh and eighth grades, and collection 2 were in the seventh, eighth and ninth grade, respectively.

Measurements

Autonomy Support

The Learning Climate Questionnaire (LCQ; Williams and Deci, 1996) was used to determine the students' perception of their teachers. As recommended by Núñez et al. (2012) we applied the short version, with the adaptation for Brazilian studies in Fin et al. (2017). This scale consists of 5 items preceded by the stem "My physical education teacher...," which evaluate autonomy support (e.g., "Tries to understand how I feel before suggesting a new way of doing things"). Answers were scored on a Likert-type scale ranging from 1 (*I completely disagree*) to 7 (*I completely agree*). Since this instrument has not yet been adapted to the Brazilian educational setting, Hambleton's back-translation method was used (Hambleton, 1996). Items were first translated into Portuguese and then translated back into English. Next, the questionnaires were applied to a small group of students to check for understanding and make any necessary corrections. Internal consistency of the scale was calculated using Cronbach's alpha, obtaining an alpha value of 0.91. We tested the factor structure by means of a confirmatory factor analysis. All standardized loadings were between 0.661 and 0.870. Regarding the CFA, the χ^2 value and fit indexes were: χ^2 (199, 5) = 9.686 (p = 0.08), RMSEA = 0.068 [0.000, 0.133] and CFI = 0.986.

Control Support

The Controlling Teacher Questionnaire (CTQ), from Jang et al. (2009), was used in its modified version for physical education (Cheon et al., 2014). This scale consists of four items, preceded by "My physical education teacher is . . .," which assesses teacher control during class (e.g., "Seeks/intends to control everything I do"). The answers were scored on a Likert-type scale, whose scores varied from 1 (*I completely disagree*) to 7 (*I completely agree*). The same procedure used in the previous scale was followed. Cronbach's alpha was 0.86. All standardized loadings were between 0.270 and 0.565. Regarding the CFA, the χ^2 value and fit indexes were: χ^2 (199, 2) = 0.130 (p = 0.94), RMSEA = 0.000 [0.000, 0.035] and CFI = 0.999.

Basic Psychological Needs

A questionnaire was applied to assess basic psychological needs in physical education (NPBEF), adapted for Portuguese by Pires et al. (2010) from the Basic Psychological Needs in Exercise Scale (BPNESp) (Vlachopoulos and Michailidou, 2006). The questionnaire consists of 12 items encompassing three

dimensions: autonomy (e.g., "I feel I do activities the way I want to"), competence (e.g., "I feel I complete class activities successfully"), and relatedness (e.g., "I feel good with my classmates"). Items are preceded by the statement "Generally, in physical education..." and are scored on a 5-point Likert scale from 1 (*I completely disagree*) to 5 (*I completely agree*). Cronbach's alpha was 0.73, 0.71, and 0.85, respectively.

Self-Determined Motivation

The Perceived Locus of Causality Questionnaire (PLOCQ) (Goudas and Biddle, 1994) was used, translated into Portuguese and validated for the Brazilian population (Tenório, 2014). The questionnaire contains 20 items and is subdivided into five dimensions: intrinsic motivation, identified regulation, introjected regulation, external regulation, and amotivation. Items are preceded by the stem "I do physical education..." and are scored on a 7-point Likert scale ranging from 1 (*I completely disagree*) to 5 (*I completely agree*). Internal consistency was 0.81, 0.76, 0.76, 0.69, and 0.74, respectively. To determine the total score of self-determined motivation, the index of self-determination (IAD): (2 × intrinsic motivation + identified regulation) – [(introjected regulation + external regulation) / 2 + 2 × amotivation] (Vallerand and Rousseau, 2001) was used.

Physical Activity Enjoyment Scale

We applied the Physical Activity Enjoyment Scale (PACES) (Motl et al., 2001), translated by Montanha (2013), to measure the enjoyment for physical activity. The scale consists of 16 items preceded by the statement "When I am physically active..." which are divided into two groups composed of eight items each. The items of one group directly assess enjoyment, with affirmative sentences (e.g., "I enjoy it," "It's very pleasant," "It gives me energy"), and the items of the other group indirectly assess enjoyment, with negative sentences (e.g., "It makes me sad," "I dislike it," "It's not fun at all"). Answers were scored on a Likert-type scale, rated from 1 (*I completely disagree*) to 5 (*I completely agree*). Cronbach's alpha was 0.91.

Procedure

Questionnaires were administered individually during class time on two time points. The first data collection took place in March 2015 and the second in December 2016. Both evaluations had the same sample of individuals, so the data represent the answers of the students at the beginning of the 2015 school year while they were in the sixth, seventh and eighth grades. At the end of 2016, these same students were in the seventh, eighth and ninth grade, respectively. It is important to consider that the Brazilian school year begins in February and ends in December. The questionnaires were applied in the classroom under the supervision of the authors of this study. Prior authorization was requested from management staff and teachers at the schools involved in the study, as well as the parents and/or guardians of the participants. The questionnaires were answered during physical education (PE) classes under the supervision of the researcher, who explained how subjects should complete the instrument and remained available to answer any questions that might arise during the process. Completion time was approximately 35 min, and anonymity was respected to ensure sincere answers. Whilst the students answered the questionnaire, only the researchers were in the classroom. The teachers were not present during the data collection. This study was carried out in accordance with the recommendations of Human Research Ethics Committee of Unoesc/Hust with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Research Ethics Committee of Unoesc/Hust, under protocol number 937.597 on December 19, 2014.

Data Analyses

Preliminary Analyses

Descriptive analyses were conducted, including Pearson's correlations between major variables at both time points (see **Table 1**). To compute variables' indicators, we began by estimating the mean for each variable. Then, we computed the difference between each time point (T_2 – T_1). Finally, to ease interpretation, we standardized these values (mean = 0 and standard deviation = 1).

Latent Profile Analysis

To explore the number and nature of clusters, we relied on Latent Profile Analysis (Collins and Lanza, 2010; Berlin et al., 2014). Following the recommendations of Marsh et al. (2009) and Collins and Lanza (2010) to choose the number of clusters, we analyzed: (1) Akaike information criterion (AIC), (2) sample-size-adjusted Bayesian information criterion (SSA-BIC),

TABLE 1 | Descriptive statistics and Pearson's correlations.

	M T_1	*M* T_2	1	2	3	4	5	6	7
(1) Self-determination	10.061	8.175							
(2) Autonomy	3.059	3.041	0.294						
(3) Competence	3.931	3.695	0.381	0.208					
(4) Relatedness	4.071	3.898	0.190	0.120	0.182				
(5) Enjoyment +	4.002	3.525	0.614	0.198	0.369	0.167			
(6) Enjoyment –	1.625	1.834	−0.609	−0.269	−0.273	−0.129	−0.574		
(7) Autonomy Support	4.519	3.753	0.454	0.311	0.280	0.082	0.487	−0.360	
(8) Control Support	2.404	2.840	−0.220	−0.160	−0.124	−0.053	−0.007	0.172	−0.142

All correlations were significant ($p < 0.05$).

TABLE 2 | Goodness of fit for models with latent groups.

Clusters	AIC	SSA-BIC	PB-LRT (*p*)	% smallest cluster
1	2286.352	2287.394	–	–
2	2231.420	2233.113	0.000	20.000
3	2224.502	2226.846	0.030	14.106
4	2226.495	2229.490	1.000	0.500

AIC, Akaike Information Criteria; SSA-BIC, Sample size adjusted Bayes Information Criteria; PB-LRT, Parametric Bootstrapped Likelihood Ratio Test.

(3) Parametric Bootstrapped Likelihood Ratio Test (PB-LRT), and (4) because solutions with few participants (e.g., 1 or 5% of the total sample) may not truly represent a unique cluster, we also relied on the percentage of cases in the smallest latent subgroup of each model. All of the calculations were done with Mplus 7.4 (Muthén and Muthén, 2017).

RESULTS

Preliminary Analyses

Means for variables at both time points and correlations between the standardized differences scores (T_2–T_1) are displayed in **Table 1**. The means varied between 1.625 (T_1 Negative Enjoyment) and 10.061 (T_1 Self-determination). With regard to correlations, they ranged from 0.614 (Self-determination with Positive Enjoyment) to −0.007 (Teacher Controlling Style with Positive Enjoyment). All correlations were significant ($p < 0.05$).

Latent Profile Analysis

Identification of Latent Groups

We compared models between one and four clusters. As can be seen in **Table 2**, the solution with lower values for AIC and SSA-BIC is the 3 clusters option, which is significantly better than the four clusters option. Based on these results, the three-factor model best represented the dataset.

Description of Latent Groups

Figure 1 and **Table 3** present the cluster solution. When the changes between the collection 1 and the collection 2 are compared, it is observed that for the first cluster, comprised of 28 students (14%), there were decreases in self-determination and need-fulfillment (low). The second cluster, comprised of 46 (63%), is characterized by no changes in self-determination and need-fulfillment (average). And the third cluster, comprised of 126 (23%), is characterized by increases in self-determination, autonomy, competence, but not in relatedness (high).

Comparison of Autonomy Support and Positive Enjoyment Across Latent Groups

Subjects in the low cluster had the lowest Autonomy Support ($M = -1.238$) and Positive Enjoyment ($M = -1.261$) variation,

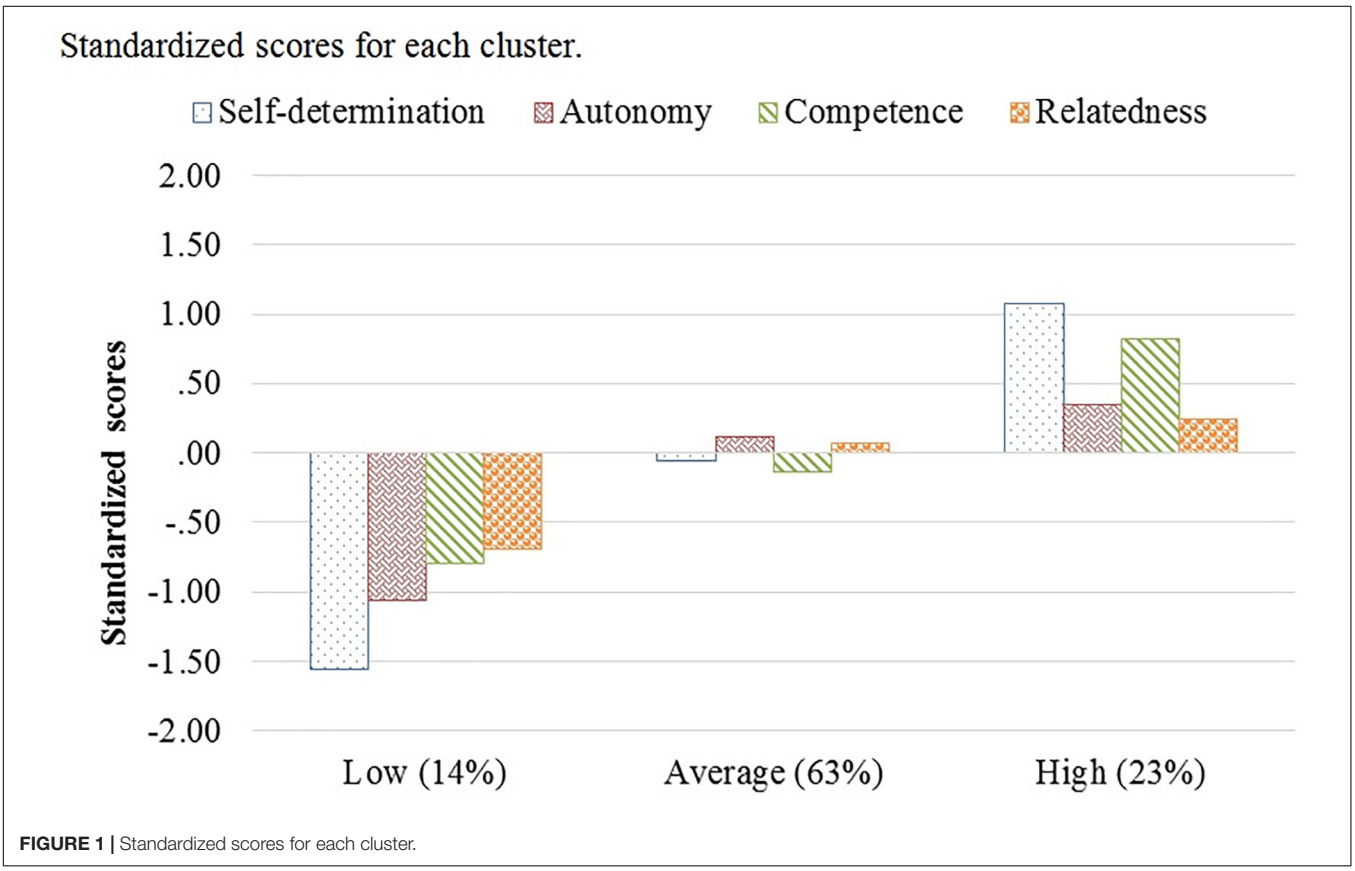

FIGURE 1 | Standardized scores for each cluster.

TABLE 3 | Mean and standard error for variables in each cluster.

		Low	Average	High
		14%	63%	23%
Self-determination	*M*	−1.559	−0.067	1.065
	SE	0.222	0.116	0.131
Autonomy	*M*	−1.069	0.111	0.338
	SE	0.261	0.095	0.168
Competence	*M*	−0.803	−0.143	0.821
	SE	0.292	0.121	0.185
Relatedness	*M*	−0.699	0.065	0.239
	SE	0.203	0.107	0.185

M, Mean; SE, Standard error.

subjects in the average cluster showed more Autonomy Support (M = 0.0881) and Positive Enjoyment (M = −0.024) than subjects in the low cluster, while subjects in the high cluster were characterized by more Positive Enjoyment (M = 0.786), but not more Autonomy Support (M = 0.493). Results are displayed in **Table 4**.

Comparison of Control Support and Negative Enjoyment Across Latent Groups

As can be seen in **Table 5**, subjects in the low cluster had the highest Teacher Controlling style (M = 0.773) and Negative Enjoyment variation (M = 1.351), subjects in the average cluster showed less Teacher Controlling style (M = −0.092) and Negative Enjoyment (M = 0.009) than subjects in the low cluster, while subjects in the high cluster were characterized by less Negative Enjoyment (M = −0.800), but not less Teacher Controlling style (M = −0.214).

TABLE 4 | Mean and standard error for autonomy support and positive enjoyment in each cluster.

		Low (1)	Average (2)	High (3)
Autonomy Support	*M*	$-1.238^{2,3}$	0.088^{1}	0.493^{1}
	SE	0.183	0.092	0.189
Enjoyment +	*M*	$-1.261^{2,3}$	$-0.024^{1,3}$	$0.786^{1,2}$
	SE	0.184	0.088	0.185

M, Mean; SE, Standard error. Numbers in superscript refers to groups significantly different (Confidence level = 95%).

TABLE 5 | Mean and standard for error teacher controlling style and negative enjoyment in each cluster.

		Low (1)	Average (2)	High (3)
Control Support	*M*	$0.773^{2,3}$	-0.092^{1}	-0.214^{1}
	SE	0.211	0.105	0.163
Enjoyment–	*M*	$1.351^{2,3}$	$0.009^{1,3}$	$-0.800^{1,2}$
	SE	0.190	0.084	0.188

M, Mean; SE, Standard error. Numbers in superscript refers to groups significantly different (Confidence level = 95%).

Comparison of Gender Across Latent Groups

With regard to gender differences, all clusters were significantly different. The low and high cluster were characterized by more females, while more males were observed in the average cluster (**Table 6**).

DISCUSSION

The aim of this longitudinal study was twofold: (1) explore different clusters based on autonomy, competence, relatedness and self-determined motivation changes over a 22-months period, and (2) test how these clusters differ on enjoyment and on teacher style (autonomy/control support). The hypothesis presented seeks for the existence of patterns of change and for the association of these patterns with an outcome and a determinant.

Patterns on Students' Self-Determined Motivation

Three patterns were identified: decreases in all psychological needs and self-determination; no changes, and increases in self-determination, competence, autonomy but no changes in relatedness. The downward pattern was associated with more Negative Enjoyment and a Controlling Style, while the upward pattern, with more Positive Enjoyment and Autonomy Support. In a similar study, Kimiecik and Horn (2017) observed three different clusters: low, moderate and high, being that the students in the high cluster reported the highest levels of moderate-to-vigorous physical activity and most healthy eating patterns. Kimiecik and Horn's (2017) findings are significant to understand adolescent self-determined motivation for the participation in health-promoting behavior.

Importantly, those three patterns differed on the outcome (positive and negative enjoyment), and the determinant (autonomy/control support). In a cross-sectional study Wang et al. (2016), observed that students in clusters characterized by the highest levels of autonomous regulation were associated with teachers with a more autonomy-supportive style. Wang et al. (2016) as well as the present study support the SDT since different subtypes of self-determined motivation differentially predict students' engagement and experience of physical education. It is possible to affirm that it is not only how motivated students are, but in what ways they are motivated, that explain their persistence in physical activities.

The group with low self-determination also showed low fulfillment of the basic psychological needs of autonomy, competence and relatedness with moderate self-determination

TABLE 6 | Proportion of males and females in each cluster.

		Low (1)	Average (2)	High (3)
Gender	Male	0.271	0.589	0.000
	Female	0.729	0.411	1.000

exhibited moderate fulfillment of these needs. Changes in satisfaction related to basic psychological needs were associated with self-determined motivation, and it is important to examine competence, autonomy and the relationship with others separately, in order to obtain a more complete understanding of the role played by the teaching style, fulfillment of psychological needs and enjoyment of physical activity in adolescents.

Earlier studies gathered evidence of the positive influence of teaching style on autonomy on self-determined motivation, basic psychological needs and satisfaction with physical activity (Lim and Wang, 2009; Cheon et al., 2012; Ntoumanis et al., 2018), since these students are also prone to participate in the proposed tasks, more satisfied with their life and more committed to their activities, in addition to having greater perception of competence (Reeve et al., 2004).

Patterns on Students' Enjoyment of Physical Activity

It is important to underscore the relationship between enjoyment and physical activities, given that the sense of fulfillment and pleasure in performing an activity promotes adherence and regularity (Vlachopoulos and Michailidou, 2006). Enjoying physical education classes may increase student commitment to overall physical activities, which increases their desire to remain active outside the school environment, and later become active adults concerned about their health (Ntoumanis, 2005).

There is also an intergroup difference between the sexes. In this study the boys obtained moderate results, while the girls showed both extremes (low and high self-determination). Most of the studies that analyze self-determined motivation to attend physical education classes, considering the students' sex, found that boys are more intrinsically motivated than girls (Cairney et al., 2012). Moreno-Murcia et al. (2006) observed that the attitudes of female adolescents or adults regarding sport and physical activity follow a more esthetic pattern, while boys display attitudes in relation to team and competitive sports. These differences may be related to attitudes labeled according to sex, stimulating a more competitive attitude in boys, which could cause a feeling of lower competence and less fun during activities.

Educational programs that stimulate the development of self-determined motivation may lead students to habitually engage in physical activities so that they are less likely to discontinue them after the school year ends. Thus, enjoyment in physical education classes results from a more self-determined behavior and fulfillment of the basic psychological needs for competence, autonomy and relationships with others, with an influence of interpersonal teaching style. The present findings support the use of educational strategies that favor autonomy and influence self-determined motivation, thereby promoting commitment to physical activity. In this respect, a number of aspects are important in improving self-determined motivation in physical education classes, such as varying activities, transmitting the feeling of responsibility, enabling student decision making, and recognizing efforts and personal improvement (González-Cutre et al., 2011). As such, it might be interesting for physical education teachers to concentrate on activities that students deem important, interesting and useful as well as stimulate feelings of competence, thereby promoting the perception of success during activities (Gu and Solmon, 2016).

Limitations

A study limitation was the fact that the authors used the self-determination index, as observed in earlier studies. However, recent research indicates that using a continuum as a general index may dilute the richness of the findings obtained, given that the model can be tested by considering each type of self-determined motivation or separating it into two large categories (autonomous and controlled). Nevertheless, the information collected may help future studies, where it will be important to design interventions with teachers in order to enhance experiences in physical education classes, thereby improving adherence to physical activities.

CONCLUSION

In conclusion, more self-determined motivation is related to greater teacher support for autonomy, greater fulfillment of basic psychological needs and increased enjoyment with physical activity. It is important to underscore that the group characterized by high self-determination exhibited high competence values. As such, it is recommended that physical education teachers use a style that supports autonomy, applying strategies that improve student self-determined motivation as well as feelings of pleasure and satisfaction with physical education.

AUTHOR CONTRIBUTIONS

JM-M conceptualized the study, supervised and managed the data, drafted and revised the manuscript, approved the manuscript to be published, and agreed to be accountable for all aspects of the work in this manuscript. GF and EB analyzed and managed the data, drafted and revised the manuscript, approved the manuscript to be published, and agreed to be accountable for all aspects of the work in this manuscript. JL and authors conceptualized the study, analyzed and wrote the results, approved the manuscript to be published, and agreed to be accountable for all aspects of the work in this manuscript. RNJ supervised the data, drafted and revised the manuscript, approved the manuscript to be published, and agreed to be accountable for all aspects of the work in this manuscript.

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Improvement of Inter-Professional Collaborative Work Abilities in Mexican Medical and Nursing Students: A Longitudinal Study

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Background: Inter-professional and interpersonal relationships in collaborative work environments can prove to be critical elements in healthcare practice. When implementers fail to understand the importance of a collaborative perspective, this can lead to communication problems which ultimately harm the users.

Objectives: To improve the inter-professional collaborative work skills of Mexican students in their first year of medical and nursing degrees through the use of a training program geared toward development of interpersonal skills and interdisciplinary work.

Methods: The sample was composed of 162 students (62 males and 99 females) from the School of Healthcare Sciences of the Autonomous University of Coahuila, Mexico. The main measures used were the Jefferson Scale of Empathy (JSE); the Jefferson Scale of Attitudes toward Inter-Professional Collaborative Work between Medical and Nursing Professionals (JSAPNC); and the Jefferson Scale of Lifelong Learning (JeffSPLL). The entire sample was divided into two groups (experimental and control groups). Both groups attended an extra-curricular program using a coaching methodology. In the first case the topic focused on attitudes toward inter-professional collaborative work. In the second case, the program focused on addiction. Both programs ran for 4 months. Psychometric instruments were applied at the beginning and at the end of both programs. After analyzing the reliability of the instruments, an ANOVA test was performed.

Results: The control group of medical students showed a deterioration in the development of collaborative work skills ($p < 0.01$), whereas in the experimental group this deterioration was not present. In the experimental group of nursing students, a significant increase in the development of collaborative work skills ($p < 0.05$) was observed. The differences were clearly due to the professional area of study ($p < 0.001$).

Conclusion: There are differences in collaborative work skill development among different professional areas. These differences can be reduced through the implementation of a program aimed at developing collaborative work and interpersonal skills in the early stages of training.

Keywords: professionalism, empathy, lifelong learning, inter-professional collaborative work, medical students, nursing students, professional roles

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INTRODUCTION

In physicians and healthcare professionals, professionalism refers to the set of skills and values that characterizes the essence of humanism in professional work (Vivanco and Delgado-Bolton, 2015). This concept comprises an articulated body of professional traits and skills that constitute the essence of their professional work regardless of their geographical, social, or cultural settings. For assessment purposes, a definition from the medical research field suggests that medical professionalism is achieved by mastering three essential areas: clinical skills, communication skills, and an appropriate understanding of the ethical and legal framework of professional behavior (Stern, 2006). Communication skills are not limited to patients and their families, but also relate to other healthcare professionals who are part of the inter-professional team which works at the healthcare institutions. According to the World Health Organization (2010), this inter-professional collaboration, also called teamwork, is described as the ability of "multiple healthcare workers from different professional backgrounds to provide comprehensive services by working with patients, their families, their professional careers, and the community, in order to deliver the highest quality of healthcare across settings." Benefits of this teamwork have been described in recent publications in healthcare institutions (Pedrazza et al., 2017). Some of those benefits are associated with the improvement of certain professional competences that are important in interdisciplinary teams′ performance, such as communication, innovation, and creativity skills or with decision-making abilities (Ceschi et al., 2014). In fact, according to Veloski and Hojat (2006), teamwork, together with empathy and lifelong learning, are the three essential components of professionalism in medical and healthcare sciences.

Despite important social, political, and economical differences among Latin American countries, there are no big differences in terms of medical and nursing educational curricula (Andrade, 1978) nor in the public health policies (Atun et al., 2015) among them. This has been evidenced by findings among Latin American physicians and nurses regarding professionalism, measured first from the scope of empathy (Alcorta-Garza et al., 2016), and most recently from the assessment of attitudes toward inter-professional collaborative work and lifelong learning (San-Martín et al., 2017a). With regard to inter-professional relationships, and specifically to the role that professionals play, two studies in Mexican healthcare institutions were the first to report the prevalence of a "hierarchical" model in physician-nurse work relationships in Latin America (Hojat et al., 2001, 2003). Fifteen years later, two international studies, one carried out among healthcare professionals who worked at public institutions in four Latin American countries (San-Martín et al., 2017a), and the other regarding Latin American medical students enrolled in training programs at Spanish teaching hospitals (San-Martín et al., 2017b), were published. Both studies not only confirmed the pervasiveness of a hierarchical model in the physician-nurse work relationships of new generations of Latin American healthcare professionals, but also remarked on the important role that some cultural stereotypes, mainly associated with professional roles, play in the development of three critical aspects of professionalism: empathy, inter-professional collaborative work, and lifelong learning.

Based on these findings, this study was designed in a Mexican university with two main objectives. The first was to confirm whether differences related to professional roles, previously reported in Mexico between physicians and nurses, are already evident in first-year medical and nursing students at two time-points, during the first month of their studies and upon completion of the first academic semester (4 months). According to this first objective, two research hypotheses were established. The first hypothesis was that first-year Mexican students attending medical and nursing schools develop different attitudes (that can be psychometrically measured) very early on, regarding the dynamics of physician-nurse collaborative work, due to the influence that culture and social stereotypes play on professional roles. The second hypothesis was that the differences observed in the development of attitudes toward physician-nurse collaborative work among students are not related to sex differences.

The second objective of this study was to prove the efficacy of a training program, based on coaching methodology, to improve attitudes toward physician-nurse collaborative work in first-year students enrolled in Mexican medical and nursing schools. A closer look at this second objective led to the creation of two more research hypotheses. The third one was that first-year students who attended a training program on inter-professional collaborative work improved their inter-professional collaboration abilities in physician-nurse teamwork. The fourth hypothesis was that a greater development of these inter-professional work abilities had a positive effect on the development of empathy and lifelong learning, given that these three areas comprise the core elements of professionalism.

MATERIALS AND METHODS

Participants

Medical and nursing students from of the Autonomous University of Coahuila, in Piedras Negras, Mexico participated in the study. Two first-year undergraduate student cohorts, one of medical students from the School of Medicine and another of nursing students from the School of Health Sciences, were invited to participate in the study at the beginning of their first academic semester. Of these, 183 students (77 male, 106 female), representing 59% of both cohorts, agreed to participate.

Design and Procedure

In the first week of class, two cohorts of first-year students (one from the medical school and the other from the nursing school, both from the same university) attended a workshop led by an external researcher. In the workshop, students were informed that this study was performed for the purpose of measuring the development of some competencies associated with professionalism in healthcare workers. Participants were randomly divided into two groups, maintaining a similar distribution according to their degree coursework and

sex, in accordance with an experimental design previously developed. One group (experimental group) received a 4-month training program on inter-professional collaborative work abilities, whereas the other one (control group) received a 4-month training program on drug addiction prevention. A multiple-choice questionnaire including specific psychometric measurements (see below) was administered before and after the training programs. The questionnaires were on paper and were returned in sealed envelopes. The students' participation was anonymous, and this anonymity was maintained through the use of pseudonyms. There was no potential harm to participants. Finally, those participants who completed both groups of questionnaires were included in the statistical analysis. A summary of the study design is shown in **Figure 1**.

An independent ethical committee for clinical research (Comité Ético de Investigación Clínica de La Rioja) approved the study design (Ref. CEICLAR PI 199) in Spain. The study was carried out in accordance with recommendations and authorization of the participating institution's administration (Autonomous University of Coahuila, Piedras Negras). In accordance with the Declaration of Helsinki, all students who agreed to participate in this study brought a written informed consent.

Main Measures

The Jefferson Scale of Attitudes toward Physician-Nurse Collaboration (JSAPNC) was used. The JSAPNC was developed in response to the need for a valid instrument to measure an important aspect of professionalism, namely teamwork and inter-professional collaboration between physicians and nurses. According to the authors of the JSAPNC (Hojat et al., 1999), physician-nurse collaboration is defined as an ability of nurses and physicians to work together cooperatively, sharing responsibilities for solving problems and making decisions to formulate and carry out plans for patient care. The JSAPNC is composed of 15 Likert scale items that are scored from 1 (strongly disagree) to 4 (strongly agree). This instrument has demonstrated a high reliability and validity among medical and nursing students in different cultural contexts (Ward et al., 2008; Hojat et al., 2015; Zakerimoghadam et al., 2015).

Two versions of the Jefferson Scale of Empathy (JSE) were used to measure students' orientation toward empathetic relationships with patients (Hojat, 2016): the medical student version (JSE-S), for medical students and the healthcare student version (JSE-HPS) for nursing students. The JSE has 20 Likert scale-type items worth seven points, ranging from 1 (strongly disagree) to 7 (strongly agree). The difference between the two versions of the JSE used is in the rewording of those items where the terms "medicine" or "physician" appeared, in order to make it more applicable to students from other healthcare areas. A sample item of the JSE-S is: "Physicians should try to stand in their patient's shoes when providing care to them," while the same item in the JSE-HPS is reworded as follows: "Health care providers should try to stand in their patients' shoes when providing care to them." A higher score means that the student has a greater orientation or behavioral tendency toward empathic engagement in patient care (Hojat, 2016).

Two versions of the Jefferson Scale of Physician Lifelong Learning (JeffSPLL) were used to measure attitudes toward lifelong learning: the medical student version (JeffSPLL-MS), for medical students (Wetzel et al., 2010); and the healthcare profession student version (JeffSPLL-HPS) for nursing students (Novak et al., 2014). The JeffSPLL was originally designed to measure the development of skills related to information gathering, the use of learning opportunities, and self-motivation (Hojat et al., 2009). The JeffSPLL has 14 Likert scale-type items that are scored from 1 (strongly disagree) to 4 (strongly agree). Similarly to the two versions of the empathy scale, the differences between the two versions of the JeffSPLL are in the rewording of those items where the term "medicine" is used, in order to make it more applicable to students from other healthcare areas. The JeffSPLL tool has demonstrated a high reliability and validity in both student groups in different cultural contexts (Muliira et al., 2012; San-Martín et al., 2016).

Information regarding degree coursework, sex, and age were collected through a complementary form.

Training Programs

Based on coaching methodology, two extra-curricular training programs were specially developed for this study: (i) for the experimental group, a program on inter-professional collaborative work; and (ii) for the control group, a program on drug addiction prevention. Both programs included similar methodologies based on theoretical classes and practical sessions, the same number of lessons (36 lecture hours) distributed over 18 weekly workshops of 2 h each, and were offered in parallel during the first academic semester (4 months). A detailed description of the contents of each program is reported in **Appendix 1**.

Statistical Analysis

The reliability of the instruments used was measured by Cronbach's alpha coefficient. This assessment was performed twice: before and after the training programs were given.

Once the normality of the inter-professional collaborative work scores was studied, a variance analysis (Three-way ANOVA) based on the following variables was performed, both prior to and at the end of the study: study group, sex, degree coursework, and the interaction between sex and degree coursework. Furthermore, a comparative analysis using Wilcoxon tests, by study groups (experimental vs. control) and by degree coursework (medicine vs. nursing), before and after the study was performed. In order to determine possible associations among inter-professional collaborative work abilities, empathy, and lifelong learning, a correlation analysis using Spearman's coefficient (ρ) among the above-mentioned variables was also performed at the beginning and at the end of the study. Finally, a multiple regression analysis for "study group by degree coursework" variables was performed in order to determine whether the development of inter-professional collaborative work abilities at the end of the course has a different effect on the

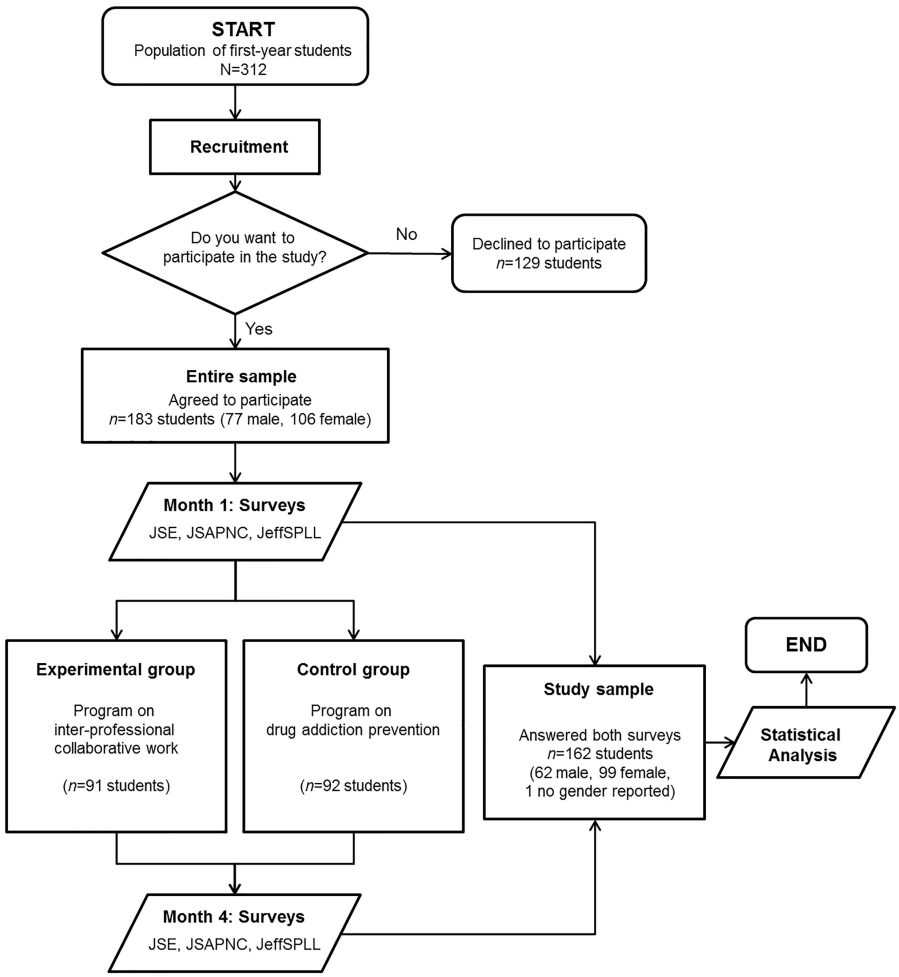

FIGURE 1 | Schematic overview of the workflow diagram followed in this study. JSAPNC, Jefferson Scale of Attitudes toward Physician-Nurse Collaboration; JSE, Jefferson Scale of Empathy (JSE-S, Medical Student version and JSE-HPS, Health Professions Student version); JeffSPLL, Jefferson Scale of Lifelong Learning (JeffSPLL-MS, Medical Student version and JeffSPLL-HPS, Health Professions Student version).

other two measured components of professionalism: empathy and lifelong learning.

Data processing was carried out with R software (R Core Team, 2017), version 3.4.1 for Windows, and included the use of *nortest* (Gross and Ligges, 2015) and *multilevel* (Bliese, 2016) statistical packages.

RESULTS

Participants

From the entire sample of 183 students, 162 students (62 males, 99 females, and one gender not stated) responded to both questionnaires. This sample was used for the statistical analysis and represented 52% of the entire population of first-year students enrolled in medical and nursing programs offered by the School of Medicine and by the School of Healthcare Sciences of the Autonomous University of Coahuila, in Piedras Negras, Mexico.

According to their degree coursework, 84 participants were first-year medical students (45 males, 38 females, and two genders not declared), and 89 were first-year nursing students (26 males and 63 females). The mean age of the entire sample was 19 years, ranging from 18 to 35 years (SD = 2).

Reliability and Descriptive Statistics

The range observed for Cronbach's alpha coefficients obtained for the five instruments used in this study was between 0.71 and 0.80. The test-retest reliability over a 4-month period yielded values ranging between 0.60 and 0.83. The score distribution summary, and reliability for all instruments used are reported in **Table 1**.

Measurement of Attitudes Toward Physician-Nurse Collaboration in First-Year Medical and Nursing Students

The first hypothesis which looked at the influential role that culture plays in students' attitudes toward inter-professional work

TABLE 1 | Descriptive analysis, reliability, and reliability of the Jefferson Scales of physician-nurse collaboration, empathy, and lifelong learning.

	Start			End (4-month)		
	n	M (*SD*)	Reliability	*n*	M (*SD*)	Reliability
JSAPNC	159	48 (6)	0.72	154	48 (7)	0.82
JSE-S	76	104 (12)	0.72	75	100 (14)	0.75
JSE-HPS	77	96 (13)	0.77	77	97 (11)	0.60
JeffSPLL-MS	78	46 (4)	0.71	76	46 (6)	0.83
JeffSPLL-HPS	76	44 (5)	0.80	82	45 (5)	0.68

JSAPNC, Jefferson Scale of Attitudes toward Physician-Nurse Collaboration; JSE-S, Jefferson Scale of Empathy- Medical Student version; JSE-HPS, Jefferson Scale of Empathy-Health Professions Student version; JeffSPLL-MS, Jefferson Scale of Physician Lifelong Learning-Medical Student version; JeffSPLL-HPS, Jefferson Scale of Physician Lifelong Learning-Health Professions Student version; n, sample size; M, mean; SD, standard deviation.

showed no differences in JSAPNC of newly admitted students when compared by degree coursework ($p = 0.06$). However, an inverse association between JSAPNC and age was confirmed ($\rho = -0.17$; $p = 0.03$), but only in newly admitted students. The second time that the JSAPNC was measured, just after the study was finished, this association disappeared ($\rho = +0.003$; $p = 0.97$). On the other hand, the second measurement of the JSAPNC showed new differences according to study group ($p = 0.0005$), and degree coursework ($p = 0.00004$), as shown in **Table 2**.

Regarding the second research hypothesis, no differences were found when the JSAPNC score was compared by gender, both at beginning ($p = 0.16$) and at the end ($p = 0.35$) of the study. A similar situation was also observed for the interaction between sexes and degree coursework, both at beginning ($p = 0.805$) and at the end ($p = 0.841$).

Enhancement of Attitudes Toward Physician-Nurse Collaboration in First-Year Medical and Nursing Students

The third research hypothesis related to the positive effect of a training program on inter-professional collaborative work abilities. A three-way ANOVA, at the beginning and at the end, revealed that students from the experimental group achieved higher scores on the JSAPNC compared to those who participated in the control group, and this difference was statistically significant ($p = 0.0005$), as shown in **Table 2**. According to degree coursework, the comparative analysis by study groups showed that JSAPNC scores of nursing students from experimental group increased over time ($p = 0.02$), while JSANPC scores of medical students of this group remained stable ($p = 0.57$). However, in the control group, JSPANC scores of medical students showed a significant deterioration over the time ($p = 0.006$), while nursing students of this group remained stable ($p = 0.16$). The summary of these findings is reported in **Table 3**.

The fourth research hypothesis related to the role that inter-professional abilities play in the development of the other two measured elements of professionalism. Correlation analysis of the entire sample confirmed a positive relationship between empathy and inter-professional collaboration, both at the beginning ($\rho = +0.27$; $p = 0.0008$) and at the end ($\rho = +0.41$; $p < 0.0001$) of the study. Although no association was observed between lifelong learning and teamwork in newly admitted students ($\rho = +0.11$; $p = 0.17$), a significant association between these two elements appeared at the end of the study ($\rho = +0.19$; $p = 0.02$). At this point, statistical differences were also observed between inter-professional collaborative work and lifelong learning according to "study group by degree coursework" ($p = 0.03$). Those differences are shown in **Figure 2**.

DISCUSSION

The scales showed adequate psychometric properties with Cronbach's alpha coefficients higher than the international recommendation of 0.70 set by the American Educational Research Association. Results observed in the present study support the use of the Spanish versions of the above-mentioned scales as consistent measures of empathy, teamwork, and lifelong learning for medical and nursing students. These findings are also similar to those previously reported for empathy in medical

TABLE 2 | Descriptive statistics and analysis of variance (Three-way ANOVA) of the scores of the inter-professional collaborative work by study group, gender, and degree coursework ($n = 162$).

	Before					After			
	n	M (*SD*)	*F*(1,154)	η^2	*p*	M (*SD*)	*F*(1,149)	η^2	*p*
Study group			2.08	0.01	0.14		11.69	0.07	<0.001
Control	82	47 (5)				46 (7)			
Experimental	80	48 (6)				50 (5)			
Sex			3.54	0.02	0.16		0.04	0.00	0.35
Male	62	48 (6)				47 (7)			
Female	99	47 (5)				48 (6)			
Degree coursework			3.66	0.02	0.06		17.95	0.11	<0.001
Medicine	78	47 (6)				46 (7)			
Nursing	83	48 (5)				50 (6)			

n, sample size; M, mean; SD, standard deviation; F, F-value; η^2, Eta-squared; p, p-value.

TABLE 3 | Summary result of Wilcoxon test of JSAPNC scores by study groups (experimental vs. control) before and after the study was performed.

Study group	*n*	Before		After		*p*
		M	*SD*	M	*SD*	
Control group						
Medicine	37	47	6	44	7	0.006
Nursing	38	47	5	49	7	0.16
Experimental group						
Medicine	34	47	7	48	5	0.57
Nursing	42	50	4	51	5	0.02

JSAPNC, Jefferson Scale of Attitudes toward Physician-Nurse Collaboration; n, sample size; M, mean; SD, standard deviation; p, p-value.

and nursing students (Hojat, 2016), and slightly lower than those reported for teamwork and lifelong learning (Hojat et al., 1999; Wetzel et al., 2010; Novak et al., 2014).

Regarding the first objective, teamwork, and cultural influence, the inverse association found between teamwork and age in newly admitted students suggests that students who start their degree coursework at a later age have a higher risk of presenting certain prejudgments related to professional roles and inter-professional work than in those who begin at a younger age. The most plausible explanation for these findings is the negative effect of dominant cultural stereotypes related to professional roles, a finding that is in accordance with research evidence reported in previous studies of Mexican (Hojat et al., 2001, 2003) and other Latin American healthcare professionals (San-Martín et al., 2017a,b). The findings of this study regarding the increasing difference in the development of this ability between medical and nursing students over time, or the deterioration in the development of this ability in the control group provide new evidence supporting the negative influence of the "hierarchical" dominant model in physician-nurse work relationships. The findings reported in this study clearly confirm that differences reported for inter-professional collaborative work among students are due to their degree coursework and not to their gender differences. This is also consistent with other studies where those differences were directly associated with professional roles (San-Martín et al., 2017a).

On the other hand, the study demonstrates that, similar to previously reported findings for empathy (Cunico et al., 2012; Hojat, 2016), collaborative inter-professional work can be enhanced and sustained in medical and nursing students by targeted educational programs. The significant increase in JSAPNC scores by the nursing students in the experimental group who were attending the training program, or the stability in the JSANPC scores of the medical students who were participating in the experimental group, evidence an antagonist tendency in comparison with those observed in medical and nursing students who were enrolled in the control group. These findings resonate with those reported in a recent study where there was a significant overlap between empathy, teamwork, and integrative patient care. The authors concluded that an improvement in inter-professional work skills is not only possible but can also positively influence the improvement of other components of medical professionalism, such as empathy (Hojat et al., 2015). In accordance with this last issue, the findings of this study confirm the positive influence that improvement of teamwork has on the other two components of professionalism. Along the same lines, a strong relationship among empathy, teamwork,

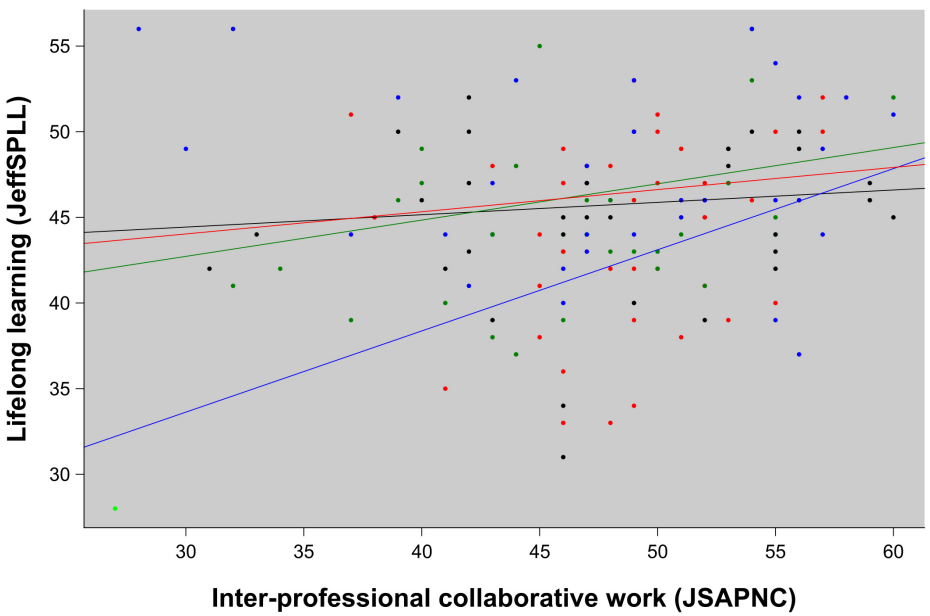

FIGURE 2 | Regression analysis of lifelong learning abilities (measured by the JeffSPLL), according to inter-professional collaborative work (measured by the JSAPNC). For "study group by degree coursework" groups: in red, medical students in the experimental group; in blue, nursing students of the experimental group; in green, medical students of the control group; and in black, nursing students of the control group ($p = 0.028$).

and lifelong learning are experimentally demonstrated. These findings coincide with others reported in previous studies with medical students (San-Martín et al., 2016) and healthcare professionals (San-Martín et al., 2017a,b; Soler-González et al., 2017). In all these studies, new research evidence supports a multi-score measurement of medical professionalism, initially proposed by Veloski and Hojat (2006) in clinical settings with a cross-cultural scope. This study supplies new evidence in an undergraduate population. Additionally, this study provides new evidence supporting the importance of improving inter-professional skills in the development of attitudes toward lifelong learning in nursing students. This issue is especially remarkable given that the development of lifelong learning skills as a professional competence is an important gap in nurses who are working under the influence of dominant "hierarchical" work models, as has been reported in recent studies in healthcare institutions located in developing countries in Africa (Muliira et al., 2012) and Latin America (San-Martín et al., 2017a).

AUTHOR CONTRIBUTIONS

LV was in charge of the study's overall design. GT-G oversaw development of the training programs and carried out the study in the participating institutions in Mexico. LV and MS-M statistically processed the data. LV and RD-B prepared the draft manuscript. All authors contributed to the present work, participated in the interpretation and processing of results, and reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.00005/full#supplementary-material

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REVIEW
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Avian Emotions: Comparative Perspectives on Fear and Frustration

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Emotions are complex reactions that allow individuals to cope with significant positive and negative events. Research on emotion was pioneered by Darwin's work on emotional expressions in humans and animals. But Darwin was concerned mainly with facial and bodily expressions of significance for humans, citing mainly examples from mammals (e.g., apes, dogs, and cats). In birds, emotional expressions are less evident for a human observer, so a different approach is needed. Understanding avian emotions will provide key evolutionary information on the evolution of related behaviors and brain circuitry. Birds and mammals are thought to have evolved from different groups of Mesozoic reptiles, theropod dinosaurs and therapsids, respectively, and therefore, their common ancestor is likely to be a basal reptile living about 300 million years ago, during the Carboniferous or Permian period. Yet, birds and mammals exhibit extensive convergence in terms of relative brain size, high levels of activity, sleep/wakefulness cycles, endothermy, and social behavior, among others. This article focuses on two basic emotions with negative valence: fear and frustration. Fear is related to the anticipation of dangerous or threatening stimuli (e.g., predators or aggressive conspecifics). Frustration is related to unexpected reward omissions or devaluations (e.g., loss of food or sexual resources). These results have implications for an understanding of the conditions that promote fear and frustration and for the evolution of supporting brain circuitry.

Keywords: emotion, fear, frustration, birds, aggression, response suppression, conflict, comparative psychology

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INTRODUCTION

In one of his epistles to Lucius, the Roman philosopher Seneca wrote that "No animal, when it enters upon life, is free from the fear of death" (Seneca, Epistle CXXI, 19, trans., Seneca, 1925). This conclusion is consistent with the general observation that most animals seem to have evolved withdrawal, escape, and avoidance behaviors from threatening situations, and seek and approach situations that promote their well-being and fulfill their reproductive potential. For example, in the mollusk *Tritonia diomedea*, tactual stimulation by a starfish (a natural predator) triggers a series of dorsoventral contractions that allow the animal to escape to a distant location (Wyeth and Willows, 2006). In some cases, however, animals seem to be willing to die, especially in defense of their colony. For example, when the colony is attacked by a predator, postreproductive females of the aphid *Quadrartus yoshinomiyai* attach themselves to the predator and secrete a sticky substance that glues the intruder and also themselves to the plant. This kills the predator, but also results in the aphid's death (Uematsu et al., 2010).

Are these behaviors accompanied by a subjective emotional experience?

Educated people may find it difficult to believe that organisms considered simple, such as a mollusk and an aphid, have the neural sophistication sufficient to produce emotional responses. But what about animals with more complex nervous systems, such as mammals and birds? Comparative psychologists have struggled with the problem of emotion because, as many other important concepts, they cannot be directly observed or measured. Darwin (1871/1965) took a different approach by emphasizing emotional *expressions*, that is, the facial and bodily movements that accompany situations that seemed akin to those that would generate emotional responses in humans. Darwin cited examples mainly drawn from mammals (e.g., apes, dogs, and cats). For example, "animals which live in society often call to each other when separated, and evidently feel much joy at meeting; as we see with a horse, on the return of his companion, for whom he has been neighing" (Darwin, 1871/1965, pp. 84–85). In Darwin's book, descriptions of emotions in birds are reduced to a few examples of negative valence. Birds "ruffled their feathers when angry or frightened" (p. 97) and are "accustomed, when in danger, either to squat on the ground or to sit motionless on a branch, so as to escape detection" (p. 100). Whereas contemporary researchers generally reject such an anthropomorphic language (but see, Safina, 2015), most would still recognize Darwin's two descriptions of avian emotion given above as examples of agonistic behavior and fear-induced freezing. Curiously, Darwin provided no examples of avian emotions that could be related to frustration, disappointment, or loss, although the example with reunited horses also given above points to the effects of social separation in a mammal.

In defining these emotions, we continue to have limits similar to those encountered by Darwin in 1871. First, we still cannot study animal emotions directly because we have no access to an organism's subjective experience. Therefore, we must depend on animal behavior and physiology—emotional expression. Second, we have to use standards developed by those who study emotional behavior in mammals and apply them to other taxonomic groups. Third, the human emotional repertoire seems too broad to start a discussion of animal emotion. It seems more effective to center our attention in a few emotions that have clear protective function. In this review, we focus on two such emotions: fear and frustration.

Animals are said to be under the influence of fear if they "avoid when they can, flee if avoidance has not been successful, and defend themselves, usually aggressively, when flight is impossible or difficult" (Gray and McNaughton, 2000, p. 38). The avoidance of threat may involve response suppression (usually called "freezing"), which, as noted by Darwin, helps the organism to avoid detection. Frustration tends to evoke similar behaviors, but in response to the unexpected failure of rewards to occur. Thus, frustration "is a temporary state that results when a response is nonreinforced (or nonrewarded in more neutral language in the appetitive case) in the presence of a reward expectancy" (Amsel, 1992, p. 1). Amsel characterizes the behavioral consequences of frustration in terms of four descriptive concepts: invigoration, suppression, persistence, and regression. When either fear or frustration is combined with approach responses triggered by the same location or stimulus, they also contribute to anxiety. For example, fear of a location previously paired with pain (electric shock) does not induce anxiety, unless the organism must approach that location for some other reason (e.g., food or social proximity). Traditionally, this is characterized as an approach-avoidance conflict (Miller, 1944). Anxiety is dependent upon the conflict induced by an ambivalent goal.

This article reviews evidence from studies with birds based on situations designed to study fear and frustration, and the ensuing states of anxiety and conflict. But first, we ask what is a bird and what do we know about their evolutionary history?

WHAT IS A BIRD?

Although birds and mammals have evolved from different reptilian lineages (theropod dinosaurs and therapsids, respectively; Benton, 2014), there are many phenotypic similarities that originated independently by convergent evolution (Kemp, 1988). Some characters common to birds and mammals that are not found in extant reptiles include relatively large brain size, similar general activity levels, cycles of sleep and wakefulness, endothermy, and complex patterns of reproductive behavior, among others. Convergent evolution in learning has been explicitly postulated for movement mimicry among psittacine birds and primates (Moore, 1992). Is it possible that evolutionary convergence extends to the emotions of fear and frustration?

The fossil record suggests that by the late Jurassic period, about 140–150 million years ago, there were several lineages of theropod dinosaurs with feathers. A specimen from China, *Anchiornis huxleyi*, dated 155 million years old, was a small theropod dinosaur covered with feathers, although not likely capable of flight (Hu et al., 2009). There is increasing evidence that feathers first evolved in dinosaurs for functions unrelated to flight, such as thermoregulation or display. Consistent with their use in communication displays, a study of melanosomes, molecules responsible for feather color in living birds, suggested that *Anchiornis* was brightly colored with gray, reddish-brown, white, and black feathers (Li et al., 2010). Although not considered a bird, *Anchiornis* and a few other fossil specimens, are classified in the same group with *Archaeopteryx lithographica*, a transition form with reptilian and avian characters, and all of them as the immediate ancestors of birds (Benton, 2014).

Archaeopteryx was not like any modern bird. It had teeth, a bony and long tail, and claws, all reptilian characters, and although the feathers had the asymmetric morphology typical of modern birds with flight capacity, whether it could take off from the ground up remains controversial (Voeten et al., 2018). Modern birds appeared in the fossil record during the Cenozoic era (Benton, 2014), but mitochondrial DNA studies suggest an origin during the late Cretaceous period, around 100 million years ago (Brown et al., 2008). *Vegavis iaai* is a fossil bird from the Late Cretaceous of Antarctica, dated 66–68 million years ago and considered to be related to modern

birds (Clarke et al., 2005). This evidence suggests that the ancestors of modern birds survived the mass extinction event of the Cretaceous-Paleogene boundary, dated to about 65 million years ago, and then underwent an adaptive radiation during the early portion of the Cenozoic era that parallels that of the mammals.

Both birds and mammals show evidence of encephalization, that is, relatively large brain size, compared to reptiles (Striedter, 2005; Northcutt, 2011). *Archaeopteryx* also appears to have had a relatively large brain (Balanoff et al., 2013), although perhaps not as large as that of modern birds (Alonso et al., 2004). Encephalization in birds and mammals has proceeded independently from reptilian ancestors that, as far as we know, have a relative brain size that, on average, was about 10 times smaller (Northcutt, 2011). Avian encephalization may be related to a number of characters, such as endothermy, flight, cognition, and sociality. Daily energy demands that could sustain some of these functions may have triggered a positive feedback loop with brain size favoring the evolution of encephalization. It is clear that the organization of the telencephalon of birds and mammals has proceeded independently to a point that it is difficult to recognize homologies at a macroanatomical level. Equally clearly, however, there are substantial telencephalic homologies that can be appreciated at the level of afferent-efferent connections, neurochemical systems, and gene-expression profiles (Montiel and Aboitiz, 2018).

Cognitive skills and social behavior have been connected to brain evolution and high levels of anatomical change in birds (Wyles et al., 1983; Iwaniuk and Wylie, 2017). For example, tool use is correlated with increased size of the avian (nidopallium) and primate (neocortex) brain (Mehlhorn et al., 2010; Lefebvre, 2013). A similar connection between brain and emotion is only beginning to be made (Panksepp, 2017). In part, the relative paucity of results is due to a large extent on the fact that behavioral data on emotion in birds are not clear. One goal of this review is to bring this issue to the foreground.

AVIAN FEAR

Fear and related emotional states are induced in response to threat, whether actual or potential. The following review is organized into four areas of research involving threatening stimuli: response suppression, active escape/avoidance behavior, aggressive behavior, and conflict.

Response Suppression

Birds exhibit freezing and corticosterone release in fear-inducing situations. In one study (de Haas et al., 2012), freezing behavior (lack of motion and vocalizations) was assessed in an open field in young chicks and the effects were measured at an adult age. Freezing levels positively correlated with adult corticosterone levels. Moreover, corticosterone increased in birds subjected to a test involving restriction of movement. Interestingly, the presence of a fearful bird increased corticosterone levels in other individuals within the group. Additional research on response suppression in birds relates to tonic immobility (TI). TI involves the suppression of the righting response, reduced vocalizations, and intermittent eye closure (Pusch et al., 2018). In a TI paradigm, the experimenter holds the hen by hand upside down for 15 s and then releases the animal. TI occurs if no movement is detected for 10 s (Hrabcakova et al., 2012; Pusch et al., 2018). According to Gallup (1973), TI is a reliable index of fear in birds. He found that a conditioned stimulus for shock is more effective than a shock to increase the duration of TI. A recent review concluded that TI remains a valid indicator of affective states in the face of threats (Fureix and Meagher, 2015). Quail strains selected for high and low TI show differences in fear responses, with the high TI strain showing more freezing and less exploration than the low TI strain (Jones et al., 1991). These differences were attenuated by exposure to environmental enrichment (colored geometric patterns painted on cards and pinned to the walls and a variety of colored objects).

The posterior arcopallium in birds (corresponding to portions of the mammalian amygdala; Jarvis et al., 2005; Hanics et al., 2017) seems to play an important role in the control of fear responses, as it does in mammals (Ressler and Maren, 2019). In Japanese quail, for example, lesions of the posterior arcopallium increase behavior indicative of fear, whereas lesions of the anterior arcopallium reduce fear responses (Saint-Dizier et al., 2009). Fear responses were tested in four tasks: TI, open-field test, hole-in-the-wall test, and novel-object test. In addition, there is evidence of lateralization of function in the arcopallium. In chickens, unilateral lesions of the right medial arcopallium have a greater effect on fear responses than equivalent lesions in the left hemisphere (Phillips and Youngren, 1986).

Neophobia is the rejection of novel objects. In birds, neophobia is usually measured in terms of the latency to approach, sit on, and finally take a food item from a novel feeder. Black-capped chickadees (*Poecile atricapillus*) and European starlings (*Sturnus vulgaris*) exhibited longer latency to approach and consume food from a novel feeder than from a familiar feeder (Apfelbeck and Raess, 2008; Roth et al., 2010). Aversive conditioning also potentiates neophobia. In an experiment with chicks (Franchina and Dryer, 1989), novel visual (red water) or taste (vinager) simuli were paired with an injection of lithium chloride, a toxin that induces sickness, or a saline injection. In subsequent neophobia tests, animals were tested with either green water (visual novelty) or saline water (taste novelty). There was evidence of neophobia in both tests, but conditioning with the vinager led to more neophobia than conditioning with red water. Bobwhite quail (*Colinus virginianus*) can be easily conditioned to suppress consumption of food with a novel color after pairings with a toxin (Wilcoxon et al., 1971).

Passive avoidance research with birds has attracted more attention. In one procedure, beads of a specific color are coated with a substance that has an aversive taste to chickens (methyl anthranilate). In later tests, young chicks are given successive tests with beads of the same or different color and the amount of pecking is recorded. Chicks peck more at the novel color than at the color paired with the aversive substance, but the

order in which the colors are presented and the time between successive tests determine the outcome of the test (Crowe and Hale, 2002).

Several aspects of passive avoidance learning in chicks are controlled by different parts of the telencephalon. For example, extensive lesions of the dorsal, lateral, and anterior hyperpallium (formerly hyperstriatum) impaired the retention and relearning of passive avoidance. Interestingly, whereas lesions of the dorsal hyperpallium disrupted acquisition of passive avoidance, anterior lesions caused no measurable deficits in passive avoidance (Benowitz, 1972). More recent research has focused on neurochemical and cellular effects of passive avoidance learning. The administration of dopamine agonists in one-day-old chicks alters memory consolidation in this passive avoidance task (Hale and Crowe, 2002). In the same species, this passive avoidance procedure resulted in an increase in cellular proliferation in the mesopallium ventrale (formerly hyperstriatum ventrale) and in the olfactory tubercle (Dermon et al., 2002). However, passive avoidance has also been reported to produce a decrease in hippocampal synaptic density in chickens (Nikolakopoulou et al., 2006). This may imply that passive avoidance is a stressful event leading to dendritic atrophy and thus a reduced spine density. Others reported the opposite result, namely, an increase in spine density in the right hemisphere of the hippocampus of male chicks trained in passive avoidance (Unal et al., 2002). The source of these differences is unclear. What is clear is that passive avoidance training in chickens gives rise to an aversive memory that has measurable neural correlates similar to those observed in mammals. For example, the density of hippocampal synaptic spines is also modified by Pavlovian fear conditioning in mice (Abate et al., 2018).

Circadian factors are also important in passive avoidance. Chickens that received a single pairing between a red bead and methyl anthranilate at 16:00 h had poor discrimination (i.e., they generalized responding) in red-versus-blue tests administered 24 h later. By contrast, chicks trained at 08:00 h and 12:00 h discriminated red from blue beads more accurately (Radford et al., 1981). Experiments with mammals show similar sensitivity to circadian factors (Barbachano et al., 2017). Caging conditions also affect passive avoidance learning. Using electric shocks as reinforcer, chickens caged in groups exhibited better learning than animals housed in individual cages (Brown, 1976). Group caging is known to also facilitate aversive learning in mammals (Penagos-Corzo et al., 2015).

Active Escape/Avoidance Behavior

Active escape/avoidance behavior has been studied in a variety of situations. This section covers research on alarm signals, the flight initiation distance (FID), and active escape/avoidance learning.

The social learning of predator avoidance can be conceptualized in conditioning terms. The predator is the conditioned stimulus and the social alarm of a conspecific is the unconditioned stimulus. Accordingly, stimuli associated to social alarm are tagged as threatening. However, at least in some birds, learned social avoidance is temporarily more flexible than in laboratory paradigms of conditioning in which a stimulus predicts important biological events (Griffin, 2004, 2008). A natural predator not always generates automatic avoidance. For instance, there is evidence from several bird species that some animals are attracted to the predator and the experience derived from these encounters is used by conspecifics. Observing a predator model with a dead gull (*Larus argentatus*) increased the avoidance distance of gulls in a later encounter with the model (Kruuk, 1976). In many species, the encounter with a predator releases specific behaviors that conspecifics perceive and later use to tag the stimulus as threatening. Social alarm in birds is based on calls and also on the noise of the wings at take-off flight (Hingee and Magrath, 2009). Social alarm in birds may be conspecific or heterospecific (Dawson Pell et al., 2018). In the case of Japanese tits (*Parus minor*), birds that have learned to respond to alarm calls become more responsive to objects resembling the predator—a sign of a search image (Suzuki, 2018). It is also worth noting that birds show the acquisition of passive avoidance through observation. For example, chickens can learn about an aversive object observing the responses of another chicken (Johnston et al., 1998). Thus, chicks that observed through a wire mesh the aversive reaction of another chick when pecking at a bead coated with methyl anthranilate later avoided a similar bead. Avoidance learning by observation has also been reported in natural environments in great tits (Landová et al., 2017; Thorogood et al., 2018).

The FID has also been used in natural environments to assess escape/avoidance behavior (Møller, 2010). Increased fear in birds, as in mammals, is associated with adrenal cortex activation (Davis et al., 2008; Tilgar et al., 2010). Neuroendocrine responses involve hypothalamic–pituitary–adrenal (HPA) axis activation and corticosterone secretion (Cockrem, 2007). Even if there is evidence supporting the relationship of both FID and corticosterone with stress and fear, the relationship between these two variables is not always direct. For example, in a comparison between urban and rural birds, despite finding differences in the FID between these two populations, a relationship with corticosterone was not found (Rebolo-Ifrán et al., 2015).

In the traditional instrumental paradigm of active avoidance learning, a signal precedes an aversive stimulus, unless the animal makes a specific response during the signal, in which case, the signal is terminated and the aversive stimulus is prevented. There are two versions of active avoidance learning, one in which the animal moves to a different compartment when the warning signal is presented. This procedure is called two-way avoidance because either compartment is both safe and dangerous depending on the trial. In the second procedure, called one-way avoidance, the animal also moves to another compartment, but each compartment is always either safe or dangerous. Macphail (1968) provided evidence of both types of avoidance learning in pigeons, albeit with only a few animals and with sequential training starting with one-way avoidance and ending with two-way avoidance training. Pigeons also exhibited efficient acquisition of a treadle-pressing response to avoid shock in an unsignaled Sidman avoidance procedure (Smith and Keller, 1970). This procedure is analogous to active

avoidance, except that no explicit signal is presented (shocks and response feedback provide signals). Instead, shocks occur at regular intervals and a response postpones the next shock for a period longer than the shock-shock interval. Using a visual + auditory compound and a treadle response, Foree and LoLordo (1975) reported that pigeons learned to respond to prevent punishment for pecking at a key for food. Moreover, post-training tests determined that the treadle response was controlled predominantly by the visual element of the compound. Interestingly, when pigeons pressed a treadle just to avoid shock or just to obtain food, then behavior was controlled predominantly by the auditory or visual component, respectively (Foree and LoLordo, 1973). Lesions of the arcopallium (previously archistriatum) impaired both active two-way avoidance and Sidman avoidance learning in pigeons (Dafters, 1975), just as they also impaired passive avoidance (see above). Two-way active avoidance performance is actually enhanced by section of the olfactory nerves, even though the warning signal was visual (Hutton et al., 1974). Opioid receptors in the olfactory tubercle (previously associated to the lobus paraolfactorius) have also been detected in chicks trained in the passive avoidance paradigm. Post-training infusions of mu and delta opioid-receptor antagonists in the olfactory tubercle impaired retention of the passive avoidance task, but kappa and opioid receptor-like receptor antagonists had no effect (Freeman and Young, 2000).

Aggressive Behavior

Experiments with several mammalian species have shown that aggressive behavior can be elicited by painful stimuli, such as electric shocks (Ulrich, 1966). Pigeons, which readily peck at other pigeons (or at stuffed pigeons or mirror images) during periods of nonreinforcement (see below), do not seem to respond with aggressive behavior toward a stuffed conspecific when receiving shocks (Rashotte et al., 1974). Such discrepancy can be understood in terms of selective pressures related to foraging and predator avoidance. Pigeons compete with conspecifics for access to food, but they rarely engage in intraspecific aggressive behaviors. Consistent with this characterization, pigeons show aggressive responses to conspecifics in the context of foraging (e.g., appetitive extinction), but not in the context of defensive behavior (e.g., shock-induced pain).

Aggressive behavior occurs in chickens reared in animal production facilities. For a variety of reasons, chickens show an aggressive behavior described as feather pecking that results in bleeding and even cannibalism (Fossum et al., 2009). Feather pecking has been associated to fear responses in the target animal. Although birds show agitation after the initial attacks, this behavior is followed by periods of freezing. Physiological changes suggest that feather pecking induces pain (Gentle and Hunter, 1991). In a line of birds selected for low mortality from feather pecking, Kops et al. (2013) reported lower levels of noradrenaline activity in the arcopallium relative to a control line and, consistent with this finding, lower levels of fear in the manual restriction test (in this test, the animal is tilted on its right side while its legs are gently pulled; response movements are recorded).

Conflict

Conflict situations usually involve a convergence of two contingencies of opposite hedonic value (Miller, 1944). This can be implemented in a Skinner box situation, where pigeons trained in a fixed-ratio or variable-ratio schedule for food reinforcement (which generates high rates of key pecking) are concurrently exposed to punishment with shock contingent on some key-pecking responses. A number of studies show that while punishment leads to behavioral suppression, there is also a tendency for recovery of response levels within the session (Dardano, 1970; Powell, 1970).

There is extensive psychopharmacological research on the effects of a variety of drugs on punished responding in birds. Chlordiazepoxide (CDP), an anxiolytic drug that binds to the benzodiazepine site in the $GABA_A$ receptor and facilitates the influx of chloride ions induced by GABA (Meyer and Quenzer, 2018), increases punished responding in rats, monkeys, and pigeons (Barrett and Gleeson, 1991). McMillan (1973) reported that CDP and diazepam (also a benzodiazepine anxiolytic) increase response rate under punished responding relative to matched unpunished behavior. Punished responding is also increased by bretazenil (a partial agonist of the benzodiazepine site of GABA receptors), which does not affect matched nonpunished responding, and the effect was eliminated by the benzodiazepine antagonist flumazenil (Witkin et al., 1997).

A variety of serotoninergic drugs have been used in the treatment of anxiety and depression in humans, but have little or no effects in established animal models of either disorders with nonhuman mammals. Yet, pigeons respond to these drugs much like human patients (Barrett et al., 1994). One example is buspirone (a serotonin-1A-receptor agonist with anxiolytic effects). Buspirone does not affect punished responding in rats (Howard and Pollard, 1990) or squirrel monkeys (Wettstein, 1988), and it also fails to eliminate consummatory negative contrast in rats (Flaherty et al., 1990). However, buspirone eliminates the suppressive effects of punishment in pigeons (Barrett and Witkin, 1991). The mechanism of action is benzodiazepine-independent (Barrett et al., 1986), but serotonin-dependent (Witkin et al., 1987; Gleeson et al., 1989).

The evidence reviewed in this section is consistent with the hypothesis that at least some avian species experience emotional states akin to mammalian fear. The behavioral, neurobiological, and psychopharmacological results reviewed above suggest interesting parallels between mammals and birds in emotional behavior.

AVIAN FRUSTRATION

Following the lead from experiments with rodents, this review focuses on a variety of procedures involving some form of surprising reward omission (SRO; Papini and Dudley, 1997). Such events are labeled as "surprising" because they occur in the presence of signals or in situations previously associated

with reward presentation. SROs can lead to a variety of behavioral and physiological effects in rodents, including response invigoration, response elicitation, escape, response suppression, and persistence. In this section, we ask whether similar behavioral effects have been observed in analogous experiments with birds. This evidence will provide a base to test the hypothesis that birds experience emotions akin to frustration when exposed to SROs. The following sections are organized along six areas of research: response invigoration, response suppression, escape/avoidance behavior, persistence, aggressive behavior, and conflict.

Response Invigoration

Amsel and Roussel (1952) reported that rats ran faster after a surprising nonreward event than after a surprising reward event in a double-runway apparatus. Their interpretation is that a SRO induces an emotional state (called primary frustration), which energizes behavior by increasing motivation to engage the dominant response. A major problem of interpretation has been to distinguish between response invigoration after the SRO (frustration) versus response suppression after the reward presentation (transient demotivation). Subsequent research has shown that both processes, response invigoration and suppression, actually occur in situations involving food omission (Stout et al., 2003). The emotional interpretation is consistent also with the elimination of response invigoration during early appetitive extinction trials in rats deprived of adrenal glands and, therefore, of reduced circulating glucocorticoids (Thomas and Papini, 2001). Adrenalectomized rats learn and extinguish lever-pressing behavior, but lack the response invigoration that usually occurs when food is initially withheld in extinction.

Response invigoration (increased key-pecking behavior) after occasional food omissions has produced inconsistent results in pigeons. In free-operant procedures, Staddon and Innis (1969) reported response invigoration when rewards were omitted in fixed-interval schedules. However, Wilton et al. (1969) reported inconsistent results also using free-operant schedules in pigeons, although the same procedures produced response invigoration in rats. Interestingly, Wilton et al. (1969) observed pacing behavior following reward omission, which they interpreted as indicating an emotional effect of the omission. In an additional phase, Wilton et al. (1969) introduced a limited hold on responding, such that pigeons had to peck the target key within a 0.9-s period to be able to obtain a reward. This limited hold was introduced to reduce the influence of pacing. Under these conditions, the results were again inconclusive. One pigeon showed response invigoration, one showed response suppression, and a third one exhibited no clear effect of reward omissions. However, even if a consistent difference in responding after reward versus after nonreward would have been obtained, the issue of whether it was invigoration driven by frustration or suppression caused by transient demotivation would have remained unresolved.

More conclusive effects of reward omissions were reported in discrete-trial experiments. For example, during fixed-interval performance, pigeons exhibited higher key-pecking rates after reward omission than after reward presentations. However, increasing the intertrial interval from 2 to 12 s eliminated the effect by increasing performance after both outcomes relative to a group receiving continuous reinforcement (Papini and Hollingsworth, 1998). A frustration account would have been supported by a reduction in responding during the long intertrial interval, but, instead, performance was increased, a result pointing to transient demotivation. Thus, pigeons responded less after food presentation, rather than more after food omission. Similar results were obtained in a runway experiment. Pigeons could anticipate rewarded and nonrewarded trials when discriminative stimuli were used, but not when outcomes were unpredictable. However, they ran faster after nonreward than after reward whether the outcomes were expected or unexpected (Stout et al., 2002). Again, this effect seems to have been caused by transient demotivation to respond for food. Furthermore, key pecking was higher immediately after nonreward than after reward, a difference that was eliminated by introducing a delay for responding. However, the difference was eliminated by response recovery after surprising reward, rather than by response decay after surprising nonreward, as a frustrative interpretation anticipated (Stout et al., 2002). Thus, there is no evidence that pigeons (or any other avian species, as far as the authors are aware) exhibit response invigoration after a SRO event that is attributable to primary frustration.

Response Suppression

As with rats (Logan, 1960; Rashotte and Amsel, 1968), pigeons find it difficult to inhibit responding for food when response spacing is required for reinforcement. For example, pigeons have difficulty adjusting to a schedule of differential reinforcement of low rates in which key pecking needs to occur once every 20 s for reward to be delivered (McMillan and Campbell, 1970). In addition, both d-amphetamine (a drug with multiple synaptic effects, including inhibition of monoamine uptake; Meyer and Quenzer, 2018) and CDP had a small, disrupting effect on key pecking during such schedules. These drug effects are difficult to interpret due to the small number of birds used with each dose (n = 2) and to the inconsistency of performance. Perhaps, the most parsimonious explanation for the small disrupting effect of both drugs would appeal to a motor impairment, rather than an emotional component. In general, animals find it difficult to inhibit responding for food, even when a cost (i.e., reward loss) is inflicted upon such impulsive behavior. In pigeons, however, key pecking shows greater impulsivity than treadle pressing (pushing a lever with a foot) in a differential reinforcement of low rates situation (Hemmes, 1975). Whereas pigeons continued operating a treadle with up to a 35-s delay requirement for responding, key pecking was disrupted by a delay requirement as short as 14 s. Nonetheless, examples of response suppression in appetitive situations are especially interesting because the behavior has to overcome the tendency toward impulsive responding.

Response suppression is expected if an animal learns to anticipate, based on prior experience, either the forthcoming omission of a reward or the frustrative response such an omission caused, both having negative emotional connotations. Such learning occurs, for example, during appetitive extinction when approach responses decrease to a minimum, as has been shown since Pavlov (1927) in a variety of situations. Emotional

responses can modulate the speed of the response decrement occurring during appetitive extinction. So, rats trained with large versus small rewards and shifted to extinction exhibit differential rates of response decrement—faster after large reward than after small reward acquisition (Wagner, 1961; Papini et al., 2001). One explanation of this phenomenon, called the magnitude of reinforcement extinction effect (MREE), is that the omission of a large reward conditions a stronger anticipation of frustration than the omission of a small reward and, therefore, a stronger avoidance of the goal that accelerates extinction (Amsel, 1992).

Pigeons trained to traverse a runway or to peck at a key in a spaced-trial situation (one trial per day) showed evidence of a reversed MREE, that is, faster extinction after training with a small reward than with a large reward (Papini, 1997; Papini and Thomas, 1997; Thomas and Papini, 2003). Interestingly, pigeons trained with different cues signaling the large and small magnitudes in a successive, spaced-trial discrimination procedure (a spaced-trial version of a simultaneous contrast paradigm) also extinguished more slowly after acquisition with the large-reward cue, than with the small-reward cue (Papini, 1997). A similar reversed MREE was reported in Japanese quail (*Coturnix japonica*) trained in a sexual conditioning paradigm (Baquero et al., 2009). In this case, groups of quail received access to either eight females or one receptive female in the goal box of a runway. When shifted to extinction (no females present at the goal), large reward led to slower extinction than small reward.

In the MREE paradigm, groups trained with different reward magnitudes (large vs. small) are shifted to the same magnitude in extinction (no reward). This is formally similar to the successive negative contrast (SNC) paradigm in which the shift is to a lower, but nonzero, magnitude. In this situation, a reward downshift from a large to a small magnitude has a transient suppressive effect on approach behavior relative to an unshifted control always trained with the small reward (Flaherty, 1996). SNC is related to a variety of indicators suggesting that the effect is accompanied by an aversive emotional reaction—primary and anticipatory frustration, depending on the situation (Papini et al., 2015).

Pigeons trained to peck at an illuminated key for 15 food pellets showed shorter response latencies than pigeons trained to peck for one food pellet, a fact indicating that the magnitudes were discriminable. However, a 15-to-1 pellet downshift caused only a gradual adjustment of response latencies without any signs of SNC (Papini, 1997). This result is consistent with the reversed MREE effects described above; in both cases, pigeons exhibited difficulty to suppress behavior following SROs. An experiment with chickens using a wet cereal reward (more preferred) versus the same cereal mixed with orange oil (less preferred) in a runway also produced evidence of a reversed SNC effect (Davis et al., 2015). These results may not be general across avian species since evidence of SNC was reported in starlings (*S. vulgaris*). In one experiment (Freidin et al., 2009), two groups of starlings received either mealworms (a preferred food) or turkey crumbs during preshift trials. Mealworm animals consumed more food than turkey-crumb animals, thus showing that the rewards affected consummatory behavior. Then, the mealworm animals were downshifted to turkey crumbs, whereas the unshifted controls continued to receive turkey crumbs. Now, downshifted animals consumed less than unshifted controls, thus showing evidence for SNC. In addition, probing the target (i.e., inserting its beak into the bowl that used to contain mealworms in preshift trials) produced no evidence of SNC. Such probing behavior is likely to have been instrumentally reinforced by access to mealworms, in which case, a failure to observe SNC in probing is potentially significant.

Instrumental behavior provided evidence of reversed MREE and SNC in three avian species (pigeons, quail, and starlings), but a regular SNC effect was observed in terms of consummatory behavior in starlings. In rats, consummatory SNC effects tend to be more robust than instrumental SNC effects. For example, several experiments in which rats were trained with sucrose solutions of different concentrations in the goal box of a runway have produced evidence of consummatory, but not instrumental, SNC in the same animals (Flaherty and Caprio, 1976; Sastre et al., 2005). Similarly, the same rats that produced no evidence of SNC in single-option training yielded evidence of SNC in free-choice trials in which they could respond to two levers previously paired with large and small rewards (Conrad and Papini, 2018). In free-choice trials, rats preferred the lever associated to the large reward during preshift, but switched preference to the lever associated with the unshifted reward during postshift trials. As a result, it seems plausible that implementing a SNC manipulation using either consummatory or free-choice procedures would yield evidence of frustration in birds.

Escape Behavior

The previous section provided scanty evidence for response suppression in situations involving reward downshifts. Suppression of approach behavior is akin to goal avoidance. One can also ask whether birds demonstrate evidence of escaping from a stimulus situation in which they are either exposed to surprising nonreward (escape from frustration effect; Norris et al., 2009) or to a signal for nonreward (escape from the S− effect; Terrace, 1971). To our knowledge, there are no demonstrations of the escape from frustration effect in birds, but there is evidence for the escape from the S− effect in pigeons.

The aversive properties of surprising nonreinforcement can be assessed directly. For example, pigeons learn a key-pecking response when its only consequence is to terminate the stimulus predicting no reward (S−), in an S+/S− successive discrimination (Rilling et al., 1969; Terrace, 1971; Gonzalez and Champlin, 1974). For example, Rilling et al. (1969) trained pigeons in a multiple schedule in which one component delivered rewards at a high rate, whereas the other component was either extinction or had a lower reward rate. Pigeons had the opportunity to peck at a second key at any time during the session and turn off all the lights in the conditioning box. Under these conditions, pigeons pecked more frequently at this second key when they were either in extinction or reinforced at a lower rate. Since the escape key did not affect the rate of reinforcement, these results cannot be interpreted in terms of reducing the delay to the next reward. Terrace (1971) also showed that the removal of the escape contingency results in the extinction of this response. Interestingly, Terrace (1971) reported that escape from the S− does not develop following errorless discrimination training in

which the pigeon has never experienced nonreinforcement for responding to the S− and, therefore, should not have experienced frustration. These results were interpreted uniformly as suggesting that the S− has aversive emotional properties.

Pigeons also learn to postpone a time-out period from reinforcement much as they learn to postpone an electric shock. Such responses may be considered to be examples of active avoidance, as different from the response suppression examples discussed in the previous section. Pigeons were trained under a concurrent schedule in which pecking at one key produced rewards on a variable interval schedule while pecking at a second key postponed a time-out period during which all lights were turned off. Consistent with previous studies with rats and chimpanzees, DeFulio and Hackenberg (2007) reported that pigeons acquired responding to the second key that postponed a time-out period, but reduced such responses when they did not affect the occurrence of the time-out period. In addition, they showed that stimuli paired with effective postponement of the time out induced higher response rates than stimuli that were less effective in postponing the time out. The authors view this procedure in terms of the aversive properties of the time-out period. To the extent that a time out postpones food delivery, it has the potential to induce frustration. Escape from S− and time-out avoidance offer interesting parallels with fear in the shock escape/avoidance situation and are thus potentially useful to shed light on emotional processes related to frustration in pigeons.

Persistence

The partial reinforcement extinction effect (PREE) is defined as greater resistance to extinction after acquisition with partial rather than continuous reinforcement. It is a ubiquitous effect that occurs under a wide range of conditions and in many species (Papini, 2014). In birds, the PREE has been found also under a variety of conditions, including massed and spaced training trials. Under massed conditions, training is administered either in a free-operant situation with the key light continuously available for responding (e.g., Nevin, 1988) or with intervals between trials in the order of seconds to a few minutes in discrete-trial situations (Jenkins, 1962; Gonzalez and Champlin, 1974). Massed training conditions can produce behavioral effects that are consistent with emotional memory, but based upon nonemotional mechanisms. When trials are administered in closed temporal proximity, the carry-over sensory or short-term memory traces of rewards consumed in one trial can acquire control over responding during the following trial. Partially reinforced animals can learn to respond in the presence of carry-over events involving both reward and nonreward, whereas continuously reinforced animals only learn to respond in the presence of reward carry-over events. As a result, a shift to extinction is less disruptive to partially reinforced animals than it is to continuously reinforced animals—a matter of stimulus generalization decrement (Hull, 1952).

There is evidence that short intertrial intervals promote the PREE under conditions that do not yield this effect when training is widely spaced. For example, toads (*Rhinella arenarum*, formerly *Bufo*) trained to traverse a runway for water reinforcement exhibit the PREE with 15-s intertrial intervals, but not with 300-s intertrial intervals (Muzio et al., 1992). Couvillon et al. (1980) tested the carry-over hypothesis by interpolating nontarget stimuli between trials with the target stimulus. Pigeons showed single alternation behavior when reinforced and nonreinforced trials alternated, but behavioral patterning was eliminated when the interpolated task was introduced. However, the same interpolated task still yielded a PREE to the target stimulus in pigeons, suggesting that carry-over stimuli may not prevent pigeons from reactivating the memory of previous trial outcomes, a mechanism that is assumed to explain the spaced-trial PREE.

This result is consistent with reports of the spaced-trial PREE in pigeons under both key-pecking (Papini et al., 2002) and runway situations (Roberts et al., 1963; Thomas and Papini, 2003). A similar runway effect was observed in quail with food reward (Buriticá et al., 2013). In these experiments, 50% partial reinforcement training administered at a rate of one trial per day led to greater resistance to extinction than continuous reinforcement. Thomas and Papini (2003) extended these findings in three directions. First, they reported that training with variable reward magnitudes (large and small reward intermixed during acquisition) also yield increased resistance to extinction in widely spaced training in pigeons. Second, they asked whether the pigeon PREE was based on the same mechanisms underlying the PREE in rats, and provided evidence based on drug effects. Three drugs were selected based on published research with rats (see Thomas and Papini, 2003, for references): CDP (a GABAergic drug that eliminates the PREE), haloperidol (a dopamine D2 receptor antagonist that has no significant effect on the PREE), and nicotine (an agonist at acetylcholine receptors that enhances the PREE). Although these drugs affected the spaced-trial runway PREE in pigeons, their effects differed relative to those described in rats. For example, CDP retarded the emergence of the PREE in pigeons, but it did not eliminate it; haloperidol and nicotine, however, did eliminate the PREE in pigeons. Thus, although behaviorally analogous, it is plausible that the PREE is based upon different neurotransmitter systems in rats and pigeons, a possibility that remains to be fully analyzed.

Finally, Thomas and Papini (2003) tested the co-variation of the PREE and MREE within the same experiment, using widely spaced training in the runway situation. Four groups of pigeons received acquisition training with large, partial reinforcement (L/P); large, continuous reinforcement matched for reinforcement (L/Cr); large, continuous reinforcement matched for trials (L/Ct); and small, continuous reinforcement (S/C). L/P acquisition yielded increased resistance to extinction compared to L/Cr and L/Ct acquisition and thus yielded evidence of a PREE. However, S/C extinguished faster than L/Ct, thus revealing a reversed MREE. Such a dissociation between these two effects involving SROs is consistent with the hypothesis that these behavioral phenomena are based upon different mechanisms.

It seems clear that birds show a PREE behaviorally similar to that observed in rats. However, whether the effect is dependent upon the emotional memory of frustrating events remains to be determined. The information available thus far suggests

that the mechanisms underlying the PREE in pigeons and rats are different.

Aggressive Behavior

Evidence consistent with an emotional component stemming from SROs comes from experiments assessing aggressive behavior in birds. Azrin et al. (1966) provided a detailed description of the aggressive responses of pigeons trained in intermittent schedules of food reinforcement, during period of food omission:

Visual observation revealed that attack consisted of strong pecks at the throat and head of the target bird, especially around the eyes. The feathers of the target bird were often pulled out and the skin bruised. The attack was often preceded by a brief period of pacing in front of the wall on which the response key was mounted. Occasionally, the pecking attack was preceded by striking movements of the wing or by a slow swaying approach to the target bird with the head lowered. Frequently, the attack was preceded and accompanied by a deep-throated sound (pp. 194–195).

During appetitive extinction, aggressive responses are directed at another pigeon present in the conditioning box (Azrin et al., 1966; Gentry, 1968; Rilling and Caplan, 1973), at a picture of a pigeon projected on a screen (Yoburn and Cohen, 1979), and at a mirror reflecting the image of the pigeon (Cohen and Looney, 1973). Chickens also behave aggressively when food is present, but made inaccessible (Duncan and Wood-Gush, 1971), and also during appetitive extinction (Kuhne et al., 2011). Azrin et al. (1966) found that the intensity of aggressive responses in pigeons increased with the number of rewards in acquisition and decreased when pigeons were given free access to food. Pigeons also display aggressive behavior in the presence of cues that predict reward omissions (Terrace, 1972). Interestingly, wing flapping, an aggressive response, is prominent during the early stages of visual discrimination training when differential responding has not yet emerged. However, as the pigeon learns the discrimination, wing flapping is reduced and eliminated. This result is consistent with the hypothesis that frustration mediates the relationship between unexpected reward failures and aggressive behavior (Amsel, 1992). Frustration is expected to weaken when the discrimination develops and, as a result, nonreward becomes expected in the presence of the S− and so wing flapping is reduced.

There is also evidence that the vocalizations described by Azrin et al. (1966) in the previous quotation reflect emotional arousal. Rashotte et al. (1975) reported evidence of a vocalization (called "Vocal A" by the authors) that usually accompanied aggressive responses, such as during episodes of wing-striking and pecking at the head region of the target pigeon. These were low-frequency vocalizations in the range between 0 and 2 kHz.

In poultry, it has been observed that preventing the occurrence of natural behaviors may redirect behavior. For example, chickens in animal production settings can peck and remove feathers from other birds to the point of causing bleeding. Feather pecking is usually interpreted as resulting from prevented natural behaviors, including foraging or dust bathing behavior. Careful monitoring of the topography of feather pecks in hens concluded that such pecks resemble those involved in foraging, rather than sand bathing, and thus likely develop out of frustrated opportunities for displaying feeding behaviors (Dixon et al., 2008).

Aggressive responses are routinely induced by periods and signals of nonreinforcement, thus offering a potentially fertile ground to test the notion that birds experience frustration. What is missing in this research is a systematic analysis of the nonreward-induced aggressive behavior in terms of underlying neurobiological factors. Drug and brain manipulations are needed to determine the extent to which these behavioral effects can be attributed to emotional activation.

Conflict

A number of experiments with pigeons have looked at choice in situation involving different reward magnitudes or probabilities. For example, McDiarmid and Rilling (1965) first reported that pigeons prefer a schedule associated to a less frequent reward to one associated to a more frequent reward if the former involves a shorter delay than the latter. Similarly, pigeons trained to withhold key pecking to earn a large reward, rather than pecking immediately to earn a small reward, failed to inhibit responding and, thus, lost a substantial amount of food (Ainslie, 1974). Such performance can typically be reversed by manipulating either the length of the delay or the magnitude of the reward. Variations of this procedure, known as delay discounting, suggest that pigeons, like other animals, are sensitive to temporal delays such that a large reward is reduced in value if it is presented only after a temporal delay. Although delay discounting is usually interpreted in cognitive terms, it is potentially amenable to an interpretation based on frustration (Amsel, 1992). When there is an immediate reward available in the same session, delayed reward options become less acceptable if they induce frustration.

Research on delay discounting has shown that individual neurons located in the pigeon's nidopallium caudolaterale, a region considered homologous to the mammalian prefrontal cortex, encode information of both reward delay and magnitude (Kalenscher et al., 2005). Additional unit recordings from the nidopallium caudolaterale indicate that some neurons are maximally active during the interval between the onset of the stimulus and the production of the response. A task that required rapid responding to one stimulus (as fast as possible), but postponing responding to a second stimulus for 1.5 s from stimulus onset, revealed units that increased activity with a rate appropriate to these two types of trials—steeper for the rapid-response stimulus than for the waiting-response stimulus (Kalenscher et al., 2006). Moreover, trials in which the pigeon responded too early or too late, thus missing a reward, were characterized by the unit responding at the incorrect rate. Delay discounting offers an interesting arena to test emotional learning in pigeons.

FURTHER COMMENTS

These additional comments on avian emotions are organized according to three significant questions suggested by the present review. First, *what is the strength of the evidence for fear and*

frustration in birds? The evidence for fear in birds seems stronger than the evidence for frustration. As noted in the introduction, the search and evaluation of evidence for fear in birds starts from a comparison with analogous experiments with mammals, since mammalian fear is perhaps the emotion that is best understood. Like mammals, birds show evidence of similar responses to analogous stimulus conditions. TI, freezing, the acquisition of passive and active avoidance responses, and the effects of benzodiazepine anxiolytics in punished responding all point to substantial similarities with mammalian fear. However, these are just a few pieces in a large puzzle. To have a comprehensive view of the degree of similarity in underlying mechanisms, a starting approach would be to systematically compare two species, under analogous conditions, and in terms of at least four levels of analysis: behavioral, neurobiological (neural circuitry), neurochemical (synaptic transmission), and cell-molecular (synaptic plasticity) (Papini, 2002, 2003, 2008). A case for homology of emotional behavior would be strengthened if the same mechanisms were uncovered at all levels of analysis. By contrast, similarity in behavior supported by different underlying mechanisms would be consistent with independent evolution by homoplasy. Of all the preparations for studying emotional behavior reviewed above, the one that seems most suitable for such a detailed analysis would seem to be punished responding. There are extensive behavioral and neurochemical (psychopharmacological) data in both pigeons and rats that set the basis for a systematic evolutionary approach to a search for avian/mammalian homology in fear.

Although the term "frustration" has been used extensively in the literature, especially in applied research with birds, the evidence for extensive similarities in the adjustment to SROs in birds and mammals is not as strong as it is in the case of fear. In some cases, the behavioral effects are simply different (e.g., SNC and related effects). In other cases, pigeons and rats produce similar behavioral effects, but apparently based upon different mechanisms, as revealed by drug manipulations (PREE). A promising phenomenon that would need to be rescued for analysis with current behavioral and neural techniques is that of extinction-induced aggressive behavior in pigeons (Papini and Dudley, 1997; Papini, 2014).

Another result in need for further analysis is the behavioral dissociation between PREE and MREE in pigeons. For example, a comparison between pigeons and rats in their adjustment to SROs suggests different scaling properties of their behavior. For rats, reward downshift is controlled by the ratio of the reward magnitudes obtained in postshift and those expected from preshift experience, rather than by their absolute magnitude (Papini and Pellegrini, 2006; Pellegrini and Papini, 2007; Pellegrini et al., 2008). For pigeons, however, postshift key-pecking performance is controlled by the absolute magnitude of the preshift reward (Pellegrini et al., 2008). The behavior of pigeons in reward devaluation tasks seems to be under tight control by the long-term memory of the preshift reward magnitudes. Instead, rats exhibit behavioral flexibility in analogous situations. Negative emotions such as anticipatory frustration induced by prior experience with SROs may accelerate the detachment from a signal or location previously paired with reward, a process known as incentive disengagement (Papini, 2003). One function of negative emotions may be to facilitate the disengagement from incentives that are no longer yielding sufficient rewards to support survival and reproductive success.

Second, *what is needed to characterize the relationship between brain and emotion in birds*? A model to imitate is provided by research on spatial learning and hippocampal size and function in birds and mammals (Sherry et al., 1992; Broglio et al., 2015). In this case, there is a fit between structure and function that permits a direct visualization of brain-behavior relationships. This is facilitated by the development of analogous training techniques that can be applied to different species (teleost fish, amphibians, birds, and mammals) and a structure that can be clearly identified in terms of homology across a wide range of species. A similar argument could be made for the relationship between active and passive avoidance learning (in relation to fear), or runway performance under conditions of reward devaluation and extinction (in relation to frustration). Such training techniques have been applied to a variety of vertebrate species using analogous procedures. The key structure in this case would be the amygdala, which, like the hippocampus, has identifiable homologs across vertebrates (Moreno and González, 2007; Vargas et al., 2012). For example, studies with Roman high- and low-avoidance rat strains, selected for active avoidance learning since the 1960s (Driscoll and Bättig, 1982), provide a potential approach to follow. In addition to differences in avoidance learning, these strains also exhibit differences in a variety of emotional behaviors, including other types of avoidance learning, anxiety situations, and also situations involving SROs (Torres and Sabariego, 2014). In general, Roman low-avoidance rats show higher levels of anxiety, SNC, PREE, and the emotional self-medication effect involving increased voluntary consumption of anxiolytics immediately after episodes involving SROs, compared to Roman high-avoidance rats. Gómez et al. (2008) reported that poor one-way avoidance in Roman low-avoidance rats was correlated with low cell density in the basolateral amygdala. Similar selective breeding protocols could be applied to some avian species, including Japanese quail and chickens, for which there are also behavioral techniques to study analogous phenomena (e.g., selective breeding for TI; Jones et al., 1991).

Third, *what information do these studies in birds offer to understand the origin of negative emotions in vertebrates*? The study of fear and frustration in mammals has suggested that the underlying neural mechanisms overlap extensively—the fear = frustration hypothesis (Wagner, 1969; Gray, 1987; Gray and McNaughton, 2000). Comparative research in other vertebrates can help determine whether brain circuits dedicated to these emotions arose simultaneously or sequentially during vertebrate evolution. Based on data on avoidance learning in goldfish (*Carassius auratus*), Papini (2003) suggested that the brain mechanisms underlying frustration could have evolved from fear mechanisms in early mammalian ancestors by a combination of gene duplication and co-option. This is based on several sources of evidence, including the comparative

distribution of the SNC effect in vertebrates. SNC has been described in several mammalian species and it has been shown to correlate with behavioral and physiological indicators of emotional activation (Papini et al., 2015). Analogous experiments with teleost fish, amphibians, turtles, and birds have provided no evidence of SNC, with the exception of a single experiment with starlings (Papini, 2014). However, some of the same species that show no evidence of SNC readily provided evidence for avoidance learning: goldfish (Portavella et al., 2003), amphibians (Daneri et al., 2007), and birds (see above). Interestingly, avoidance behavior in goldfish (Portavella et al., 2004) and in toads (Puddington et al., 2016) depends on activity in brain areas homologous to the mammalian amygdala.

Much remains to be done to develop lab techniques to study behaviors clearly related to emotion, following the lead of such studies with mammals. This review suggests several potential candidates for behavioral targets. But, in addition, behavioral effects need to be analyzed at lower mechanistic levels from a comparative perspective to uncover clues on the evolution of vertebrate emotional systems. These goals could be best approached at this time using fear and frustration as model emotions.

AUTHOR CONTRIBUTIONS

All the three authors contributed, to varying degrees, in each of the following items: conception of the article, writing of the manuscript, review of form and content, and approval of the final manuscript.

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BRIEF RESEARCH REPORT
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Hands Up! Atypical Defensive Reactions in Heavy Players of Violent Video Games When Exposed to Gun-Attack Pictures

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Threatening cues and surrounding contexts trigger specific defensive response patterns. Posturography, a technique for measuring postural strategies, has been used to evaluate motor defensive reactions in humans. When exposed to gun pointed pictures, humans were shown to exhibit an immobility reaction. Short and long-term exposure to violent video games was shown to be a causal risk factor for increased violent and aggressive behavior. Assaultive violence with a gun is a major trigger for motor defensive reactions, and post-traumatic stress disorder (PTSD) is the most characteristic psychiatric sequelae. Recent studies point to links between PTSD symptoms and emotional shortfalls in non-clinical trauma-exposed samples. The present study investigated defensive reactions to gun threat and PTSD symptoms in heavy players of violent video games compared to non-players. Male university students were screened according to use of violent video games and divided in three groups: non-players, moderate players, and heavy players. Stimuli were pictures depicting a man pointing a gun directed at the participant. In matched control pictures, non-lethal objects replaced the gun. Posturography was recorded and PTSD symptoms were assessed. When exposed to the threat pictures, non-players exhibited the expected reduction in amplitude of body sway (immobility), heavy players presented atypical augmented amplitude of body sway, and moderate players showed intermediate reactivity. Heavy players presented a significant distinct reaction compared to non-players. They also scored significantly higher in PTSD symptoms than non-players. Disadvantageous defensive reactions and higher vulnerability to PTSD symptoms, revealed in the present study, add to other shortcomings for heavy players.

Keywords: violent games, posturography, defensive reactions, PTSD, immobility, video games, gun, emotion

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INTRODUCTION

"Violence is an extreme form of aggression that has the potential to produce severe physical harm, such as injury or death, to another" (Anderson et al., 2017, p. 143). Interpersonal violence involving guns has increased across the world (WHO, 2014; Grinshteyn and Hemenway, 2016), and populations living in large urban centers are specially affected (Ribeiro et al., 2013;

Fowler et al., 2015). Assaultive violence with a gun is a major trigger for motor defensive reactions in humans, and posttraumatic stress disorder (PTSD) is the most characteristic psychiatric sequelae. Patients with PTSD were shown to be more susceptible to the hazardous effects of repeated activation of stress mediators on dysregulation of brain and body allostasis (McEwen and Gianaros, 2010). Recent studies point to links between PTSD symptoms and emotional shortfalls in non-clinical trauma-exposed samples (Miles et al., 2017).

The Global Status Report on Violence Prevention (WHO, 2014) proposed adopting a number of preventive strategies. Besides the reduction of access to guns and knives, changing of cultural and social norms that support violence was stressed. Guns figure prominently in the culture of violence, with a heavy contribution from the media (Smith et al., 2004). Indeed, "violence in screen entertainment media (i.e., television, film, video games, and the Internet), defined as depictions of characters (or players) trying to physically harm other characters (or players), is ubiquitous" (Anderson et al., 2017, p. 142).

Extensive research has shown that media violent content is a causal risk factor for increased violent and aggressive behavior (Bender et al., 2018). Playing video games, more than passively watching violent TV and films, has an even greater effect. Exposure to violent scenes in video games occurs in a more active context and the player is rewarded by acting violently (Anderson and Gentile, 2014).

Through posturography, a technique to study body sway, distinct defensive reactions were revealed (Volchan et al., 2017). Bastos et al. (2016) recorded defensive reactions to pictures depicting realistic gun attack and showed a reduction in amplitude of body sway indicating an immobility reaction to pictures of men pointing guns straight at the participant. The authors excluded heavy players of violent video games from the analyses, considering the literature on their proneness to aggression (Anderson et al., 2010; Greitemeyer, 2014).

Given the importance of studying the hazardous effects of exposure to violent video games, the present study investigated if those heavy players of violent video games present atypical defensive reactions to gun threat and whether they show differences in PTSD symptoms compared to non-players.

MATERIALS AND METHODS

Participants

The database for the present study derived from Bastos et al. (2016). The sample consisted of 88 (48 men) undergraduate and graduate students who reported no neurological, psychiatric, or orthopedic disorders and were not under medications with nervous system action. The Ethics Institutional Review Board of the Federal University of Rio de Janeiro approved the study and participants provided written informed consent.

Questionnaires

Frequency of Violent Video Game Play

Participants had to answer the following question "Do you play video games with violent contents (people using firearms)?". The alternatives were "never", "sometimes", "often", and "almost always".

Posttraumatic Stress Symptoms

Participants completed the Trauma History Questionnaire (Green, 1996; Fiszman et al., 2005). This questionnaire lists life-threatening events. Participants indicated the event they considered the most intense and completed the PTSD Checklist for DSM-IV (PCL-C for DSM-IV) (Weathers et al., 1993; Berger et al., 2004) based on this event. The PCL-C for DSM-IV is a 17-item self-report measure of the severity of symptoms. Using a 5-point Likert scale (1 = *not at all*, 5 = *extremely*), participants rated the extent to which each symptom has disturbed them in the past month, providing a total symptom score and a score for each cluster (re-experiencing, avoidance and numbing, and hyperarousal). Participants scoring above the cutoff for clinical PTSD were excluded. Thus, in the sample of 88 participants, none reached the criterion for possible PTSD.

Visual Stimuli

Sixteen pictures showed a man pointing a gun directed toward the participant (threat set). Sixteen other pictures (control set) showed, instead of a weapon, a man carrying a non-lethal object directed toward the participant. Within each set, individual pictures were presented for three seconds with no interval between them. Each set lasted for 48 s and the control set preceded the threat set. See **Supplementary Material** for more details.

Data Collection

Details of posturographic recordings are described in Bastos et al. (2016). Briefly, amplitude of body sway was estimated by recording center of pressure displacement using a force platform. Participants stood motionless on the force platform looking at the presentation monitor while control and threat pictures were presented and posturographic signals were recorded. The posturographic parameter analyzed here was the standard deviation of center of pressure displacement (amplitude of body sway) in the anterior-posterior direction.

Data Analysis

The difference in the anterior-posterior amplitude of sway during exposure to threat and control sets of pictures defined a reactivity index (Δ = *threat – control*). Total scores on the PCL-C for DSM-IV and scores in the three clusters (hyperarousal, reexperiencing, and numbing/avoidance) were computed.

Statistical analyses were performed with Kruskal-Wallis H and Mann-Whitney U tests. The threshold for significance was $p < 0.05$.

TABLE 1 | Distribution of the three groups separated by gender.

GROUP GENDER	Non-players	Moderate players	Heavy players
Male	15	21	12
Female	24	11	5

RESULTS

Frequency of Violent Video Game Play

Participants were grouped according to the frequency of violent video game play. Participants reporting never playing violent video games were classified as "non-players" and the ones who reported playing "sometimes" were classified as "moderate players". "Heavy players" were those that reported using violent video games "often" or "almost always". The distribution of the three groups separated by gender is presented in **Table 1**.

To avoid bias due to the very different proportion of men and women in each group and the low number of heavy players who were women, the analyses focused on men.

The final sample of 48 men had age (23.5 ± 4.11 y.o.) and weight (70.1 ± 11.17 kg) evenly distributed among the three groups (Age: $F(2,45) = 0.90$, $p = 0.40$; weight: $F(2,45) = 0.36$, $p = 0.70$).

Posturography

Figure 1 illustrates the reactivity index (*threat – control*) associated with the anterior-posterior amplitude of body sway for the three groups. It can be observed that non-players exhibited a reduction in amplitude of body sway (negative reactivity index), heavy players presented an augmented amplitude of body sway (positive reactivity index), and moderate players showed intermediate reactivity.

Analysis of the reactivity index revealed a significant main effect for group ($H(2,48) = 7.39$, $p = 0.02$). Follow-up *post hoc* analyses showed a significant difference between the reactivity of non-players and heavy players ($Z = -2.56$, $p = 0.01$). Heavy players presented a distinct reaction compared to non-players.

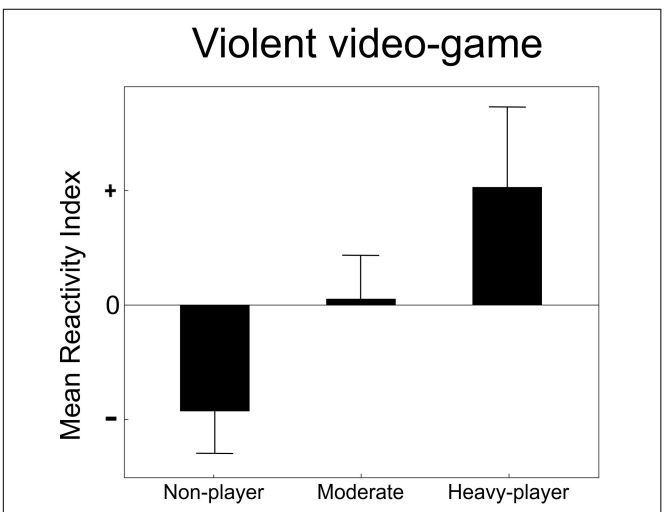

FIGURE 1 | Exposure to violent video games and defensive reactions to pointing gun pictures. Anterior-posterior amplitude of body sway during presentation of threat (gun) minus control (non-lethal object) pictures, the reactivity index, is displayed according to participants' frequency of violent video game play. Bars illustrate means and standard errors. Non-players exhibit reduced anterior-posterior amplitude of sway (immobility), heavy players exhibit a reverse reaction (augmented anterior-posterior amplitude of sway), and moderate players exhibit intermediate reactivity.

PTSD Symptoms

Given the postural differences between heavy players and non-players, scores on the PTSD symptoms scale (PCL-C for DSM-IV) were compared between the two groups. Participants who anchored their PCL-C responses on a life-threatening trauma were included in the present analysis (14 non-players and 8 heavy players).

Total scores on the PCL-C were significantly higher for heavy players compared to non-players ($Z = -2.06$, $p = 0.04$). Scores on the PCL-C hyperarousal cluster were also significantly higher for heavy players ($Z = -2.08$, $p = 0.04$). Scores on the numbing/avoidance cluster, although higher for heavy players, did not reach statistical significance ($Z = -1.77$, $p = 0.08$). Scores on the reexperiencing cluster did not differ ($Z = 0.78$, $p = 0.41$).

The difference in PTSD symptoms between heavy players and non-players remained statistically significant (PCL-C total score ($Z = 2.58$, $p = 0.01$)) when participants who did not anchor their PCL-C responses on a life-threatening trauma were included in the analysis.

DISCUSSION

Previously, we studied defensive reactions in a sample that did not include heavy players (Bastos et al., 2016). That work showed a reduction in anterior-posterior amplitude of sway under exposure to threatening pictures depicting a man pointing a gun at the participant. Here, non-players presented this expected immobility reaction. Heavy players showed, as hypothesized, an atypical reaction (augmented anterior-posterior amplitude of sway). Furthermore, heavy players presented higher scores on the PTSD symptoms scale.

Motor Defensive Response Patterns

Across species, threatening cues and surrounding contexts trigger specific defensive response patterns. Motor reactions, either overt actions or immobility, are the core of the observable defensive behaviors. Research on rodents (Blanchard and Blanchard, 1971, 1989; Blanchard et al., 1986) showed that, under attack by a predator or co-specific, flight is the dominant response when escape is available. When escape routes are blocked, immobility ensues. This action inhibition is considered an evolutionary adaptive strategy embedding preparatory states for action to be released only when appropriate context conditions trigger an action shift, like jump-attack seen in rodents (Blanchard et al., 1986).

In the present study, an attack-like condition was created by the presentation of pictures depicting a man pointing a gun at the participant. Non-players exhibited an immobility-like reaction. Heavy players behaved as if "jumping the gun", increasing mobilization, instead of immobilizing and waiting for the best chance to get rid from danger. Indeed, "... the simple sight of a weapon increases the likelihood of aggression if the person has mentally paired a weapon such as a gun

with killing or hurting people..." [Anderson and Warburton (2012), p. 72]. This is the case for heavy players of violent video games who are frequently exposed to weapon use for the purpose of killing and hurting others. The contrasting reaction of heavy *vs.* non-players to attack-like pictures add to converging evidence showing that exposure to violent video games is a causal risk factor for increased violent and aggressive behavior [for meta-analyses, see Anderson and Bushman (2001), Anderson et al. (2010), Bushman and Huesmann (2006), Greitemeyer and Mügge (2014)]. No previous study had employed posturography to investigate reactions of heavy-players to gun attack pictures.

PTSD Symptoms

There are several potential drawbacks associated with a high frequency of violent video game playing. Heavy exposure to violent video games can lead people to see the world as more dangerous, to be more fearful, and initiate more self-protective behaviors such as carrying guns (Anderson and Gentile, 2014). Several studies have shown that civilian gun ownership, rather than protecting, increases the risk of both perpetrating and being a fatal victim of violence (Bangalore and Messerli, 2013; Branas et al., 2009; Cukier and Eagen, 2018; Monuteaux et al., 2015).

Heavy players scored higher than non-players on the PTSD symptoms scale, especially on the hyperarousal cluster, which includes feeling irritable or having angry outbursts, being "super-alert" or watchful or on guard, and feeling jumpy or easily startled. Symptoms such as angry outbursts may be related to the results of numerous studies that point to the effects of violent video games on increasing aggressiveness (Anderson et al., 2010; Anderson and Gentile, 2014; Greitemeyer and Mügge, 2014). Being on guard and feeling jumpy, along with angry outbursts, are consistent with heavy players adopting a precipitated (and possibly disadvantageous) response to an attack-like context.

LIMITATIONS

This is a secondary analysis of previously collected data. The experiment was originally designed to investigate basic defensive reactions in humans. A convenience sample was recruited, ending up with a relatively small proportion of heavy players of violent video games. Characterization of heavy players was based on the high use of violent video games depicting guns, whereas other forms of violent content were not assessed. Furthermore, a standard validated questionnaire was not employed. The sample was not screened by trait questionnaires that assess aggressiveness. Finally, the present research is based on a cross-sectional sample and does not allow conclusions to be made regarding causality.

CONCLUSION

Accumulating evidence led to a clear consensus that a high frequency of exposure to violent video games significantly alters important interpersonal behaviors in negative ways (Bender et al., 2018). Atypical disadvantageous defensive reactions and higher vulnerability to PTSD symptoms, revealed in the present study, add to other shortcomings for the heavy players themselves.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Declaration of Helsinki. The protocol was approved by the Ethics Institutional Review Board of the Federal University of Rio de Janeiro. All subjects gave written informed consent.

AUTHOR CONTRIBUTIONS

MS, AB, JO, SG, MP, EV, and FE designed the research. MS, AB, JO, IF, EV, and FE performed the research and analyzed data. AB, JO, SG, EV, and FE wrote the paper. All authors reviewed draft versions and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.00191/full#supplementary-material

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CONCEPTUAL ANALYSIS
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A Non-mediational Approach to Emotions and Feelings

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The present analysis proposes a non-mediational approach to the study of affective phenomena. It starts off with the common recognition that "emotion" is not a technical term. Even so, researchers often treat it as if it were, confusing ordinary language with technical language. This leads to two problems: first, a referentialist bias, according to which we assume emotions to be something unapparent that one must infer and describe; and second, the nominalist fallacy, according to which we assume that emotions have causal effects on actions by the fact of naming them. I review some proposals to solve the problem, among which are some behavioral alternatives. Although these alternatives overcome many of the problems mentioned, they do not completely avoid them. I conclude that a strict non-mediational approach is possible and necessary. It supports the analytical separation of ordinary and technical language. Technical language abstracts relevant properties of ordinary language that become relevant parameters to model certain emotions, as they are referred to in ordinary language. I present some possible parameters and examples for consideration and conclude that the non-mediational approach is a plausible alternative that can stimulate research programs to find natural regularities in affective phenomena.

Keywords: emotions, feelings, non-mediational approach, referential bias, nominalist fallacy, behaviorism

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THE PROBLEM

Emotions and feelings are a central topic in the history of psychology. Nonetheless, the study of emotion is not a well-demarcated field and, thus, leads to confusion. For example, Kleinginna and Kleinginna (1981) collected 92 definitions of the concept "emotion." Likewise, Plutchik (1980) concluded that "there is no sense of the definitions moving in a certain direction with time" (p. 80). Recently, Izard (2010) acknowledged, after asking scholarly experts, that it is "difficult or impossible to conclude that emotion meets the standards of a scientific construct" (p. 368). He recommends that references to emotions in scientific literature should be specifically defined.

Some theorists (e.g., Widen and Russell, 2010; Scarantino, 2012) consider the problem to be a confusion between everyday and scientific uses of emotional words. The former are lay concepts, multivocal, with diffuse limits, and dependent on the cultural context (Russell, 1991, 2015). We use them to express ourselves and to communicate with others. When we say "the anger I felt made me hit him," we understand well what we are talking about, but that expression is not so usual in other cultures. For example, Levy (as cited in Kassinove, 2013) reports that Tahitians have 46 different terms to talk about anger. Each use corresponds with a different property of the action. Likewise, Briggs (as cited in Kassinove, 2013) suggests that the Utku do not express

anger. Linguistics, history, anthropology, history, philosophy, and, sometimes, psychology classify, describe, and define emotions with qualitative or quantitative tools. The work of Fehr and Russell (1984), and Cowen and Keltner (2017), illustrates this approach. Technical uses, for their part, delimit the field. They describe natural types and prescribe the inclusion and exclusion of cases. Their goal is to achieve consensus and guide scientific inquiry, facilitating prediction and control (Scarantino, 2012). In the field of emotions, there are no prescriptive definitions (see Izard, 2010; Widen and Russell, 2010), and, thus, there is confusion.

The main implication of omitting such distinctions between both languages is that scientists use ordinary terms as if they were technical. That is, they use terms that only make sense in everyday usage, as if they were descriptive terms for entities or natural kinds. Doing this implies at least two types of errors: (1) a referentialist bias and (2) the nominalist fallacy.

Referentialist Bias

Referentialist bias is the assumption that ordinary words always refer to entities or activities, whether they are observable or not. Wittgenstein (1953) was especially critical of referentialism. As the noun "apple" refers to the fruit we see on the table, we could assume that the noun "emotion" refers to an entity as well. As the verb "to run" refers to the activity we see a person doing in the park, we use "feel" as if it were also an activity. We use these terms as if we had to identify and describe them in reality.

The displacement of the reference from the observable to the unobservable sustains the bias—for example, "apple" could refer to the apple on the table or to an apple in another room. We assume that the referenced unobservable items exist, but they are not accessible to our observation. Another example is having a stone in my shoe, which I refer to but cannot be observed by others. In cases like this, we rely on some other evidence, or the help of extra instruments, to confirm its existence. I call entities that are not apparent now, but could be visible later, *unobserved observables*. We can infer their functioning from direct indicators.

The usage of emotional terms exemplifies the referentialist bias when we assume they describe discrete, unobserved, but potentially observable entities. We assume that we only observe one part (i.e., physical indicators) and infer the rest because it is assumed to occur under the skin. That location is inaccessible to us, but not for the person experiencing the emotion. This person would have privileged access through a kind of internal perception. We refer to it as "to feel" or "to have the experience of," as if it were a kind of observation that we report.

Treating emotions as if they were entities or processes happening within the skin is committing what Ryle (1949/2009) calls a "category mistake." The example of the university illustrates the concept. A visitor comes to the campus for the first time, and the host shows him the colleges, libraries, and departments. The visitor then says that he was already acquainted with all that, but he has not yet seen where the university is. The host explains to him that the university is not a place, but a way in which those things—the colleges, libraries, and departments—are organized. The category mistake is treating the university as if it were one of the buildings. The visitor cannot see the university in the same way he sees a building—not because it is behind something, but because it is an abstract concept. Concepts are not the kind of things that we observe. One can interpret a concept, but not observe it directly—even though to interpret a concept like university one must observe things (such as buildings, boards, books, etc.). Emotions are concepts, not entities or processes occurring within an organism. The referentialist bias often implies the reification fallacy when we attribute to concepts the properties of entities, such as location, duration, and action (see Hayes and Fryling, 2017, for an akin relational non-organismic account of feelings). I now turn to the nominalist fallacy.

Nominalist Fallacy

The nominalist fallacy consists of assuming that naming a thing also serves to explain it. For example, when someone does not get out of bed for several days and prefers to be alone, we describe it as depression. It is a label that summarizes what is happening. But we often accept it as an explanation of what we see: "He does not get up because he suffers from depression." In general, we accept emotions and feelings as explanations of the acts that help us to identify them. It is circular reasoning because the evidence for depression is the behavior we observe (Schlinger, 2013).

We commit the nominalist fallacy and the referentialist bias when we refer to emotion as the cause of action. This is often the case in folk psychology (Ong et al., 2016). We accept it when we wish to understand a situation, because we usually accept reasons as explanations. But we cannot demand that lay people conform to the logical demands of scientific language. Instead, if we want to explain depression, we ask why people do not get out of bed and what led them to that condition. We discover causal explanations when the answer points to something other than the depression itself.

Some Examples

Several examples show that it is common for scientists in the so-called affective science to fall into the problems described above. The following cases illustrate these tendencies.

In Finucane's study (2011), researchers presented a scene from a movie that caused anger. Then, they presented images from the scene at regular intervals during a task to measure attention. The participants evaluated how much anger they felt. The researchers verified that the participants rated higher levels of anger in front of the anger-inducing scene, and that the reaction times in the task were faster than in the control condition. Those results would show enhanced focusing of attention. The author concluded: "These results support the general prediction that high arousal negative emotional states inhibit processing of non-target information and enhance selective attention" (p. 973). Thus, anger is an unobserved state that we recognize through words and actions. It is something inferred beyond the measured behavior, which exemplifies the referentialist bias. However, we expect in science that entities have clear limits, in order to achieve replicable generalizations. But which of the 46 forms of anger described by the Tahitians correspond to the generalization reached by Finucane (2011)? Does the generalization not apply

to the Utku, who, according to anthropological evidence, do not express anger?

On the other hand, attributing causal effects (inhibit and enhance) to the concept of "anger" on a property of the action (selective attention) illustrates the nominalist fallacy. If the author does not specify anger as an entity or activity distinct from the action that serves to identify it, then anger could not be the cause of the action. Indeed, if we wish to replicate the effect on selective attention, then we would manipulate the film, not the emotional state. Thus, what is relevant for scientific explanation is to describe how some parameters of the film affect the performance in the task.

Polman and Kim (2013) provide another example. They asked their participants to describe something they had done the previous day, and then report whether their current emotions and feelings were angry, disgusted, sad, or neutral, on a scale of 1–5. Finally, they responded to a social dilemma in which they could keep chips, give them to a group pool, or take them from the pool. Depending on whether there was an excess or deficit of chips in the pool compared with the number taken, they received a bonus or not. The authors measured the effect of certain emotions on the amount of chips participants gave or took. They found that the participants who had reported sadness donated more resources to the group, but also took more from it than the participants who reported an emotionally neutral state. The authors concluded that: "This indicates that sadness has mixed effects on decisions to cooperate in a social dilemma. Sadness causes individuals to give more shared resources to others, but also take more shared resources for themselves" (pp. 1688–1689).

Unlike in the study by Finucane (2011), in Polman and Kim (2013), emotion was not induced by a manipulated external event. They began their analysis from the participant's report. However, the logic of the analysis was the same: sadness is a discrete phenomenon different from adding chips to, or removing chips from, the group pool. We do not have access to the sadness except through the participants' reports. Intuitively, we agree that what we call sadness is something, and therein lies the referentialist bias. We encounter the nominalist fallacy when authors explain giving or taking resources by naming and appealing to sadness. The only evidence we have that sadness is something is that we treat it as one in our linguistic practices. But practices are variable and circumstantial. For example, in some African languages, there is only one word that covers what in English is both "anger" and "sadness" (see Bamberg, 1997). In conclusion, affirming that sadness causes action is an expression that makes complete sense in ordinary language, and we can analyze it as such. But it would not be the kind of conclusion one hopes to get from a scientific study of emotions.

SOME ALTERNATIVE SOLUTIONS

The solution to this problem lies in studying emotions without treating ordinary language as if it were technical. Several authors have proposed different solutions, and I will review some of them below. The behavioral alternative will be analyzed in more detail because it is the direct antecedent of the non-mediational approach suggested later. The success of the proposed solutions depends on how much they avoid the two errors discussed in the previous section. The thesis is that none of the proposed solutions eliminates either error completely, and that we need a strict non-mediational approach instead.

Scarantino's Scientific Emotion Project

Scarantino (2012) distinguishes between the Folk Emotion Project and the Scientific Emotion Project. The former describes the membership conditions of ordinary emotion categories. The latter prescribes membership conditions of natural kinds of emotions. A natural kind is a category containing the maximum class of items that share properties for a scientific explanation by causal mechanisms. Most members of a natural kind are also part of the ordinary categories, so the relationship between the two is one of *subordination*. For example, the categories "anger_x" and "anger_y" are different natural kinds of the ordinary category "anger." So most of the members of the first category are also members of the second one. One issue is that most members of a natural category also belong to an ordinary category. But if the limits of the latter are fuzzy, culturally dependent, and do not refer to a given entity, then the limits of the former are also quite undefined. It goes against the aspiration that they serve as a strict scientific language.

Russell's Constructionist Perspective

Russell (2015) proposes a unit of analysis called "emotional episode," which is not qualitatively different from a non-emotional episode. Thus, it is not a technical term but a class of phenomena ordinarily defined. Psychology explains the emotional episode using the same technical language that would be used to study any other kind of episode. An emotional episode is a one-time event, constructed out of simpler components. A component is whatever an observer takes as a sign of emotion—for example, core affect, emotional meta-experience, affective quality, appraisal, or instrumental action. Therefore, the relationship between ordinary and scientific language is one of *application*. The former defines the phenomenon under investigation, and scientists apply the latter to understand it. At first glance, one problem is the *a priori* assumption that an "emotional episode" includes concepts like core affect and appraisal, which carry an important theoretical load. The referentialist bias lies in the supposed components of an episode ordinarily conceived.

Behavioral Approaches

Behavioral approaches consider emotions as properties of behavior, rather than as another kind of phenomena. The function of technical-scientific language is to describe behavior. Thus, behavioral scientists translate any ordinary reference to emotions into that behavioral, technical language. The relationship between both languages, then, is one of *translation*.

Duffy's (1941) classic ideas are compatible with this approach. She suggests that "emotion" is not a natural category. Instead, the term designates extreme values of the vigor of responses in situations interfering with the achievement of a goal. These ideas did not lead to an experimental program, so it is difficult

to check its heuristic scope. However, the Skinnerian behavioral tradition offers us enough material for analysis due to its vast theoretical and experimental production.

Skinner laid his groundwork for the relationship between ordinary and technical language in a seminal paper (1945), which he further developed in *Verbal Behavior* (1957), and revised in his last years (Skinner, 1984). His alternative was the functional analysis of ordinary psychological terms: dealing with ordinary terms as verbal responses, and finding out the conditions under which people utter them and the verbal community reinforces them. Therefore, through functional analysis, we translate ordinary terms into the scientific language of the occasion-response-consequence schema. This was his alternative to classic operationism, which "[has not] improved upon the mixture of logical and popular terms usually encountered in casual or even supposedly technical discussions of scientific method" (Skinner, 1945, p. 270).

In particular, Skinner (1953) proposed translating ordinary emotional terms as predispositions to act in certain ways. For example, saying that a man is "angry" can mean that he is more likely to hit or insult someone in certain conditions. Translation is not one-to-one: "Even an apparently well-marked emotion like anger may not be reducible to a single class of responses or attributable to a single set of operations" (Skinner, 1953, p.164).

The functional analysis of ordinary terms surpasses both the referentialist bias and the nominalist fallacy. Apropos of the first, Skinner (1953) affirmed: "The names of the so-called emotions serve to classify behavior with respect to various circumstances which affect its probability... by describing behavior as fearful, affectionate, timid, and so on, we are not led to look for *things* called emotions" (p. 162). As regards the second, the author stated:

> A man does not neglect his business because of anxiety or worry. Such a stamen is at best just a way of classifying a particular kind of neglect. The only valid cause is the external condition of which the behavior of neglect, as part of an emotional pattern known as anxiety or worry, can be shown to be a function (p. 168).

Thereby, functional analysis has hitherto been a reliable alternative to overcome these logical problems. However, we need another non-mediational approach to the study of emotions for two reasons: (1) operant functional analysis alone seems to be insufficient and (2) certain modalities of the referentialist bias persist in this approach, although not in a reificationist mode. Specifically, sometimes behavioral scientists have treated emotions as states that operate concurrently with behavior, or as affective experiences.

Emotions as States in Functional Relationships

At times, Skinner included emotions as part of a functional relationship: "We discover the variables of which emotional states are a function—as we discover any variables—by looking for them [...] Continued physical restraint or other interference with behavior may generate 'rage"' (Skinner, 1953, p. 164). Consequently, the emotional states are no longer ordinary translatable terms. Instead, Skinner uses "emotion" as a concept that is part of the scientific scheme of behavior. It is an event that is a function of something, or can be generated by something else.

Skinner (1974) also says that "[behavior] does not change because [the individual] feels anxious; it changes because of the aversive contingencies which generate the condition felt as anxiety. The change in feeling and the change in behavior have a common cause" (Skinner, 1974, pp. 61–62). It is clear that Skinner does not consider the feeling of anxiety to be the cause of the behavioral change. Instead, both are co-occurring products of the same contingency. However, it is also evident that both are two different things: one is a felt condition, and the other is overt behavior.

One more example of this usage is evident in the classic study by Estes and Skinner (1941). They evaluated the effect of anxiety on the steady response rate in a positive reinforcement schedule, and defined anxiety as an anticipatory emotional state. A Pavlovian tone-shock pairing procedure served to establish the conditioning of a state of anxiety. The effect found was a decrease in the response rate. The authors explained: "Anxiety is an emotional state arising in response to some current stimulus which has been followed by a disturbing stimulus. The magnitude of the state is measured by its effect upon the strength of hunger-motivated behavior" (p. 400).

Affective Experiences

Skinner (1953, 1957) addressed what in ordinary language we call affective experiences. He showed us how to translate them into the scientific schema of behavioral science. The distinction between public and private events served this purpose. The criteria of distinction between both were location and accessibility. If events occur within the skin, they are private and thus are difficult to access; if they occur externally, they are public and accessible. Of course, he took pains to make it clear that the same laws governed private and public events, and ended up yielding to the idea that something happens under our skin, and we have to account for how it comes under the control of contingencies of reinforcement (see a critical analysis in Hayes and Fryling, 2009).

Skinner (1957) treated both unobserved observables and concepts as private events. For example, he states:

> If we could say precisely what events within the organism control the response *I am depressed*, and especially if we could produce these events at will, we could achieve the degree of prediction and control characteristic of verbal responses to external stimuli (p. 131).

Skinner considered that an event within the organism controls the expression "I am depressed," as a stone controls the expression "I have a stone in my shoe." The person describes something happening under the skin, which we are accustomed to refer to as "depression." This creates a category mistake by confusing a concept with an unobserved observable.

Perhaps Skinner did not intend to do a philosophical treatment of privacy. Instead, he wanted to show that we can functionally analyze the way we learn to talk about private events. However, the logical consequences of his approach are

remarkable, even today. Privacy remains a matter of controversy among behavioral analysts (Schlinger, 2011). The way the study of emotions and feelings has evolved suggests that the approach has not changed substantially. For example, Baum (2011) analyzed the inaccuracy of introspection and stated: "People often express confusion or uncertainty about private events (Is that a pain or an itch? Am I embarrassed or angry?)" (p. 190). Thus, Baum exemplifies private events (events occurring within the skin, p. 186) with concepts like embarrassment and anger, tacitly suggesting that they are something we could refer to, and that the verbal community infers (p. 190).

Several contemporary behavior analysts ratify the referentialist bias when studying emotions and feelings. They place feelings within the field of experience, as behavior controlled by private stimuli. In doing so, they mix the categories of ordinary language categories with those of the operant conditioning. For example, Layng (2017) distinguishes between emotions as private experiences and overt behavior. Likewise, Moore (2000) explains the relationship between private experiences and behavior. According to him, some contingencies result both in a change to the probability of responses, and the feeling of bodily conditions (note here the connection to W. James' theory—James, 1884). What is "feeling" here, apart from behavior? Whatever it is, what the person feels acquires a discriminative function. The verbal community reinforces certain expressions when the person is feeling. That expression then acquires discriminative functions for further behavior, and so on. So, feelings (an ordinary category) become intertwined with behavior (a scientific category). Authors such as Friman et al. (1998) present an even more complex picture. They deal with private experience as a consequent event: "Consistent with our experiential avoidance approach to anxiety disorders, the disruptive washing has important functional properties: reducing, avoiding, or escaping private events (experience)" (p. 148).

Keller and Schoenfeld (1950) approached emotions as behavioral changes resulting from certain operations—for example, interrupting a behavioral chain. Authors such as Dougher and Hackbert (2000) and Lewon and Hayes (2014) have developed this idea further. They use the concept of establishing operations (Michael, 1993) and that of motivating operations (MOs) defined by Laraway et al. (2003). This approach coincides with a non-mediational one in its parametric emphasis. Even so, it still falls into a referentialist bias because it includes feelings and emotions as something operating in contingencies of reinforcement. For example, Lewon and Hayes (2014) consider that events that serve as MOs "are often correlated with the report of subjective feelings we learn to describe emotions or moods [...] the same events that are said to produce the subjective feelings of emotions or moods also serve as MOs...." (p. 817).

Finally, other behavioral traditions fall into a referentialist bias when they translate emotions as behaviors or properties of behaviors. That bias is not "inward," as a reference to an unobservable private event, but "outward," as a reference to a public event. As a consequence, they understand emotions as emotional behavior—which is different from a supposed non-emotional behavior—or as properties of responses specified episodically. This approach is especially noticeable in some classical authors, such as Watson (1914, p. 185), and Kantor (1933, p. 201).

A NON-MEDIATIONAL APPROACH TO EMOTIONS AND FEELINGS

The present analysis is akin to other non-mediational approaches, such as Watkins' (1996) for memory and Gibson's (1979) for perception. Watkins (1996) shows that scientists use "memory trace," which is a metaphor, as a hypothetical construct causing remembrances. This construct illustrates both the referentialist bias and the nominalist fallacy. The former by assuming that it is something simply because we refer to it; the latter by suggesting that we are explaining remembering when we name it in a causal relation. The result is an inexhaustible source of theories with no critical test to discriminate among them. Watkins' alternative is to discard concepts of this sort and instead proposes "... identifying, weighing, and deciphering the relation between the key variables operating at the time of an event's occurrence and at the time of its recollection" (p. 331).

For his part, Gibson (1979) remarks, critically, that the traditional psychology deals with the concept of "sensation" as a descriptor of something for which we must account. This information would be the data on which a process ending in perception operates. The result is the explosion of sophisticated theories to explain how we perceive from these supposed sensations. Thus, a concept that makes sense within social practices enters into causal relations as if it were an empirical entity. Gibson's alternative involves a richer conception of the environment and the perceptual systems, and the description of the direct relationship between both.

Mediationalism posits that perception results from operating on visual sensations, while remembering is the outcome of processing on memory traces. In contrast, non-mediationalism considers perception to be the direct relationship with places, objects, and events currently occurring, while remembering is the direct relationship with events which have already occurred. This alternative focuses on describing that relationship, and we must specify the relevant environmental parameters. The relationship involves not "stimuli" but, rather, more complex properties of environmental events such as persistence and change, gradients, and the proportion of classes of cues. Despite the epistemological closeness with behaviorism, Gibson and Watkins consider it too narrow to cover such matters sufficiently.

Regarding the relationship between ordinary and technical language, the approach proposed here holds that it is one of *abstraction*: technical language abstracts specific properties in ordinary language (cf. Ribes, 2018). I suggest that this way of conceiving that relationship would reduce the risk of falling into the problems mentioned. It will be further developed in the following sections.

The Grammar of Ordinary Expressions

Ordinary language is as good as it could be because it has evolved to communicate meaningfully, not to do science. "I feel angry with him," "I experienced a deep frustration with the project," and

"She has been feeling depressed" are expressions that make sense. Ryle (1949/2009) and Wittgenstein (1953) give us some tools for analyzing those expressions. The result is to recognize in them some properties relevant to scientific study.

Based on the ideas of Wittgenstein (1953) and Ter Hark (1990), it is possible to state that words are different concepts if their usage is also different (see also Slaney and Racine, 2011). A *language game* (Wittgenstein, 1953) or field of application defines a word's use. When we learn to talk about emotions and feelings, we learn concepts (not definitions), just as we learn concepts such as *poverty*, *justice*, *university*, *belief*, and so on. Learning to talk about emotions is no different from learning to talk about other concepts. Thus, "I am depressed," which Skinner (1957) used as an example of a reference to private events, seems to be more of an abstract tact than a reference to a private event. The reason is that it expresses subtle properties of a social practice and not an event under the skin.

The relationships between concepts, expressions, and actions in a language game are internal relations to such a game. Ter Hark (1990) defines internal relationships by three characteristics. First, it is impossible for the two relata not to have such a relation. The reason is that the criterion for the identity of one is the criterion for the identity of the other. When we talk about emotions, it is not possible to identify an emotion without referring to some degree of action. It is also not possible to identify an action without some degree of emotion (e.g., "kiss with passion," "write with frustration," and so on). Second, a third term does not mediate the relationship between the two relata. The relation is direct. That is, it does not need to include a term that makes an adverbial relation possible. By doing that, it externalizes the internal. This would create hypothetical constructs leading to a regression to infinity. Finally, the relation exists within a practice. A social practice establishes the relation between emotion and action. It is not something generic, because both are concepts coined in particular cultures in a certain way. So the internal relations of a language game constitute the grammar of ordinary expressions.

The description of the grammar of ordinary emotional expressions aims to understand, not to explain (von Wright, 1971). Thus, art, anthropology, philosophy, and linguistics can do it successfully. Statistical classification of ordinary emotional concepts (e.g., Cowen and Keltner, 2017) is also an example of this type of inquiry.

Natural Regularities

External relations have the three characteristics opposed to those of internal relations. That is: (1) the relata are independent, and their relation is contingent; (2) a third element could mediate the relation; and (3) the relation exists beyond a particular social practice. Natural sciences aim to find external relations, and causality is a type of them (von Wright, 1971). We commit both the referentialist bias and the nominalist fallacy when we include a concept within an external relationship.

Now I can revisit the studies that illustrate the problems analyzed above. In our terms, Finucane (2011) found that a person who is angry performs the attentional task in a certain way. Performing in that way is a property of anger, not an effect of it. Based on Finucane's study, we know that solving that task in that manner could be a criterion to interpret that a person is angry. However, we still do not know how or what is the cause. To know that, we need to look at something external to that relationship—in this case, the film. Proposing a hypothetical extra mechanism does not solve the problem but complicates it. Though this analysis could be extended to Polman and Kim's (2013) study, they do not offer enough information about possible external relations.

The Relationship Between Ordinary Language and Scientific Language

The relationship between ordinary and scientific language is one of *abstraction* (Ribes, 1991, 2018). We abstract some properties in the internal conceptual relations entailed in the expressions of ordinary language through the lens of the scientific language. Those properties will allow us to find orderly external causal relations. The result is that we achieve an *experimental model* of emotion, in whatever form ordinary language expresses it. Ordinary language provides the phenomena that attract the interest of psychology. Scientific language offers concepts to isolate relevant properties in order to find scientific regularities. Thus, the latter must include concepts referring to independent entities. Otherwise, they could not be part of external relations, and the logical problems already analyzed would persist.

The Basic Analytical Unit

The unit of analysis is an organism and an object or event with which it is in a relationship. Therein, I agree with the behavioral tradition. It is not necessary to the analysis that such a relationship is operant behavior, although the analysis could include it. Some authors, such as Roth and Gewirtz (1998) and Layng (2017), defend the need for research on emotion as operant behavior. Such research focuses on studying the roles of discriminative stimuli, responses, consequences, and establishing operations. The alternative considers that this analytical unit does not frame all the relevant problems. As Kantor (1970) points out:

> We may not regard conditioning or any other single kind as the necessary and sufficient way to deal with all behavior. To do so means uniformly to reduce all behavior to a single class adaptable to arbitrary chosen patterns of manipulation and specialized apparatus (p. 102).

For example, to develop an experimental model of Schadenfreude (joy in others' misfortune, Smith, 2013), response rate may be insufficient or even irrelevant. Skinner (1950) argued that it is the best alternative to study learning, but other phenomena may need other types of measures.

An organism relating with an object or event as the unit of analysis is common to the study of any psychological phenomenon. The particularity in the analysis of emotions is what we observe in such a relationship.

The Relevant Parameters to Study Emotions

The properties abstracted from the ordinary language are considered parameters of the individual and the events.

Parameters are qualitative and quantitative dimensions modulating the relationship between individuals and events.

The individual's parameters are any property of their reactivity. We can measure them as magnitude, duration, persistence, amplitude, variation, effectiveness, precision, latency, organization, and direction, among others (Ribes, 2018). A single parameter can hardly suffice for an experimental model of emotions. Authors like Sherman (as quoted in Keller and Schoenfeld, 1950 and Russell, 2015) report that we cannot identify a particular emotion based on one reactive pattern. Thus, it is helpful to return to Kuo's (1967) concept of *behavioral gradients*. This refers to the qualitative and quantitative variations of the different reactive systems involved in the behavior of an organism. In some cases, certain systems are dominant, and in others, other systems will be dominant. However, there is always activity in all the reactive systems (Kantor, 1933).

On the other hand, choosing the parameters of the relevant event is critical to developing an experimental model of emotions. This is the crux of the matter. For example, Keller and Schoenfeld (1950) think that removal of reinforcers is relevant to the study of anger. We could refer to them as MOs (Laraway et al., 2003) or as setting factors (Kantor, 1933), if this facilitates the analysis. However, rather than thinking about a list of operations, the critical issue is to abstract parameters related to affective expressions in ordinary language.

Roseman (2001) proposed parameters that he considered appraisal dimensions of emotions. Using them as such entails hypothetical causal processes and thus falls into the nominalist fallacy. Instead, we can consider those "dimensions" as situational parameters. For his part, Turkia (2009) offers other parameters in context of the computational modeling of emotions and feelings. Turkia's epistemological assumptions do not match those of the present proposal either. However, the author thought of those parameters as algorithms, which makes it easier to incorporate in the present analysis. Thus, I offer an adapted and completed list of event parameters that could be useful to the development of experimental models of emotions and feelings. Some of them were borrowed from Roseman (2001) and Turkia (2009):

(1) *The nature of the event:* It is that with which the individual interacts; it can be an event or an object.
(2) *Direction:* If it is an event, whether it is directed toward oneself or others.
(3) *Intensity:* Magnitude of occurrence.
(4) *Time:* If it is an event, the moment of its occurrence: whether it already happened, is currently happening, or will happen.
(5) *Probability:* The degree of certainty (certain or uncertain) that the individual has about the future occurrence of the event.
(6) *Predictability:* The extent to which the event belongs to a pattern or regularity. It can be high or low predictable.
(7) *Delay:* Time elapsed since occurrence and affectation.
(8) *Agency:* What or who produced the event. It can be produced by circumstances, other individuals, or oneself.
(9) *Potential control:* Whether or not the individual can alter the occurrence of the event and its properties. Potential control could be high or low.
(10) *Motivational condition:* Whether the individual would continue interacting with the event or object or not.
(11) *Motivational compatibility:* Whether an event or object is compatible with the motivational condition. It can be consistent, inconsistent or irrelevant. If it is inconsistent, then the problem type needs to be specified (see below).
(12) *Problem type:* Whether inconsistency arises because an event blocks the completion of an interaction, or by some other variable intrinsic to the event.

Some Examples

We could model some ordinary episodes expressed as hope, desire, or impatience. For instance, consider a situation in which an individual interacts with an event, which has the following properties (parameters):

(1) It is highly predictable.
(2) Its probability of occurrence is uncertain.
(3) It is consistent with a condition relevant to the individual at that moment.
(4) If established, it would lead to the individual remain in the situation.
(5) It is caused by the circumstance or by another individual.
(6) It is not under the control of the individual.

If we vary the probability of occurrence, ceteris paribus, we would map other ordinary situations usually expressed, such as joy or delight.

On the other hand, ordinary episodes that could be called "disappointment" could entail a situation in which an event has already occurred, and it was directed toward the individual, and was predictably consistent with a motivational condition at a lesser magnitude than predicted. A similar emotion in ordinary language, such as "bewilderment," may require changing the parameters for its experimental modeling. In this case, we would design a situation in which a highly predictable event stops occurring or occurs in a different way than usual for circumstantial reasons or because other individuals, it was directed at the individual, and the individual had little control of its occurrence, regardless of its motivational compatibility.

In Estes and Skinner's study (1941), instead of anxiety affecting the strength of behavior, we can design the situation parametrically: an event highly regular, directed to the rat, motivationally inconsistent, whose probability is uncertain, caused by the circumstance, and with a very low potential control. Possibly, that the decrease in the response rate depends on the degree of predictability of the event—which we could manipulate, the uncertainty of its occurrence, or the degree of potential control over its occurrence.

We can model expressions referred to emotions, passions, feelings, moods, and so on, combining some parameters. As

Ryle (1949/2009) noted, in ordinary language we refer to different types of things when talking about emotions—we mean states, dispositions, occurrences, or relationships. We can model them through infinite measures and manipulations. For example, when someone causes an event that blocks the achievement of a highly preferred condition, and we measure the magnitude, duration, direction, persistence, and organization of the activity, this could be a model of "feeling anger" as a sudden and momentary state. The temporal evolution of those measurements, while we hold the parameters constant, or increase them, could correlate with a range of denominations of anger. If we then arrange the situation so the individual continues the interrupted task and we continue measuring those behavior properties, a different pattern might emerge. It is no longer a temporary state but a disposition to do things in a certain manner. It would no longer be an emotion in ordinary language, but a "feeling of determination," for example.

Finally, the systematic analysis of the behavioral gradient development raises a number of productive questions: how are different components of the same reactive pattern differentially affected among themselves? How does the induction of certain responses (facial expressions, postures, gaze, and so on) mobilize a complete pattern in which they are already integrated (see Laird, 2007)? How can certain instrumental skills alter the parameters of the situation modulating certain properties of the behavioral gradient? The answers to all of these questions could also have enormous clinical potential.

CONCLUSION

It seems that a strict non-mediational analysis of emotions and feelings is possible. Its core is the search for functional relationships between parameters, i.e., conceptual properties abstracted from ordinary emotional expressions. Using this approach eliminates the most common problems in the experimental analysis of emotions. This analysis' aim is the explanation of natural phenomena, since it does not introduce ordinary concepts as part of functional relationships. However, this approach recognizes the usefulness of the interpretative analysis of such ordinary concepts for understanding how we talk about emotions and feelings, but not for explaining them. Finally, we could formulate law-like regularities, which are still absent in the literature on emotions and feelings.

AUTHOR CONTRIBUTIONS

RP-A conceived and developed entirely the ideas exposed in the document.

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Emotional Reactivity to Incentive Downshift in Adult Rats Exposed to Binge-Like Ethanol Exposure During Adolescence

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Alcohol use in adolescents is often characterized by binge-like ethanol consumption pattern, which is associated with long-term health consequences and even with important harms to his developing brain. Among this, ethanol exposure induces long-lasting alterations in anxiety-related neurobiological systems such as corticotropin releasing factor (CRF) or melanocortin system (MC). Recently, it has been demonstrated that adult rats exposed to adolescent intermittent ethanol (AIE) exposure exhibited anxiogenic-like behavior. Given that it has been demonstrated that negative affective state is relevant to development of addictive behavior, it is tempting to suggest that increased risk of adult abusive alcohol use exhibited in rats exposed to ethanol during adolescence may be related with differences in anxiety-related behavior. We conducted a study investigating the emotional reactivity after a reward devaluation (12-to-1 pellet or 32-to-4% sucrose downshift) in adult rats exposed to binge-like ethanol exposure during adolescence. For this aim, adolescent Sprague-Dawley rats were treated with ethanol (2.5 g/kg ip; AIE) or saline (AIS) for 2 consecutive days at 48-h intervals over a 14-day period (PND30-PND43). Following 25 free-ethanol days, adult rats were trained in consummatory and instrumental successive negative contrast task (cSNC and iSNC). Our data shows that both AIE and AIS groups exhibited suppression of the consummatory and instrumental behavior after reward devaluation relative to unshifthed control. Also, adult rats exposed to alcohol during adolescence exhibited a particularly strong negative affective state (lower sucrose consumption) with regards to the AIS group in the cSNC. This data demonstrated that adolescent binge-like ethanol exposure might trigger a greater emotional reactivity following incentive downshift, which might be linked to higher vulnerability to substance use disorder.

Keywords: binge-like ethanol exposure, adolescence, intermittent-access ethanol paradigm, emotional state, successive negative contrast

INTRODUCTION

According to National Institute on Alcohol Abuse Alcoholism [NIAAA] (2017), almost 90% of alcohol consumed by adolescents is in the form of binge-drinking, especially during leisure time and weekends; namely, drinking 4/5 standard alcohol drinks (women/men, respectively) in a 2 h timeframe, that usually results in high blood alcohol concentration (about 0.8 mg/dl). The prevalence of binge drinking among adolescents (15–19 years) in Europe and America is 24.1 and 18.5% respectively (World Health Organization [WHO], 2018). However, in some countries of South America such as Argentina, Chile or Peru this pattern of ethanol consumption is particularly prevalent with one out of every two students showing binge drinking in the last month (Organization of American States [OAS], 2013; Pillati et al., 2017). Even though alcohol use increases with age, and sometimes even the total amount of alcohol consumed per month is greater in adults than in adolescents, it has been estimated that adolescents consume larger amount of alcohol per occasion than adults (Carvajal and Lerma-Cabrera, 2015).

Underage binge-drinking is particularly dangerous because adolescence is an important period for brain development where ethanol exposure causes long-lasting neuroadaptative changes in neural pathways critically involved with neurobehavioral responses to ethanol (Pascual et al., 2007, 2009, 2012; Maldonado-Devincci et al., 2010; Lerma-Cabrera et al., 2013a). Several studies had associated early alcohol use with increased likelihood of abusive alcohol use and other anxiety-related disorders in adulthood (Pascual et al., 2009; Maldonado-Devincci et al., 2010; Moaddab et al., 2017). Importantly, adolescent intermittent ethanol exposure alters basal α-MSH, NPY and CRF activity in the amygdala and hypothalamic areas (Przybycien-Szymanska et al., 2011; Gilpin et al., 2012; Lerma-Cabrera et al., 2013a; Kokare et al., 2017). Given that these neurobiological systems are involved not only in the regulation of ethanol consumption but also in anxiety and emotional stress (Koob, 2013; Berkel and Pandey, 2017), several studies have suggested that dysregulation of emotional processing, similar to the observed in adolescent binge drinkers, may drive compulsivity in ethanol intake (Koob, 2013, 2015).

It has been demonstrated that binge alcohol-drinking elicits symptoms of negative affect such as ethanol withdrawal-induced anxiety in adult mice (Lee et al., 2015) or social anxiety (Varlinskaya et al., 2014). In many case, the emergence of a negative emotional state (e.g., dysphoria, anxiety and irritability) leads to seek the drug to remove it. It has been called the "dark side" of addiction and plays an important role in the maintenance of drug dependence (Koob, 2013, 2015). However, the adverse impact of binge-like ethanol exposure during adolescence on other aspects of negative emotional states like frustration has received relatively limited attention.

Successive negative contrast (SNC) is a reward downshift or incentive downshift procedures widely used to evaluate the emotional state in animal models (Flaherty, 1996). The discrepancy between the actual reward of low value and the expected reward of higher value causes a reduction in instrumental or consummatory behavior and elicits an aversive emotional, cognitive and behavioral state called frustration (Amsel, 1958, 1992). In this way, it has been found that exposure to reward devaluation activates the hypothalamic–pituitary–adrenal (HPA) axis and increases the release of stress hormones (i.e., ACTH and corticosterone) (Papini and Pellegrini, 2006; Pecoraro et al., 2009). Also, behavioral response induced by reward devaluation is reduced by anxiolytic drugs such as diazepam (Mustaca et al., 2000; Kamenetzky et al., 2008) or ethanol (Manzo et al., 2015).

Genetic and lesion studies also suggest the role of emotional behavior in reward devaluation situations. In this way, several studies have shown that rats who are selectively bred to exhibit differences in emotional reactivity, fearfulness or anxiety also differ in their response to reward loss. This is the case of Roman high-avoidance (RHA) vs. low-avoidance (RLA) rats (Rosas et al., 2007; Gómez et al., 2008; Cuenya et al., 2015), Lewis vs. Fischer rats (Brewer et al., 2016) or spontaneously hypertensive vs. Wistar-Kyoto rats (Bentosela and Mustaca, 2005) among others. Consistent with genetic studies, lesions in brain areas involved in emotional responses such as nucleus accumbens, the hippocampus, the medial prefrontal cortex or the amygdala disrupt the SNC effects (Becker et al., 1984; Flaherty et al., 1998; Leszczuk and Flaherty, 2000). Specifically, lesions of the hippocampus decrease the occurrence of instrumental SNC (iSNC), while local inactivation of the centromedial amygdala reduce (Bentosela et al., 2001; Kawasaki et al., 2015). The excitotoxic lesion of basolateral amygdala eliminate the consummatory SNC (cSNC) effect (Kawasaki et al., 2017). These studies suggest that the neural circuitry underlying both procedures (iSNC and cSNC) are different.

Recent studies suggests a bidirectional interaction between reward loss and addictive behavior (see review Ortega et al., 2017). In this way, it has been shown that the reward devaluation increases ethanol consumption and preference (Manzo et al., 2015). Besides, Matson and Grahame (2015) reported that High Alcohol Preferring (HAP) mice, which are selectively bred to prefer ethanol, exhibited larger cSNC effects compared to their corresponding replicate Low Alcohol Preferring (LAP) lines. Also, pharmacological studies have shown that drugs with addictive potential (e.g., opioids or dopaminergic drugs) have an impact on reward loss (Barr and Phillips, 2002; Daniel et al., 2009). Thus, it has been suggested that SNC could be a useful tool to better understanding the initiation and maintenance of alcohol use disorders (AUD).

Previous studies have reported that exposure to alcohol during adolescence resulted in alterations of neuropeptides involved in processing of negative emotional reactions (Koob, 2013; Berkel and Pandey, 2017). Given that it has been demonstrated that negative affective state is relevant to development of addictive behavior, it is tempting to suggest that an increased risk of adult abusive alcohol use exhibited in rats exposed to ethanol during adolescence (Pandey et al., 2015) may be related with differences on negative emotional states. To address this issue, the main objective of the present study was to evaluate whether binge-like ethanol exposure during adolescence alters

response of frustration in adulthood through a task of SNC. Additionally, we evaluated whether binge-like ethanol exposure during adolescence differentially affect the iSNC and cSNC in adulthood.

MATERIALS AND METHODS

Animals

Sixty-one male Sprague-Dawley rats on postnatal day (PND 30) (Pontificia Universidad Católica de Chile, Chile) were used as subjects in these experiments. The rats remained housed in groups of three/four rats per cage and maintained in an environmentally controlled room (22°C temperature in a 12:12 h light-dark cycle). Standard rodent chow and water were provided *ad libitum*. In adulthood (PND 60), the animals were housed individually and assigned randomly to reinforcement groups. Throughout the experiment, rats were deprived to 80–90% of their *ad lib* weight via daily feedings of lab chow, approximately 30 min after the end of the experimental session. Animals had free access to water throughout the experiment. Behavioral procedures were approved by the University of Autonoma of Chile Bioethical Animal Care and Use Committee.

Apparatus

iSNC Task

The apparatus was a black straight Plexiglas runway measuring 120 × 11 × 14 cm (L × W × H), and covered by clear Plexiglas lids. The runway was divided into three sections. The start and goal box were 20cm long and were separated from the running section by two guillotine doors. As reward a 45-mg pellets (formula P; Research Diets, Lancaster, NH, United States) [either 1 or 12 pellet(s), depending on the group] were placed on the floor at the distal end of the goal box. Response latencies (in seconds) were measured with the help of a chronometer (Extech, model 364410) as time from the moment the guillotine door was raised to the moment the rat had all four its legs inside the goal box.

cSNC Task

Consummatory training was conducted in Plexiglas boxes (measuring 30 × 15 × 30 cm) with a central orifice through which animals had access to a bottle containing a 32% or 4% (w/v) sucrose solution during 5 min. After this period, sucrose consumption (g) was assessed.

Procedure

Ethanol Exposure

Morning doses of either 25% (w/v) ethanol (2.5 g/kg) in isotonic saline (adolescent intermittent ethanol group, AIE), or saline (adolescent intermittent saline group, AIS) were intraperitoneally administered (i.p.) to 30-day old rats on two consecutive days with gaps of 2 days without injections, during 2 weeks. Specifically, rats were injected at PND 30, 31, 34, 35, 38, 39, 42, and 43 (Lerma-Cabrera et al., 2013a; Carvajal et al., 2017).

SNC Task

Experiment 1: incentive downshift through iSNC task

On PND 66, thirty-one rats (16 AIE and 16 AIS) were tested in the instrumental SNC following the prior described procedure (Flaherty et al., 1998; Rosas et al., 2007). Specifically, the experiment was conducted in three phases: habituation, preshift, and postshift phases.

Habituation. On the first day, rats were allowed to freely explore the entire runway for five trials of 2 min each. On the second day, rats were given two 2-min trials of free-access followed by three feeding in the goal box (i.e., the animal was confined to the goal box and given the appropriate number of pellets either 12 or 1 depending on the group). The last habituation session consisted of three goal-box feeding trials in which subjects have a maximum of 30 s to consume the pellets. After that, they were removed from the goal box and taken back to their home cage.

Preshift training phase. After this habituation, each animal was placed in the start box with the door closed and the goal door opened. Each session began with the opening of the start door and finished when the rat reach the goal box to obtain the food reward [1 or 12 pellet(s), depending on the group]. A maximum time of 20 s was allowed for the rat to complete the trial. If the rat did not reach the goal box before 20 s had elapsed, it was gently pushed into the goal box by the experimenter and 20 s was recorded as response latency. When the rat reached the goal box, the goal door was quietly closed and a stopwatch was stooped. The rat was given a maximum of 30 s to consume the food reward. As soon as the rat had finished eating or 30 s had elapsed, it was removed from the goal box and placed back in its home cage between trials.

Postshift phase. On day 10, the last trial of training, the rats receiving 12 pellets were shifted to 1 pellet while the rats receiving 1 pellet remained unchanged. Thus, from the 10th day of training onward, all rats received 1 pellet upon arrival to the goal-box. The postshift phase continued for 2 days.

Experiment 2: incentive downshift through cSNC task

Consummatory SNC task were based on previous procedures (Cuenya et al., 2015; Manzo et al., 2015). On PND 66, rats ($n = 15$ for AIE group and $n = 14$ for the AIS group) were familiarize with the consummatory box and allowed to freely explore the box for 5 min. On Days 2 and 3 of the habituation period, the animals also have access to a bottle containing water.

Preshift phase. On Days 4–13, half of the animals in each group (AIE or AIS) received free access to 32% sucrose solution and the remaining half of animals received 4% sucrose solution in the consummatory box. Immediately after the first contact with the sipper tube, sucrose intake (ml) was recorded for 5 min in each session.

Postshift phase. During the next two sessions (days 14–15), all animals were exposed to 4% sucrose for 5 min.

Data Analysis

Kolmogorov–Smirnov–Lilliefors (K–S–L) test indicated that, across datasets, the assumptions of homogeneity and normality

were maintained. The sphericity assumption was tested with W of Mauchly's statistic. In case of not fulfilling the assumption, it was used as a corrector index Huynh-Feldt (H-F).

Repeated measures of ANOVA using Days as the repeated measure factor and Treatment (AIE vs. AIS) and Reinforcement (12-1 and 1-1 pellets or 32-4 and 4-4 percentage of sucrose) as between-subjects measures was used to analyze preshift data both in iSNC and cSNC. A similar repeated measures of ANOVA was used to analyze the effect of reward downshift, including the last preshift and postshift session. When significant differences were found ($p \leq 0.05$), pair wise comparisons were conducted with *post hoc* Newman–Keuls test. Partial eta-squared (η_p^2) was used as the measure of effect size for all analyses of variance (ANOVAs), with small ($\eta_p^2 = 0.009$), medium ($\eta_p^2 = 0.09$), and large ($\eta_p^2 = 0.25$) effect sizes (Levine and Hullett, 2002). All data in this report are presented as means ± SEM.

RESULTS

Experiment 1: Incentive Downshift Through iSNC Task

Figure 1A shows the mean latency (sec) to reach the goal-box during the preshift phase as function of receiving 12 or 1 pellet(s) for AIE and AIS group. Mauchly test indicated that the assumption of sphericity had been violated [$\chi 2(44) = 199.233$, $P < 0.0001$]; therefore, degrees of freedom were corrected using Huynh-Feldt estimate of sphericity. A Treatment × Reinforcement × Days analyses conducted for the first ten sessions (preshift phase) showed that rats reinforced with 12 pellets exhibited lower latency than those exposed to only 1 pellet, as shown by significant effects for Reinforcement [$F(1,28) = 24.99$, $p < 0.0005$; $\eta_p^2 = 0.47$] and for the Reinforcement × Days interaction [$F(3.968,111.11) = 4.54$, $p < 0.00001$; $\eta_p^2 = 0.14$]. There was also a Days effect [$F(3.968,111.11) = 78.98$, $p < 0.00001$; $\eta_p^2 = 0.73$] and Treatment × Reinforcement × Days interaction [$F(3.968,111.11) = 5.10$, $p < 0.00001$; $\eta_p^2 = 0.15$]. *Post hoc* analysis aimed to further analyze the triple interaction indicated greater latency in the AIS 1-1 group than the rest of the groups during day 1 ($p < 0.05$).

When we compared the latency to reach the final box during days 10 and 11 (when the downshift occurred), there was a significant effect for the Reinforcement × Days interaction [$F(1,28) = 24.007$, $p < 0.00005$; $\eta_p^2 = 0.461$]. *Post hoc* test revealed that rats that had been trained to run for 12 pellets showed higher latency in the post shift phase (day 11) than the rats that had been trained to run for 1 pellet from the outset ($p = 0.03$). Given that no other main effect or interaction was significant, this data suggests that binge-like ethanol exposure in adolescence did not alter differently the emotional response induced by the downshift in reward in iSNC task during adulthood. **Figure 1B** shows the average latency of each group in the last day of preshift (day 10) and postshift phases of iSNC.

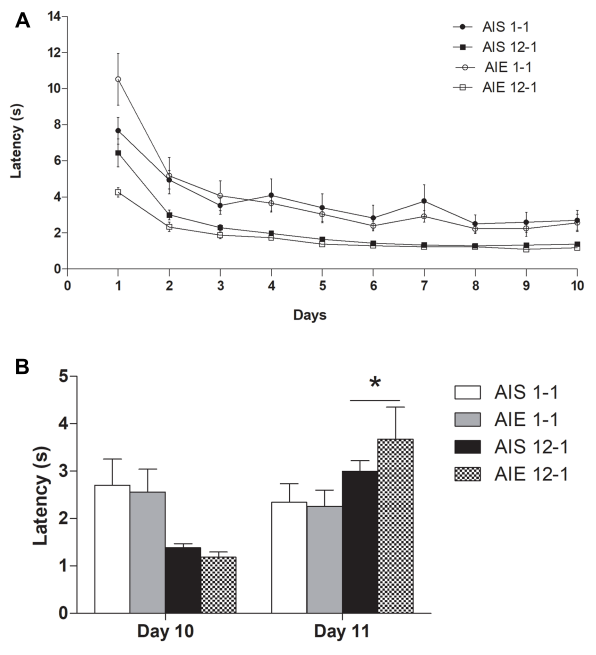

FIGURE 1 | (A) Mean latency (s) to reach the goal-box on each of the training sessions in the straight alley in preshift phase and **(B)** in postshift phase in groups AIE12-1, AIE1-1, AIS12-1, and AIS1-1. All values are means ± SEM. *$p < 0.05$.

Experiment 2: Incentive Downshift Through cSNC Task

Figure 2A shows the mean sucrose intake (g) in the four groups (AIE 32-4%, AIE 4-4%, AIS 32-4%, and AIS 4-4%) during the preshift phase. A 2 (Treatment) × 2 (Reinforcement) × 10 (Days) ANOVA conducted in the preshift phase revealed a significant effect of Treatment [$F(1,25) = 14.727$, $p \leq 0.001$; $\eta_p^2 = 0.37$], Reinforcement [$F(1,25) = 92.198$, $p \leq 0.00001$; $\eta_p^2 = 0.79$] and Days [$F(3.968,202.128) = 31.567$, $p \leq 0.00001$; $\eta_p^2 = 0.56$]. Also, Treatment × Days [$F(3.968,202.128) = 2.2266$, $p \leq 0.02$; $\eta_p^2 = 0.08$], and Reinforcement × Days interaction [$F(3.968,202.128) = 2.1288$, $p \leq 0.02$; $\eta_p^2 = 0.07$] were statistically significant. However, the Treatment × Reinforcement and the triple interaction were not. The *post hoc* analysis of the Treatment × Days interaction revealed that animals pre-exposed to binge-like ethanol exposure during adolescence exhibited lower sucrose intake than AIS group from day 4 to day 8 of the preshift phase. In addition, the effect of the sessions was significant in both AIE [$F(9,126) = 21.876$, $p \leq 0.001$; $\eta_p^2 = 0.61$] and AIS groups [$F(9,117) = 13.373$, $p \leq 0.01$; $\eta_p^2 = 0.51$] showing an improvement in performance of the task across session. Additional *post hoc* tests aimed to further analyze Reinforcement × Days interaction revealed that animals receiving the 32% sucrose solution performed better than animals exposed to 4% sucrose throughout the training.

Data showing sucrose consumption (g) during the last preshift session and the postshift session (day 11) are represented in **Figure 2B**. The ANOVA performed on reward devaluation found a significant main effect of

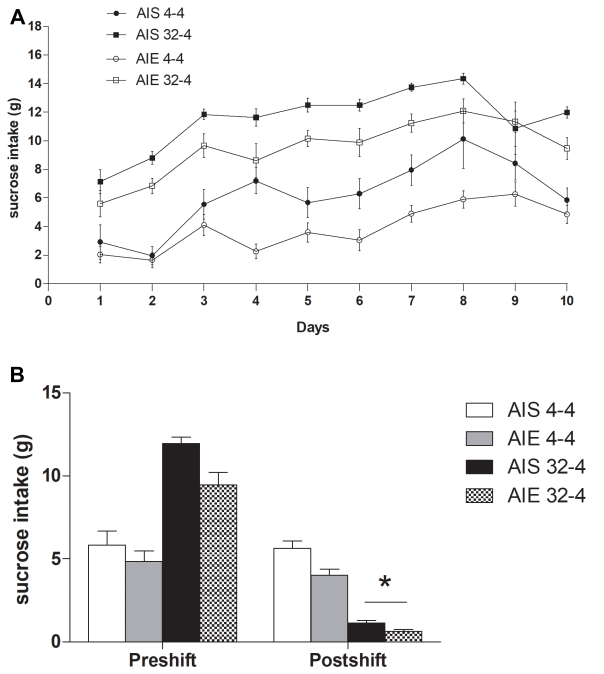

FIGURE 2 | (A) Mean values of sucrose consumption (g) on each of the training sessions in preshift phase and **(B)** in postshift phase in both groups AIE32-4, AIE4-4, AIS32-4, and AIS4-4. All values are means ± SEM. *$p < 0.0001$.

Treatment [$F(1,25) = 10.226$, $p \leq 0.004$; $\eta_p^2 = 0.29$] and Days [$F(1,25) = 306.09$, $p \leq 0.00001$; $\eta_p^2 = 0.924$]. The interaction Reinforcement × Days [$F(1,25) = 247.54$, $p \leq 0.00001$; $\eta_p^2 = 0.908$] and Treatment × Reinforcement × Days [$F(1,25) = 4.8447$, $p \leq 0.03$; $\eta_p^2 = 0.162$] were statistically significant. As was observed in training, AIE group drink less sucrose than AIS group also in the postshift phase ($p = 0.004$). Finally, on day 11 downshifted groups (AIE 32-4 and AIS 32-4) performed significantly below their respective unshifted control (AIE 4-4 and AIS 4-4) ($p = 0.0001$ in both AIE and AIS group).

Taking together, this data suggests that although binge-like ethanol exposure during adolescence did not alter the response of incentive downshift through cSNC task during adulthood, it reduced sucrose intake in both AIE 32-4 and AIE 4-4%.

DISCUSSION

Binge drinking, which is the most common pattern of ethanol consumption during adolescence, is a significant risk factor for the development of alcoholism. It is known that aversive emotional states may drive escalated ethanol intake in rodents, suggesting that increased likelihood of abusive alcohol use in rats exposed to ethanol during adolescence may be related with differences in anxiety behavior. This study assessed for the first time the effect of adolescence binge-like ethanol exposure on the response of frustration following a situation of reward devaluation in adulthood. To this end, AIE and AIS groups received training in the instrumental (iSNC) and consummatory successive negative contrast (cSNC) procedure. The results indicated that both AIE and AIS rats showed similar emotional reactivity to incentive downshift in instrumental and consummatory task. Thus, our results replicate previous studies on successive negative contrast following reward downshift and provide more evidence about the fact that negative successive contrast can be produced using reinforcers from different modalities (i.e., liquid vs. solid food). Also, AIE exhibited a generalized decrease of sucrose consumption in cSNC when compared with AIS group, suggesting that exposure to alcohol in adolescence might increase anhedonic behavior or palatability changes in adulthood.

In experiment 1, the results obtained during the preshift phase showed that all groups learned the task during the days shorter latencies were exhibited. Consistent with previous studies (Leszczuk and Flaherty, 2000; Rosas et al., 2007; Binkley et al., 2014), rats receiving 12 pellets showed lower latency than rats receiving 1 pellet. Binge-like ethanol exposure during adolescence had not an effect on latency during the preshift phase, with the exception of day 1 in which AIE/1-1 exhibited the major latency to reach the goal-box, in comparison with the rest of the groups. Several studies had shown that ethanol exposure during adolescence reduce spontaneous locomotor activity (Pascual et al., 2009; Teixeira et al., 2014; Fernandez and Savage, 2017). Thus, the increase in latency showed by group AIE/1-1 could be due to a low exploratory behavior in a novel context. However, as on this first day AIE/12-1 group exhibited lower latency, rather than higher, this explanation is unlikely. Conversely, it is tempting to postulate that in day 1, different reinforcing values of 1 pellet vs. 12 pellets in AIE group may have been a factor in the time spent to reach the goal-box on day 1. However, those differences are only observed on the first day and, given that exclusive measures of latency were taken, it is difficult to defend this idea. Additional measures as operant behavioral procedure among others are needed to specifically evaluate this hypothesis.

Some interesting data obtained on cSNC task also point to possible basal differences in the AIE group in the response to the reinforcing properties of reward. Although both groups (AIE and AIS) showed higher sucrose intake of the greater concentration of solution, animals exposed to binge-like ethanol during adolescence drinks significantly less sucrose compared to AIS group. This effect was independent of the magnitude of the presented reinforcer. Interestingly, these differences had been observed not only during the preshift phase but also during postshift phase. Generally, reduced sucrose intake is considered an index of anhedonia in animal models (Katz, 1982). Recently, it has been proposed that anhedonic behavior might arise from dysfunctional interaction between the stress and reward system (Pizzagalli, 2014; Bolton et al., 2018).

In this sense, exposure to alcohol during adolescence increases adult CRF mRNA expression in the hypothalamic paraventricular

nucleus, central nucleus of the amygdala and the prefrontal cortex (Przybycien-Szymanska et al., 2011; Boutros et al., 2018, 2016). Such alterations are important due to the implication of CRF in anxiety (Binder and Nemeroff, 2010) and stress response (Deussing and Chen, 2018). Adolescent alcohol exposure also alters other anxiety-related neuropeptides such as α-MSH or NPY (Lerma-Cabrera et al., 2013a; Kokare et al., 2017). An increase in anxiety levels might interfere with the ability to experience pleasure during normally rewarding activities, such as the consumption of sucrose (Bolton et al., 2018). In relation with this idea, for example, it has been demonstrated that inbred RLA rats, which were selectively bred to display enhanced anxiety/fearfulness, exhibits elevated CRF gene expression in the extended amygdala in comparison with RHA rats (Carrasco et al., 2008). Besides, RLA 32-4% rats consume less sucrose than RHA 32-4% rats in the preshift phase of cSNC task (Gómez et al., 2008). Thus, our data of the reduction of sucrose intake in AIE groups could reflect an anhedonic response mediated by CRF or even by α-MSH given that stress-elicited anhedonia requires α-MSH/MC4R signaling (Lim et al., 2012). In addition, given that α-MSH has the ability to modulate non-homeostatic aspect of a reward (Lerma-Cabrera et al., 2013b), it should be considered that reduction of sucrose intake might be determined by changes in taste palatability or hedonic value of sucrose. Future studies analyzing the temporal microstructure of the consumption response (Dwyer, 2012) or using the taste reactivity test (Cuenya et al., 2018) are needed to evaluate the hedonic value of sucrose in AIE and AIS group. Otherwise, additional pharmacological or genetic studies directed to manipulate CRF or MC system will provide information for a deeper understanding of the involvement of these systems on anhedonia.

In the last decade, some studies had shown that neural circuit engaged in consummatory and instrumental SCN are, almost partially, different. Lesion studies suggests hippocampus and nucleus accumbens affect behavior in the iSNC situation (Leszczuk and Flaherty, 2000), whereas cSNC was affected by lesion of the amygdala and cortical areas (Bentosela et al., 2001; Ortega et al., 2013; Kawasaki et al., 2017). This neuroanatomical dissociation between iSNC and cSNC suggests that these tasks could involve relatively different emotional and cognitive mechanisms (Papini and Pellegrini, 2006). The amygdala has an important role not only in anxiety responses but also in reward learning and addiction (Janak and Tye, 2015; Kawasaki et al., 2017); for that, and as showed in our study, cSNC seems to be more sensitive than iSNC to evaluate changes in incentive salience after adolescent intermittent ethanol exposure.

The present study had shown a successive negative contrast effect in two testing situations (consummatory behavior and instrumental learning) in both AIE and AIS groups. In the potshift phase of iSNC, rats expecting a high-value reward (12 pellets) exhibited greater latency when given a low-value reward (1 pellet) relative to unshifted rats always receiving 1 pellet. It has been demonstrated that iSNC involving the devaluation from 12 to 1 pellet induce a negative emotional state due to incentive loss (Manzo et al., 2012; Papini et al., 2015). Secondly, and in accordance with other studies (Gómez et al., 2008; Manzo et al., 2015) a response of reduced intake of sucrose was observed when the concentration was severely reduced (from 32 to 4%) in the postshift phase of the cSNC. These results showed that the effect of deterioration in the performance of the shifted group was not related with previous history of alcohol intermittent exposure during adolescence. Several studies had shown that adolescent binge-drinking has long-lasting effects on anxiety-like behavior (Varlinskaya et al., 2014; Lee et al., 2015), however, in our study, at least under our experimental conditions, AIE and AIS animals did not exhibit differences in their responses when the amount of reinforcement was unexpectedly reduced in a consummatory or instrumental appetitive learning task. Other studies have also found controversial results: decreased anxiety or increased impulsivity (Gilpin et al., 2012) or even, no signs of anxiety (Marco et al., 2017) after adolescent intermittent alcohol exposure. The contrasting results in these studies may be related to the use of different protocol of ethanol exposure during adolescence, as well as the paradigm used to evaluate anxiety-related responses. Further studies are needed to clarify the effect of binge-like ethanol exposure on anxiety in adulthood. One possible explanation of our data is that a negative contrast effect is too powerful to be sensitive to distinguish between the performance of AIE and AIS group. Maybe, the reward downshift was aversive enough to cover an effect of adolescent intermittent ethanol exposure on the magnitude of SNC. Therefore, a ceiling effect cannot be ruled out. Bearing this in mind, reducing the reward disparity between the pre- and postshift phase (i.e., 12-2 in the iSNC and 22-4% in the cSNC) could increase the sensitivity of the tasks as suggested in Rosas et al. (2007) and Gómez et al. (2008). Also, identifying profiles of recovery from successive negative contrasts could help us test in major detail the effect of binge-like ethanol exposure during adolescence on negative emotional state has. It has been demonstrated that the higher level of suppression after the devaluation shown by animals, the lower level of recovery (Bentosela and Mustaca, 2005; Rosas et al., 2007; Gómez et al., 2008; Brewer et al., 2016).

In summary, the present study demonstrates that negative contrast in consummatory and instrumental behavior occurred after reward devaluation, regardless of binge-like ethanol exposure during adolescence. However, as showed in cSNC, animals exposed to binge-like ethanol during adolescence exhibited reduced sucrose intake. Besides, cSNC may be more sensitive to evaluate emotional changes induced by adolescent intermittent ethanol exposure than iSNC. The primary limitation to the generalization of these results could be the small group size (n = 8-7). But also, it is possible that small group size prevented us from findings more significant differences. Still, the small population did not negate recognition of the importance of binge drinking during adolescence on response of frustration in adulthood. Many similar studies evaluating emotional reactivity to incentive downshift in the instrumental and consummatory devaluation have reported a similar group size (Rosas et al., 2007; Gómez et al., 2008) or even a smaller number of animals (Cuenya et al., 2015). Future studies characterizing the motivational aspects and emotional state associated with reward loss could contribute to further understanding in which

conditions intermittent ethanol exposure during adolescence leads to substance use disorder in adulthood.

AUTHOR CONTRIBUTIONS

JL-C and FC were responsible for the study concept and design. CA-R, GC-T, and AA-C conducted the experiments. FC, JL-C, CA-R, and GC-T analyzed the data. All the authors critically reviewed content and approved final version for publication.

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BRIEF RESEARCH REPORT
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Good Relationships, Good Performance: The Mediating Role of Psychological Capital – A Three-Wave Study Among Students

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Academic Psychological Capital, or PsyCap, – a set of positive psychological resources encompassing hope, efficacy, resilience, and optimism – has begun to gain attention in academia, showing positive relationships with desirable academic outcomes. However, studies in the field have paid limited attention to the social factors that may increase PsyCap and therefore may lead to positive outcomes. In the present study, we examine whether academic PsyCap mediates between teacher-student relationships and academic performance as assessed by student's GPA, using a three-wave longitudinal design. Through structural equation modeling, as expected, a statistically significant indirect effect was found between teacher-student relationships and academic performance *via* academic PsyCap. Theoretical and practical implications of the results are discussed; strengths and weaknesses are mentioned; and future research directions are proposed.

Keywords: teacher-student relationships, academic psychological capital, PsyCap, academic performance, high school students

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INTRODUCTION

Despite previous studies showing positive relationships between academic PsyCap and desirable academic outcomes (e.g., academic adjustment – Liran and Miller, 2017; study engagement – Siu et al., 2014; satisfaction with life – Riolli et al., 2012; and academic performance – Luthans et al., 2012), there are no previous studies of the social factors that might play a role in sparking this positive relationship. This is an important limitation because – based on Goldstein (1999) – academic settings are considered a relational zone in which the quality of the interactions promotes students' motivation, wellbeing, and performance (Furrer and Skinner, 2003). Thus, providing empirical evidence about the interaction between teacher-student relationships (TSR), academic PsyCap, and academic performance (AP) could be an important step in understanding the role of social factors in building students' PsyCap and in developing future evidence-based interventions programs to foster students' PsyCap, wellbeing, and performance.

Self-determination theory (SDT) recognizes the importance of feeling connected to others as a basic psychological need (the so-called need for relatedness) and is considered a fundamental ingredient for functioning at optimal levels (Ryan and Deci, 2000, 2017). Hence, a student who perceives an emotional connection with his/her social environment, believes that s/he is

cared for and loved, and feels special to his/her key social partners (e.g., teacher-student relationships) has satisfied his/her need for relatedness. Along this line, previous research has demonstrated the relevant role that interpersonal relationships play in students' success in terms of engagement, achievement, and wellbeing (Furrer and Skinner, 2003; Cornelius-White, 2007; Roorda et al., 2011; King, 2015; Datu, 2017). The explanation is that high-quality relationships with significant others provide students with the necessary emotional security to actively explore and effectively deal with their (academic) world (Martin and Dowson, 2009).

The conservation of resources (COR) theory recognizes the importance of accumulating resources in the biological, cognitive, and social domains as a strategy to preserve and foster their health and wellbeing, the so-called resource caravan (Hobfoll, 1989, 2002, 2011; Hobfoll et al., 2018). Thus, a student who accumulates personal resources (e.g., hope, efficacy, resilience, and optimism) is more likely to possess the specific skills and attitudes necessary to meet academic requirements and, therefore, achieve academic success. In line with this reasoning, previous research has identified academic PsyCap as a predictor of academic performance (Luthans et al., 2012; Datu et al., 2016; Ortega-Maldonado and Salanova, 2017; Carmona-Halty et al., 2018). The explanation is that academic PsyCap facilitates the processes necessary for students' attention, interpretation, and retention of positive and constructive memories that are conducive to wellbeing and good performance (Luthans and Youssef-Morgan, 2017).

Overall, the proposed mediational model assumes that students with high-quality TSR will be in a better position to persevere in their objectives (i.e., have hope), rely on their own abilities (i.e., be efficacious), overcome obstacles (i.e., be resilient), and be optimistic about their future (i.e., feel optimism); in turn, these set of four resources would foster AP. In other words, when students satisfy their need for relatedness, they are more likely to accumulate personal resources in the form of PsyCap that can help them to achieve good academic performance. Hence, we tested a structural equation model that assumes that Academic PsyCap mediates between TSR and AP.

MATERIALS AND METHODS

Participants

The sample consisted of 771 high school students attending three Chilean educational institutions. Participants ranged in age from 12 to 18 years (M = 14.25, SD = 1.60), and 51% of the sample was female. Of the 771 students, 18% were 12 years old, 18% were 13 years old, 19% were 14 years old, 21% were 15 years old, 14% were 16 years old, 7% were 17 years old, and 2% were 18 years old at the time of data collection.

Procedure

This study was part of a project designed to examine the role of non-intellectual variables in academic performance. The school principals, students, and students' parents granted a written informed consent. The adolescents voluntarily completed a questionnaire twice: once at the end of the regular academic semester (Time 1: TSR) and once 9 weeks later (Time 2: academic PsyCap). In addition, AP was assessed at the end of the next academic semester, 9 weeks later (Time 3: AP). The online data collection was carried out in a group sessions of about 25 students, and we used the Spanish version of the scales because it is the official language. This study was carried out in accordance with the recommendations of Comité Ético y Científico (CEC-UTA) with written informed consent from all subjects in accordance with the Declaration of Helsinki.

Instruments

At time 1, TSR was measured using the Teacher-Student Relationships Scale (Martin et al., 2007). This scale has four items (e.g., "My teachers give me the help and support I need") rated on a scale from 1 (*strongly disagree*) to 7 (*strongly agree*).

At time 2, Academic PsyCap was measured using an adaptation of the Psychological Capital Questionnaire (Avey et al., 2011) to the academic context. This questionnaire has 12 items (e.g., "Right now I see myself as being pretty successful in my studies") rated on a scale from 1 (*strongly disagree*) to 6 (*strongly agree*).

At time 3, AP was assessed using the grade point average (GPA) provided by the educational institutions. The GPA was based on three mandatory subjects in the Chilean education curriculum: math, language, and history. According to the Chilean grading system, GPAs range from 1 (*poor*) to 7 (*excellent*). The three subjects are offered in both semesters (March-June and July-November), with a total of 6 h per week. For the objective of this study, the GPA was included of the end of the semester before the data collection.

Data Analysis

All data analyses were conducted using JASP 0.9.01 and SPSS AMOS 23. We used maximum likelihood estimation methods, and goodness-of-fit was evaluated using absolute and relative indexes: χ^2 and normed χ^2, incremental fit index (IFI), comparative fit index (CFI), root-mean-squared error of approximation (RMSEA) with a confidence interval (90%), and standardized root mean residual (SRMR). To determine the fit of the model, we followed the European Journal of Psychological Assessment (Schweizer, 2010) and previous recommendations (Schreiber et al., 2006). Finally, we tested the statistical significance of the indirect effects by computing the bias-corrected and accelerated method (BCa) around the indirect effect, as obtained from bootstrapping analysis (Williams and MacKinnon, 2008).

RESULTS

Table 1 shows means, standard deviations, Cronbach's α and McDonald's Ω indexes, and Pearson's correlations among the variables. The internal consistencies obtained for the scales

TABLE 1 | Means (*M*), standard deviation (*SD*), α and Ω indexes, and correlations for the study variables (n = 771).

	M	*SD*	α	Ω	1	2	3
1. Teacher-student relationship (T1)	5.34	1.39	0.893	0.895	–		
2. Academic PsyCap (T2)	4.06	1.00	0.915	0.916	0.382**		
3. Academic performance (T3)	5.31	0.76	0.777	0.780	0.162**	0.281**	–

**$p < 0.001$.

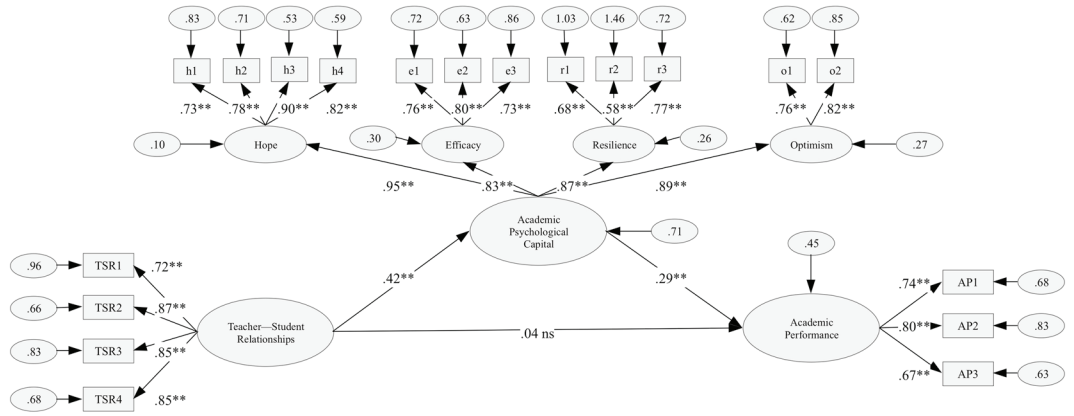

FIGURE 1 | Single mediation model shows the effect of teacher-student relationships on academic performance through academic psychological capital. Standardized coefficients are presented. ** = $p < 0.001$; ns = non-significant effects.

used were good, and the pattern of correlations revealed significant direct relationships for all the measures in our sample.

The hypothesized model consisted of 7 latent factors and 19 indicators. That is, the latent TSR one factor reflects TSR with four indicators; academic PsyCap is composed of one-high order factor and four lower-order factors, which, in turn, are formed by 12 indicators, and three indicators make up the latent AP factor. The results showed that this model exceeded the recommended standards and was a good representation of the sample relations, explaining 17.5% of the academic PsyCap variance and 9.8% of the AP variance: $\chi^2 = 694.289$; $\chi^2_{/df\,(145)} = 4.788$; *IFI* = 0.929; *CFI* = 0.929; *RMSEA* = 0.070, 90% *CI* (0.065, 0.075); *SRMR* = 0.053. In addition, as **Figure 1** shows, the factor loadings were uniformly moderate to high and statistically significant, and exceeded the factor-loading criterion of 0.35 by far (Byrne, 2010).

For the mediation analysis, we implemented a bootstrapping procedure with 5,000 new samples taken from our sample, and indirect effects were calculated (Hayes, 2009). The results led us to conclude that: (1) TSR is significantly related to academic PsyCap [a = 0.418, SE = 0.039, BCa 95% *CI* (0.339, 0.492), $p < 0.001$]; (2) academic PsyCap is significantly related to AP after controlling for TSR [b = 0.295, SE = 0.063, BCa 95% *CI* (0.158, 0.403), $p < .001$]; and (3) the indirect effect between TSR and AP – *via* academic PsyCap – is statistically significant [ab = 0.123, SE = 0.031, BCa 95% *CI* (0.066, 0.186), $p < 0.001$]. In addition, TSR is not significantly related to AP [c = 0.039, SE = 0.066, BCa 95% *CI* (− 0.082, 0.177), $p > 0.050$]. Hence, we can conclude that academic PsyCap fully mediates the relationship between TSR and AP.

DISCUSSION

The present study makes an innovative contribution to the scarce research on the antecedents of academic PsyCap (i.e., a set of resources composed of hope, efficacy, resilience, and optimism) and the interactions among TSR, academic PsyCap, and AP.

Consistent with the previous research on SDT – particularly studies on the need for relatedness – we found that students who perceive high-quality relationships with their teachers are more likely to report higher levels of academic PsyCap. In a similar vein, our findings confirmed – in agreement with COR theory – that students who have high levels of academic PsyCap are more likely to achieve better academic performance. Taken together, integrating both SDT and COR theories, we hypothesized and confirmed that academic PsyCap is a mediator between TSR and AP. In other words, we found that the path from good relationships to good performance is fully mediated by academic PsyCap.

The practical implications of the current study are first, rather than focusing exclusively on increasing academic knowledge and skills, teachers should also focus on the affective elements of high-quality relationships with their students; that

is, getting along with them, caring about them and showing interest, and providing help and support, among others. According to our results, this focus will help – through increased academic PsyCap – to achieve better academic performance. Second, PsyCap interventions (PCIs) have been carried out by increasing its four components using a within-individual approach (see Luthans et al., 2008; Luthans et al., 2010). However, our results suggest that it might be necessary to include the teachers in an academic PCI as well in order to develop his/her social skills and stimulate developing a high-quality relationship with students. Thus, a between-individual approach could be an important step in future of PCI programs.

The strengths of the current study are first, we used a longitudinal approach, which is scarce in academic PsyCap research; second, we included an objective measure of performance (i.e., GPA); and third, we successfully integrated STD and COR theories in an academic setting. However, there are also some weaknesses that have to be acknowledged. First, to assess TSR and academic PsyCap, we used students' self-reports. In future research, we could include teachers' reports about the students' TSR and their perceptions of their PsyCap. Second, only unidirectional effects were examined (i.e., TSR ➔ academic PsyCap ➔ academic performance). In future research, bidirectional effects using cross-lagged models could be included. Third, only adolescent high school students were recruited. In future research, we could include different academic levels (e.g., undergraduate university students).

Finally, some avenues for future research can be mentioned. First, based on Furrer and Skinner (2003), parent-child and peer-student relationships could be incorporated in our model in order to obtain a better understanding of the role of significant others in academic PsyCap. Second, based on broaden-and-build theory (Fredrikson, 1998), the mediator role of study-related positive emotions between teacher-student relationships and academic PsyCap could be considered. Third, based on Ryan and Deci (2000), basic need satisfaction could be explored as an antecedent of academic PsyCap.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MCH conceived the idea for the study, conducted the analyses, and wrote the manuscript. MS and WS contributed to the interpretation of results and revised the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Psychology

BRIEF RESEARCH REPORT
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Influence of Family Involvement and Children's Socioemotional Development on the Learning Outcomes of Chilean Students

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There is an extensive body of evidence to support both family involvement and students' socioemotional development as key factors in the promotion of learning outcomes. However, there is insufficient evidence to establish exactly what this impact is when both factors are considered simultaneously. Therefore, the aim of this study is to analyze the influence of family involvement and socioemotional development on learning outcomes of Chilean students, identifying the structure that most correctly identifies the influence of the predictor variables (family involvement and socioemotional development) on learning outcomes. We present the following three hypotheses that consider possible basic interrelation structures: (1) The influence of family involvement on learning outcomes is mediated by students' socioemotional development (mediation hypothesis); (2) The influence of family involvement on learning outcomes is moderated by students' socioemotional development (moderation hypothesis); (3) Family involvement and students' socio emotional development directly affect learning outcomes (covariance hypothesis). The structures were evaluated by means of a structural equation model analysis. The study included 768 students who attended second and third elementary grades in Chilean schools. The children were between 7 and 11 years old (M = 8.29, SD = 0.86); 41.3% were girls and 58.7% were boys. The results show that family involvement and students' emotional development directly affect learning outcomes (CFI = 0.995, TLI = 0.993, RMSEA = 0.016). From the results, we can conclude that the data support the hypothesis that both family involvement and socioemotional development are predictors of learning outcomes, thereby rejecting that the impact of family involvement on learning outcomes is mediated or moderated by socioemotional development.

Keywords: family involvement, children's socioemotional development, learning, family and school relation, child development

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INTRODUCTION

The concept of family involvement has consistently emphasized the importance of and need for family support in children's education (Baker et al., 2016), which allows for a broader conceptualization of the roles of families and schools, their relations and the impact on children's development (Christenson and Sheridan, 2001; Patrikakou et al., 2005; Christenson and Reschly, 2010; Yamauchi et al., 2017). *Family involvement* is understood as the family's willingness to become involved with the school and their children's learning, including behavioral and verbal practices in the home and school activities (Anderson-Butcher, 2006).

Several studies indicate that *family involvement* has a positive influence on *children's socioemotional development.* (Gutman and Midgley, 2000; Fan and Chen, 2001; Garbacz et al., 2017). Several studies also indicate that *family involvement* has a positive influence on the development of children's abilities (Epstein and Sander, 2000; Vélez, 2009; Chavkin, 2017) and particularly on school learning outcomes (Brody et al., 1999; McWayne et al., 2004). Most of the studies in this area come from Anglo-Saxon (Garbacz et al., 2017) while in the Latin American context research is still scarce.

In his meta-analysis of 51 studies into family involvement programs, Jeynes (2012) concluded that reading programs shared between parents and children, programs focused on effective alliances, and programs focused on improving the communication between home and school had the greatest impact on children's academic performance. Another meta-analysis, after analyzing 46 studies identified that the key aspects explaining the positive correlation between learning outcomes and parental participation were the school-home connection (Ma et al., 2016). Finally, in a meta-analysis of 37 studies, Castro et al. (2015) found that the type of family participation that most affected students' academic performance was the parents having high expectations of their children, developing and maintaining a fluid communication about what happened at school, and promoting the development of reading habits.

In addition to the literature on the positive impact of family involvement on *children's socioemotional development and learning outcomes*, there is ample evidence of the relation between *children's socioemotional development* and learning outcomes (Jiménez and López-Zafra, 2009). It has generally been observed that positive emotions (e.g., pleasure in learning) are positively related to academic success, whereas negative emotions (e.g., anxiety) have an inverse relation (Goetz and Hall, 2013; Pekrun and Linnenbrink-Garcia, 2014). There are also studies that have found a statistically significant relationship between Emotion Quotient Inventory EQi- scores and academic performance (Bar-On, 1997; Parker et al., 2004) crucial for the education-learning process (Humphrey et al., 2007; Pekrun et al., 2017), nevertheless the results of some studies that have analyzed the relation between academic success and socio-emotional competence present ambiguous results (Newsome et al., 2000).

Due to the findings reviewed previously, we may conclude that there is evidence to support both family involvement and students' socioemotional development as key factors in the promotion of learning outcomes. We may also conclude that there is insufficient evidence to establish exactly what this impact is when both factors are considered simultaneously. Therefore, the aim of this study was to analyze the influence of family involvement and socioemotional development on learning outcomes of Chilean students, identifying the structure that most correctly identifies the influence of the predictor variables (family involvement and socioemotional development) on learning outcomes. The following three hypotheses were considered possible basic interrelation structures (**Figure 1**):

(A) The influence of family involvement on learning outcomes is mediated by students' socioemotional development (mediation hypothesis);
(B) The influence of family involvement on learning outcomes is moderated by students' socioemotional development (moderation hypothesis);
(C) Family involvement and students' socio emotional development directly affect learning outcomes (covariance hypothesis)

MATERIALS AND METHODS

Participants

The study included 768 students who attended second and third elementary grades in 18 schools in regions in Chile (O'Higgins, El Maule and La Araucanía). The children were between 7 and 11 years old (M = 8.29, SD = 0.86); 41.3 and 58.7% were girls and boys, respectively.

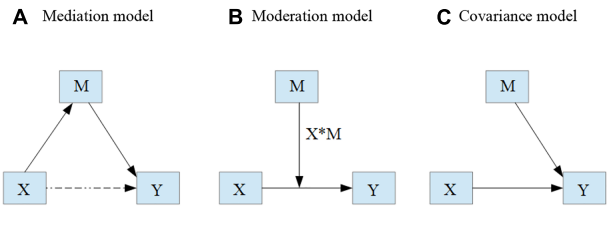

FIGURE 1 | Diagrams depicting possible relations between family involvement and children's socioemotional development on learning outcomes. **(A)** Mediation model. **(B)** Moderation model. **(C)** Covariance model. X, family involvement; M, children's socioemotional development; Y, learning outcomes.

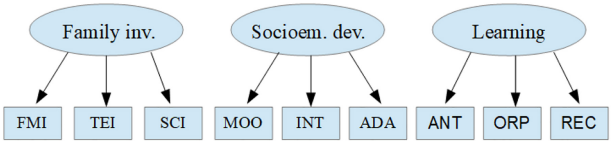

FIGURE 2 | Constructs and subdimensions. Family inv., components of family involvement; FMI, father/mother involvement; TEI, teacher involvement; SCI, school involvement; Socioem. dev., components of socio-emotional development; MOO, mood; INT, interpersonal; ADA, adaptability; Learning, components of learning; ANT, analogical thinking; ORP, organizational perspectives; REC, reading comprehension.

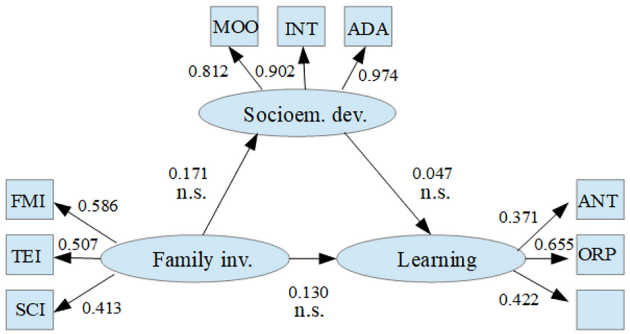

FIGURE 3 | Path diagram of the theoretical mediation model. Family inv., components of family involvement; FMI, father/mother involvement; TEI, teacher involvement; SCI, school involvement; Socioem. dev., components of socio-emotional development; MOO, mood; INT, interpersonal; ADA, adaptability; Learning, components of learning; ANT, analogical thinking; ORP, organization perspectives; REC, reading comprehension; n.s., non-significative difference.

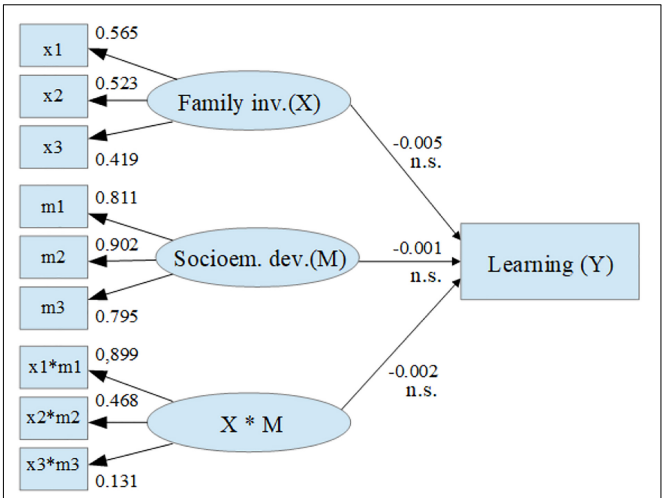

FIGURE 4 | Path diagram of the moderation model. Family inv.(X), components of family involvement; x1, father/mother involvement; x2, teacher involvement; x3, school involvement; Socioem. dev.(M), components of socioemotional development; m1, mood; m2, interpersonal; m3, adaptability; Learning (Y), learning; X*M, multiply of indicators variables; x1m1, father/mother involvement by mood multiply; x2m2, teacher involvement by interpersonal multiply; x3m3, school involvement by adaptability multiply; n.s., non-significative difference at 5%.

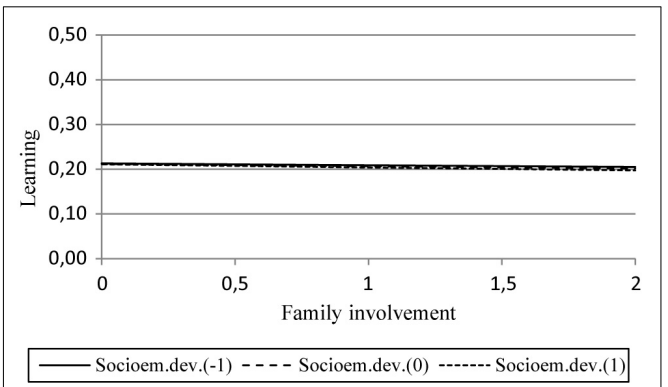

FIGURE 5 | Graph of regression lines for the moderation model. Socioem. dev., socioemotional development.

Participants were selected using non-probability purposive sampling (Kerlinger and Lee, 2002), as is the case of the students included in this study, who come from schools that offer elementary education and have been described as having high levels of vulnerability according to the student vulnerability index issued by the Chilean Ministry of Education.

This study was carried out in accordance with the recommendations of the Chilean National Commission for Scientific and Technological Research. The protocol was approved by the Ethics Committee of the Universidad de La Frontera (Acta 066-2017, Folio 036-17). All the subjects provided written informed consent in accordance with the Declaration of Helsinki

Instruments

Assessment of Family Involvement

Three scales of the Hoover-Dempsey and Sandler (2005) Parental Involvement Scale translated into Spanish and validated by a panel of experts in Chile (Reininger, 2014) were used in this study: The Parental Involvement forms (with two subscales: home based involvement, 5 items, and school based involvement, 5 items); the teacher invitations for involvement scale (6 items); and the general school invitations scale (6 items). The fist scale has a four-point Likert response scale, from 1 (never) to 4 (always), while the rest was a 5-point scale Likert response, from 1 (strongly disagree) to 5 (strongly agree).

Evaluation of Learning Outcomes

Three subtest of the educational psychology battery EVALÚA (García and González, 1999; García et al., 2006) were used in this study: Reading Comprehension (22 items) and two subtest of reasoning, Analogical Thinking (20 items) and Perceptual Organization (34 items).

Assessment of Socioemotional Development

Three dimensions of the EQ-I: YV questionnaire (Bar-On and Parker, 2000) adapted and validated in Spanish (Ferrándiz et al., 2012) were used in this study: interpersonal (12 items), adaptability (10 items) and general mood (14 items). The response scale ranged from 1 (rarely) to 4 (nearly always).

The coefficient omega average of MacDonald to ordinal scales was 0.96 to Socio-Emotional (0.78–0.97), 0.88 to Family involvement (0.78–0.84) and alpha 0.81 to Learning.

Procedure

This study is part of a wider project focusing on the effectiveness of interventions to strengthen the link between families and schools.

TABLE 1 | Estimations of the structural parameters of the mediation model.

		Effect of.	Est.no std.	S.E.	Est./S.E.	Sig. Est.Std.	Sig.
Learning	<- Family inv.	0.130	0.085	1.523	0.128	0.132	n.s.
Learning	<- Socioem. dev.	0.047	0.038	1.225	0.221	0.092	n.s.
Socioem. dev.	<- Family inv.	0.171	0.113	1.513	0.130	0.088	n.s.

Family inv., family involvement; Socioem. dev., socioemotional development; Learning, learning; n.s., non-significative difference at 5%.

TABLE 2 | Confidence intervals for the estimations of the structural parameters of the mediation model.

Confidence interval		inf.99%	inf.95%	inf.90%	Estim.	sup.90%	sup.95%	sup.99%
Learning	<- Family inv.	−0.023	−0.011	0.027	0.130	0.319	0.366	0.460
Learning	<-Socioem. dev.	−0.020	−0.008	−0.002	0.047	0.124	0.141	0.179
Socioem. dev.	<- Family inv.	−0.125	−0.034	0.004	0.171	0.373	0.413	0.497
Ef.ind.specific		−0.013	−0.008	−0.005	0.008	0.021	0.024	0.029

Family inv., family involvement; Socioem. dev., socioemotional development; Learning, learning; Ef.ind.specific, specific indirect effect.

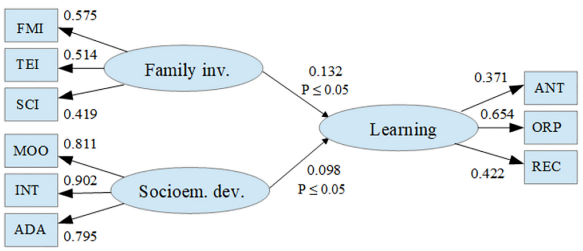

FIGURE 6 | Path diagram of the covariance model. Family inv., components of family involvement; FMI, father/mother involvement; TEI, teacher involvement; SCI, school involvement; Socioem. dev., components of socio-emotional development; MOO, mood; INT, interpersonal; ADA, adaptability; Learning, components of learning; ANT, analogical thinking; ORP, organization perspectives; REC, reading comprehension. $P \leq 0.05$, significative difference at 5%.

The data referring to the students (evaluation of learning outcomes and assessment of socioemotional development) was collected during school hours and were registered in digital format in the schools' computer rooms during three sessions. The data referring to the families (family involvement) were collected in paper format during parent teacher meetings.

Analysis Plan

In the first phase of data processing, inverse items were recoded, response rates were verified and corrected, unanswered records were identified and eliminated and non-parametric multiple imputation of classification and regression trees with random-forest resampling was used for missing data (Stekhoven and Bühlmann, 2012), which enables imputation for ordinal variables. In order to center the focus of the causal model on the predictive structure of the factors, the factorial scores of the subdimensions for the family involvement scale and those of socioemotional development and school learning were calculated (**Figure 2**), these consisting of the subscales with loads greater than 0.40 (Stevens, 2009; Brown, 2015).

Once the factorial scores of the selected subscales were calculated, these subsequently occupied the role of indicator variables for each construct. Using this configuration of components, several structural equation models were fit to determine the structure of the causal relationship between the two factors considered as antecedents (family involvement and socioemotional development) of school learning. The structures of the trajectories studied were a relation of mediation, moderation and covariance as shown in **Figure 1**.

In order to evaluate the fit of the models to the data, the following indices were used: comparative fit index (CFI), Tucker-Lewis index (TLI) and root mean square error of approximation (RMSEA); for the first indices, CFI and TLI, values above 0.90 or 0.95 are considered an adequate fit of the model (Schreiber et al., 2006), while for the RMSEA values below 0.08 are considered a reasonable fit (Hooper et al., 2008).

For the case of the mediation effect, statistical significance was used and the estimation of the confidence intervals by means of resampling of the specific indirect effect attributable to the presence of the mediator variable (Muthén and Asparouhov, 2015). In order to evaluate the moderation effect, a multiplicative model was used that included the product of the indicator variables of the two factors used as antecedents (Marsh et al., 2004). Finally, to evaluate the presence of a structure with covariance effect, an analysis was performed of the statistical significance of regression slopes applied to the trajectories of the latent variables of the model. The statistics software used was Mplus 7.11 as well as the miss Forest, laavan and sem Tools packages in R.

TABLE 3 | Regression slopes for the moderation model.

Socioem. dev.	Mod	Slope	SE	Wald	Sig.
Low	−1	−0.0024	0.021	−0.12	0.91
Medium	0	−0.0049	0.014	−0.36	0.72
High	1	−0.0074	0.021	−0.36	0.72

Socioem. dev., socioemotional development; Mod, moderator variable level (socioemotional development).

RESULTS

The mediation model (**Figure 3**) revealed low levels of indirect relation of family involvement mediated by socioemotional development to the response of school learning; these results allow the presence of an indirect and mediating effect to be ruled out, leaving open the possibility that the effect of family involvement on learning can take the form of a moderated relation or interaction, or assume a direct and independent role of socioemotional development.

The results of the model demonstrated high levels of goodness of fit, with CFI and TL values of 0.995 and 0.993, respectively; in turn, a low error of estimation was observed with a RMSEA of 0.016, which confirms the stability of the results. On the other hand, the estimations of the parameters of the mediation model showed low and non-significant levels of the mediation effect, which leads to the conclusion that mediation is not the structure of relation between the study variables (**Table 1**).

This was also corroborated by the estimation of the confidence intervals for significance levels of 1 and 5%, both for the sum of the mediation effect and for the estimation of its specific effect (**Table 2**).

Based on the results of the evaluation of the mediation effect, it is possible to conclude that there is no significant evidence in any of the parameters of the model ($p > 0.05$) to corroborate that the relation between the study variables is a model with mediation effect. Therefore, with the hypothesis of the mediation effect being discarded, it becomes necessary to evaluate the models corresponding to the effects of moderation and covariance.

In relation to the moderation hypothesis, the results showed that incorporating the interaction effect of the exogenous variables means that all the effects are statistically non-significant (**Figure 4**).

This is confirmed through an analysis of the slopes of each level of socioemotional development, which are not statistically different from zero (**Table 3**). The intercept analysis was dismissed, as the indicator variables were centered prior to the statistical analysis of the moderation model.

These findings were also corroborated by the presence of parallelism in the graph of the regression lines (**Figure 5**), resulting from the interaction between the independent variable of family involvement and the variable that acted as moderator in the model, which in this case was socioemotional development.

Based on the evidence verified in the evaluation of the interaction model, it is possible to conclude that the hypothesis regarding a moderation effect of socioemotional development on the relation of family involvement and school learning is rejected.

Finally, due to the rejection of the two previous hypotheses corresponding to the mediation and moderation models, in this section the results obtained in the verification of the statistical significance of a covariance model are described. The results showed that family involvement and students' emotional development directly affect learning outcomes (CFI = 0.995, TLI = 0.993, RMSEA = 0.016), explaining 69% of the learning variance. This allows to conclude that in the context of the analyzed data, socioemotional development participates directly and independently in school learning in a similar way although to a lesser degree ($\gamma_{21} = 0.098$, $p = 0.049$) than family involvement ($\gamma_{23} = 0.132$, $p = 0.032$) (**Figure 6**).

DISCUSSION

International literature indicates that the degree of family involvement in school processes is a critical element in the development and learning outcomes of children during their first school years (Hoover-Dempsey and Sandler, 1997; Caspe et al., 2006) making relevant the need to generate scientific evidence from the Chilean context for eventual future replications in other Latin American countries.

The results of the present study support the hypothesis that both family involvement and socioemotional development are predictors of learning outcomes, thereby rejecting the notion that the impact of family involvement on learning outcomes is mediated or moderated by socioemotional development. In this sense, both factors are positioned as dimensions with a direct effect on learning outcomes in the case of Chilean students.

One of the main contribution of this study is its focus on Latin America due to the lack of literature from this region. For example, in a recent systematic review, only one Mexican study from 1998 appeared, which was strongly influenced by U.S. interventions (Eichin and Volante, 2018). In this context, Chile has acknowledged the importance of collaborative relationships between families and schools leading to the development a National Policy for Father, Mother and Legal Guardian Participation. Nevertheless, the majority of research in the country has thus far been of a qualitative nature with a focus on describing family school relations and identifying tensions between these two spheres (Gubbins, 2011). Thus, this study aimed to make progress in the analysis of the effect of parental involvement in school and children's socioemotional development on learning outcomes of Chilean students.

One of the main weaknesses is that the study utilized a thematic or convenience sample. Therefore, one of the main challenges for future research in Chile and Latin America is the need for studies with probabilistic samples.

AUTHOR CONTRIBUTIONS

MS developed the study concept and the study design. LL and DM substantially contributed to the study concept and the study design. DM, TR, and LL performed the data collection. HM and EM-Z performed the data analysis and interpretation under the supervision of MS and LL. MS, LL, and DM drafted the

manuscript. DM, TR, LL, HM, and EM-Z substantially contributed to the interpretation of the data and provided important critical revisions. All authors approved the final version of the manuscript and also agreed to be accountable for all aspects of the work.

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Neurophysiological Effects Associated With Subliminal Conditioning of Appetite Motivations

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When attempting to encourage eating, explicitly providing statements like "eating is pleasant" may produce little effect. This may be due to subjective, negatively-valenced narratives evoked by perception of the verb "eating" (e.g., *eating* → *fat* → *lonely*), overriding any explicitly provided *eating-pleasant* valence information. In our study, we presented eating-related verbs under subliminal visual conditions to mitigate the onset of *eating*-associated deliberation. Verbs were linked with neutral or positively valenced terms across independent blocks. Modulations of event-related magnetoencephalographic (MEG) components and parietal activations in the alpha range (8–12 Hz) illustrated a significant effect of valence during pre-lexical time windows. We found significantly greater saliva production and declarations of increasing hunger after eating-related verbs were linked with positive terms. Orally reported preferences did not vary between conditions.

Keywords: subliminal conditioning, appetite, eating disorders, motivation, learning theory, magnetoencephalography

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INTRODUCTION

In classical conditioning, associating a minimally salient (neutral) stimulus with emotionally salient events [unconditioned stimuli (US)] can transform the former's hedonic valences, making it a conditioned stimulus (CS; Staats and Staats, 1957; Mowrer, 1980). The establishment of valenced CS following systematic CS–US correlations is ubiquitous to human language. Propositions can function as context-specific "conditioning devices" due to their ability to transform the *valence* of the terms qualified (Mowrer, 1980). Within this framework, the proposition "eating is good" is conceptualized as a CS–US relation, where the positive valences from *good* (US) can transform the valences of *eating*-associated representations (Mowrer, 1980, pp. 112–116). Positively transforming the valences of *eating* (CS) can enhance appetite motivation given that valence is intrinsically linked to a motivational state (Peterson, 2002; Custers and Aarts, 2010).

In practice, however, such clear-cut US-to-CS valence transformations are not always observed. We conjecture that self-generated (subjective) narratives of high valence may counteract explicit CS–US valence information (e.g., Bar-Anan and Moran, 2017, p. 10). We illustrate this idea in **Figure 1**, where we describe how the proposition "eating is pleasant" may fail to augment actual eating. We posit that mere awareness of the word "eating" (CS) evokes the self-generation of a negatively valenced narrative associated with the CS representation (*eating*). These narratives can function to negatively valence the derived *eating* representations (e.g., to the effect "eating will make

me fat and ugly and therefore I will become unattractive and lonely"), overriding any positively valences projected from the explicit *eating-pleasant* (CS–US) information. Our hypothesis explains how the proposition "eating is pleasant" may even induce reduced eating in certain individuals (i.e., those who tend to self-generate excessively negatively valenced narratives about eating; Merwin et al., 2010).

A representative study by Hensels and Baines (2016) provides evidence for our hypothesis. In that study, images of food (CS) were linked with happy or angry faces (US) during a CS–US conditioning task. After conditioning, participants evaluated CS via implicit association tests (IATs; Greenwald and Nosek, 2001) and decision-making tasks to determine, first, whether the valences for the food CS had transformed in accordance with their linked US and, second, whether the direction of the valence transformations (i.e., positive versus negative) influenced the motivation to consume CS-associated foods. Hensels and Baines found that subjects who were less likely to engage in emotional thinking were more receptive to external CS–US valence information, where evaluations toward CS foods shifted predictably (i.e., linking certain foods with happy faces led toward increased preferences toward those foods). Alternatively, subjects more likely to engage in emotional thinking were less susceptible to explicit valence information (CS–US pairings produced no significant effects on food preferences). These results showcase how subjective narratives can counter explicit CS–US valence information. It also may explain why strategies to counteract pathological behavioral patterns through talking strategies may not always alter the pattern in question (e.g., telling an anorexic person that "eating is good" and seeing no effect – again, see Merwin et al., 2010).

In the present study, we aimed at minimizing potential interference from CS-evoked narratives during CS–US conditioning by presenting CS too briefly (subliminally) to permit conscious identification (Custers and Aarts, 2010; Aarts and Custers, 2012). We assumed that narrative generation requires lexical processing of a CS representation. We also assumed that CS representations take longer to be redintegrated when based on partial visual information (Kouider et al., 2010). Briefly, "redintegration" describes how a significate becomes reinstated "as a memory or idea" in response to the presentation of a "partial constituent" of said significate (Warren, 2018). The notion resembles "feature integration" concepts across contemporary binding accounts of S-S learning, but without any a priori requirements for selective attention processes (Walther et al., 2018). A redintegrated CS representation may evoke valence responses prior to additional processing. By preventing conscious awareness of CS and their associated narratives, we hoped to enhance the probability of visible US directly transforming the redintegrated CS representations. That is, reducing the chance of seeing the verb "eating" should mitigate the onset eating-associated narratives. We addressed two questions in our present study. First, we asked whether US-to-CS valence transformations could be detected following the presentation of visible US and subliminal CS using magnetoencephalographic (MEG) source imaging. Next, we explored whether augmenting the valences of eating-associated CS would correlate with salivary, performance-based, and orally reported measures of appetite motivation.

We decided on stimulus-evoked MEG activations as our exclusive measure of valence for two reasons. First, assuming that awareness of a CS could suffice for a subject to generate a valenced CS-associated narrative, *any* behavioral measure of CS valence, whether explicit/implicit, incurs the possibility of CS-evoked narratives influencing evaluations (Gawronski and Hahn, 2018; March et al., 2018). By restricting our valence measurements to CS- and US-evoked electrophysiological components and never having subjects evaluate CS behaviorally, we minimized the possibility of CS-evoked narratives moderating our measurement of CS valence (Amd et al., 2013).

In spite of such precautions, it can still be the case that *some* ongoing narrative can influence CS valence expression. To increase the likelihood that we were assessing stimulus-evoked (as opposed to narrative-associated) valences, it is required to identify stimulus valence effects before lexical processing begins (typically within ∼200 ms of stimulus onset – Barber and Kutas, 2007). MEG and EEG provide the millisecond temporal resolution. MEG has the added advantage that magnetic brain signals do not get distorted by head tissues, which significantly enhance the topographical interpretation and source modeling of brain responses (Baillet, 2017). The combination of high spatial and temporal resolution is the second reason for selecting MEG to assess stimulus valence.

Previous research has demonstrated valence-associated event-related components (ERCs) within 100–200 ms of stimulus onset using EEG, typically over regions associated with language processing and feature redintegration (Amd et al., 2013; Kuchinke et al., 2015; Blechert et al., 2016, p. 14; Bayer et al., 2017). As our CS and US were naturally occurring words, we predicted ERCs localized over language areas would discriminate between neutral and positive US within 200–300 ms of US onset. Observing similar ERCs discriminating neutral from positive CS would indicate US-to-CS valence transformations.

We focused on positive versus neutral contrasts for two reasons. First, earlier investigations on event-related effects between positive, negative, and neutral terms highlighted positive/negative versus neutral items as typically being the most discernible (Rozenkrants et al., 2008; Hinojosa et al., 2009; Amd et al., 2013; Kissler and Herbert, 2013). This is because early electrophysiological responses are driven by overall stimulus salience, and positive/negative terms are typically more salient than neutral items (Olofsson et al., 2008; Hofmann et al., 2009). Another reason for employing only positive and neutral US is because we do not know about any long-term effects following the current procedure, hence it could be unethical to employ negative US. Specifically, if our present hypotheses are valid and appetite motivations can be subliminally influenced, employing negative US could pose the risk of disrupting normative eating patterns across otherwise healthy individuals.

Modulations of brain rhythmic fluctuations in the 8–12 Hz alpha frequency band also correlate with manipulations of stimulus valence (Simons et al., 2003; Aftanas et al., 2004; Uusberg et al., 2013; Amd and Roche, 2016, 2017; Marshall et al., 2018). Alpha oscillations may reflect inhibition in anticipation

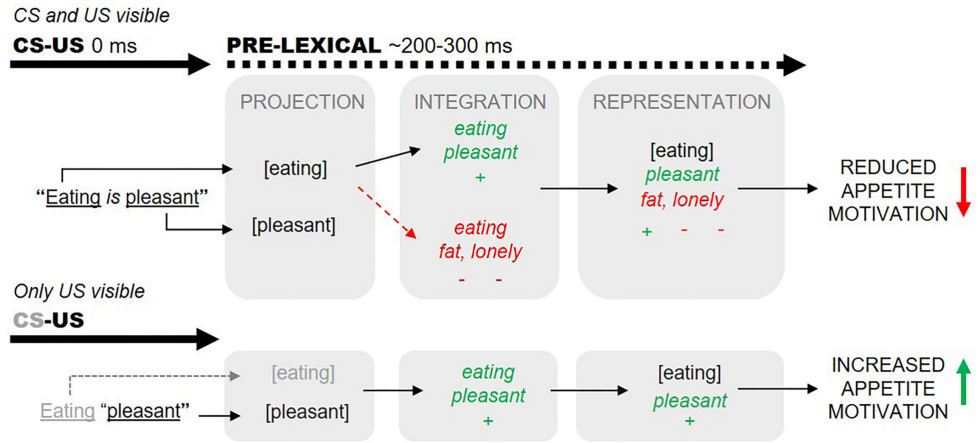

FIGURE 1 | Valences predicated through the proposition "eating is pleasant" can be countered by subjective narratives evoked by conscious projection of the word "eating" (dashed arrows, top panel). Subjective and explicit valences are assumed to integrate within 200–300 ms of word onsets (before lexical processing). We aimed to minimize subjective eating-associated narratives by presenting "eating"-related words subliminally (bottom panel).

to salient events, where more salient stimuli are predicted to invoke greater selective cortical inhibition (augmented alpha power) over task-irrelevant regions (Onoda et al., 2007; Klimesch, 2012). Note that alpha modulations are not associated exclusively with stimulus valence and can be observed across multiple tasks that manipulate response inhibition (e.g., action observation versus action execution – Babiloni et al., 2016). We predicted that positively valenced US would induce greater alpha activity relative to neutral US. Similar alpha activations in the presence of CS would provide additional evidence of US-to-CS valence transformations. Since our task involved natural words, valence-associated ERCs and alpha activations were predicted over left central-parietal-temporal regions, which are key nodes of a distributed network for semantic (valence) comprehension (Binder, 2017; also see Brownsett and Wise, 2010; Meyer et al., 2013).

We employed three measures of appetite motivation in our study. First, after each conditioning trial, subjects had to answer Yes/No to the question "Are you getting hungry/sleepy?". Our second (saliva) and third (oral) measures were implemented between blocks of conditioning trials. At the end of each block, we placed dental rolls in the subjects' mouths to measure salivary volume. We predicted that increased saliva would follow conditioning trials where eating-related CS were linked with positive US, since increased saliva production is positively correlated with greater appetite motivation (Nirenberg and Miller, 1982; Epstein et al., 1996). After saliva was collected, subjects orally reported their preferences toward various activities (e.g., to the question "how much would you like to *run* right now?" presented on a screen). Some of the displayed activities functioned as CS during conditioning. We asked subjects to verbally respond to a visual prompt with minimal time/movement restrictions to maximize the probability our subject would deliberate (i.e., generate narratives) before responding. Assuming self-generated narratives can counteract explicitly provided valence information (**Figure 1**), we predicted that orally provided ratings would be least sensitive to our procedural manipulations, relative to all other measures of appetite motivation. We also included a measure of CS visibility after each conditioning trial to determine whether conscious CS identification is necessary for valence acquisition (Heycke and Stahl, 2018).

MATERIALS AND METHODS

Subjects

Six males and seven females were recruited for the present study through personal invitation. Inclusion criteria for the study included normal/corrected-to-normal vision, proficiency in English, age (above 18), and no confounding medical histories (e.g., legal/illegal drug use, pre-existing medical conditions like schizophrenia). All subjects provided informed and written consent prior to participating. The data of two females were excluded as they turned out to be novel English speakers who were unfamiliar with nearly three quarters of the stimuli used; a third female was discarded due to extensive artifacts caused by her dental fillings. This left a final sample of $n = 10$ (27.5 ± 9.5 years). Seven of our 10 subjects had never undergone a MEG study previously, and nine among those 10 reported having never participated in psychological research. Everyone received CAD $50.00 for their time and were instructed not to eat two hours prior to the session. We did not want our subjects to arrive to the experiment satiated, which would reduce the probability of augmenting appetite motivations even if our eating-related CS had been positively valenced (Booth and Toase, 1983). All subjects were informed during the study's onset that they were to commence a task measuring "attention," with specific instructions to identify the CS word that appeared after the fixation. All subjects were fully de-briefed at the end of the study. The procedures were approved by the Research Ethics Boards at the McGill University Health Center and Montreal

Neurological Institute (Approval # 2018-4166) and correspond with the guidelines provided in the Declaration of Helsinki. The duration of experimental sessions, including MEG setup and debriefing, was 90–120 min per subject.

Materials

Stimuli designated to be CS included eight sleeping-related (resting, yawning, relaxing, slumbering, napping, snoring, dreaming, snoozing) and eight eating-related (snacking, nibbling, munching, chewing, consuming, devouring, gobbling, feasting) verbs. Stimuli designated to be US included eight positively valenced attributes (happy, rich, nice, pleasant, lovely, wonderful, enjoyable, great) and eight neutral nouns (chime, gray, door, building, lamp, bell, wall, window) terms. For positive and neutral US, the mean ± SD valences reported by Warriner et al. (2013) were 7.4 (±1.8) and 5.4 (±1.4) respectively (see **Table 1** for arousal and dominance scores). These were corroborated in a separate investigation by the authors, where 15 subjects produced valences of 7.8 (±1.2) and 5.4 (±0.8) for the positive and neutral US, respectively. All ratings reflect scores on scales ranging from 1 (sad) to 10 (happy). The mean number of characters for positive and neutral US words was not significantly different (p = 0.156). US were not controlled along lexical frequency, imageability, and other topographical characteristics as these do not significantly mitigate subliminal conditioning (Greenwald and De Houwer, 2017). We also included "distractor" verbs (CS-) that were not used during conditioning trials (running, reading, dancing, writing, talking, swimming, jogging, speaking). Distractors were used during the two-alternative-forced choice (2AFC) tasks and between-condition oral sessions (see section "Procedure"). All CS were sandwiched by masks constituting of three X' as these were not semantically relatable with our CS/US (Jaśkowski and Przekoracka-Krawczyk, 2005). Masks were of Arial black size 34 font. CS and US appeared in regular Arial size 22 font. Characters of size 34 and 22 fonts have em heights of 0.85 and 0.55 in each ("em" is a unit of measurement in typography). Corresponding em widths were 0.78 and 0.35 in, respectively. The total area spanned by three 34 font X's equaled (0.85*3*0.78) = 1.99 square inches. The maximum area covered by a US (the longest of which had 11 characters) was (0.55*11*0.35) = 2.12 square inches. Despite the different number of characters, the maximal visual discrepancy between the masks and stimuli was less than 0.13 square inches. We assessed subjects' ability to identify words at this distance by having them loudly read off unrelated words presented in a smaller font prior to the beginning of the conditioning task. All stimulus presentations were projected onto a screen from a video projector with a 60-Hz refresh rate. All statistical analyses were conducted on the R platform (R Core Team, 2014). All MEG pre-processing and analyses were completed with Brainstorm (Tadel et al., 2011) following guidelines for group analysis (Tadel et al., 2019).

TABLE 1 | Mean ± SD of US valence, arousal, and dominance.

Condition*	ID**	Word	Valence	Arousal	Dominance
Positive	5596	Happy	8.5 ± 1.3	6.1 ± 2.1	7.2 ± 2.0
Positive	10380	Rich	6.8 ± 2.3	6.8 ± 2.0	6.8 ± 2.4
Positive	8178	Nice	7.0 ± 2.0	3.5 ± 2.4	6.5 ± 2.0
Positive	9153	Pleasant	7.2 ± 1.5	2.9 ± 2.5	6.7 ± 2.0
Positive	7250	Lovely	7.6 ± 1.7	4.0 ± 2.8	6.4 ± 2.4
Positive	13793	Wonderful	7.4 ± 1.8	4.6 ± 2.5	7.2 ± 2.1
Positive	4121	Enjoyable	7.6 ± 1.5	4.8 ± 2.2	7.0 ± 1.9
Positive	5375	Great	7.5 ± 1.9	4.1 ± 2.7	6.7 ± 1.8
		Average	*7.4 ± 1.8*	*4.6 ± 2.4*	*6.8 ± 2.1*
Neutral	2042	Chime	6.1 ± 1.2	4.1 ± 2.2	6.2 ± 2.2
Neutral	5369	Gray	3.7 ± 1.7	2.8 ± 1.9	4.8 ± 2.2
Neutral	3727	Door	5.4 ± 1.2	3.2 ± 2.0	6.1 ± 2.1
Neutral	1570	Building	5.5 ± 1.1	3.4 ± 2.2	6.0 ± 2.2
Neutral	6904	Lamp	5.7 ± 1.1	2.7 ± 1.8	6.0 ± 2.2
Neutral	13532	Wall	5.1 ± 1.7	2.9 ± 1.9	4.2 ± 1.7
Neutral	13738	Window	6.5 ± 1.6	3.3 ± 1.9	5.4 ± 2.6
Neutral	1054	Bell	5.7 ± 1.8	4.7 ± 2.3	6.2 ± 2.4
		Average	*5.4 ± 1.4*	*3.4 ± 2.0*	*5.6 ± 2.2*

*Neutral and positive terms appeared as USs across separate blocks.
**IDs refer to the dataset from Warriner et al. (2013).

Procedure

Overview

Subjects were fitted with non-magnetic scrubs upon arrival and comfortably seated in an upright position under the MEG helmet. The experimenter provided the subject with two sterile dental rolls to place in their mouth. After a minute, the rolls were collected and weighed within 10 s. The difference in dental roll weights before and after being placed in the subject's mouth was recorded as salivary volume. Next, subjects viewed prompts on the computer screen to orally call out a rating between 1 and 10 in response to the questions "How much would you like to (activity) right now? (Where '1' means 'not at all' and '10' means 'a lot')" presented in no specific sequence. Subjects provided preferences for four activities, two of which resembled our eating- and sleeping-related CS.

Salivary volume and oral preferences were collected five times for each subject over the course of the experiment; at baseline before the procedure began, and once after each of the four conditioning blocks. Across two of these blocks, CS were exclusively linked with neutral US. Across the two remaining blocks, CS were exclusively linked with positive US. The sequence of neutral (N) and positive (P) trial blocks were counter-balanced across participants, so that half our subjects underwent an N→P→N→P sequence, whereas the remaining half underwent a P→N→P→N sequence. Each block contained 80 conditioning trials. Each conditioning trial commenced with a black fixation cross inside a white box on the left or right sides of the screen. Subjects had to produce a button press corresponding to the left/right position of the cross (Amd et al., 2017). An accurate button-press (1 = Left, 2 = Right – see **Figure 2**) produced a jittered forward mask for 202 ± 51 ms, followed by an eating/sleeping related verb (CS+) for ∼17 ms and a backward mask for 260 ms. This was followed by a second blank screen for 900 ms with a white fixation point in place of the box that had appeared earlier. The box next re-appeared in the same location with a US for 160 ms. Our US remained visible in order to reliably

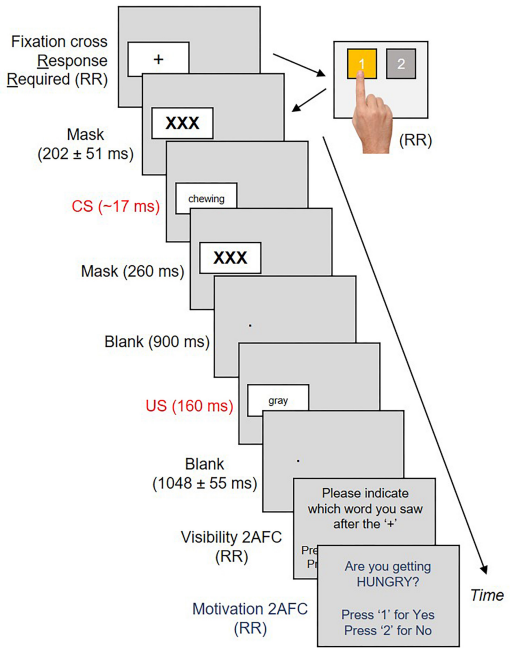

FIGURE 2 | Each conditioning trial commenced with a fixation cross on the left or right sides of the screen. Subjects had to respond to the location of the cross by pressing "1" or "2" on a button box. This produced a CS sandwiched between masks, a blank interval and a US in the same location as the cross. US offsets produced a second blank interval, followed by two two-alternative forced choice tasks measuring CS visibility and motivational state, respectively.

evoke valenced responses (Lähteenmäki et al., 2015). The US was replaced with a second blank screen (1048 ± 55 ms) and our first 2AFC (visibility check – **Figure 2**). Subjects had to select from two options (CS+/CS−) which word they thought had appeared earlier. A response produced a second 2AFC, where subjects had to respond Yes/No to the question "Are you getting hungry (sleepy)?". A response here initiated a third blank screen for 500 ms, marking the end of that trial.

MEG Acquisition and Pre-processing

Magnetoencephalographic activity was recorded using a 275-channel whole-head MEG system (CTF/VMS, BC, Canada) with a sampling rate of 2400 Hz and a 0–150 Hz filter bandwidth. We measured vertical (VEOG) and horizontal (HEOG) eye movements by placing two electrodes ∼1 in above and below the right eyeball, and two electrodes near the left and right temporal bones. Cardiac activity (ECG) was recorded by placing one electrode near the second interspace left midclavicular line, and a second electrode near the eighth interspace midclavicular line. VEOG, HEOG, and ECG activity was recorded to inform artifact correction during analysis. Head position was determined by placing three head localization coils at three fiducial points (nasion, left ear, right ear). Anatomical registration with the default MRI anatomy (Colin27_2016 template; Brainstorm) was facilitated with the manual addition of ∼120 digitized points using a 3-D Polhemus Isotrack digitizer system along the top surface of the head and nose for all our subjects. Head position was continuously recorded with a sampling rate of 150 Hz to ensure that subject head movements did not exceed 1 cm during and between conditioning trials. CS and US onset durations were confirmed to fall within ±1 ms of 16.6 and 160 ms, respectively, through timing information provided by photodiodes. The photodiodes were not visible to subjects during stimulus presentations.

We pre-processed our data according to the recommendations of Gross et al. (2013). All data were visually inspected for head movement and environmental noise artifacts, which were corrected during analysis through the use of signal-space projectors (SSPs; Tadel et al., 2011). A notch filter of 60 Hz was applied to remove powerline contamination. Heart and eye movement artifacts were detected from the EOG and ECG electrodes, respectively. We calculated SSPs from segments of data centered around the artifacts of interest. SSPs were defined following principal component analysis of artifact-contaminated segments, filtered between 10–40 and 1–15 Hz for heartbeats (150 ms segment duration) and eye-blinks (400 ms segment duration), respectively. We rejected 13/1600 and 81/1600 trials from the neutral and positive conditions, respectively, which allowed us to retain >95% of the collected data. We bandpass filtered our data between 0.5–1 (high-pass) and 40–46 Hz (low-pass) using a linear phase finite impulse response filter with stopband attenuation at 60 dB. We down-sampled our data to 400 Hz for analysis, which retained a frequency resolution of 0.012 Hz. We epoched our data into 2400-ms windows [−200, 2200 ms], centered around CS [0 ms] and US [1170 ms] onsets, for analysis of ERCs and alpha power.

Source Estimation

We produced a MEG head model to estimate the cortical sources underlying the magnetic fields detected by the sensors during recording. The forward model was computed from a default cortical surface representation with 15,000 vertices using the overlapping-spheres analytical method (Huang et al., 1999). We derived MEG source maps from the weighted minimum-norm estimate available in Brainstorm using default parameters (Baillet et al., 2001). Noise covariance statistics were derived from a 200-ms pre-CS [−200, 0 ms] baseline taken across trials. To account for inter-subject variability, the orientation of elementary cortical current dipoles was not constrained to the cortical surface template. Source maps were produced from trial averages for each condition (neutral X positive). **Figure 3** illustrates cortical activations following CS/US onsets.

RESULTS

MEG Correlates of US-to-CS Valence Transformations

ERCs

We first defined sensor clusters of interest over ten cortical sites. These included left (LF = 32 sensors) and right frontal

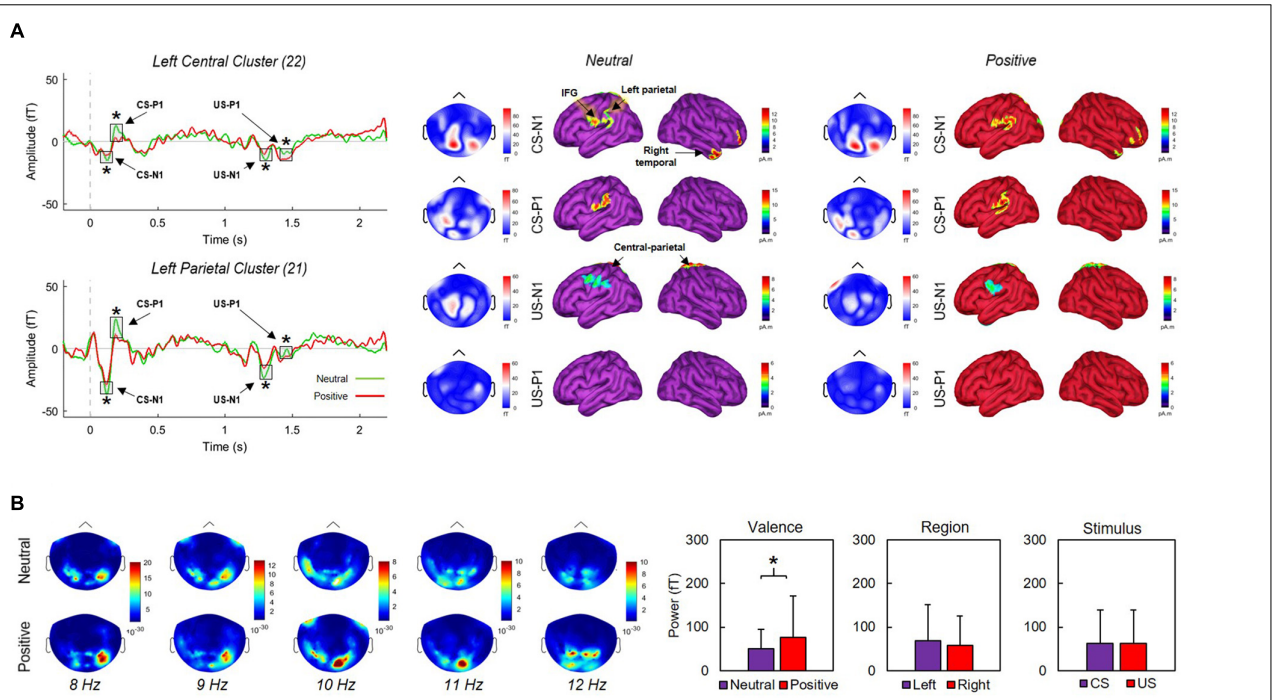

FIGURE 3 | (A) Grand averaged activity over left central and parietal clusters over a 2400 ms window (left) and corresponding source activations during neutral (middle) and positive (right) blocks. CS and US onsets were at 0 and 1170 ms, respectively. Early negative (N1) and positive (P1) event-related components produced by CS and US onsets differed significantly between neutral and positive conditions (asterisks indicate p's < 0.02) across the displayed clusters. **(B)** Mean activation maps across five 1-Hz bands corresponding to the alpha range. Alpha power did not vary significantly between left and right regions, or between stimulus types. Significantly greater alpha ($p = 0.001$) appeared during positive conditions only. Error bars indicate SDs.

(RF = 32), parietal (LP = 21, RP = 21), central (LC = 22, RC = 22), temporal (LT = 32, RT = 32), and occipital (LO = 18, RO = 18) sites. Second, we extracted absolute peak/trough amplitudes across each cluster along epochs. Third, we identified N1 and P1 components within 80–130 (CS-N1) and 170–220 ms (CS-P1) of CS onset, and within 90–140 (US-N1) and 200–250 ms (US-P1) of US onset, following inspection of ERC topographies over parietal regions. Finally, we contrasted each component between neutral and positive conditions using Welch's t-tests. We found significant effects for ERCs over left-central (LC) and left-parietal (LP) clusters (**Figure 3**). Over the LP cluster, N1 components were significantly more negative-going during neutral conditions for CS, $t(30.8) = 3.97$, $p < 0.001$, $d = -1.22$, and US, $t(38.9) = 5$, $p < 0.001$, $d = -1.54$; P1 components were significantly more positive-going during neutral conditions for CS, $t(32.6) = 12.53$, $p < 0.001$, $d = 3.87$, and US, $t(36.8) = 14.83$, $p < 0.001$, $d = 4.58$. Similar modulations were observed over the LC cluster; N1 components for CS, $t(34.7) = 2.56$, $p = 0.015$, $d = -0.79$, and US, $t(39.9) = 3.21$, $p = 0.003$, $d = -0.99$, were significantly more negative-going during neutral conditions. P1 components for CS, $t(32.2) = 13.45$, $p < 0.001$, $d = 4.15$, and US, $t(32.9) = 8.24$, $p < 0.001$, $d = 2.54$, were significantly more positive-going during neutral conditions. Finally, we found significant N1/P1 effects over our right parietal cluster following US onsets (all p's < 0.001), but not CS onsets (all p's > 0.05). None of our remaining contrasts reached significance.

Alpha Oscillations

We computed power spectral densities (PSDs) over 2400-ms windows (−200 to 2200 ms where CS onset was at 0 ms) using Welch's method with a 1000-ms sliding window and 50% overlap along and across individual trials from each condition (positive X neutral). Cortical maps illustrating the location of peak alpha power across individually defined bands between 8 and 12 Hz are presented in **Figure 3**. We extracted PSDs in the alpha band from sensor time series over 0–1000 and 1000–2000 ms windows, corresponding with CS and US onsets, respectively. The 40 selected sensors were located over the left (20) and right (20) parietal sites. We ran three Welch's t-tests to determine whether PSDs significantly differed as a function of stimulus type (CS, US), region (left, right), and/or valence (neutral, positive). We found no significant effects for region ($p = 0.237$) or stimulus-type ($p = 1$). For valence, PSDs during positive conditions (76.3 ± 95.7) were significantly greater, $t(236.4) = 3.22$, $p = 0.001$, $d = 0.35$, than PSDs during neutral conditions (50.1 ± 44.6). Conducting similar contrasts over other traditionally defined frequency bands (beta, theta, gamma) produced no significant differences between conditions (all p's > 0.05).

Measures of Appetite Motivation

Salivary Volume

We contrasted mean ± SD grams of saliva produced after CS were linked with neutral US (2.96 ± 1.54 g) with saliva produced

after CS were linked with positive US (4.47 ± 2.54). Subjects produced significantly more saliva after positive conditioning blocks, $t(31.3) = 2.27$, $p = 0.03$, $d = 0.72$ (**Figure 4**). Saliva produced by subjects before conditioning (5.7 ± 5.38) was not included in our analysis for two reasons: first, subjects provided a single pre-conditioning saliva sample, but four post-conditioning saliva samples (two neutral, two positive). Second, as the samples were collected before conditioning, they were unrelated to our procedural valence manipulations.

Oral Ratings

Subjects orally rated four activities at the end of each block. Two activities were related to our CS (eating, sleeping). Remaining activities were distractors (running, reading) that were not used during conditioning. Higher scores correspond with increased preference. CS preference between positive (4.08 ± 1.89) and neutral (3.7 ± 1.88) blocks did not significantly vary ($p = 0.376$). Preferences for distractors between positive (5.25 ± 0.38) and neutral (5.5 ± 2.57) blocks did not vary ($p = 0.655$). See **Figure 4**.

Motivation Check

Subjects responded Yes/No to the questions "Are you getting hungrier (sleepier)?" after each conditioning trial. We found the mean proportion of Yes responses between positive (0.62 ± 0.49) and neutral (0.57 ± 0.5) blocks was significantly different, $t(2553.2) = 2.48$, $p = 0.013$, $d = 0.09$. Response times (RTs) during positive (1274 ± 667.5 ms) and neutral (1531.1 ± 865.9) significantly differed, $t(2155.5) = 8.62$, $p < 0.001$, $d = 0.34$. See **Figure 4**.

CS Visibility

Subjects selected from two verbs during the visibility check 2AFC across each trial. A response was scored as a (correct) hit if subjects accurately identified the verb that had previously appeared as a CS. Subjects produced significantly more hits during (0.47 ± 0.5) relative to neutral (0.43 ± 0.5) conditions, $t(2594.7) = 2.08$, $p = 0.038$, $d = 0.079$, although the number of correct detections across both conditions was lower than would be expected by random chance (0.5). RTs did not significantly differ between neutral (1188.8 ± 691.6 ms) and positive (1231.7 ± 692.7 ms) conditions ($p = 0.101$). See **Figure 4**.

DISCUSSION

Our study linked eating- and sleeping-related CS with neutral and positively valenced US across four blocks of CS-US conditioning trials. The CS were presented subliminally and not reliably identified beyond chance levels. Analysis of time-resolved electrophysiological components showed significant valence-associated disassociations of stimulus-evoked cortical activations within 200 ms of CS onset, i.e., before the expected engagement of lexical processing. These disassociations were significant over left parietal and central regions of interest, which are involved in lexical-semantic comprehension (Binder, 2017). We found alpha activity over parietal regions to be significantly more pronounced during positive conditioning trials. Finally, we observed greater production of saliva and Yes responses (to the questions *Are you getting hungrier/sleepier?*)

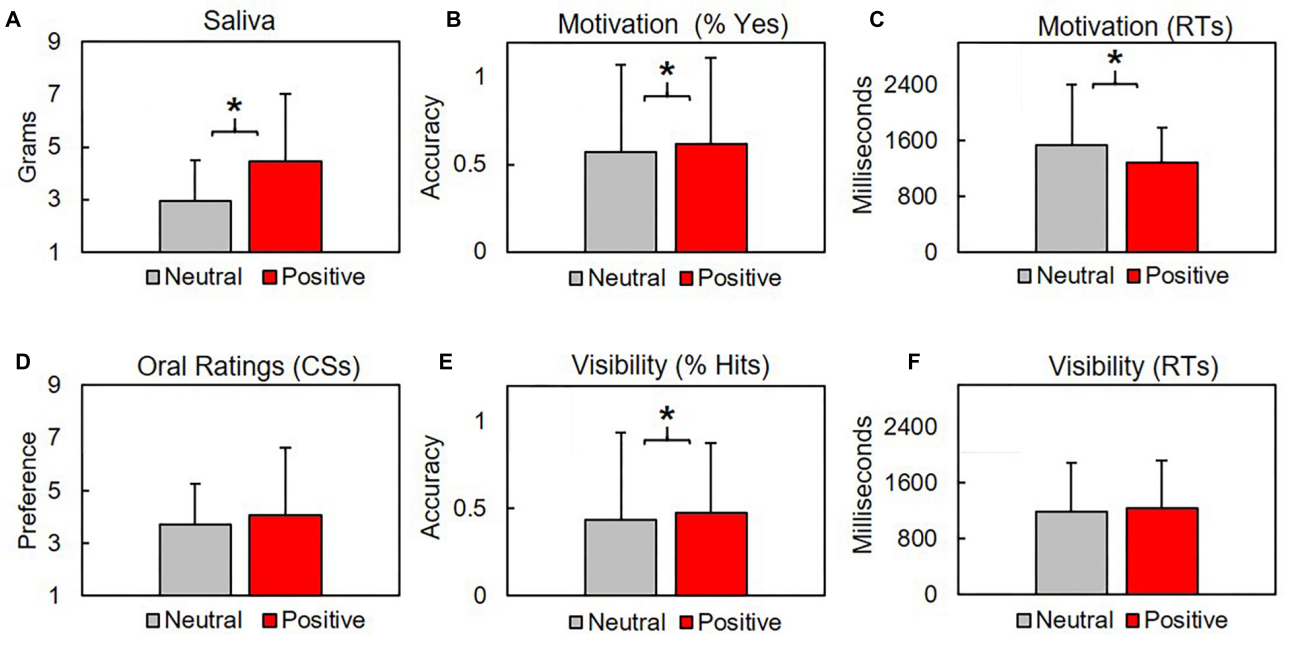

FIGURE 4 | Subjects produced significantly more saliva after positive conditioning blocks **(A)**. Subjects responded *Yes* significantly more times **(B)** and faster **(C)** in response to the question "Are you getting hungry?" during positive conditioning trials. Oral preferences did not differ between conditions for CS **(D)** or distractors (not shown), where higher values indicate increased preference (*y*-axis). CS were identified significantly more times during positive conditioning trials **(E)**, although hit rates across both conditions fell below chance levels. Response times for visibility checks did not vary across conditions **(F)**. Asterisk indicate *p*'s < 0.03.

following blocks of positive conditioning trials. Only orally provided preferences did not vary between conditions.

Our results show that subliminally enhanced CS valences can augment CS-associated motivational states, as previously claimed by Aarts and Custers (2012). Our study is the first to demonstrate the disassociation of valence-associated ERCs following the presentation of *subliminal* CS and supraliminal US within pre-lexical windows. We claim that the absence of effect across our oral reports actually supports our initial hypothesis. Indeed, our oral preferences task was designed to be biased by self-narratives: they maximized the possibility of participants deliberating (generating narratives) prior to responding. This interpretation is compatible with the observation that it took ∼4 s between presentation of a question during the oral ratings task (*How hungry are you?* for example) and the contingent response. For comparison, our second behavioral measure of motivation produced RTs under 2 s and was far less likely to have been driven extensively by deliberative processes. This illustrates how CS-associated narratives can mitigate explicit CS–US valence information.

Our framework posits that (i) motivational states, like hunger, can be significantly modulated through the construction and integration of self-generated emotionally salient narratives (Peterson, 2002; also see Van Vugt et al., 2018), (ii) the representational content from which self-narratives are constructed is a function of externally available relational information and earlier learning histories (Osgood, 1980; Bar-Anan and Moran, 2017; Fields and Arntzen, 2018), and (iii) external valence information can be encoded without a perceiver's complete awareness of the valence-specifying stimulus/relation perceived (Kouider et al., 2010). We found physiological evidence in support of this hypothesis, given that observed physiological effects incorporated semantic and motor regions (Boulenger et al., 2008).

Our present findings corroborate embodied-cognition perspectives that propose how language comprehension relies on the internal reenactment of sensorimotor activations associated with a specific stimulus (Vigliocco et al., 2009). The embodiment of stimulus-associated response mechanisms resembles earlier neo-associationist accounts of symbolic behavior, where fractional stimulus-response (sG-rG) associations exclusive to a representational class were hypothesized to probabilistically mediate response tendencies through spreading activation mechanisms, such as those involving stimulus convergence, response divergence, and secondary generalization (Hull, 1930; Berlyne, 1965; Osgood, 1980).

These findings also provide a roadmap for possible future intervention strategies to address (for example) eating disorders resistant to explicit valence information, such as anorexia nervosa (Merwin et al., 2010). Taken together, our results suggest that urges to engage in pathological eating practices may be mitigated through preventing the conscious perception of eating-associated stimuli/cues (Custers and Aarts, 2010). Future research could extend these findings to subjects prone to "emotional eating" (Hensels and Baines, 2016) to determine whether subliminally augmenting the valences of eating representations motivates actual eating, and whether such motivations may be transitory or cumulative (e.g., Mattavelli et al., 2017). If the latter, the logical next step would be to adapt the proposed procedure for use with anorexic individuals, whose maladaptive belief systems regarding eating constitutes a core element of their pathology (Merwin et al., 2010).

We conclude our discussion by noting some potential limitations of the current design. First, neutral and positive US appeared in separate blocks instead of being mixed within the same block. In fact, US were always visible to subjects, whereas CS were visible only in 50% of trials or less. We reasoned that if positive and neutral US had appeared during the same block of trials, and these were the only items subjects could consciously identify, then their simple co-occurrence (e.g., *gray* with *pleasant*) could have consequated the unprovoked derivation of a relational qualifier between the differentially valenced terms (e.g., *gray* "co-occurs with" *pleasant* – De Houwer, 2018, p. 5). In other words, neutral US could have transformed into valenced CS. By separating US across blocks, any potential US-to-US valence "contamination" effects were mitigated (Pastor et al., 2015). Future investigations could nevertheless use a mixed design to determine whether neutral US remain neutral. A second concern may be raised regarding the relatively small sample size (n = 10). In response, we point out that our ERC effects were quite robust, replicating earlier EEG findings (Amd et al., 2013; Bayer et al., 2017). We also report large effects for our saliva and performance-based motivation checks. The small sample size nevertheless is an important limitation that requires us to be cautious regarding any substantial interpretations – future replication studies should employ larger samples to determine the generality of the present findings.

One criticism may be our present focus on central-parietal ERCs, since earlier works have demonstrated valence-specific ERCs over frontal motor regions following the presentation of action words, although at time windows too late to reflect pre-lexical processes (Aarts et al., 2013, p. 969). Additionally, our US were non-action terms and thus not predicted to engage motor regions differentially during pre-lexical windows. Given that frontal/pre-frontal regions engaged by verb representations also employ central-parietal networks during pre-lexical windows (Hauk and Pulvermüller, 2004, p. 197), future works could employ valenced action words as US to determine whether pre-lexical differences appear over frontal motor regions (e.g., M1, BA6). This would illustrate whether the sensory-motor/fractional response components associated with activities may be augmented independent of their associated significate.

Another criticism may be leveled at our ascription of valence to CS given that subliminally presented stimuli do not reliably evoke valences isomorphic with the functions implied by the actual stimulus topographies/features (Kouider et al., 2010). For those concerned with that label, imagine "CS valence" as a summary description of the affective response components associated with redintegrated pre-lexical representations evoked by CS onsets. In support of the notion that our CS representations were *pre*-lexical, note that our examined effects appeared within 200 ms of stimulus onset, whereas lexical processing onsets within ∼250 ms of stimulus

perception (Schacht and Sommer, 2009; Palazova et al., 2011). Fragments of a percept may redintegrate into meaningful representations based on partial visual information, even if the reconstructed representations do not structurally cohere with the actual stimuli they represent (Kouider et al., 2010; Tonneau, 2013). Indeed, the time courses of our ERCs provide compelling evidence that bottom-up stimulus valence produced the observed effects (Kissler and Herbert, 2013).

A final issue can be raised regarding our interpretation of early ERCs as indicative of stimulus valence instead of, say, the engagement of attentional resources (Vogel and Luck, 2000). In contrast, we propose that *orienting* (non-volitional attending – see Maltzman, 1979; Amd et al., 2017) constitutes the initial affective discrimination toward valence gradients embedded in perceived stimulus objects, however minute such gradients may be (Lebrecht et al., 2012). From this perspective, early ERCs thought to reflect "selective attention, object recognition, and categorization" (Blechert et al., 2016, p. 16) are conceptualized as anticipatory response links pre-empting stimulus redintegration (Berlyne, 1965; Osgood, 1980). Specifically, orienting is the first non-volitional emotional response anteceding the start of a contextualized stimulus-response chain (Hull, 1930). The notion of a S-R chain anticipated by a valenced representation allows the theoretical separation of stimulus-evoked and narrative-associated valences. This is supported by the early time window of our pre-lexical ERCs, since higher order processing vis-à-vis attention/recognition/categorization takes more processing time and resources.

Regardless of one's preferred theoretical flavor, a majority of perspectives agree that the integration of self-generated and explicitly-provided valence representations manifest in actual, experienced valence (Peterson, 2002). When representations are action-oriented (as evoked by the verb "eating"), their evocations generalize to response mechanisms associated with said action (increased saliva production). Our study demonstrates how subliminally augmenting the valences of eating-related representations can significantly moderate associated motivational states.

AUTHOR CONTRIBUTIONS

MA designed the study and collected and analyzed the data. Both authors contributed toward the writing of the manuscript.

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Emotional Induction Through Music: Measuring Cardiac and Electrodermal Responses of Emotional States and Their Persistence

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Emotional inductions through music (EIM) procedures have proved to evoke genuine emotions according to neuroimaging studies. However, the persistence of the emotional states after being exposed to musical excerpts remains mostly unexplored. This study aimed to investigate the curve of emotional state generated by an EIM paradigm over a 6-min recovery phase, monitored with valence and arousal self-report measures, and physiological parameters. Stimuli consisted of a neutral and two valenced musical excerpts previously reported to generate such states. The neutral excerpt was composed in a minimalist form characterized by simple sonorities, rhythms, and patterns; the positive excerpt had fast tempo and major tones, and the negative one was slower in tempo and had minor tone. Results of 24 participants revealed that positive and negative EIM effectively induced self-reported happy and sad emotions and elicited higher skin conductance levels (SCL). Although self-reported adjectives describing evoked-emotions states changed to neutral after 2 min in the recovery phase, the SCL data suggest longer lasting arousal for both positive and negative emotional states. The implications of these outcomes for musical research are discussed.

Keywords: emotional induction, music, emotion, skin conductance level, heart rate, emotional persistence

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INTRODUCTION

Emotional responses have been reported as one of the primary motivations to listen to music (Schäfer et al., 2013; Shifriss et al., 2015; Reybrouck and Eerola, 2017), and several studies have shown that music can evoke genuine basic emotions, such as happiness, sadness, and fear (Västfjäll, 2002; Fritz et al., 2009; Egermann et al., 2015).

In this context, emotion is a set of homeostasis-related body alterations that involve changes in the brain activations (Damasio, 2004). Therefore, an effective emotional stimulus can trigger hormonal and autonomic responses that prepare the body for various actions or complex behaviours. The most common model of emotion used across studies is the valence–arousal since it enables a dimensional description (Eerola and Vuoskoski, 2011; van der Zwaag et al., 2011). The valence designates the extent to which an emotional stimulus is positive/happy or negative/sad. This dimension is usually assessed by self-report measures, while arousal refers to the degree of excitation

elicited by a stimulus in an individual, which produces a physiological measurable increase or decrease of the autonomic system activation. The arousal dimension is often measured also by self-reported measures and physiological parameters, for instance, skin conductance level (SCL) and heart rate (HR) response (Lang et al., 1997; Russell, 2003; Mauss and Robinson, 2009).

Specifically, the SCL is referred to as a reliable measure of the sympathetic nervous system (Boucsein et al., 2012). In fact, the SCL increment is associated with higher physiological arousal, which is usually caused by emotional stimuli (Lang et al., 1993; Kreibig et al., 2007). Furthermore, HR decelerations are associated with unpleasant stimuli (Palomba et al., 1997) and increased attentional responses. For instance, if a negative arousing stimulus is more attention demanding, it may elicit a deceleration of HR (Bradley and Lang, 2000; Sammler et al., 2007; Taelman et al., 2011).

Some studies have already benefited from physiological measures confirming the effectiveness of music in eliciting genuine emotions (Krumhansl, 1997; Khalfa et al., 2002; Etzel et al., 2006; Lundqvist et al., 2009; White and Rickard, 2016; Mori and Iwanaga, 2017). Krumhansl (1997) used a range of physiological measures, including HR and SCL, while participants were listening to 3-min songs, intended to induce sad, fearful, and happy emotions. The author found that Albinoni's Adagio in G Minor and Barber's Adagio pour Cordes songs, rated as sad excerpts by participants on a rating from 0 to 8 on valenced scales, produced slower HR, and decreased SCL in comparison to happy excerpts, namely La Primavera (Spring) from The Four Seasons (composed by Antonio Vivaldi) and Midsommarvaka (composed by Hugo Alfven). Congruently, Etzel et al. (2006) when using erudite music found HR deceleration during a sad excerpt with length varying from 74 to 189 s and HR acceleration during fear inductions.

Khalfa et al. (2008) investigated whether psychophysiological differences between happy and sad music would be attributable to tempo or rhythm variations. At a psychophysiological level, the authors showed that 1-min happy excerpts with the explicit instruction to participants focus on feeling the emotions evoked by the musical stimuli, were able to increase the *change values* signals (calculation of mean SCL activity during musical listening minus the mean signal of recovery phase) of SCL activity compared to the sad ones. However, no HR differences between sad and happy music were observed. According to the authors, more extended excerpts are more likely to induce HR differences in response to happy and sad music. This argument is explained by the musical expectations, which are based on the listener's prior experiences with music. Like language, music comprises perceptually discrete elements that are preorganized according to rules and emotional reaction happen when expectations are somehow disrupted in the excerpts compositions (Juslin and Västfjäll, 2008).

Consistently, Lundqvist et al. (2009) investigated the impact of happy and sad pop songs on a variety of physiological measures, including HR, and SCL. In this study, they noticed that the happy song elicited significantly greater SCLs than the sad one. Regarding the HR, a deceleration–acceleration activity pattern was observed at the beginning of the emotional stimuli, regardless of its valence. The authors explained these HR findings as an orienting response that might be associated with a shift on the participant's attention toward the task.

When it comes to the musical features that may convey emotions to listeners, some authors have advocated that the autonomic responses underlying the emotional response induced by musical stimuli could result from intrinsic and/or extrinsic musical properties (Sloboda and Juslin, 2001). The intrinsic properties of music are those that elicit an emotional response in the listener as a direct consequence of musical structural features, such as intensity, tempo, and mode. The extrinsic properties are the emotional responses elicited as a consequence of the association of the musical structure with previous personal events, experiences, or contextual associations of the sound, i.e., how subjects relate music to a particular setting, context, or circumstance (Sloboda and Juslin, 2001). Focusing on the intrinsic properties, music evaluated as happy is typically composed in major mode and fast tempo, while sad music is slower and written in the minor tone (Hodges, 2010; White and Rickard, 2016; Taruffi et al., 2017). Congruently, some studies found positive correlations between valence–arousal parameters and musical structures (Balkwill and Thompson, 1999; Egermann et al., 2013).

Nevertheless, fast *tempo* is often related to highly arousing music, while a piece of slow *tempo* music is linked to low arousal, and these different rhythmic patterns would influence the listener emotional perception (Gabrielson and Juslin, 2003). Physiological functioning tends to mimic the musical expression, i.e., the rhythmic patterns, and through afferent physiological feedback, it elicits the emotion conveyed by the *tempo* (Scherer and Zentner, 2001; Dibben, 2004). Then, specifically for music, the positive (happy) and negative (sad) emotions effects in arousal might depend on musical elements composing the excerpt and not only to the quality of the emotion.

Current studies also have found that sad songs can evoke tears and chills; in which tears were accompanied by HR acceleration, while chills showed to increase electrodermal activity and subjective arousal (Mori and Iwanaga, 2017). Additionally, women were reported as being more likely to feel chills with increased SCL than men (Panksepp, 1995). Nevertheless, sad songs can also be reported as unpleasant and not relaxing, which can produce increases in the SCL (Baumgartner et al., 2006; Nater et al., 2006). Moreover, the ability of sad music to generate pleasure or displeasure might be influenced by its esthetics and also by personality, mood, and learned associations of who listen to it (Sachs et al., 2015).

Assuming that music can generate happy and sad emotions, it is possible to argue that valenced music could also generate moods. In this perspective, the mood can be defined as a general affective background that persists over time without a specific stimulus (Beedie et al., 2005). To assess the musical influence on mood, Eich and Metcalfe (1989) produced continuous musical induction throughout a 90-min session, while participants performed an encoding and retrieval task. Mood states were measured throughout

different moments in the session. Results showed that, in the beginning, positive (or negative) songs made participants feel happy (or sad) as measured by self-report, and with repeated exposure, the mood manipulation lost some of its intensity. However, continuous music induction produced statistically reliable data since there were still differences between the positively and negatively induced participants at the end of the experiment, which show relatively stable changes by musical mood induction.

The literature on the persistence of the emotional effects after musical exposure on HR and SCL signals are scarce, yet critical. If an induced emotion persists after its induction, one may also expect to observe a variation in physiological responses of the subjects induced by music. One of the few studies investigating the persistence of emotions used four different valence–arousal film clips with its original soundtracks to induce positive high-arousal, positive low-arousal, negative high-arousal, and negative low-arousal. These mood inductions lasted 10 min; they were followed by a computer task (online shopping) aiming to observe whether induced emotions would last throughout the cognitive task. The authors reported lower SCL for the negative videos during the accomplishment of the task. In contrast, the HR results for those participants who watched the two positive videos did not present significant differences. Also, after approximately 9 min of the computer task, no self-reported arousal effects were observed (Gomez et al., 2009).

Another study carried out by Kuijsters et al. (2016), similarly selected sad videos with their original soundtracks as inductors for 10 min and subsequently assessed mood maintenance throughout 8 min at recovery phase, measuring emotional states through self-report, HR, and SCL. The results showed that SCL was above the baseline until the fourth minute of the recovery phase, and the HR results were similar to the baseline. The authors observed that the self-reported arousal decayed fast after the first minute and slowly returned to baseline. Conversely, the SCL suggested longer-lasting arousal effects, as such the above-quoted study (Gomez et al., 2009).

Although the previous two studies investigated the effects of emotional persistence, they included videos with soundtracks to induce emotion. As demonstrated by other authors, this junction of video and audio stimuli may lead to mixed emotional messages and elicitation, such as simultaneous positive and negative emotions, since it requires an added degree of elaborative processing when compared to non-combined stimuli (Koelstra et al., 2010).

According to Koelsch (2010), a particular advantage of using music to evoke emotions, in relation to other stimuli, is that it enables researchers to study positive emotions (e.g., fun and enjoyment) or negative emotions (e.g., sadness), which are challenging to evoke in experimental settings. Moreover, such emotions evoked by musical stimuli are meaningful to music therapy, and rehabilitation since music allows emotions to be experienced and communicated indirectly, i.e., without verbalization.

Despite the remarkable body of work providing evidence for the effectiveness of music in inducing emotions and moods (Krumhansl, 1997; Khalfa et al., 2002; Etzel et al., 2006; Lundqvist et al., 2009; White and Rickard, 2016; Mori and Iwanaga, 2017), little is known about how emotions evoked through music evolve over time. As shown by the cited studies, a significant difference in valence and arousal ratings after musical exposure is used as an indication of successful induction, but scarcely any quantification of its persistence has been detailed in the literature (Koelsch, 2010). For this reason, the present study aimed to investigate the persistence of sad and happy emotions evoked by an Emotional Induction through Music (EIM) procedure originally designed for the current study comprised by erudite instrumental music, and analysis of the progress of the emotions until a return to baseline after the EIM.

We hypothesized that the EIM procedure would be effective in inducing positive and negative emotions, happy and sad, respectively, taking into account self-reported mood responses and physiological measures. Specifically, positive (happy) emotions evoked through music should produce higher arousal, while negative (sad) emotions should cause lower arousal in contrast to neutral states. We expected to detect these differences by the physiological measurement, and consequently observe longer lasting moods during the recovery phase after both EIM procedures.

MATERIALS AND METHODS

Participants

Thirty-eight Portuguese college students participated in this investigation in exchange for partial course credit. They were screened and ruled out if they reported any history of neurological or psychiatric disorders or severe levels of depression or anxiety, or hearing or visual impairments that could not be corrected. Four people out of 38 participants were ruled out: two of them presented high levels of anxiety; the other two were excluded due to procedural problems. The final sample included 34 healthy participants, as displayed in **Table 1**, with a mean age of 23.38 (SD = 4.68; 18 females). None of them had received formal musical training and were naïve to the purpose of the experiment. This study was approved by the University of Minho Ethics Commission in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

TABLE 1 | Mean scores and standard deviations (in parenthesis) for screening.

Screening (n = 34)	Mean (SD)
Beck Anxiety Inventory	7.32 (4.87)
Beck Depression Inventory	4.68 (3.11)
Profile of mood states	
Tension	5.23 (3.42)
Depression	3.18 (3.51)
Anger	3.53 (3.56)
Vigor	13.32 (3.47)
Fatigue	5.29 (4.10)
Confusion	1.47 (2.03)
Total	105.38 (14.52)

Materials

Screening

Beck Anxiety Inventory (Beck and Steer, 1993). It is a 21-item self-report scale that measures the intensity of anxiety symptoms. The items must be assessed by the participant on a four-point scale: 0 – nothing (not at all); 1 – mild (mildly, but it didn't bother me much); 2 – moderate (moderately, it wasn't pleasant at times), and 3 – severe (severely, it bothered me a lot). The total score is calculated by the sum of each response item and can vary between 0 and 63. Higher total scores indicate more severe anxiety symptoms. The standardized Portuguese cut-offs are: minimal anxiety level: 0–10 points; mild level: 11–19 points; moderate level: 20–30 points; and severe level: 31–63 scores (Oliveira and Yoshida, 2009).

Beck Depression Inventory (Beck et al., 1996). The Beck Depression Inventory is a 21-item self-application instrument that measures depression severity symptoms in adults and adolescents at age 13 years or older. Items are measured on a four-point scale in which each answer is scored on a scale value of 0–3 with a maximum score of 63. Higher total scores designate severe depressive symptoms. The standardized Portuguese cut-offs are: minimal depression: 0–13 points; mild depression: 14–19 points; moderate symptoms: 20–28 points; and severe depression: 29–63 points (Gomes-Oliveira et al., 2012).

Profile of Mood States (McNair et al., 1992). It contains 42 adjectives that measure six mood dimensions of adults aged 18 years and older: tension, depression, anger, vigor, fatigue, and confusion. The adjectives are assessed on a scale value of 0–5 points. The Tension (T) dimension is composed of adjectives describing increases of musculoskeletal tension and preoccupations; Depression (D) represents an emotional state of discouragement, sadness, unhappiness, and loneliness; Anger (A) dimension corresponds to a mood of anger and dislike compared to the others; Vigor (V) is related to a physical energy state and psychological vigor; Fatigue (F) represents a state of tiredness, inertia, and low energy; and finally, Confusion (C) is characterized by a state of confusion and low lucidity. Moreover, a total mood disturbance result is calculated adding the five negative dimensions (T+D+A+F+C), subtracting the Vigor scale result, and summing a constant of 100 to avoid a negative result. Normative values for the Portuguese population were published by Viana et al. (2001).

Emotional Induction Through Music Procedure (EIM)

The emotional induction procedure was carried out with erudite music selected from previously published works (Västfjäll, 2002; Grewe et al., 2010). However, these studies did not specify the arousal levels of songs. Besides, no valence–arousal standardized ratings of erudite music were published for the Portuguese population. For this reason, we carried a pilot study including three positive, four negative, and seven neutral excerpts that allowed us to identify which musical excerpt was the most effective in each condition (provided in the **Supplementary Material**).

Musical excerpts

The length of each music excerpt was precisely 3 min, and each musical excerpt was edited using Audacity® 2.1.2 to avoid startling participants. "Fade out" and "fade in" were applied when necessary and normalized to the same Root Mean Square loudness level. The musical excerpt used for negative induction was *Albinoni "Adagio" composed in G-minor with 3/4 time signature*. For positive mood induction, we used *Bach "Brandenburg concert n∘2" composed in F-Major with 2/2 time signature.* The neutral excerpt was *"Steve Reich-Variations for winds, strings, and keyboard" composed in C-minor/C-flat, and B-major, with varied time signatures* (for more details regarding excerpts editions see **Supplementary Material**).

Valence–arousal self-report measure

Participants rated their emotional state on the valence dimension selecting one out of nine adjectives (adapted from Plutchik, 2001). There were three adjectives related to positive mood (*happy*, *excited*, and *euphoric*), three to negative mood (*sad*, *melancholic*, and *distressed*), each one corresponding to a low, medium, or high-intensity level, respectively. We also selected three adjectives to rate neutral mood (*neutral, indifferent*, and *unresponsive*)[1]. Although the valenced adjectives were rated as low, medium, and high intensities, participants were not informed about these intensities.

The arousal dimension was measured immediately after valence, in which participants were requested to rate the arousal of the previously selected adjective on a 7-point analog scale about how they felt at that moment. One corresponded to "I feel very little aroused" and 7 to "I feel very much aroused." Moreover, to assess ratings of musical pleasantness, a 7-point scale was also included, with 1 meaning unpleasant and 7 meaning very pleasant. All self-reported measurement were computer-based administered using SuperLab 4.5 Software (Cedrus Corporation, San Pedro, CA, United States).

For this study, induction was achieved whenever participants chose one of the three adjectives congruent with the EIM condition (e.g., "this song made me feel . . . sad," and the condition of the EIM was negative/sad music). On the other hand, unachieved induction refers to incongruent responses to the induction condition (e.g., "In general this song made me feel . . . neutral," and the condition of induction applied was a positive/happy excerpt).

The mood self-report measure was complemented with physiological measurements, i.e., the cardiac (measured by the heart rate – HR) and electrodermal (measured by the skin conductance level – SCL) responses, which are non-invasive measurements.

Psychophysiological Measures

A sampling rate of 1000 Hz was chosen for both HR and SCL channels. The psychophysiological measures were recorded using the wireless BioNomadix System (BN-PPGED module for the SCL and BN-RSPEC module for the HR) (BIOPAC Systems Inc.,

[1]Portuguese words for positive (feliz, entusiasmado, and eufórico), negative (triste, melancólico, and angustiado), and neutral (neutro, alheio, and indiferente) self-report.

Santa Barbara, CA, United States). The acquisition system was connected to a computer running AcqKnowledge 4.4 software.

Skin conductance level

The disposable silver/silver chloride electrodes (BIOPAC Type EL 507) were attached to the palmar surface of the medial phalanges of the index and middle fingers of the non-dominant hand. Then, the BioNomadix transmitter was placed on the participants' non-dominant wrist by the experimenter. The transmitter passes a constant voltage of 0.5 V between the two sensors and transfers the difference in charge (i.e., the conductance afforded by the sweat glands on the palm) back to the BioNomadix data acquisition unit. The raw SCL data were filtered using the recommended standard filter settings for the acquisition device, an FIR low-pass Blackman filter of 1 Hz with the number of coefficients set at 4000 (Coutinho et al., 2017). Then, 1-min epoch means (for the 3 min during each MIP and the 6 min during recovery phase) were calculated for each participant and exported to SPSS for further analysis.

Heart rate

The HR measured in beats per minute was achieved from participants' raw Electrocardiogram (ECG) using an adjusted three-electrode Lead-II configuration. The HR electrodes were filled with an electrode gel that is specifically intended for use in the recording of bioelectrical potentials. The disposable Ag–AgCl electrodes (BIOPAC Type EL 503) were placed on the participants' left acromial and sternal end of the clavicle, and a third one on the left spine of the scapula and a transmitter was attached to a belt placed around the participants' thorax region. Before electrode placement, the skin was cleaned with alcohol and dried with cotton to diminish impedance and to improve signal quality. The raw ECG data were filtered using the recommended standard filter settings for the acquisition device, an IIR high-pass filter of 1 Hz and an IIR low-pass filter set at 35 Hz (Coutinho et al., 2017). HR was calculated offline from the filtered ECG trace using the Acknowledge 4.4 software. Furthermore, within-subject means were calculated based on 1-min epochs within each period of interest for the HR measure, such as for the SCL. The data were exported to SPSS for further analysis.

Procedure

The experiment was presented to the participants as a study to explore the physiological correlates of emotion and memory in adults, but they were not informed about the specific aims of the study to avoid biased responding. Besides, participants were told that physiological responses would be recorded during the tasks and received basic information on the psychophysiological devices (BIOPAC equipment). Then, participants were asked to sign a written consent form; fill out a demographic questionnaire; and the anxiety, depression, and mood scales.

The electrodes were placed and recording started subsequently. After a 3-min waiting period (Baseline) participants were asked to choose one of the nine adjectives (balanced per valences), followed by a 1–7 analogic scale to assess arousal (the valence–arousal self-report measure). The initial EIM instruction was then displayed on the computer screen, instructing participants to close their eyes as they listened to the song, to increase emotionality (Lerner et al., 2009). Next, they proceeded to the first of the three EIMs. The musical excerpts were presented via headphones at a comfortable volume level (below 60 dB). A valence–arousal self-report measure followed each EIM and by a 6-min silence (Recovery phase), in which participants were asked to wait in silence for the next task. During the recovery phase, participants were requested to evaluate their emotional state with the valence–arousal self-report measure every 1 min.

Once the first block (EIM plus recovery phase) was completed, participants were immediately induced for a second and third time (blocks 2 and 3) following the same steps as previously described. The experimental procedure is depicted in **Figure 1**.

The order of the three musical excerpts of the EIM was counterbalanced between subjects (negative–neutral–positive,

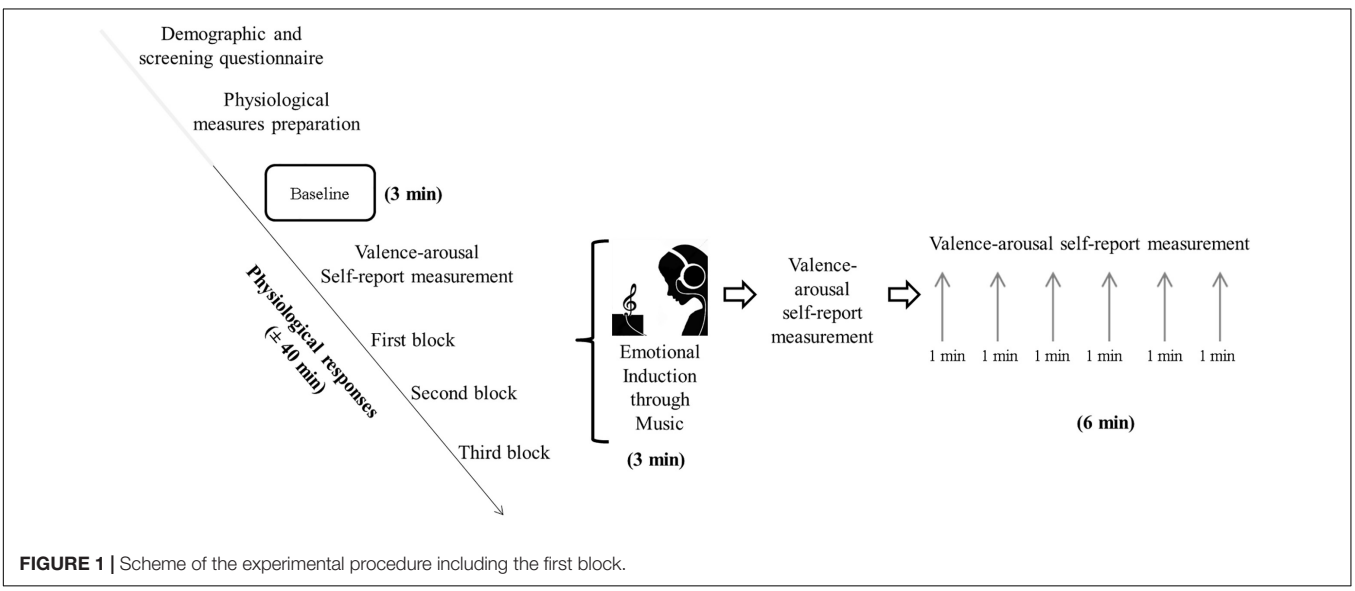

FIGURE 1 | Scheme of the experimental procedure including the first block.

neutral–positive–negative, or positive–negative–neutral). The physiological measures were continuously recorded during all moments of the procedure, and the total length of the experiment was around 40 min per participant. All participants were assessed individually.

RESULTS

We will first report an analysis of the valence–arousal self-reports for all the EIM conditions, including the number of participants congruently induced by the EIM procedure, to observe its effectiveness. Second, we will report exploratory analyses of the valence–arousal self-reports data immediately after each EIM condition and after each minute of recovery phase for those participants induced congruently, and the SCL and HR results during baseline and each EIM condition. Finally, we will report valence–arousal self-reports, SCL, and HR data of those participants induced congruently on the positive and negative conditions of the procedure and the results during the 6-min recovery phase.

EIM Efficacy

According to the valence–arousal self-report measurement, it was possible to detect that positive and negative EIM conditions were effective since they achieved more than 79% of congruent emotional responses. The neutral EIM condition was not effective since only 32% of participants were induced. The effectiveness of each EIM condition is displayed in **Figure 2**.

Thus, we observed that only four participants were congruently induced across the three conditions, while the other 24 participants were induced congruently by the positive and negative EIM conditions. After the exclusion of non-induced participants, we certified that the conditions were counterbalanced to avoid presentation order effects. We carried out two separated sets of exploratory analyses: the first included the SCL and HR responses for the four participants congruently induced across the positive, negative, and neutral EIM conditions, and the second comprising the SCL and HR responses for those subjects inconsistently induced by the positive and negative EIM in order to observe possible variations in physiological signals.

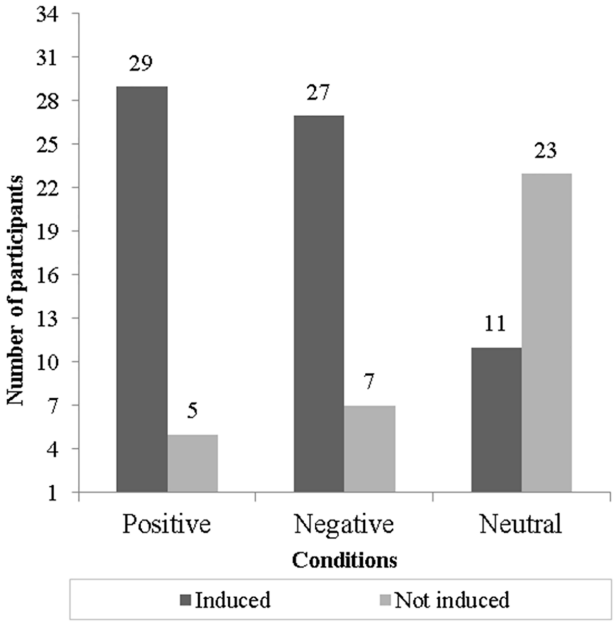

FIGURE 2 | Induction effectiveness for each condition (bars show the number of participants "induced" or "not induced" congruently to each one of three conditions).

Valence–Arousal Self-Report, SCL, and HR Responses for Participants Congruently Induced Across EIM Conditions

Table 2 displays the valence–arousal self-report descriptive results for the four congruently induced participants and shows that the adjectives related to positive and negative EIM conditions change quickly for the neutral ones after the first minute, while the neutral EIM adjectives are constant over time.

Nevertheless, non-parametric tests were used because the data did not assume normal distributions or homogeneity of the variances. The Friedman test rendered no significant results for the HR responses, $\chi^2(3) = 0.60$, $p = 0.89$. However, we observed a significant difference between conditions for the SCL responses, $\chi^2(3) = 10.20$, $p = 0.02$. The *post hoc* non−parametric Wilcoxon signed-rank test was used to compare the baseline scores with each EIM condition reported with a Bonferroni correction for multiple tests resulting in a significance level of =0.016 (0.05/3).

As displayed in **Table 3**, the Wilcoxon signed-rank test showed that the EIM conditions did not elicit a statistically significant change in comparison to baseline [positive EIM: $W(4) = 2.74$, $Z = -1.83$, $p = 0.07$; negative EIM: $W(4) = 2.74$, $Z = -1.83$, $p = 0.07$; and neutral EIM, $W(4) = 2.73$, $Z = -0.73$, $p = 0.46$].

As expected, SCL and HR responses during the neutral EIM condition did not differ from baseline, as shown by the above Wilcoxon signed-rank test. The neutral excerpts were poorly rated as having a neutral valence, then we initially contrasted the positive and negative EIM conditions in further within-subject analyses.

Valence–Arousal Self-Reported, SCL, and HR Responses for Participants Congruently Induced in Positive and Negative EIM Conditions

First, we carried out a power analysis through G*Power 3.1.9.2 (Faul et al., 2007) to verify how many participants would be necessary to perform paired *t*-test analyses (two-tailed). This analysis indicated that with an $n = 24$ there would be sufficient statistical power ($\beta = 0.80$) to observe a significant effect ($\alpha < 0.05$) with a large effect ($dz = 0.60$). For this reason, we included 24 participants in the main analyses, which comprised participants congruently induced on positive and

TABLE 2 | Number of participants that remained induced and mean arousal ratings after EIM and during 6 min recovery phase assessed by the self-report.

	After EIM	1 min	2 min	3 min	4 min	5 min	6 min
Conditions (number of participants that remained induced)							
Positive	4	4	2	1	0	0	0
Negative	4	1	0	0	0	0	0
Neutral	4	4	4	4	4	4	4
Arousal (mean rates of the four participants)							
Positive	5	3.8	3.5	4	0	0	0
Negative	3.5	3	0	0	0	0	0
Neutral	4	3.8	4.5	3.8	4	4	4.5

TABLE 3 | Raw SCL and HR responses for the four participants congruently induced across the three EIM conditions.

	Baseline	Negative EIM	Positive EIM	Neutral EIM
Skin conductance level (μS)				
Participant 1	8.00	9.37	10.04	8.07
Participant 2	8.60	9.13	10.23	8.89
Participant 3	9.00	11.08	9.39	8.61
Participant 4	6.22	9.22	9.27	6.79
Heart rate (bpm)				
Participant 1	66.23	64.47	67.54	65.79
Participant 2	82.22	76.52	77.49	82.94
Participant 3	65.68	67.84	64.06	66.34
Participant 4	62.62	68.30	68.49	63.93

TABLE 4 | Mean and standard deviation (in parenthesis), and confidence intervals (CI) for participants' SCL (μS) and HR (bpm) raw responses for baseline, positive, and negative EIM.

	SCL (μS)	95% CI		HR (bpm)	95% CI	
		LB	UB		LB	UB
Baseline	6.87 (3.71)	5.30	8.44	76.06 (10.39)	71.67	80.45
Positive EIM	8.10 (4.32)	6.28	9.93	74.95 (9.13)	71.09	78.81
Negative EIM	8.29 (4.58)	6.35	9.12	74.50 (9.12)	70.64	78.35

LB, lower bound; UB, upper bound.

TABLE 5 | Mean and standard deviation (in parenthesis), and confidence intervals (CIs) for participants' SCL and HR change values on 3 min epoch of the positive and negative EIM.

	SCL (μS)	95% CI		HR (bpm)	95% CI	
		LB	UB		LB	UB
Positive EIM						
First minute	1.73 (2.48)	0.68	2.78	−1.60 (6.08)	−4.17	0.97
Second minute	1.11 (2.32)	0.13	2.09	−1.96 (6.08)	−4.53	0.60
Third minute	0.85 (2.23)	−0.09	1.80	−1.51 (5.54)	−3.85	0.83
Negative EIM						
First minute	2.04 (2.42)	1.02	3.06	−1.44 (5.59)	−3.81	0.91
Second minute	1.50 (2.25)	0.55	2.45	−2.32 (5.82)	−4.78	0.13
Third minute	1.02 (2.13)	0.12	1.92	−2.66 (6.22)	−5.29	−0.03

LB, lower bound; UB, upper bound.

negative EIM conditions (M_{age} = 24.12, SD = 5.11; 15 women), with self-reported arousal immediately after the induction as the dependent variable and positive and negative EIM as independent variables. The results revealed higher arousal ratings during the positive EIM (M = 4.67, SD = 1.24) over the negative EIM condition (M = 3.96; SD = 1.23), $t(23) = -2.24$, $p = 0.04$, $d = 0.46$, 95% CI [−1.36, −0.06]. These results reveal that the EIM procedure used in this study, beyond being effective in inducing

happy and sad emotions, which was demonstrated by the valence self-report, it was also accompanied by arousal differences.

Nevertheless, we also conducted power analysis before carrying out repeated-measures within subjects with three (baseline, positive, and negative EIM) and seven measurement times (baseline and 6 min of recovering phase) related to the analyses carried out in the induced emotion across time section, in which the G*Power (Faul et al., 2007) indicated for the first that with $n = 12$ and for the latter with a $n = 8$, there would be sufficient statistical power ($\beta = 0.80$) to observe a statistically significant effect ($\alpha < 0.05$) with an observed medium-to-large effect ($f = 0.40$). In this sense, we included in these analyses the 24 participants mentioned above.

It is important to note that participants underwent only one baseline followed by the counterbalanced positive and negative EIM. For this reason, we applied two independent one-way repeated measures ANOVA including in as independent variables baseline (baseline 3 min mean in silence), positive (EIM 3 min mean), and negative EIM (EIM 3 min mean), and as dependent variables SCL or HR raw responses, in order to confirm whether physiological responses were congruent with self-reported arousal ratings. The results regarding SCL raw responses showed significant differences, $F(2,46) = 6.62$, $p = 0.003$, MSE = 2.16, $\eta_p^2 = 0.22$, in which negative (Bonferroni; $p = 0.01$) and positive EIM 3-min mean (Bonferroni; $p = 0.04$) showed higher raw responses compared to baseline mean. These findings may be interpreted as a validation of the paradigm itself since it demonstrates the inductive effect of musical stimuli. On the other hand, no significant differences were found for HR raw responses mean, $F(2,46) = 2.58$, $p = 0.09$, MSE = 5.98, $\eta_p^2 = 0.10$ (**Table 4**).

For every participant's SCL and HR responses, we calculated the *change values* for each 3-min mean during EIM and each one of the 6 min in the recovery phase after the EIM conditions. Change values determine how much each individual changed during EIM in relation to their own baseline. To achieve the change value, we subtracted the baseline total mean from 3-min mean raw responses, of each EIM, e.g., positive EIM:

$$mean_{(\text{positive EIM})} - baseline_{(\text{total mean})}.$$

Similar calculations were carried out for the recovery phase, including each one of the 6 min of the recovery phase, after positive and negative EIM. These *change values* served as dependent variables for further analysis.

The effects of EIM conditions after starting musical presentation on SCL and HR *change values* were investigated by two mixed 2 (positive and negative EIM) × 3 (1, 2, 3 min) repeated-measures ANOVAs. A significant effect of minutes was found, $F(2,46) = 34.52$, $p < 0.001$, MSE = 0.32, $\eta_p^2 = 0.60$, for the analysis including SCL *change values*. Bonferroni pairwise comparisons showed higher SCL responses for the first EIM minute in comparison to the second EIM minute and the third minute ($ps < 0.001$). As well as between the second EIM to the third EIM minute ($p = 0.004$). However, no differences were found between positive and negative EIM, $F(1,23) = 0.85$, $p = 0.36$, MSE = 3.51, $\eta_p^2 = 0.03$, nor was there a significant interaction, $F(2,46) = 0.74$, $p = 0.48$, MSE = 0.21, $\eta_p^2 = 0.03$.

Concerning the HR change values, there was no effect of minutes, $F(2,46) = 0.89$, $p = 0.41$, MSE = 6.26, $\eta^2{}_p = 0.03$; condition, $F(1,23) = 0.54$, $p = 0.46$, MSE = 13.63, $\eta_p^2 = 0.02$; or interaction, $F(2,46) = 0.97$, $p = 0.38$, MSE = 5.32, $\eta_p^2 = 0.04$ (**Table 5**).

Moreover, to investigate gender differences between HR and SCL change values, we conducted exploratory statistical analyses using a non-parametric test, the Mann–Whitney *U*-Test, and no SCL *change values* differences between genders were observed.

The Induced Emotion Across Time

We carried out Friedman test analyses for the self-reported arousal rates before the EIM, immediately after the EIM, and after each minute of the recovery state. No differences were observed neither for positive EIM, $\chi^2(7) = 8.03$, $p = 0.33$, nor negative EIM $\chi^2(7) = 8.36$, $p = 0.30$.

Furthermore, **Figure 3** shows the subjective valence (adjectives reported) and arousal responses for each of the seven valence–arousal self-report measurements for both positive and negative EIM conditions of the induced participants. The valence of adjectives reported for both positive and negative EIM changes quickly to neutral ones after the first 2 min. Moreover, the graph with arousal self-reported rates shows the results of participants that remained induced (indicated the same valence adjective).

In order to investigate whether the influence of positive and negative EIM conditions remain during the recovery phase (i.e., at the six specific recovery phase moments – 1 min each), since the assumption of normality was not met across all six recovery phase moments, we performed the analyses with the non-parametric Friedman test, as can be seen below.

The SCL and HR baseline raw responses were compared to the recovery phase raw data after positive and negative EIMs. These analyses were done to confirm whether emotional activations continued throughout the recovery phase after the EIM procedure. The Friedman's test comparison showed that activations during recovery phase increased with statistical significance for SCL compared to baseline after positive, $\chi^2(6) = 31.56$, $p < 0.001$, and negative EIM, $\chi^2(6) = 29.05$, $p < 0.001$ (**Table 6**).

Post hoc analyses with Wilcoxon signed-rank tests were conducted with a Bonferroni correction applied, which resulted in a significance level set at $p < 0.008$. Increased SCL raw scores were detected for recovery phase after negative EIM compared to baseline for minute 1, $W(23) = 248.00$, $Z = 3.61$, $p < 0.001$; minute 2, $W(23) = 249.00$, $Z = 3.54$, $p < 0.001$; minute 3, $W(23) = 247.00$, $Z = 3.89$, $p < 0.001$; minute 4, $W(23) = 250.00$, $Z = 3.82$, $p < 0.001$; minute 5, $W(23) = 250.00$, $Z = 4.36$, $p < 0.001$; and minute 6, $W(23) = 250.00$, $Z = 4.64$, $p < 0.001$. Similar results were observed during recovery phase after positive EIM procedure in comparison between baseline and minute 1, $W(23) = 245.00$, $Z = 4.57$, $p < 0.001$; minute 2, $W(23) = 245.00$, $Z = 4.50$, $p < 0.001$; minute 3, $W(23) = 245.00$, $Z = 4.64$, $p < 0.001$; minute 4, $W(23) = 243.00$, $Z = 3.34$, $p < 0.001$; minute 5, $W(23) = 246.00$, $Z = 3.61$, $p < 0.001$; and minute

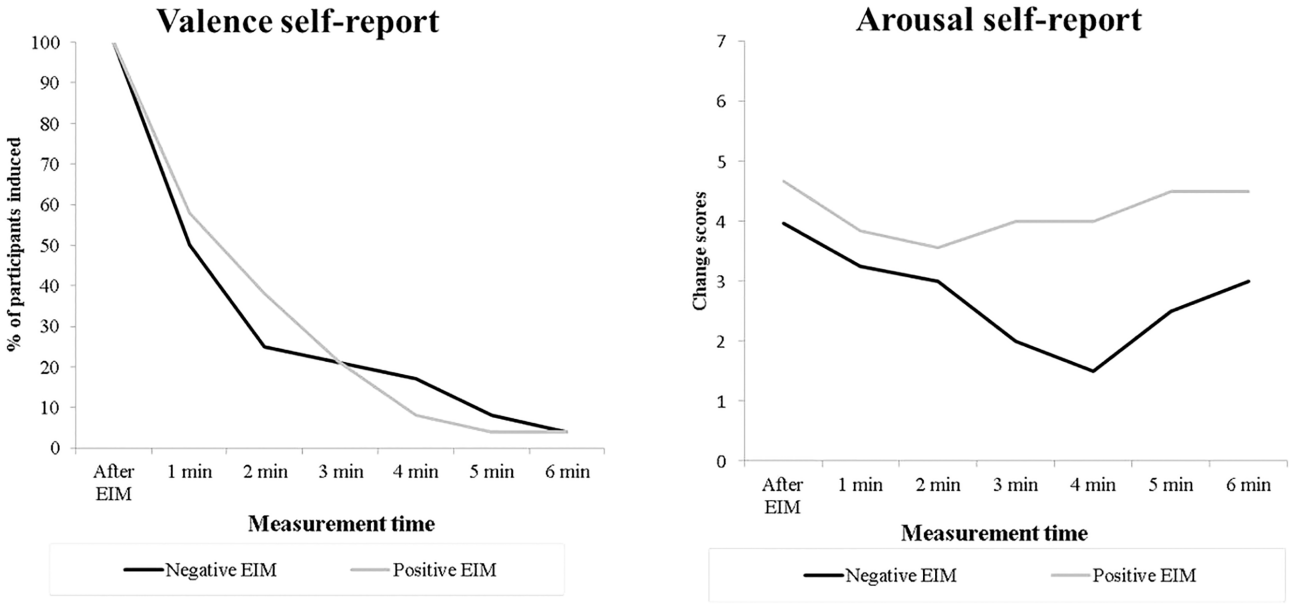

FIGURE 3 | Percentage of participants that remained induced (left) and arousal self-reported rates induced for induced participants (right) across measurement times.

TABLE 6 | Mean and standard deviation (SD) for participants' SCL and HR raw data in 6 min epoch for the entire duration of the recovery phase for positive and negative EIM.

Recovery phase min	SCL (μS)		HR (bpm)	
	Mean	***SD***	**Mean**	***SD***
Recovery phase per minutes after positive EIM				
First	8.64	4.64	75.93	8.55
Second	8.68	4.59	75.70	8.45
Third	8.71	4.70	74.96	7.99
Forth	8.70	4.87	75.31	8.80
Fifth	8.56	4.83	74.67	8.36
Sixth	8.71	5.04	75.37	8.36
Recovery phase per minutes after negative EIM				
First	8.45	4.98	76.87	9.02
Second	8.34	4.48	75.55	9.32
Third	8.39	4.42	76.25	9.83
Forth	8.40	4.42	76.06	9.45
Fifth	8.50	4.55	75.99	10.06
Sixth	8.63	4.53	76.68	9.44

6, $W(23) = 244.00$, $Z = 4.57$, $p < 0.001$. No significant results were found for HR raw responses in comparison to baseline, after positive $\chi^2(6) = 6.17$, $p = 0.40$ and negative EIM condition, $\chi^2(6) = 3.20$, $p = 0.78$.

Furthermore, the subsequent four statistical analyses were conducted separately, in which one included the SCL *change values* during the 3 min of positive EIM and their respective 6 min recovery phase, another with the 3 min of negative EIM and following recovery phase. The same pattern of analyses was carried out for HR *change values*. These statistical analyses did not identify any differences, neither for positive and negative EIM SCL *change values* compared to respective recovery phases (positive EIM, $\chi^2(6) = 1.84$, $p = 0.93$, and negative EIM, $\chi^2(6) = 4.36$, $p = 0.62$, nor for change values for HR, positive EIM, $\chi^2(6) = 8.61$, $p = 0.19$, and negative EIM, $\chi^2(6) = 4.01$, $p = 0.67$). This means that SCL and HR responses remained stable across all minutes of the recovery phase.

In summary, these results showed that positive and negative MMI conditions increased autonomic sympathetic activity assessed by SCLs, and activations remained observable during the 6 min recovery phase after EIM finishing for positive and negative EIM conditions.

The Neutral EIM

Exploratory analyses were carried out only for the neutral EIM condition in order to see if the results found in the intra-subject analyses would also be observed in the HR and SCL in an inter-subject analysis. Out of the total sample ($n = 34$), 12 participants reported positive valence adjectives, while 11 reported negative adjectives, and only 11 participants responded to the neutral EIM with neutral adjectives. Non-parametric analyses were carried out to compare arousal ratings, SCL, and HR responses as the dependent variable, between groups, those participants that felt positively, negatively, and neutrally induced by the neutral EIM.

A Kruskal–Wallis *H*-test showed that there was a statistically significant difference in arousal ratings for those participants that felt positive, negative, and neutral, $\chi^2(2) = 11.33$, $p = 0.003$, with mean rank arousal ratings of 19.25 for positive, 9.86 for negative, and 23.23 for neutral. Moreover, we conducted Mann–Whitney's *U*-tests to evaluate the difference in arousal ratings. To adjust for multiple comparisons using the Bonferroni

correction significance was considered for $p < 0.016$. We found a significant effect for positive and negatively induced groups and to negative and neutrally induced. The mean ranks of positive and negative induced were 15.42 and 8.27, respectively; $U(23) = 25$, $Z = -2.66$, $p = 0.008$, and the mean ranks of negative and neutral were 7.59 and 15.41, respectively; $U(22) = 17.50$, $Z = -2.90$, $p = 0.004$. These results showed that negative arousal ratings were lower than positive and neutral.

Regarding physiological measures, we carried out a Wilcoxon signed-rank test to compare SCL and HR baseline and during musical listening for each group positive, negative, and neutral. Results showed that positively induced ($M = 6.57$, $SD = 3.61$) participants had higher SCL responses compared to baseline ($M = 5.94$, $SD = 3.26$), $W(12) = 65.00$, $Z = -2.04$, $p = 0.04$, and the negatively induced group ($M = 9.00$, $SD = 4.92$) showed increased SCL responses in comparison to baseline ($M = 7.55$, $SD = 4.53$), $W(11) = 64.00$, $Z = -2.76$, $p = 0.006$, while the HR responses for the negatively induced group ($M = 77.98$, $SD = 11.56$) revealed lower HR in relation baseline ($M = 80.29$, $SD = 11.84$), $W(11) = 0$, $Z = -2.93$, $p = 0.003$. No significant differences were observed for participants that reported neutral adjectives to neutral EIM. These results reveal that SCL and HR responses are dependent on how participants interpreted the music, and not simply from musical listening *per se*.

Recovery Phase

With Friedman test, we compared baseline and every 6 min recovery phase, we found that only the negatively induced group showed higher SCL responses across 6 min, $\chi^2(6) = 25.55$, $p < 0.001$. A separate analysis for baseline and each minute, with the p-value set at $p < 0.008$ (Bonferroni correction), revealed that subjects had higher SCL responses in all recovery phase minutes compared to baseline (ps = 0.005). These results indicated that SCL responses were maintained, even if the adjectives reported were no longer congruent with the initially felt valence.

DISCUSSION

To the best of our knowledge, this is the first study investigating whether positive and negative EIM would evoke happy and sad emotions, respectively, assessed via valence–arousal self-report and autonomic activation measurements, specifically SCL and HR. The originality of the present study lies in the fact that we investigated the persistence of negative and positive emotions during 6 min of recovery phase after each EIM with no emotional instructions. Furthermore, we registered valence and arousal self-report together with SCL and HR physiological measures recognized as sensitive to emotion and emotional changes (Kreibig et al., 2007). The present study is a significant update on the existing literature because no previous study has examined how long the induced mood persists and how the mood would turn back to baseline. Besides, we investigated the effects and the persistence of emotions after a newly designed EIM procedure using songs that were tested regarding its capacity to elicit emotions, especially in Portuguese undergraduate students, instead just selecting musical excerpts from previous studies; otherwise, data generated in other countries with different cultural background could bias our results (Lim, 2016).

Consistent with our first predictions, participant's self-reported responses revealed that both positive and negative EIM conditions were effective in producing happy and sad emotions, respectively. Besides, higher subjective arousal responses were observed for the positive excerpt when compared to negative ones (Krumhansl, 1997; Etzel et al., 2006; Khalfa et al., 2008; Lundqvist et al., 2009). These findings contradict other studies using emotional stimuli, such as images (Yang et al., 2016). However, it is in congruence with Khalfa et al. (2002) that revealed that musical excerpts evoking fear and happiness are strongly arousing emotions, mainly due to its temporal structure.

Regarding the physiological measurement, the analyses for raw data and change values of HR activations did not show differences between baseline and post-induction, nor between positive and negative EIM conditions, only a tendency for HR deceleration. These results are in line with previous ones (Etzel et al., 2006; Khalfa et al., 2008; Lundqvist et al., 2009), which suggests that listening to musical excerpts was not an attentional demanding or a stressing activity to participants (Taelman et al., 2011). Other potential explanation may be related to the difference between these two autonomic responses. Although the literature has focused on the commonalities among the different physiological modalities (i.e., HR, SCL, cortisol, etc.) treating them as interchangeable measures of the physiological component of an emotion, the different physiological responses are mediated by the interaction between different brain circuits (Norman et al., 2014).

However, our results contrast with Krumhansl (1997) which also used the *Adagio in G minor* from Albinoni composer excerpt with a duration of 3 min. Then, after having cautiously observed the performed induction, we noticed that the author included another sad excerpt from Samuel Barber, the *Adagio for Strings*, *Op. 11*, which has a different minor tone, which, compared to *Adagio in G minor*, is much slower. We can suggest firstly that divergence in the characteristics of the excerpts might be crucial for HR results since musical features interplay in modulating emotions (Peretz et al., 1998; Sloboda and Juslin, 2001; van der Zwaag et al., 2011). Secondly, cross-cultural comparisons in the literature suggest that emotional responses can be quite differently felt by dissimilar musical cultures (Walker, 1996; Balkwill and Thompson, 1999). Finally, the participants in Krumhansl's study had formal musical training, which can alter the perception of the musical stimuli, consequently the emotions induced (Kawakami et al., 2013).

The SCL measurement revealed a clear differentiation between baseline (silence) and both positive and negative EIM. These results validate that listeners experienced the emotion reported rather than perceived the emotion in the music (Khalfa et al., 2002; Lundqvist et al., 2009). Nevertheless, our results partially replicate previous studies (Krumhansl, 1997; Khalfa et al., 2002; Khalfa et al., 2008; Lundqvist et al., 2009; van der

Zwaag et al., 2011), since the sad excerpt did not decrease SCL responses; instead, it revealed analogous activations of happy excerpt. This negative EIM SCL increase cannot be attributed to gender since both men and women obtained similar activations (Panksepp, 1995).

A critical observation of those studies that detected decreased SCL responses for negative EIM was that they explicitly instructed participants to feel in a specific way prior to the emotional induction. For instance, Lundqvist et al. (2009) in their procedure explained to participants the goals of their study. While Khalfa et al. (2008) and Krumhansl (1997) included in their design a training on how to respond emotionally to music. It is worthwhile to mention that previous investigations showed that telling the participants that they will be asked to rate their mood after an emotional stimulus presentation is sufficient to induce an expected emotional reaction when compared to no guidance before the emotional induction (Westermann et al., 1996).

Another aspect to be taken into account is the multi-faceted emotional experience, which underlies sad music since it is often described by participants as melancholic yet pleasant, and often as unpleasant. In the case of our sample, they felt the negative EIM as sad and unpleasant in congruence with previous studies (Baumgartner et al., 2006; Nater et al., 2006). Otherwise, it would be perceived as calmer and decreases SCL (Mori and Iwanaga, 2017). Future studies should include an investigation regarding pleasantness and chills of sad excerpts to have a broad view of the musical evoked emotions.

Regarding neutral EIM condition, mixed results related to valence were observed, in other words, neutral EIM generated positive and also negative emotions. This confirms the assumption that musical neutrality is rare (Krumhansl, 1997; Peretz et al., 1998) since people listen to music to change how they feel (Shifriss et al., 2015). Interestingly, our exploratory results showed that the subjective-report corroborated SCL since no differences were found just for those participants that responded that they were in a neutral state after hearing the "neutral" song. For this reason, we recommend experimenters to carefully test the participant's responses and consider for the neutral condition just those that were in a neutral state after listening to the song. Further studies should consider inquiring participants about thoughts generated during EIM to evaluate its valence, and consequently its influence on the evoked emotions.

This study was the first to examine the persistence of an EIM paradigm. According to our valence–arousal self-report, the valenced adjectives changed quickly to neutral ones after the second minute of the recovery phase, which is congruent with Gomez et al. (2009) and Kuijsters et al. (2016), both using videos. However, over time, SCL remained increased at least 4 min more for positive and negative EIM. One possible explanation for this longer-lasting arousal effect could be that, contrary to the physiological measures, the self-report rates are discrete measurements that cannot continually capture all the variations of the emotions felt (Mauss and Robinson, 2009) or participant's attention to the stimulus decreases.

According to our results, carefully selected positive and negative excerpts can be effective in inducing happy and sad states, respectively. However, positive and negative emotions seem to change quickly to the neutral state after musical exposure. It raises a concern about research undertaking emotion-induced effect on a subsequent cognitive task since hedonic adaptation modulates the emotional state continuously. Based on our findings, upcoming studies should investigate the effects of musical induction on following cognitive functions by applying a task lasting no more than 2 min. Otherwise, performance shall not be related to the emotions evoked by music. In addition, researchers should also report the cognitive task lengths, a factor rarely accounted.

The present study has certain limitations, which constraint the scope of our conclusions. Firstly, findings may be considered preliminary given that in some of the analysis the sample size was small, specifically for the physiological responses since they presented a significant variation among participants. However, we did our best to include in each analysis the number of participants estimated by the power analysis. Secondly, despite our control over musical stimuli and several individual characteristics, it is possible that individual differences linked to musical preferences, and the impact of personality on EIM could influence outcomes as both might be predictors of emotional elicitation through music (Sachs et al., 2015). In this sense, future studies should also address whether the musical preferences, personality, different ages would impact the persistence of emotions evoked by music, and how explicit valence–arousal reports might induce changes by themselves. Finally, the persistence of those emotions could also be assessed during diverse cognitive tasks, for instance with high and low cognitive demands, since it also seems to influence the persistence of evoked emotions (Ribeiro et al., 2018).

ETHICS STATEMENT

The research was approved by Institutional Review Board of the University of Minho (SECSH 009/2014) and was in accordance with the declaration of Helsinki. All Participants gave their written, informed consent prior to inclusion in the study.

AUTHOR CONTRIBUTIONS

FR and FS designed the study. FR and PO-S analyzed the data. FR drafted the initial manuscript. All authors contributed to the interpretation of the results, revised the manuscript, and approved its final version.

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SUPPLEMENTARY MATERIAL

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A Quantitative Account of the Behavioral Characteristics of Habituation: The Sometimes Opponent Processes Model of Stimulus Processing

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Habituation is defined as a decline in responding to a repeated stimulus. After more than 80 years of research, there is an enduring consensus among researchers on the existence of 9–10 behavioral regularities or parameters of habituation. There is no similar agreement, however, on the best approach to explain these facts. In this paper, we demonstrate that the Sometimes Opponent Processes (SOP) model of stimulus processing accurately describes all of these regularities. This model was proposed by Allan Wagner as a quantitative elaboration of priming theory, which states that the processing of a stimulus, and therefore its capacity to provoke its response, depends inversely on the degree to which the stimulus is pre-represented in short-term memory. Using computer simulations, we show that all the facts involving within-session effects or short-term habituation might be the result of priming from recent presentations of the stimulus (self-generated priming). The characteristics involving between-sessions effects or long-term habituation would result from the retrieval of the representation of the stimulus from memory by the associated context (associatively generated priming).

Keywords: habituation, priming, SOP, stimulus processing, stimulus intensity

INTRODUCTION

The predominant consequence of stimulus repetition is a systematic decrease in the frequency or amplitude of the response to the stimulus. When it is proved that this decrement is not caused by physiological changes at the sensory or motor levels, it is inferred that a learning phenomenon, known as habituation, has occurred. Habituation has been experimentally studied since the early twentieth century (Humphrey, 1933; Prosser and Hunter, 1936; Harris, 1943) and its core behavioral regularities were soon compiled by Thompson and Spencer (1966) and Groves and Thompson (1970) into a list of nine characteristics or parameters of habituation. This list has remained relatively uncontroversial and has oriented most of the research in the field over the years. Indeed, 40 years after the publication of these characteristics, a group of recognized researchers in the area gathered in a symposium where one of the goals was to revisit the empirical status of these features. With minor amendments and the addition of

one characteristic, the conclusion of the symposium was essentially confirmatory (Rankin et al., 2009; Thompson, 2009).

No similar agreement has been reached, however, concerning theories of habituation. Three approaches have dominated the field over the years: Groves and Thompson's (1970) dual process theory, Sokolov's (1960) comparator theory, and Wagner's (1981) Sometimes Opponents Processes model (SOP). Although there is not a plethora of choices, these theories have not been systematically compared. This is likely due, in part, to the fact that they differ in their level of formalization and emphasis on different subsets of empirical data. Certainly, these theories have each their respective merits (see, Mackintosh, 1987; Hall, 1991; Siddle, 1991 for critical reviews); but, in our opinion, only SOP is formulated with sufficient quantitative detail to make relatively unambiguous descriptions of a broad spectrum of phenomena and testable predictions.

In an early chapter, Whitlow and Wagner (1984) exposed in detail the potential of SOP on this topic. However, their analysis was more conceptual than quantitative. Alternatively, Donegan and Wagner (1987) and Wagner and Vogel (2010) presented a quantitative analysis of SOP, but they focused primarily on the kind of response decrement that might be attributed to associative factors. In this paper, we attempt to complement these efforts by evaluating the quantitative performance of the model on a relatively larger set of phenomena. We also propose possible instantiations of some mechanisms that were left unspecified in previous formulations of SOP.

In the first part, we briefly describe the major principles of SOP emphasizing those more closely related to habituation. We show the theoretical mechanisms by which the habituation of any stimulus can be understood as the result of two types of memorial priming: a transient memorial effect due to recent exposure to the stimulus (Davis, 1970; Whitlow, 1975; Vogel and Wagner, 2005) and a more persisting memorial effect due to the context carrying a relatively stable association with the habituated stimulus (e.g., Jordan et al., 2000). Then, we proceed to demonstrate, by computer simulations, how these mechanisms account for the 10 parameters of habituation accorded by Rankin et al. (2009). In the last part, we discuss the potential of the model to embrace the related phenomenon of sensitization, and we comment on the limitations of our current analysis.

THE SOP MODEL

The SOP model is described in more detail elsewhere (e.g., Wagner, 1981; Mazur and Wagner, 1982; Vogel et al., 2018), so we present only its essentials here. As shown in **Figure 1A**, the model states that the representation of any stimulus (i.e., "s") comprises a large set of elements that can be in one of three states of activity: inactive (I_s), primary activity ($A1_s$), and secondary activity ($A2_s$). Upon presentation of the stimulus, a proportion of the inactive elements are promoted to the $A1_s$ state according to the probability $p1_s$, which might be taken to be a function of the intensity of the stimulus. Once in the $A1_s$ state, the elements decay, first to the $A2_s$ state, with probability $pd1_s$, and then back to inactivity with probability $pd2_s$, where they remain unless a new presentation of the stimulus occurs. Thus, the momentary theoretical processing of the stimulus can be characterized by the proportion of elements in each of the three states, that is, by the vector (PI, PA1, PA2) where PI + PA1 + PA2 = 1 (Donegan and Wagner, 1987). It is assumed that the primary response to the stimulus is a function of P_{A1} and that P_{A2} might be either behaviorally silent or add to or oppose the primary response.

Let us consider the example depicted in **Figure 1B**, which exemplifies the momentary distribution of elements across the three states of activity over time after a single introduction of a 1-moment duration stimulus, with $p1 = 0.8$, $pd1 = 0.1$, and $pd2 = 0.02$. At the moment t_0, that is, before the presentation of the stimulus, all elements are in the I state; so, the activity vector is (1, 0, 0). At moment t_1, $p1$ elements move to the A1 state, leaving the activity pattern in (0.2, 0.8, 0), and in moment t_2, $pd2$ of these elements decay to the A2 state leaving the pattern in (0.2, 0.72, 0.08). Since the stimulus is only "on" at moment t_1, no further elements are promoted to A1 at any other time and thus PA1 declines very rapidly. Since the rate of decay from A2 to I, $pd2$, is five times smaller than the rate of decay from A1 to A2, $pd1$, PA2 persists for a longer period. With these standard assumptions, the consequence of the presentation of a brief stimulus is a rapid and transient increase in the proportion of the elements in the A1 state, followed by an increase in the proportion of elements in the A2 sate and by a very protracted return of elements to inactivity.

Notice in **Figure 1B** that there is a long period after the offset of the stimulus in which a substantial proportion of elements are in the A2 state. Indeed, only at moment 250, almost all elements have decayed back to inactivity, being, thus, just then eligible for reactivation in case the stimulus was presented again at this time. This is the reason why the A2 state can be regarded as a refractory state of activity. This is illustrated in the left-hand plot of **Figure 1C**, which depicts the theoretical activity that would be generated if the same stimulus of **Figure 1B** was repeated once at an interval of 32 moments. There, it is apparent that in the second presentation, the stimulus is less effective in provoking A1 activity, which reaches a peak of about half of the size of that of the first presentation. Generally speaking, the presentation of a given stimulus may have different effects depending on the momentary distribution of elements in the three states. Since the only consequence of presenting a stimulus is through $p1$, the stimulus will have greater efficacy in provoking A1 activity the greater is the number of elements in the inactive state and the lesser in the refractory state. This feature of SOP is a quantitative rendition of priming theory which states that "when an event is pre-represented ('primed') in short-term memory (STM) further corresponding stimulation is rendered less effective than it otherwise would be" (Pfautz and Wagner, 1976, p. 107). In the case depicted in the figure, this priming is occasioned by previous presentations of the same stimulus, so it is referred as "self-generated priming" (Wagner, 1976, 1978).

It is clear, thus, that self-generated priming is the primary mechanism by which SOP accounts for within-session decrements or short-term habituation. Of course, this is a transient effect

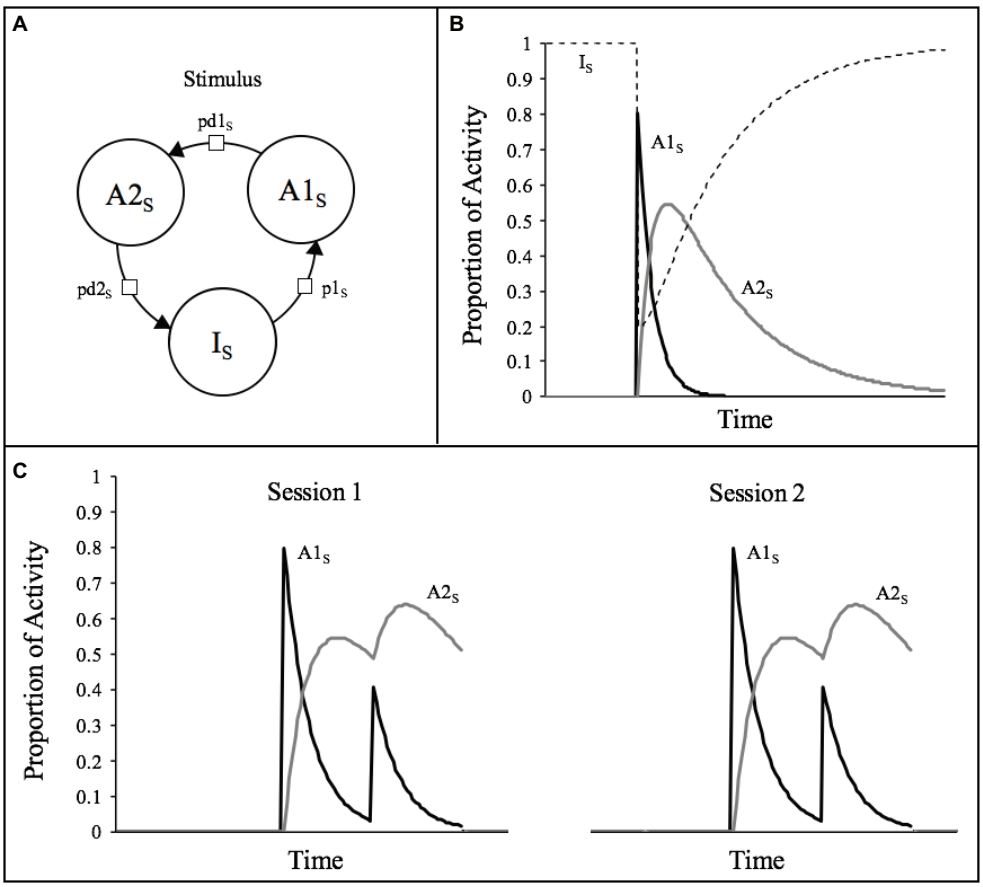

FIGURE 1 | (A) The three possible activity states for a stimulus representation assumed by SOP. **(B)** Courses of activity for a typical stimulus (s; duration = 1 moment, $p1_S = 0.8$, $pd1_S = 0.1$, $pd2_S = 0.02$) according to SOP rules of activity. **(C)** Simulations of the theoretical processes involved in two training sessions of two presentations of a 1-moment stimulus.

that disappears when sufficient time has elapsed from the last presentation of the stimulus (e.g., from one session to another). This is illustrated in the right-hand plot of **Figure 1C**, which reveals an almost total recovery of $PA1_s$ provoked by the first presentation of the same stimulus in a separate session.

Between-session effects or long-term habituation, on the other hand, require a different kind of mechanism that Wagner (1976, 1978) called "retrieval-generated priming." In this case, the supposition was that when a stimulus is repeatedly presented in a context, the context would act as a conditioned stimulus (CS) to develop an association with the habituating stimulus, which plays the role of the unconditioned stimulus (US). As the association grows, the stimulus becomes gradually more expected in the context and thus, primed, by the context. **Figure 2A** sketches how SOP conceives this by assuming that both the context and the stimulus activate a respective sequence of representational nodes, and that the context, *via* its association with the stimulus, acquires the capacity to promote elements directly from I_s to $A2_s$ *via* the variable $p2_s$. The assumption is that $p2_s$ is a function of the degree of primary activity of the context ($A1_{Ctxt}$), and the strength of the association between the context and the stimulus (i.e., $p2 = A1_{Ctxt} \times V_{Ctxt-s}$).

According to the learning rules of SOP, changes in the net association between a CS and a US are the result of excitatory minus the inhibitory associations that develop simultaneously depending on the respective states of activity of the stimuli. The development of excitatory CS-US links, $\Delta V+$, are assumed to be proportional to the momentary product of concurrent $A1_{CS}$ and $A1_{US}$ activity multiplied by an excitatory learning rate parameter, L+ (i.e., $\Delta V+ = L+ \times PA1_{CS} \times PA1_{US}$), whereas changes in the inhibitory CS-US connections, $\Delta V-$, are assumed to be proportional to the momentary product of concurrent $A1_{CS}$ and $A2_{US}$ activity, multiplied by an inhibitory learning rate parameter, L– (i.e., $\Delta V- = L- \times PA1_{CS} \times PA1_{US}$). In the standard procedure to get habituation, there are several repetitions of the habituating stimulus (US) in a distinctive context (CS), which seems to comply with the conditions of SOP for strengthening the association between them.

Although this may sound straightforward, context-stimulus associations are more theoretically challenging than they appear. Vogel et al. (2018) noticed that if the context is viewed intuitively as a long duration CS with a constant value of $PA1_{Ctxt}$ over the entire duration of the session, then SOP predicts no net association with the habituating stimulus. In this example, the

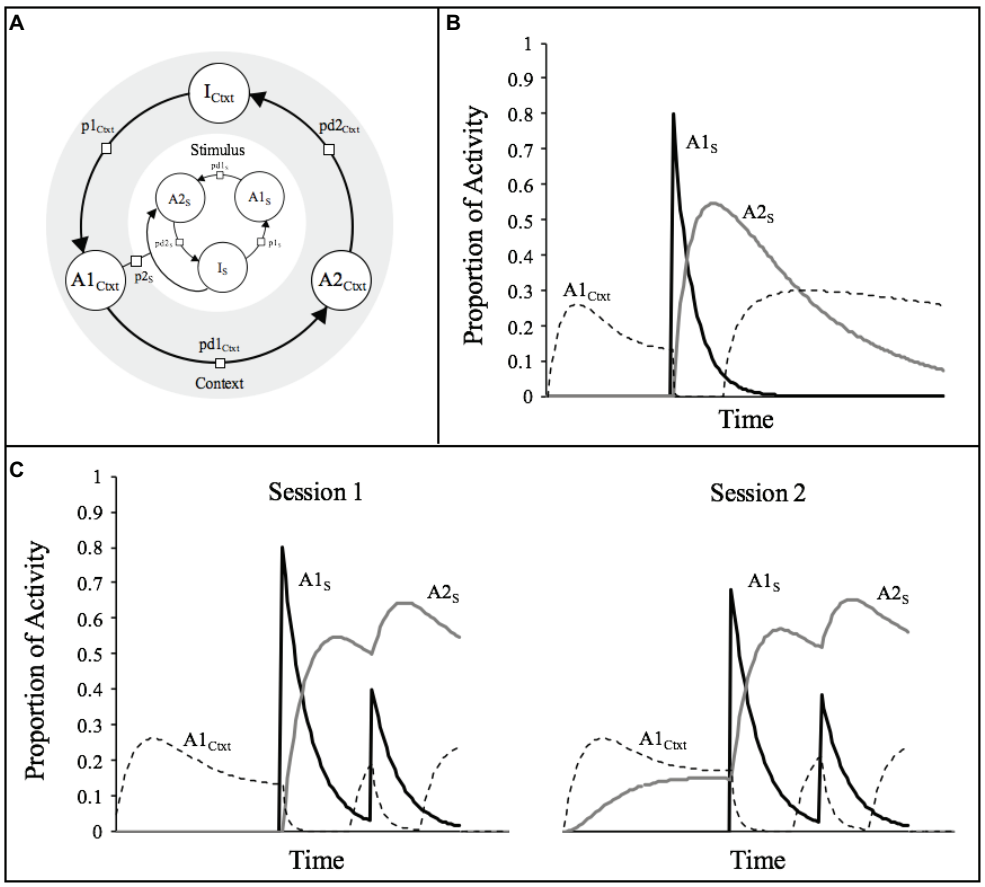

FIGURE 2 | (A) A representation of the context-stimulus relationships assumed by SOP. **(B)** Courses of activity for a typical stimulus (s; duration = 1 moment, $p1_S = 0.8$, $pd1_S = 0.1$, $pd2_S = 0.02$) and the context ($p1_{Ctxt} = 0.05$; $pd1_{Ctxt} = 0.1$; $pd2_{Ctxt} = 0.02$) according to SOP rules of activity. **(C)** Simulations of the theoretical processes involved in two training sessions with two presentations of a 1-moment stimulus within the context.

net excitatory association that would be acquired by the context during the period in which the stimulus is in its A1 state of activity will be overcome by the inhibitory associations that would be provoked during the occasions in which the context is in its primary activity and the stimulus in its secondary activity during the inter-trial intervals. In order to solve this, Vogel et al. (2018) suggested that contexts should not be represented as a long uniform stimulus with a constant primary activity. They proposed that presentation of explicit cues, like the habituating stimulus, provokes systematic changes in the subject's receptor orientation so that the processing of the context is transiently disturbed. Given the dynamics of activity of SOP, this interruption allows the context to enjoy more overlap of its A1 processing with the A1 processing of the stimulus, rather than with the later A2 processing of the stimulus. Vogel et al. posited that this is consistent with the idea that the representation of the context is very vulnerable to disruption by explicit cues.

To implement the idea of context disruption, Vogel et al. (2018) adopted the simple strategy of setting the $p1_{Ctxt}$ value to zero for some period after the presentation of explicit cues. Here, we rationalize this principle in a related but different way. First, we assume that $p1_{Ctxt}$ equals zero if $PA1_s$ is greater than some threshold. Second, we follow Wagner's (1981) distractor rules by stating that the decay rates from $A1_{Ctxt}$ to $A2_{Ctxt}$ and from $A2_{Ctxt}$ to I_{Ctxt}, respectively, are increased by the presentation of the habituating stimulus. The level of increase in these decay rates is assumed to be a function of the activity of the habituating stimulus; that is, $pd1'_{Ctxt} = pd1_{Ctxt} + A1_s/c1$ and $pd2'_{Ctxt} = pd2_{Ctxt} + A2_s/c2$, where $pd1'_{Ctxt}$ and $pd2'_{Ctxt}$ are the effective decay rates, and c1 and c2 are constant parameters of the model.

Figure 2B illustrates the effects of these assumptions on the processing of the context by simulating a situation in which the context is processed alone for some time until a 1-moment stimulus is presented. The relevant pattern of activities displayed in the figure indicates that presentation of the habituating stimulus provokes the progressive diminution of $PA1_{Ctxt}$, which remains active for a few moments when $PA1_s$ is at its maximal, but eventually gets mostly suppressed when $PA2_s$ predominates. The net result of this is more excitatory learning (which is proportional to $PA1_{Ctxt} \times PA1_s$) than inhibitory learning (which is proportional to $PA1_{Ctxt} \times PA2_s$). **Figure 2C** presents the same simulations as those of **Figure 1C**, but this time we added

our assumptions about the processing of the context. As can be appreciated, in session 1 there is a decrease in the amplitude of the $PA1_s$ generated by the second presentation of the stimulus, which is not very different than the pattern that was described in **Figure 1C**. In session 2, however, the pattern is very different in that now, even in the absence of self-generated priming, the amplitude of $PA1_S$ in the first presentation of the stimulus is considerably diminished. This diminution is caused by the anticipatory $PA2_s$ activity provoked by the context which has developed an association with the stimulus. This between-sessions decrement is thus explained by the retrieval-generated priming announced by Wagner (1976, 1978).

SIMULATIONS OF THE CHARACTERISTICS OF HABITUATION

In order to show the quantitative strength of these assumptions, in this section, we present a series of computer simulations illustrating how the model accounts for each characteristic of habituation. For this, we used the revised description of the parametric features of habituation proposed by Rankin et al. (2009).

Due to the diversity of procedures, stimuli, responses, and species underlying the corpus of research that has given rise to these characteristics, we did not attempt to mimic any specific procedure or published data in particular. Rather, we conduct all simulations with a standard procedure with minimal parametric variation from one simulation to another. Thus in all simulations, the habituating stimulus lasted 1 moment and its activation parameters were: $p1_s = 0.8$, $pd1_s = 0.1$, $pd2_s = 0.02$. In order to simulate high-, low-, and medium-intensity stimuli, we used three different values of $p1_s = 0.8$, 0.5, and 0.2. The parameters for activation of the context were identical to those of the habituating stimulus excepting for a lower $p1$ value. That is, $p1_{Ctxt} = 0.05$, $pd1_{Ctxt} = 0.1$, and $pd2_{Ctxt} = 0.02$. The context was turned on at the first moment of each simulation and stayed on according to its $p1$, $pd1$, and $pd2$ values unless the habituating stimulus is presented (which occurred at moment 60 of the simulation). Specifically, if $PA1_s > 0.07$ then, $p1_{Ctxt} = 0$. The presentation of the habituating stimulus also increases the decay parameters of the context to $pd1'_{Ctxt} = 0.1 + PA1_S/2$ and $pd2'_{Ctxt} = 0.02 + PA2_S/10$.

To simulate the transition from one session to another, all activity was set to zero at the end of the session. Only the associative values of the context were carried on from one session to the next. For the simulations, we used the software Stella® Architect (Isee systems; Lebanon, NH, United States).

Simple Within-Session Effects

The simulations described in **Figures 1C** and **2C** attempted to make clear that decrements in responding that occur within a session are mainly explained by self-generated priming. In order to illustrate the generality of this effect, we conducted a series of computer simulations in which a 1-moment duration stimulus was repeated four times at inter-stimulus intervals (ISI) of 2-, 4-, 8-, 16-, or 32-moments in a single session. The results are depicted in **Figure 3** in terms of the peak

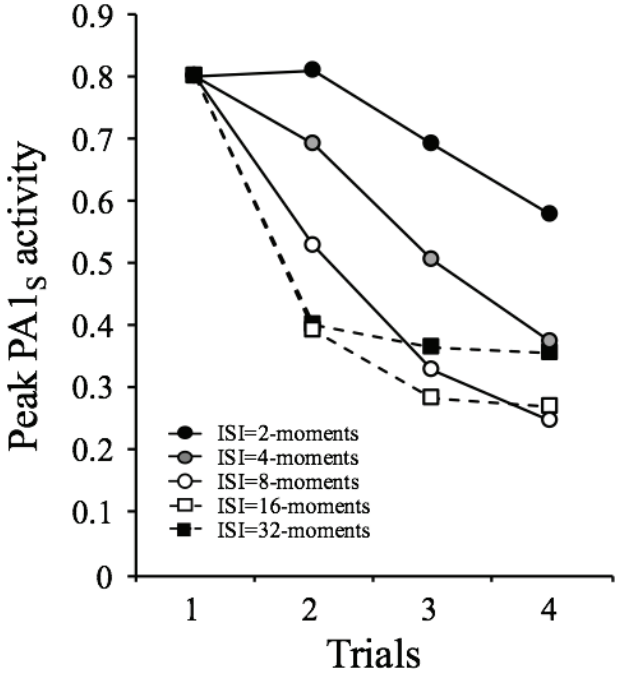

FIGURE 3 | Simulated peak $PA1_S$ activity over 4 presentations of a 1-moment stimulus at inter-stimulus intervals ranging from 2 to 32 moments.

$PA1_s$ activity provoked by each presentation of the stimulus. The general pattern is that in each occasion, the stimulus becomes less effective in provoking its $A1_s$ activity than in the previous occasions. The decrease in most cases approximates an exponential function except for the shortest ISI, in which there is transient facilitation. According to SOP, this facilitation occurs only when the two presentations of the stimulus are sufficiently close in time to produce a summation of $PA1_s$. Beyond the cases of very short ISI (2 and 4 moments), the model predicts that the longer the interval, the less pronounced is the decrement at the end of the session.

This pattern approximates well to the first empirical feature of habituation listed by Thompson and Spencer (1966) and reviewed by Rankin et al. (2009) as follows:

"Repeated application of a stimulus results in a progressive decrease in some parameter of a response to an asymptotic level. This change may include decreases in frequency and/or magnitude of the response. In many cases, the decrement is exponential, but it may also be linear; in addition, a response may show facilitation prior to decrementing because of (or presumably derived from) a simultaneous process of sensitization." (Characteristic #1, p. 136).

Spontaneous Recovery and Long-Term Habituation

Here, we analyze SOP's account of several related facts of habituation listed by Rankin et al. (2009). One refers to the fact that "if the stimulus is withheld after response decrement, the response recovers at least partially over the observation time ('spontaneous recovery')." (Characteristic #2, p. 136).

Another is the observation that "Some stimulus repetition protocols may result in properties of the response decrement (e.g., more rapid rehabituation than baseline, smaller initial responses than baseline, smaller mean responses than baseline, less frequent responses than baseline) that last hours, days or weeks. This persistence of aspects of habituation is termed long-term habituation." (Characteristic #10, p. 137).

According to the model, the self-generated priming effects that occur within a session of habituation tend to disappear with the passage of time. This gives rise to the prediction of "spontaneous" recovery of the response from one session to another. The retrieval-generated priming caused by the context, however, does not depend on temporal factors but on the use of the same context in the two sessions. This gives rise to the prediction of a long-term decrement from one session to the next. Thus, in principle, it seems relatively straightforward to conclude that the model predicts a partial recovery of responding from session to session, which would result from the combination of the natural termination of self-generated priming and the persistence of retrieval-generated priming.

Another characteristic of spontaneous recovery that is consistent with this analysis is that "after multiple series of stimulus repetitions and spontaneous recoveries, the response decrement becomes successively more rapid and/or more pronounced (this phenomenon can be called potentiation of habituation)." (Characteristic #3, p. 136). According to SOP, every repetition of the stimulus will lead to an increase in the association between the context and the cue, so more decrement and less spontaneous recovery are expected over extensive training.

The simulations presented in **Figure 4** illustrate how SOP accounts for all the characteristics described above. The simulation involved four presentations of the stimulus at intervals of 8 and 32 moments in each of three identical sessions. The results are clear for the two conditions: there is a partial recovery in the $PA1_s$ from the last trial of one session to the first trial of the next (spontaneous recovery), and there is a diminution in the degree of spontaneous recovery in session 10 relative to session 2 (potentiation of habituation).

The data displayed in the figure also allow for the analysis of a further characteristic (#4), which states that "Other things being equal, more frequent stimulation results in more rapid and/or more pronounced response decrement, and more rapid spontaneous recovery (if the decrement has reached asymptotic levels)." (Rankin et al., 2009, p. 136). This is seen in the figure by comparing the within- and between-session decrements for the two simulated ISIs. That is, there are more within-session decrement and more spontaneous recovery for the 8-moments ISI than for the 32-moments ISI.

Finally, there is a further characteristic that can be embraced by the context-stimulus association. This property is listed as the sixth characteristic and described by Rankin et al. (2009) as: "The effects of repeated stimulation may continue to accumulate even after the response has reached an asymptotic level (which may or may not be zero, or no response). This effect of stimulation beyond asymptotic levels can alter subsequent behavior, for example, by delaying the onset of spontaneous recovery." (p. 137). SOP explains this phenomenon, also known as "below-zero habituation," by appealing to the fact that once the level of PA1s has reached a low asymptotic value within a session, further training can increase $V_{Ctxt\text{-}S}$ with no major observable effect in this session but that will be apparent in a spontaneous recovery test in another session. **Figure 5** illustrates this by displaying the result of a computer simulation in which the habituating stimulus was presented either 4 or 10 times at a 32-moments interval. As can be seen, the level of $PA1_s$ in the fourth trial of the short training condition is almost identical to the tenth trial of the extended training condition. Nonetheless, in the tests conducted in session 2, there is more decrement in the extended condition relative to the short-training condition.

Stimulus Properties

There are two characteristics of habituation listed by Rankin et al. (2009) that can be explained by some features of the habituating stimulus. One says that "within a stimulus modality, the less intense the stimulus, the more rapid and/or more

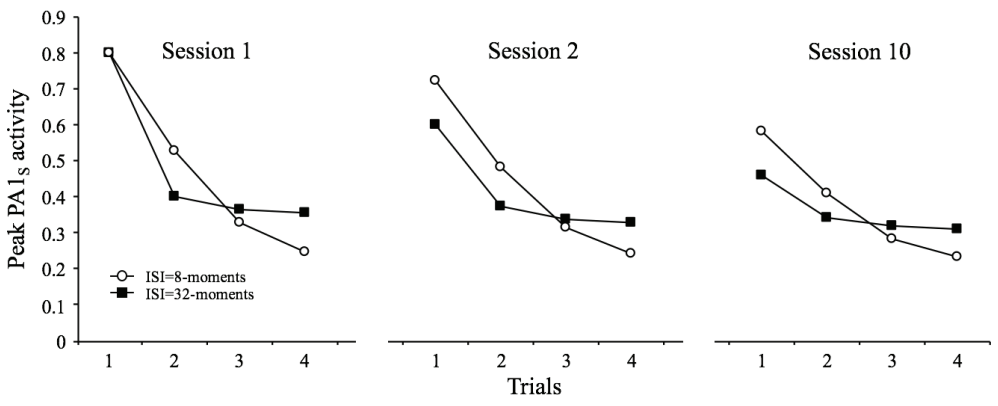

FIGURE 4 | Simulated peak $PA1_S$ activity over 4 presentations of a 1-moment stimulus at inter-stimulus intervals of 8 and 32 moments. The figure contrasts the courses of within-session decrements and spontaneous recovery for the two ISIs during the first, second, and tenth sessions of training.

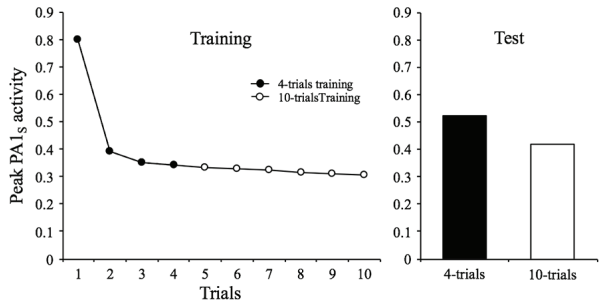

FIGURE 5 | Simulated peak $PA1_S$ activity over 4 or 10 presentations of a 1-moment stimulus at a 32-moments inter-stimulus interval. The bar graph depicts the peak $PA1_S$ activity in a single spontaneous recovery test-trial for the two training conditions.

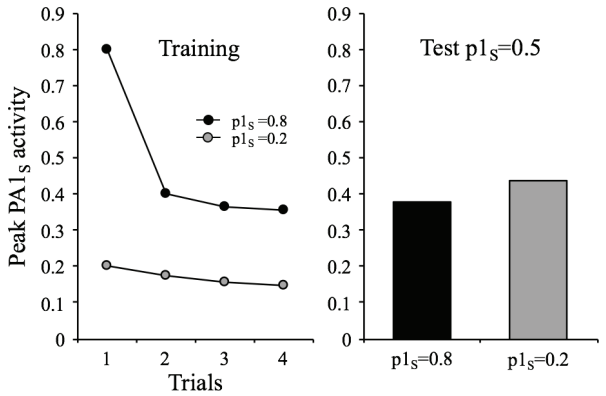

FIGURE 6 | Simulated peak $PA1_S$ activity over 4 presentations of a 1-moment duration stimulus at a 32-moments ISI, under two intensity conditions: high intensity ($p1_s$ = 0.8) and a low intensity ($p1_s$ = 0.2). The bar graph depicts the peak $PA1_S$ activity in a single spontaneous recovery test-trial for the two training conditions tested with a common $p1_s$ = 0.5.

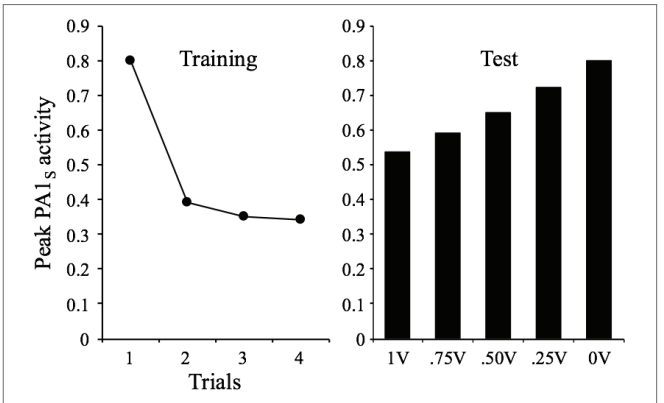

FIGURE 7 | Simulated peak $PA1_S$ activity over 4 presentations of a 1-moment stimulus at a 32-moments inter-stimulus interval. The bar graph depicts the peak $PA1_S$ activity in a single spontaneous recovery test-trial for stimuli that received a rate of 1, 0.75, 0.5, 0.25, and 0 of generalized V from the habituated stimulus.

pronounced the behavioral response decrement. Very intense stimuli may yield no significant observable response decrement." (Characteristic #5, p. 137). As mentioned before, the parameter $p1$ in the model can be assumed to represent the intensity of the stimulus. In terms of the model, $p1$ influences two relevant processes: performance and learning. That is, the higher the value of $p1$, the higher is the response and the faster is learning. The result of this is shown in **Figure 6**, which depicts the results of a simulation in which the habituating stimulus was presented four times with $p1_s$ values of 0.2 and 0.8 and then tested in a subsequent session with a common $p1_s$ of 0.5. As can be appreciated, the $p1_s$ = 0.2 condition exhibited more within-session decrements, but less between-session decrements than the $p1_s$ = 0.8 condition. Of course, the within-session effect is a mere performance effect (i.e., less responding to lower $p1_s$) while the between-session effect is a reflection of differential context-stimulus learning (more learning, and therefore, less responding for higher $p1_s$).

Another property is stimulus generalization, which is described by Rankin et al. (2009) as follows: "Within the same stimulus modality, the response decrement shows some stimulus specificity. To test for stimulus specificity/stimulus generalization, a second, novel stimulus is presented, and a comparison is made between the changes in the responses to the habituated stimulus and the novel stimulus." (Characteristic #7, p. 137). This property does not pose a special theoretical difficulty for any theory of stimulus processing. To account for it, it is sufficient to assume some generalization gradients for stimulus variation. For the sake of simplicity, here we just make the simple assumption that the context-stimulus association is generalized from one stimulus to another as a function of their similarity. **Figure 7** presents the results of these assumptions showing that after training a given stimulus for four trials at an interval of 32 moments, the peak $PA1_s$ values are proportional to the assumed percent of generalization of $V_{Ctxt\text{-}stimulus}$.

Dishabituation

There are two further characteristics listed by Rankin et al. (2009) that refer to the effects of the presentation of a novel stimulus or distractor in the middle of a sequence of presentations of the habituating stimulus. The first states that a "presentation of a different stimulus results in an increase of the decremented response to the original stimulus. This phenomenon is termed 'dishabituation.'" (Characteristic #8, p. 137). The second says that "upon repeated application of the dishabituating stimulus, the amount of dishabituation produced decreases (this phenomenon can be called habituation of dishabituation)." (Characteristic #9, p. 137).

As described above, SOP provides with a set of "distractor rules" by which the presentation of a novel stimulus shortly before a target stimulus causes increments in the decay rates of the target stimulus ($pd1$ and $pd2$). These increments are proportional to the degree of primary and secondary activity of the distractor. To exemplify this, **Figure 8** depicts the results of a simulation in which the habituating stimulus was presented four times at an interval of 32 moments in

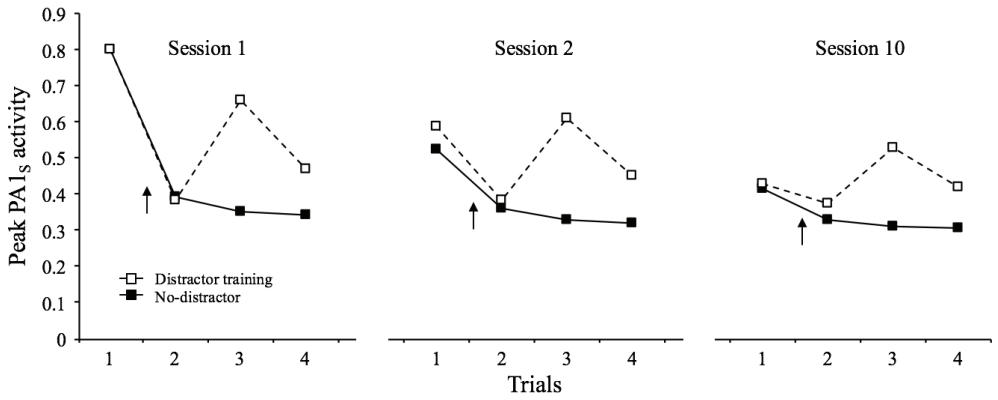

FIGURE 8 | Simulated peak $PA1_S$ activity over 4 presentations of a 1-moment stimulus at a 32-moments inter-stimulus interval over 3 training sessions. In the distractor-training condition, a novel 1-moment stimulus ($p1 = 0.8$; $pd1 = 0.1$, and $pd2 = 0.02$) was presented between trial 1 and 2 of each session, while in the other condition there was no such a stimulus in any trial The arrow indicates the distractor location in each case.

each of three identical sessions. In one condition, a distractor was presented between trial 1 and 2 of each session, while in the other condition, there was no distractor in any trial. The results indicate that the distractor provoked an increase in $PA1_s$ in the subsequent trials relative to the non-distractor condition. Although this effect was notorious in all three sessions, it became progressively less robust over sessions (habituation of dishabituation). The last effect is due, in part, to the fact that as the distractor itself is repeated, it becomes associated with the context and thus rendered less effective.

FINAL COMMENTARIES

In this paper, we showed how the major features of the historically classic phenomenon of habituation can be modeled by the quantitative instantiation of the principles embedded in an already classic theory of stimulus processing, the SOP model (Wagner, 1981). Although this may sound outdated, one reason for bringing such issues here is that theorizing in this field has been relatively neglected, especially in the domain of quantitative modeling.

In the present exercise, we preferred to keep the analysis as simple as possible for expository reasons. But, of course, we must recognize that our simulations of the 10 characteristics of habituation do not exhaust the empirical wealth of the field. Thus, and before concluding, let us make a brief reference to a couple of issues that can be taken forward in future theoretical analyses.

The first refers to the fact that every stimulus evokes several distinct types of responses. These responses may have very different topographies and be differentially susceptible to habituation. In SOP, this difference can be modeled by variations in the parameters of activation. Wagner and Brandon (1989) suggested, for instance, that emotional responses to an aversive stimulus may be represented by more delayed decay processes (i.e., smaller $pd1_s$ and $pd2_s$) than the sensory response to the same stimulus. With this parametric variation, one can expect, in principle, that emotional responses will be associated more rapidly with the context than sensory responses. This might explain, in part, the fact that different measures of habituation can show differential context specificity (e.g., Jordan et al., 2000; Pinto et al., 2014).

Furthermore, according to SOP, the repetition of an aversive stimulus, apart from leading to habituation, can also result in the conditioning of emotional responses that potentiate the response to the habituating stimulus itself. Wagner and Vogel (2010) proposed that emotive sensitization competes with sensory habituation in complex ways, such that habituation might be obscured by potentiating effects, presumably reflecting the contribution of an emotional response controlled by the same context that controls habituation. The co-existence of several types of interacting associations between the context and the stimulus is conceptually consistent with SOP. This analysis must be complemented, however, with sufficient empirical studies that succeed in dissociating the response-potentiating from the response-diminishing effects of stimulus repetition (Ponce et al., 2011; Ponce et al., 2015).

There is one further aspect of SOP that was left untreated in the present analysis of habituation. Wagner (1981) proposed that the response to the stimulus is a function of PA1 and PA2 of the stimulus; that is, $R = f \times (wl \times PAl_S + w2 \times PA2_S)$, where wl and w2 are linear weighting factors, and f is a mapping function appropriate to the response measure of interest. As Donegan and Wagner (1987) suggested, this equation provides for at least three options that have differential impact on the course of habituation. One is assuming a very low value of w2, say zero, as we did in this paper. In this case, the response would depend entirely on $PA1_s$, with $PA2_s$ contributing only indirectly *via* its priming effect on $PA1_s$. In our simulations, we have adopted this tactic because it seems to represent better the predominant types of responses that were used for the definition of the 10 characteristics of habituation (e.g., limb flexion in the spinal cat and startle response in rats).

Another possibility is to adopt a sizable and negative value for w2. In this case, the conditioned and unconditioned secondary activity is subtracted from the primary activity to produce the response. Here, both the negative contribution of $PA2_s$ to the response and its priming on $PA1_s$ would act in a synergic way to diminish the primary response to the stimulus. The use of w1 and w2 with opposite signs may be particularly advised when there are empirical reasons to believe that the response to the habituating stimulus shows a secondary response that opposes the primary response as it has been frequently reported with pharmacological stimuli (e.g., Siegel, 2005).

The third theoretical alternative is to assume that w2 is substantial and positive. Here, $PA2_s$ would have two opposite effects on the response: an augmentative effect through summation with $PA1_s$ and a diminutive effect through priming. In this more complex scenario, it would be expected to observe less behavioral habituation than in the former cases. Although it may be difficult to assess this possibility with the standard habituation procedures, it is consistent with reports of enhanced performance in some perceptual tasks when the target stimulus is preceded by the same or an associated stimulus (e.g., Posner and Snyder, 1975; Kristjansson and Campana, 2010; Henson et al., 2014).

It may be seen that, despite its complexity, the SOP model is quite well articulated and as such, it seems to be uniquely equipped to encourage further theoretical and empirical work beyond the 10 features of habitation and for a range of very distinct stimulus–response systems. It should be said also that the explanatory scope of the model is not restricted to habituation. Its usefulness has been demonstrated in a variety of phenomena, mainly in the domain of associative learning, such as occasion setting (Wagner and Brandon, 2001; Vogel et al., 2017), timing (Vogel et al., 2003), divergence of response measures (Wagner and Brandon, 1989), trial spacing (Sunsay and Bouton, 2008), cue competition (Mazur and Wagner, 1982; Vogel et al., 2015), causal learning (Dickinson and Burke, 1996; Aitken and Dickinson, 2005), mediated conditioning (Dwyer et al., 1998; Pearce and Bouton, 2001), latent inhibition (Honey and Hall, 1989), and object recognition (Honey and Good, 2000; Robinson and Bonardi, 2015).

In concluding, let us make a personal statement. This paper was prepared in response to the call for papers to be published in a special issue of Frontiers in Psychology on "Research in emotion and learning: Contributions from Latin America." The authors of this article work in Talca, Chile, and we were all tremendously influenced by Allan R. Wagner. His influence was not just intellectual but also took the form of concrete contributions to the setting up of our laboratory for the study of learning in Chile. Allan had accepted to write this paper in collaboration with us. He agreed with the general approach of the paper and with the novel instantiation for context learning, but he passed away before any of the work was completed.

AUTHOR CONTRIBUTIONS

EV and YU-B contributed to the conception of the study; FP, YU-B, and SB conducted the simulations and organized the database; and EV wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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A Descriptive Analysis of the Interactions During Clinical Supervision

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This study intends to analyze some skills trained during supervision. In it we describe sets of interactions (based on the American Psychological Association [APA], 2006; competency domains) happened between the supervisor and the supervisee during the supervision process. Interactions from twelve supervisor-supervisee dyads during asynchronous and direct, and individual and group supervision sessions were video recorded for this purpose. The recordings helped to determine, classify, and define behavioral response classes in each dyad's interactions. Percentages of time spent in each behavior class were computed. A reliability of 95% CI [0.91, 0.95] among observers was obtained. The behavior classes in which more time was spent were assessment, intervention, and conceptualization skills. Behavior classes in which less time was spent were related to emotional and interpersonal processes. These findings are discussed by linking the evidence-based theory on supervision with the time spent in each behavior class.

Keywords: clinical supervision, functional analysis, therapist training, evidence-based supervision, professional skills in clinical psychology

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INTRODUCTION

Several studies indicate the relevance of the clinical supervisor-supervisee relationship from both, the therapeutic and the pedagogical viewpoint, within the field of psychotherapy. Clinical supervision is a setting in which therapists undergoing training develop the skills needed for psychotherapy (Falender, 2014; Martino et al., 2016; Falender and Shafranske, 2017) while client protection is guaranteed (American Psychological Association [APA], 2015), and the quality of service is optimized (Reiser and Milne, 2014).

In psychology, the clinical supervision is currently known as an independent professional research-based specialization, aiming to identify best practices along with specific skills required (Borders et al., 2014; American Psychological Association [APA], 2015). The literature and research in this area have increased with such recognition (Falender, 2014). It has additionally fostered the development of therapeutic models that promote systematic supervision to address practice needs (Falender and Shafranske, 2004; Myers and Sweeney, 2008; Plakun et al., 2009; Tsai et al., 2009; Rodolfa et al., 2013; Holt et al., 2015).

Abbreviations: APA, American Psychological Association; FAP, functional analytic psychotherapy.

Several measurement and cognitive behavioral therapist training strategies rely on the supervisor-supervisee interaction (Milne et al., 2002). This is expected because this interaction constitutes the vehicle to train skills in supervisees (Cheon et al., 2009), and set the stage for learning to occur (Milne, 2018). Management and support of emotional processes related to psychotherapy and supervision itself are of capital importance within these interactions (Falender and Shafranske, 2004; Watkins, 2014; Milne and Reiser, 2017; Milne, 2018). This has led to the development of conceptualizations of the supervisory relation and of the supervision that includes emotional processes. Palomo et al. (2010) developed the supervisory relationship questionnaire to characterize and measure the relevant dimensions of the supervisory relationship. This instrument includes emotional aspects of the supervision, in a factor that explains most of the variance called "secure base." According to Milne (2018), possible actions that promote this secure base are: empathizing and connecting emotionally, offering warmth and respond to learners needs, among others. Likewise, Milne et al. (2011a) developed an observational instrument to measure the supervision competence based on his evidence-based clinical supervision (EBCS) model that includes a component called "relating" (Milne, 2018). The inclusion of this component in this assessment tool shows its relevance, since this instrument was the strategy chosen by Milne to operationalize the clinical supervision. This component involves the supervisor's interpersonal effectiveness and illustrates the importance that emotional processes have within supervision.

There is a parallelism between supervision and psychotherapy. In both cases, the relationships between the supervisor and the supervisee and between the therapist and the client play a major role in the process. Furthermore, the skills required for supervision benefit the therapist training and the client protection. Thus, the competences that a supervisor should have and the ones that should be developed by the supervisee have similarities (see American Psychological Association [APA], 2006, 2015). Emotional skills are an essential part of the relationship skills, in both cases, according to guidelines for supervisors and clinical therapists. For example, the therapists' interpersonal expertise involves interpreting non-verbal and verbal cues, empathetic responding to implicit and explicit concerns. The supervisory relationship associated with the supervisor's competencies include favoring an environment that promotes self-disclosures, and skills to repair ruptures of the supervisory relationship. The parallelism between therapist and supervisory competencies offers an opportunity for modeling therapist skills during supervision.

This paper aims to describe interactions between supervisors and supervisees relying on the evidence-based clinical practice guidelines (American Psychological Association [APA], 2006) that include the set of competencies that a clinical therapist should develop, including interpersonal skills. We will illustrate these domains of skills below.

Table 1 shows that therapists must have the skills required to provide clinical services (items a, b, and c). Therapists should seek training, be informed about the latest literature in the field, acknowledge their limitations, and use additional resources to guarantee an appropriate service (items d, e, and f). Therapists should also pursue proper training for developing skills to work with diverse population. In other words, they should be able to identify individual differences based on gender, sexual orientation, race, culture, religion among other variables. Therapists could then adapt therapy to the needs deriving from these factors (g). In addition, it is paramount the development of interpersonal skills (i.e., establishing an adequate relationship and being able to repair it when necessary) (h).

Supervision has also been studied within particular *behavior analytic therapeutic model*s (Tsai et al., 2009). It is well-known the applications of behavior analysis (BA) to contexts of clinical therapy. BA employs learning principles to treat clinical problems and to discover strategies to alleviate them (Ghaderi, 2007; Leaf et al., 2016; Welch and Polatajko, 2016). BA takes the so-called "operant behavior" principles to apply them to therapy contexts. This application involves direct observation of behavior, development of case conceptualizations, and interventions to modify behavior during therapy, introducing procedures such as contingencies, reinforcement, shaping, etc.

As one of the therapies based on BA, FAP relies on the assumption that the relationship between the client and the therapist is the vehicle of behavior change and learning (Kohlenberg et al., 2002). FAP offers the possibility for the therapist to address different situations by safely selecting and adjusting treatments or techniques to each client (Kohlenberg et al., 2002). According to a behavior analytic approach, creating or establishing adequate contingencies for the client's issues constitutes the foundations of any intervention, in FAP those contingencies might occur within the client-therapist relationship (Kohlenberg et al., 2002).

A similar rationale has been used to develop clinical skills. For example, Follette and Callaghan (1995) employed a therapist training based on FAP by using one-way mirror observation so the supervisor could indicate to the supervisee if their performance was effective, non-effective, or neutral. Supervisees improved their skills to identify their mistakes during intervention, and behaviors that are valuable but not frequently employed (for a similar and more modern approach see Carmel et al., 2016).

TABLE 1 | Evidence-based clinical practice areas of expertise according to APA guidelines.

Components of clinical expertise American Psychological Association [APA], 2006)
Assessment, diagnostic judgment, systematic case formulation, and treatment planning (a)
Having a cogent rationale for clinical strategies (b)
Clinical decision making, treatment implementation, and monitoring of patient progress (c)
Appropriate evaluation and use of research evidence in both basic and applied psychological science (d)
Understanding the influence of individual and cultural differences on treatment (e)
Seeking available resources (e.g., consultation, adjunctive, or alternative services, etc.) as needed (f)
Diversity skills (g)
Interpersonal expertise (h)

Nevertheless, other methods are usually employed during supervision. According to Milne et al. (2011b), live or video-recorded feedback, role-play and modeling proved to be the most common methods among supervisors. Both, feedback and modeling, are key elements within evidence-based supervision (Martino et al., 2016). Milne and Reiser (2012) present an evidence-based method that works not only for therapy contexts but also for therapist training. This method aims to improve the effectiveness of training and intervention by considering the main needs and the most influential elements according to literature (Milne et al., 2011b). The training includes experiential learning methods. Its efficacy can be increased by a combination of a functional BA during training and feedback from the trainee (Milne et al., 2002).

All these strategies depend on the interactions within the supervisory relationship. For this reason, understanding the different types of interactions between the supervisor and the supervisee is critical for comprehending and improving the effectiveness of the supervision (Tsai et al., 2014). In the literature, there is a supervision model for FAP training that relies on the supervisor-supervisee relationship (Callaghan, 2006b; Tsai et al., 2009). According to this model, supervisors use the supervisory relationship to improve therapists' interpersonal repertoires (see Barraca Mairal, 2009).

Given the importance of the supervisory relationship in the FAP model, Callaghan (2006b) developed the functional assessment of skills for interpersonal therapists (FASIT) as a tool for implementing five response classes involved in interpersonal relationships. These response classes are: (a) assertion of needs, (b) interpersonal feedback, (c) management of conflict, (d) disclosure of personal information, and (e) emotional expression. Response classes a, b and e involve emotional processes, since they require appropriate regulation and expression of emotions. We used this implementation in this study because it involves many elements regarded as important in the literature.

Based on the supervisor-supervisee interaction, it is possible to expect the supervisee begins to take appropriate actions during the psychotherapy practice, as the behavior promoted by the supervisor when interacting will influence the supervisees' interventions (see Callaghan, 2006a). For example, if the therapist needs to discuss difficult emotions avoided by the client (e.g., the sadness that accompanies the loss of someone important for them) but frequently postpone this discussion (because this aspect is also personally challenging for the therapist), the supervisor may talk to the therapist about the feelings that arise when talking about this topic with the client. In this case, the therapist avoids examining his feelings, similarly to the client. Therefore, the supervisor may address this issue during supervision to teach the therapist how to relate to the client's emotions differently, providing a more effective intervention. This interaction is well suited to address the emotional aspects of supervision that we mentioned earlier in this paper.

Within FAP supervision, the training focus are the specific therapist's classes of behavior regarding interpersonal repertoires. There is limited knowledge regarding other response classes that might occur during supervision, which are unrelated to the interpersonal relationship. Literature has addressed other interaction elements. However, interactions between supervisor and supervisee have not been studied using American Psychological Association [APA] (2006) guidelines for evidence-based therapy. Therefore, inquiring into the response classes that currently occur within clinical supervision is essential.

A previous research of this group studied the supervisors' opinions about certain skills that should be developed by supervisees. However, as this information relies on the supervisors' verbal reports, it might be biased because of social desirability reasons. This study aims to correct this matter by providing a comprehensive analysis of the kind of skills trained during supervision. Accordingly, we describe the response classes that take place between the supervisor and the supervisee along with the supervision process intended for supervisees to develop clinical skills. Skills suggested by American Psychological Association [APA] (2006) were organized into response classes for this purpose. Supervisor-supervisee interactions occurring during supervision were observed in order to determine the frequency of these response classes.

MATERIALS AND METHODS

Participants

The sample of the study was composed by nine supervisors and one of their supervisees, working for 3 universities in Bogotá, Colombia. Fifteen video recordings of individual, direct, and asynchronous supervisions were conducted within the universities that offer undergraduate or graduate programs in clinical psychology. In most of the cases, the supervisions lasted for 1 h.

Instruments

Following the domains of competency proposed by the American Psychological Association [APA] (2006), it was designed a *supervisor-supervisee interaction assessment manual* as an instrument to measure the response classes described by Callaghan (2006a). There were also carefully classified the interactions intended to develop skills in the supervisee. Each category comprises a response class selected based on their impact and relevance to the development of the therapist skills. **Table 2** shows the 13 chosen categories.

Two observers coded the interactions in each session using the classes definitions in the manual. The total length of the session was divided into 3-min intervals. Observers paused the recording at the end of each interval to individually assess and code the observed interaction. It was possible to code several types of interactions within the same interval since they were not mutually exclusive. Observers coded whether the supervisor or the supervisee started the interaction, as this proves to be relevant for identifying the efforts made by the supervisor to teach a skill required by the supervisee.

There were recorded four supervisions (not included in the final sample) to assess the definitions in the manual. Observers discussed their impressions and hesitations regarding these definitions after every observation interval. Whenever disagreements occurred, they were discussed with the two first

TABLE 2 | Brief description of coding categories.

Category	Description
Self-Evaluation skills	Supervisee's assessment of their own abilities and limitations
Diversity skills	Identification of the client's particularities by taking into account individual variables
Feedback	The supervisor describes one of the supervisee's skills in order for him/her to improve it
Conflict management	Descriptions of conflict situations between the supervisor and the client, or between the supervisor and the supervisee or instruction to manage those situations.
Self-Revelation/experience, and emotional expression	Reports of feelings and impressions of the therapeutic process, or of the supervision process
Interpersonal proximity	Discussions or activities that promote developing skills to strengthen the therapeutic bond
Ethical skills	Favoring decision making and acting according to legal and ethical considerations
Expression of needs	Activities that encourage expressing the therapist or the client's needs during the supervision process
Autonomy	Actions that foster autonomous decision making by the supervisee
Bidirectional communication	Activities that raise awareness of the influence the therapist behavior has on the supervisor or on the client
Conceptual management skills	Activities that promote relating case elements to theory and psychological concepts
Intervention skills	Activities that encourage an adequate use of techniques for changing and promoting the client's wellbeing
Assessment skills	Activities that favor diagnosing and understanding the issues, in addition to assess therapeutic change

authors of the article, who have clinical supervision experience, and defined the categories for observation. The definitions were subsequently adjusted and the second author provided observers with four training sessions for the implementation of the manual.

Procedure

This research was reviewed and approved by the ethics committee of the Faculty of Psychology at Fundación Universitaria Konrad Lorenz. All participants provided written informed consent. Supervisors were asked not to mention any data during supervisions that could lead to the identification of the supervisees' clients. An undergraduate student conducted the recording. The student remained in the supervision session monitoring the quality of the recording without interfering.

The videos, along with the consent forms, were stored in a safe place. Afterward, four observers that received the training, manually coded the video-recordings. Once the coding was completed, observation records were digitized for their analysis. Lastly, manually written records were filed.

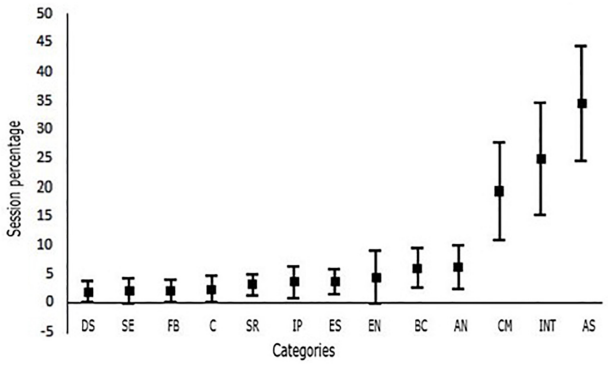

FIGURE 1 | Percentages of the interactions started by the supervisor in each category using bars to represent confidence intervals at 95% level of confidence. DS, diversity skills; SE, self-evaluation; FB, feedback; C, conflict management; SR, self-revelation; IP, interpersonal proximity; ES, ethical skills; EN, expressing needs; BC, bidirectional communication; AN, autonomy; CM, conceptual management; INT, intervention skills; AS, assessment skills.

RESULTS

The interval record used in this study allows us to determine the approximate percentage of time spent in each interaction per session. Reliability analyses of the measure of supervisor-supervisee interactions during clinical supervision were conducted. Interclass correlations were used to find the observers agreement when calculating the percentages of each session since this strategy let us find interobserver-agreement with a continuous variable. Each session agreement was used to perform a bootstrap analysis. The approximate index rate of agreement found was 0.94, 95% CI [0.91, 0.95].

Afterward, the average of the percentages of each category was calculated, and a bootstrap analysis was conducted in order to find the estimated average and the confidence interval. For this analysis, the interactions the supervisor started were differentiated from the ones the supervisee began, aiming at identifying potential variations.

Figure 1 shows the interactions began by the supervisor during the supervisee's training. Skills related to the task of the psychologist during therapy have a higher occurrence percentage whenever the supervisor starts the interaction. These interactions are intended to develop assessment, intervention, and conceptual management skills. In addition, these are the only skills with a higher percentage of spent time with 95% confidence. The remaining interactions are not highly distinguishable from each other and occur 10% of the time at the highest, with *autonomy* at the upper limit. Categories composing this group include interpersonal closeness, autonomy, ethical, diversity skills, and feedback. The last group comprises response classes with limited occurrences (average around 0%). These interactions aim at the supervisee's assessment of their own

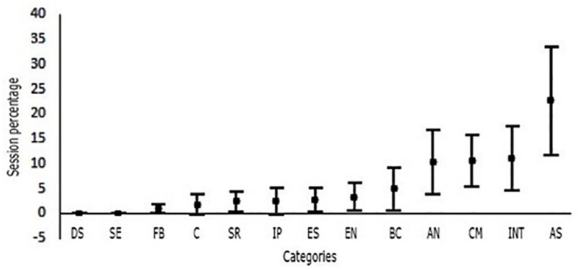

FIGURE 2 | Percentages of the interactions started by the supervisee in each using bars to represent confidence intervals at 95% level of confidence. DS, diversity skills; SE, self-evaluation; FB, feedback; C, conflict management; SR, self-revelation; IP, interpersonal proximity; ES, ethical skills; EN, expressing needs; BC, bidirectional communication; AN, autonomy; CM, conceptual management; INT, intervention skills; AS, assessment skills.

behavior, skills, and limitations during the intervention or the supervision process.

Figure 2 shows the interactions started by students. As before, the group of skills related to the tasks of the psychologist within the therapy process has a higher occurrence percentage. However, only *assessment skills* class is distinguishable from other classes. Conceptual proficiency, intervention, and autonomy skills comprise between 3 and 27%, but are only different from categories occurring with a percentage around 0 which are conflict management, feedback, and emotional expression skills. The remaining interactions occur between 0 and 16% of the time. However, they are not mutually distinguishable in terms of spent time. They comprise ethical, diversity, and interpersonal skills.

DISCUSSION

Clinical Psychologist Skills

Skills related to the clinical therapist duties have been extensively conceptualized. The Cube Model (Rodolfa et al., 2005) is useful to address this matter. This approach divides professional skills into two groups: foundational skills, which refer to the therapist's professionalism underlying practice, and functional skills, which are linked to the practical aspects of the work as a therapist (Rodolfa et al., 2013). *Assessment and intervention skills* are included in the last group. This study found that interactions aiming at developing those skills had a higher occurrence percentage than the rest. Assessment skills were particularly high within interactions started both by the supervisor and the supervisee. Such findings are similar to those of Rodolfa et al. (2013). Rodolfa et al. (2013) consider that assessment and intervention-related clusters are fundamental for clinical practice. This might be because assessment skills are used throughout the entire therapy process. According to American Psychological Association [APA] (2006), these skills facilitate establishing a diagnosis, selecting the type of treatment, assessing therapeutic change, making clinical decisions to modify treatment, and seeking for other alternative resources that improve the service provided.

The percentage of self-evaluation activities during the supervision session was very low. According to Karel et al. (2014), this skill is fundamental because supervisors notice more issues in the supervisees' performance than those perceived by themselves. This is expected because supervisees tend to overestimate their skills. Despite the encouragement to develop this skill in several discussions about supervision (Falender, 2014), this study exposes a difficulty to link scholar discussions about supervision with its actual practice, which is consistent to Falender and Shafranske's (2017) considerations.

In addition, this study found that both, supervisors and supervisees, begin a limited number of interactions aiming at fostering the supervisee's autonomy. Comparably, Rodolfa et al. (2013) reported that the Evidence-Based Decision-Making/Critical Reasoning cluster was frequently considered as a non-crucial skill for achieving positive results for the client. This cluster also included choosing relevant literature, its critical review and linking it to the assessment process. The minor importance given to this skill during the supervision, contrasts with the significance given to it by the American Psychological Association [APA] (2006) evidence-based practice guidelines. This inclination is alarming because if this skill is not considered significant and, thus, is not developed by the supervisee, it is possible they would not have the foundations to autonomously choose the most appropriate treatment, make clinical decisions, and modify the treatment in order to adjust it to each client. All these competences are essential for conducting autonomous practices (Milne and Reiser, 2012). For, if this skill is not systematically developed, supervisees might base their decisions on improper strategies, such as allowing biases or overgeneralizations to unconsciously influence their decisions (American Psychological Association [APA], 2006; Cummings et al., 2015).

Fundamental Therapy Knowledge

Theoretical and conceptual knowledge required by the supervisees is paramount within supervision. This is evident because this skill shows higher percentages than the rest, specifically considering interactions started by supervisors. These findings align with the supervision model presented by the FAP (Callaghan, 2006b), the Cube Model for Competency Development (Rodolfa et al., 2013), and the Evidence Based Clinical Supervision Model (Milne and Reiser, 2017). They consider developing theoretical and conceptual skills either as a supervision goal or as a stage for linking them to the clinical skills developed throughout the supervision process (Milne et al., 2011b; Holt et al., 2015; Watkins et al., 2015). It is worth highlighting that this skill needs to be coherent with the supervisee's approach because this is how their decisions will be guided during therapeutic intervention (Lewis et al., 2014; American Psychological Association [APA], 2015). Additionally, according to Callaghan (2006b) and American Psychological Association [APA] (2015), this skill constitutes a professional criterion within the training process because it encourages autonomous decision-making by the supervisee.

Due to the size of the study sample, it is not possible to distinguish among different stages of the supervision training.

It is expected that conceptual aspects in which therapy is based require less time dedication as the supervision goes on, and the supervisees develop these skills. To continue working on this skill during the final training stages, would imply a bias obstructing the supervisees' improvement. It is therefore significant for further research to inquire into the change of focus along the supervisees' training.

Moreover, other related skills such as legal knowledge and ethical case management, and diversity skills occupy limited supervision time. These findings can be interpreted in two directions. First, ethical knowledge is required for clinical practice. Thus, students need to address this topic during their first training stages. Consequently, addressing it during supervision should only be necessary whenever particular dilemmas or special ethical considerations arise. Second, these skills might not be incorporated due a lack of supervision training. De las Fuentes et al. (2005) and Hatcher et al. (2013) argue that ethics –as a fundamental skill of psychological practice– should occupy a broad space within the process. Therefore, exercises and activities designed to identify and solve legal and ethical dilemmas (based on reviewing both scientific data, and current regulations) are necessary for students to take coherent positions with the profession and with the client's wellbeing. Hence, ethical standards are as significant as scientific skills (knowledge and methodologies).

In addition to that, diversity skill training is especially significant considering that immigration from other parts of the country to Bogotá is common. For this reason, the reduced percentage of time spent during supervision to develop this skill might indicate that awareness regarding the need to address multicultural skills should increase. Such skills would improve adjustment processes of migrants arriving to the city, particularly of those coming from rural areas and moving permanently or temporarily to urban areas (Salas-García et al., 2016). Bastidas-Bilbao and Velásquez (2016) mention that supervision, as an educational program, works as an open system constantly being influenced by the socio-historic conditions of each specific location and by the group of people interacting within that place. Our findings differ from the results of Rodolfa et al. (2013) because the Interpersonal and Cultural Competence cluster was reported as one of the most significant. However, it has been found that despite of the fact that supervisors reported discussing diversity aspects, supervisees indicated that this was infrequent, and that often it was not well received when they proposed the topic during the supervision session (Jernigan et al., 2010; Falender, 2014). The supervisees' reports in these studies coincide with the findings of this study.

Interpersonal Relationship Skills

A general lack of dedication in developing the supervisees' interpersonal skills was identified. For instance, conflict management, bidirectional communication, and interpersonal proximity skills occurred at an average below 5%. Conflict and feedback interactions started by the supervisees had 0 occurrences. This is remarkable because both of these skills are required to establish adherence and a good therapeutic relationship. Duff and Shahin (2010) mention that conflict is an inevitable but essential part of any working relationship. Thus, conflict management skills are fundamental. Inadequate conflict management can produce dissatisfaction with the work and the supervision, which could limit the supervision's effectiveness and jeopardize the client's wellbeing. Therefore, the conflict should be addressed in a way that solutions are found based on relationship aspects like trust, respect, rapport, and empathy. This will allow a productive supervision, and a constructive learning experience. Regarding interpersonal skills, Watkins and Scaturo (2013) emphasize that aspects like a safe environment, empathy, authenticity, remoralization, session planning, and appropriate affective experiences (transmitted to the supervisee through the working relationship) take part in establishing and keeping a supervisor-supervisee relationship. These elements are crucial for developing the required skills for future professional practice. Lastly, an appropriate supervisor-supervisee relationship generates an adequate therapeutic relation (Callaghan, 2006b). This has been recognized as one of the non-specific elements that contributes the most to therapeutic change (Ellis, 1991).

Regarding interactions started by the supervisor, bidirectional communication is the skill with most occurrences within this group. Interactions focused on it aim at incorporating discrimination training or efficient supervisee responses toward the impact they have on other people. This facilitates establishing a therapeutic relationship with the client or a professional relationship with the supervisor (Callaghan, 2006b). Consequently, an authoritarian relationship with the supervisor is incompatible. These findings are encouraging because, according to numerous authors, authoritarian supervision strategies are negative aspects of supervision, and obstruct the supervisee's progress and learning (Ramos-Sánchez et al., 2002; Herschell et al., 2010; Grant et al., 2012). However, this type of interaction has a low number of occurrences, which implies that more attention should be drown to its use and development.

Comparison With Other Supervision Interaction Measurements

Examining supervision evaluation based on cognitive behavioral therapy (CBT) and EBCS approaches provides several methods to assess supervision (Milne et al., 2013; Newman et al., 2016). Assessment criteria include, first, the client's satisfaction with the therapeutic process and the results of the therapy (Grossl et al., 2014), second, the supervisee's satisfaction and their skills assessment (Cliffe et al., 2014; O'Donovan and Kavanagh, 2014; Calvert et al., 2017), third, the assessment of specific supervisor skills (Bambling and King, 2014; Berger et al., 2014; Gonsalvez and Calvert, 2014; Newman et al., 2016), and lastly, the analysis of the behavior during the supervision session (Milne et al., 2002) through a systematic observation approach (Milne and Reiser, 2011).

The instrument used for this research is aligned with Milne and Reiser's (2011) recommendations. First, it is properly designed. Second, it is optimal for application because it only requires a short training and it is clearly understood and interpreted. Finally, the findings collected with this technique are useful since they allow identifying the frequency of the

supervision subjects' interactions related to specific skills. Corrective feedback of the supervisor and the supervisee's performance can be provided using this frequency report, according to observational data from both participants' skills (Milne and Reiser, 2011). Hence, it can be combined with other complementary tools in order to determine if supervision lines up with the supervisee's learning needs. The test-retest properties of this tool still need to be assessed. However, it had an adequate reliability among raters.

Study Limitations and Future Directions

This study identified objectively the time spent developing different types of skills required for evidence-based clinical psychology, which is an advantage over other investigations based on the supervisors' oral reports (e.g., Rodolfa et al., 2013). This identification can lead to implementing corrective actions by supervisors and university's regulations. However, due to the transversal analysis carried out, the study did not examine the different stages of the supervisee's skill development, for instance, half-yearly. It is, therefore, relevant that further studies examine the different stages of supervision training in order to identify potential supervision content adjustments, and interaction elements determining this content. A functional analysis is crucial to recognize precedents and consequences of relevant interaction behavior. Finally, theory and analysis in this paper were based mainly in supervision models and theory akin to behavioral therapies which might limit the generalization of this analysis to other theories and therapies.

AUTHOR CONTRIBUTIONS

MN-G and OC-S developed the study concept and design, critically reviewed, and corrected the manuscript. NR, LS, DC, and SR contributed with data collection and analysis and drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Cognitive Benefits From a Musical Activity in Older Adults

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The aging population is growing rapidly. Proposing interventions that enhance the cognitive functions or strategies that delay the onset of disabilities associated with age is a topic of capital interest for the biopsychosocial health of our species. In this work, we employed musical improvisation as a focal environmental activity to explore its ability to improve memory in older adults. We present two studies: the first one evaluated neutral memory using the Rey Complex Figure (RCF) and the second one evaluated emotional memory using International Affective Picture System (IAPS). A group of 132 volunteers, between the ages of 60 and 90, participated in this investigation. Fifty-one of them were musicians with more than 5 years of formal musical training. After acquisition of neutral (Study 1) or emotional (Study 2) information, the groups of older adults were exposed to music improvisation (experimental intervention) or music imitation (control intervention) for 3 min. We then evaluated memory through two tasks (free recall and recognition), by means of immediate and deferred measures (after a week). We found a significant improvement in memory among participants involved in music improvisation, who remembered more items of the RCF and images from IAPS than the imitation group, both in the immediate and deferred evaluation. On the other hand, participants who had musical knowledge had a better performance in neutral visual memory than non-musicians. Our results suggest that a focal musical activity can be a useful intervention in older adults to promote an enhancement in memory.

Keywords: cognitive reserve, musical strategy, improvisation, memory, aging

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INTRODUCTION

Nowadays, there is an increase in life expectancy, which is highly positive for the human being, although it brings with it a decline in our cognitive functions (Christie et al., 2017). It is estimated that by 2050 there will be 114 million people with dementia, this condition being one of the major causes of disability and dependence in the older adult population (World Health Organization, 2012; Iuliano et al., 2015). For this reason, proposing interventions

that enhance the cognitive functions or strategies that delay the onset of disabilities associated with age is a topic of capital interest for the biopsychosocial health of our species (Kramer et al., 2004). For example, treatments that enhance cognitive abilities could be promoted in each life stage, from childhood to old age.

Memory is one of the cognitive skills most affected by aging (Nyberg et al., 2003; Park and Festini, 2017). This function could be defined as the capacity to learn, store, and retrieve information (Tulving, 2002; Squire and Wixted, 2011). There are several memory subsystems; the one mostly affected by aging is episodic memory (Friedman, 2013). At the same time, emotional memory could be considered a part of episodic memory, and it is defined as better storage and recall of the events associated with emotional factors, i.e., those events that have an emotional load are better remembered than the neutral ones (Cahill and McGaugh, 1995; Bermúdez-Rattoni and Prado-Alcalá, 2001). Evidence showed that older adults had a decrease in episodic memory, but emotions could work as enhancers and compensate for this deficit (Moayeri et al., 2010).

Several strategies or environmental interventions, in addition to lifestyles, have been investigated mainly to improve cognitive functions and to prevent and/or delay cognitive deficits. Such interventions include learning other languages (Abutalebi et al., 2015), physical activity (Loprinzi et al., 2018), and music (Schneider et al., 2018). In particular, music makes unique demands on our nervous system (Justel and Diaz Abrahan, 2012), and therefore, over the last years, music and each of its components have been used as a tool to investigate human cognition and its underlying brain mechanisms, because music affects the cortical and subcortical areas (Pantev and Herholz, 2011; Koelsch et al., 2018). Some studies show that listening to music improves cognitive skills such as fluency (Thompson et al., 2006), working memory (Mammarella et al., 2007), and recognition memory (Ferreri et al., 2013), among others. For example, background music was investigated as a focal and acute strategy that could improve cognitive skills. This technique refers to any music that is played while the listener's primary attention is focused on another task or activity (Bottiroli et al., 2014). Different studies about the effect of background music have shown some improvements on cognitive abilities. For example, Judde and Rickard (2010) performed a study in which participants listening 3 min of music after the acquisition of information and they had a better recognition memory 1 week later. However, there is some evidence of reduced cognitive performance when music is present (Kämpfe et al., 2010; Rickard et al., 2012).

Furthermore, other investigations indicate that musical production could have even more beneficial effects than musical perception (Lappe et al., 2008; Fancourt et al., 2014). There is some research about music production, as a focal intervention, in the field of neurologic music therapy (Thaut et al., 2009; Thaut and Hoemberg, 2014), but none of them focused on the effects of music production on memory. Besides, the studies distinguish how music and its components affect people with and without formal musical knowledge (Zuk et al., 2014; Schlaug, 2015; Zhao et al., 2017). In general, because of their extensive training affecting the anatomical and functional organization of their brains, musicians have been shown to have a greater cognitive reserve than non-musicians (Hanna-Pladdy and Gajewski, 2012), and hence, their memory would be less compromised over the years (Talamini et al., 2018). In addition, the protective effect of playing an instrument is greater than that of other leisure activities (Amer et al., 2013). For example, some studies indicated that music training has shown improvements in the cognitive functions of older musicians compared with non-musicians, such as memory, naming, and executive functions, among others (Hanna-Pladdy and MacKay, 2011).

Among the interventions that involve musical production, musical training is the one that has received the most attention. Training includes learning how to play an instrument, and most studies evaluate the effect of moderate or long-term learning (Barrett et al., 2013), leaving a gap as far as focal interventions are concerned. Another intervention that involves musical production is musical improvisation, which is defined as an example of musically creative behavior, conceived as an original and novel process requiring divergent thinking (Bengtsson et al., 2007; Manzano and Ullén, 2012; Diaz Abrahan and Justel, 2015). Research is scarce in this area, and most studies emphasize the use of improvisation in musicians (Limb and Braun, 2008); assuming that improvising musically implies having some degree of expertise in music. However, it is also used with people without musical training as a technique for the patient population (e.g., neurological music therapy, Thaut et al., 2009). In this perspective, music improvisation is conceived as the combination of sounds created in a specific framework inside an environment of trust, which is established to address the needs of the participant or patient (Wigram, 2004). In this sense, music improvisation is not only performed by musicians, but it is also a real-time ability that every person has (Wigram, 2004). Still, research on the use of the musical improvisation technique in people without a pathology and in non-musicians is infrequent. In addition, older people are unlikely to begin learning an instrument at an advanced age. Therefore, providing the opportunity of a focal intervention where the participants play instruments and create something novel in groups, without long-term demands, could result in low dropout rates.

The main goal of this work was to investigate the effect of a focal environmental activity as a possible memory improvement technique in older adults. We evaluated whether there were differences between neutral and emotional memory and between participants with and without formal musical knowledge. The intervention employed was musical improvisation, because it involves a musically creative behavior that may be implemented in musicians or non-musicians and because this focal/acute technique is used with older adults. We expected musical improvisation to improve memory and musicians to perform better than non-musicians in the memory evaluations. Finally, we hypothesized that information with emotional content would be better remembered than neutral information.

STUDY 1

Materials and Methods

Participants

Sixty-nine volunteers (75% female participants) between the ages of 60 and 90 (M = 74.16; SD = 1.1) participated in this study. Twenty-six were musicians (M) with more than 5 years of formal musical training (schools, institutes, music conservatories). Forty-three were considered non-musicians (NM). An *a priori* power analysis suggested that N = 57 would be adequate to provide 0.60 power (software G***power*, Faul et al., 2007). They were recruited from different senior cultural centers through online announcements. Participant exclusion criteria included visual or hearing impairment, amusia, or any music-related pathology, cognitive impairment, and depression. Each participant signed a written informed consent form and completed a questionnaire where socio-demographic and musical expertise information was requested. The procedure was approved by the University of Buenos Aires Ethics Committee.

Measures

General Cognitive State Evaluation

As depressive symptomatology may affect memory, we administered the Yesavage Geriatric Depression Scale (GDS, Sheikh and Yesavage, 1986; Martinez de la Iglesia et al., 2002), which measures depression specifically in older adults by assessing anhedonia, sadness, loss of interest, etc. Scores between 0 and 10 are considered to be within the normal range, scores 11–14 show sensitivity to depression, and scores over 14-signal show depression. The participants with a score of 11 or more were excluded. The Mini Mental State Examination (MMSE, Folstein et al., 1975) was used to rule out cognitive impairment. The MMSE is a screening test that measures dementia symptoms. Scores between 9 and 11 are considered to be within the dementia range, scores between 12 and 24-signal cognitive impairment, and scores between 24 and 26 suggest sensitivity to dementia. For schooled participants under 75 years of age, 27 points was the cut score; when the schooled participants were over 75 years old, 26 was the score selected to exclude participants (Butman et al., 2001). Both, the GDS and MMSE were administered individually.

Neutral Memory Evaluation

The material for the neutral memory task was the Rey Complex Figure (RCF; Meyers and Meyers, 1995). It is a figure with 18 different items that compose a larger image.

Instrumental Setting

For the musical experiences (imitation or improvisation), participants were allowed to choose percussion instruments (e.g., drums, maracas, bells, wood blocks, shakers, tambourine) or melodic/harmonic instruments (e.g., guitar, melodica, xylophone, flutes). These instruments were included because they were easy to handle.

Musical Interventions

Music Improvisation (Experimental Condition, EXP). The first author (a music therapist) performed a rhythmic pattern repeatedly during 3 min as a base for an improvised performance by the participants playing their instruments. This pattern was performed with a percussion instrument at a medium volume (**Figure 1**; Berkowitz and Ansari, 2008, 2010; Manzano and Ullén, 2012; Pinho et al., 2016). Participants chose any instrument and improvised musical patterns with instruments or their voices or bodies, spontaneously creating some musical feature according to the context provided by the base-pattern. The instructions included playing without restrictions: the researcher proposed a free improvisation based on the same rhythmical pattern used in REP intervention (**Figure 1**). Such a rhythmical baseline was introduced in order to guide non-musician participants because pilot studies had shown that without such a guidance participants could not follow the improvisation directions.

Imitation (Control Condition, CTRL). The same researcher who conducted the musical improvisation performed the same rhythmic pattern repeatedly during 3 min as a model to be imitated by the participants with their instruments. This pattern was performed in the same percussion instrument at a medium volume. In this intervention, the participants imitated the pattern for 3 min (Gilbertson, 2013). The instructions included imitating the pattern heard as faithfully as possible, avoiding variations or new musical materials. This intervention was meant to control for possible effects of movements, music perception, musical instruments, among others, that could explain the results.

Experimental Design

Because there were two interventions (EXP vs. CTRL) and the participants had different musical expertise (M and NM), a 2(Intervention) × 2(Training) experimental design was run, with four groups with the following number of subjects: (1) M/EXP: musicians' improvisation group (n = 15); (2) M/CTRL: musicians' imitation group (n = 11); (3) NM/EXP: non-musicians' improvisation group (n = 22); and (4) NM/CTRL: non-musicians' imitation group (n = 21). Participants were randomly and blindly assigned to the different groups, and they were always tested in groups, with a minimum of four and a maximum of 10 participants, in order to control the involvement of each participant in the music performance.

Procedure

The study was divided into two sessions with a one-week intersession interval. The first session consisted of four immediately

FIGURE 1 | Rhythmic base-pattern presented by the researcher to guide both music reproduction and improvisation that participants were asked to perform with a set of basic instruments.

consecutive phases. In the first phase (information phase, about 15 min), the participants signed the informed consent form and completed the socio-demographic and musical expertise questionnaire. In this step, we also evaluated the general cognitive state with MMSE and GDS. In the second phase (acquisition, 9 min), the participants watched the RCF and they were asked to copy it (they were supplied with pencil and paper).

In the third phase (treatment phase, about 3 min), the participants were exposed to the musical interventions (improvisation or imitation). The following directions were given during the music improvisation intervention: "*We will listen to a rhythmic base, from which you have to create something musical as a group. This rhythmic base will help you to start the improvisation at any time you want. You can use instruments, your voice or your body. It is important to listen not only to the base but also to your own group*." In the imitation intervention (control condition), the following directions were given: "*We will listen to a rhythmic base and, anytime you want, you can start to imitate me. You can use instruments, your voice or your body*." Before starting, the researcher corroborated that all the participants understood the instructions. Then, they chose freely the musical instrument that they wanted to play, and they performed the improvisation or imitation task in groups for 3 min.

Soon afterwards, in the fourth phase (test phase, about 11 min), a two-task test was run. Participants were given paper and pencil to drawn from memory the RCF (*Immediate Free Recall* task), and then 12 target items of the RCF were mixed with 12 new items and participants were asked to indicate whether they had seen the item before or not (*Immediate Recognition* task).

The second session (11 min) was held a week later, when the two-task test was run again (*Deferred Free Recall* task and *Deferred Recognition* task; see **Figure 2** for a schematic design of the procedure).

Data Analysis

Age, years of formal education, and years of musical education were analyzed independently *via* univariate analysis of variance (ANOVA), where *Intervention* (improvisation vs. imitation) and *Training* (musicians vs. non-musicians) were the between-factors.

Copy and free recall (immediate and deferred) of the RCF were evaluated by means of the following procedure: Each of the 18 components of the RCF was evaluated according to whether it was well-drawn and correctly located (2 points), well-drawn but incorrectly located (1 point), badly drawn but correctly located (1 point), badly drawn but recognizable (0.5 points), and badly drawn and incorrectly located (0 points). The maximum final score could amount to 36. Because musicians had more years of education than non-musicians and because there were differences in the copy of the RCF (data shown in Results section), recall and recognition (immediate and deferred) were independently analyzed *via* ANCOVA with *Intervention* (improvisation vs. imitation) and *Training* (musicians vs. non-musicians) as the between-factors and *Education* and *Copy* as the co-variables.

Post hoc least-significant difference (LSD) pairwise comparisons were conducted to analyze significant interactions. The partial Eta square (η_p^2) was utilized to estimate effect size. The alpha value was set at 0.05, and the SPSS software package was used to compute descriptive and inferential statistics.

RESULTS

Socio-Demographic Characteristics and General Cognitive State

The final sample consisted of 64 participants, because five evaluations were discarded due to cognitive deficit and/or depression; the final number of participants per group were

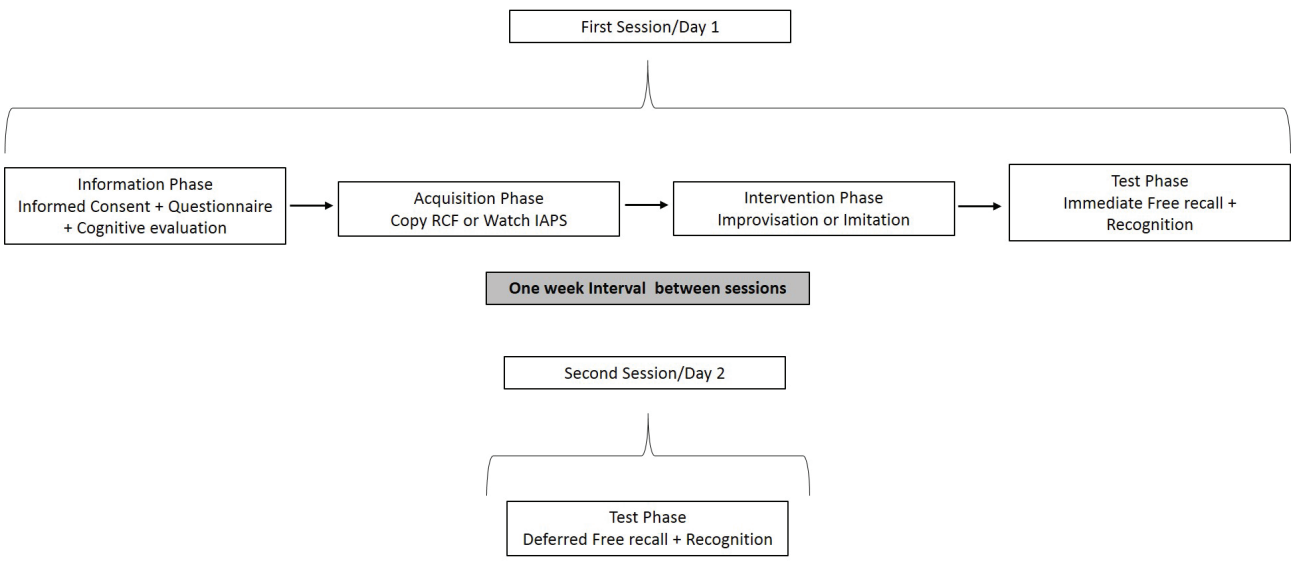

FIGURE 2 | Scheme of the experimental procedure.

as follows: (1) M/IMP = 13; (2) M/REP = 10; (3) NM/IMP = 21; and (4) NM/REP = 19. The general cognitive state values (MMSE and GDS) are depicted in **Table 1**.

Regarding the socio-demographic information (**Table 1**), no differences were found between the groups in terms of age $p > 0.05$. Nonetheless, depending on the educational level, there were differences in the Intervention factor $F(1, 68) = 5.95$, $p = 0.017$, $\eta_p^2 = 0.084$, where the improvisation groups had a higher educational level than the imitation groups. For this reason, educational level was a co-variable in the statistical analyses performed for the memory evaluations of the RCF. As regards musical expertise, there were differences in the Training factor $F(1, 68) = 61.26$, $p < 0.0001$, $\eta_p^2 = 0.485$, as expected, since we selected musicians and non-musicians for the samples. The average year of musical experience in the musicians' group was 15.24 ± 2.4 years. Non-musicians had an average musical experience of 0.96 ± 0.3 years.

Copy of the RCF

The acquisition of neutral visual information was evaluated through the copy of the RCF. The results are depicted in **Figure 3**. The ANCOVA indicated a main effect of the Intervention factor $F(1, 64) = 9.98$, $p = 0.002$, $\eta_p^2 = 0.135$. The *post hoc* test showed that the improvisation groups had higher copy scores than the imitation groups. Due to this result, copy was implemented as an additional co-variable in the subsequent memory analysis (immediate and deferred).

Immediate Measures

After being exposed to the different musical interventions, the participants were instructed to draw from memory the RCF that they had seen in the acquisition phase. The ANCOVA yielded a main effect of Training $F(1, 63) = 8.68$, $p = 0.005$, $\eta_p^2 = 0.121$. The *post hoc* analysis indicated that musicians had a better recall of the RCF than non-musicians (**Figure 4A**).

Recognition was the second task employed to evaluate memory. The participants watched 24 items, and they had to decide which ones were part of the RCF and which were new. False recognitions were subtracted from the total recognition score. The results are depicted in **Figure 4B**. The ANCOVA indicated a significant effect of the double interaction Training × Intervention $F(1, 63) = 4.889$, $p = 0.031$, $\eta_p^2 = 0.072$. The *post hoc* test showed that the musicians' improvisation group had a better recognition score than non-musicians' improvisation group. Also, this test indicated that the non-musicians' imitation group had a better recognition score than the musicians' imitation group.

Deferred Measures

After 7 days, free recall and recognition were again evaluated (deferred measures). Regarding free recall, the ANCOVA indicated a main effect of Intervention $F(1, 57) = 8.36$, $p = 0.005$, $\eta_p^2 = 0.128$. The *post hoc* test showed that improvisation groups had a better recall of the RCF than imitation groups (**Figure 5A**).

After the free recall evaluation, participants watched 24 items, and they had to decide which ones were part of the RCF and which were new. False recognitions were subtracted from the total recognition score (**Figure 5B**). The ANCOVA yielded a main effect of Training $F(1, 57) = 4.696$, $p = 0.034$, $\eta_p^2 = 0.076$. The corresponding *post hoc* indicated that the participants with formal musical knowledge had a better recognition score than the non-musicians.

STUDY 2

Materials and Methods

Participants

Sixty-three *new* volunteers (76% female) between the ages of 60 and 90 ($M = 71.94$; $SD = 0.91$) participated in this study. Twenty-five were musicians (M) with more than 5 years of musical formal training (schools, institutes, music conservatories). Thirty-eight participants were considered non-musicians (NM). An *a priori* power analysis suggested that $N = 57$ would be adequate to provide 0.60 power

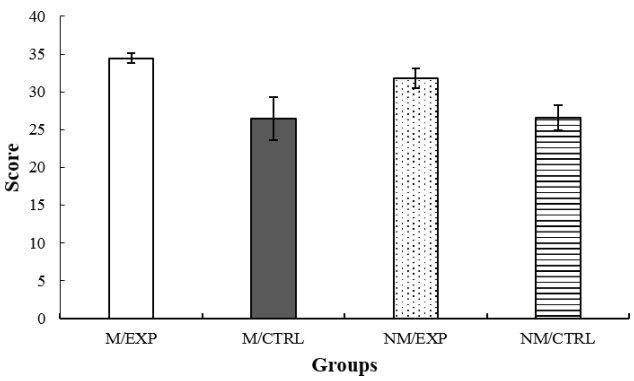

FIGURE 3 | Score for the copy of the RCF. M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group.

TABLE 1 | Socio-demographic and general cognitive state data.

Groups	Age	Education	Musical educ.	MMSE	GDS	Copy
M/EXP	70.93 ± 3.51	15.27 ± 0.99	13.87 ± 3.41	28.17 ± 0.35	4.75 ± 0.72	34.47 ± 0.65
M/CTRL	74.55 ± 2.92	12.64 ± 1.55	16.45 ± 3.22	28.2 ± 0.42.9	4.4 ± 0.99	26.45 ± 2.87
NM/EXP	74.47 ± 1.55	13.95 ± 0.62	0.56 ± 0.41	28.14 ± 0.26	6.24 ± 0.88	31.77 ± 0.33
NM/CTRL	76.05 ± 1.6	11.9 ± 0.88	1.43 ± 0.33	28.11 ± 0.25	6.47 ± 0.7	26.52 ± 1.64

Median ± standard deviation, for age, years of education, years of musical education, MMSE, and GDS. M/EXP, musicians' improvisation group; M/CTRL, musicians' imitation group; NM/EXP, non-musicians' improvisation group; NM/CTRL, non-musicians' imitation group.

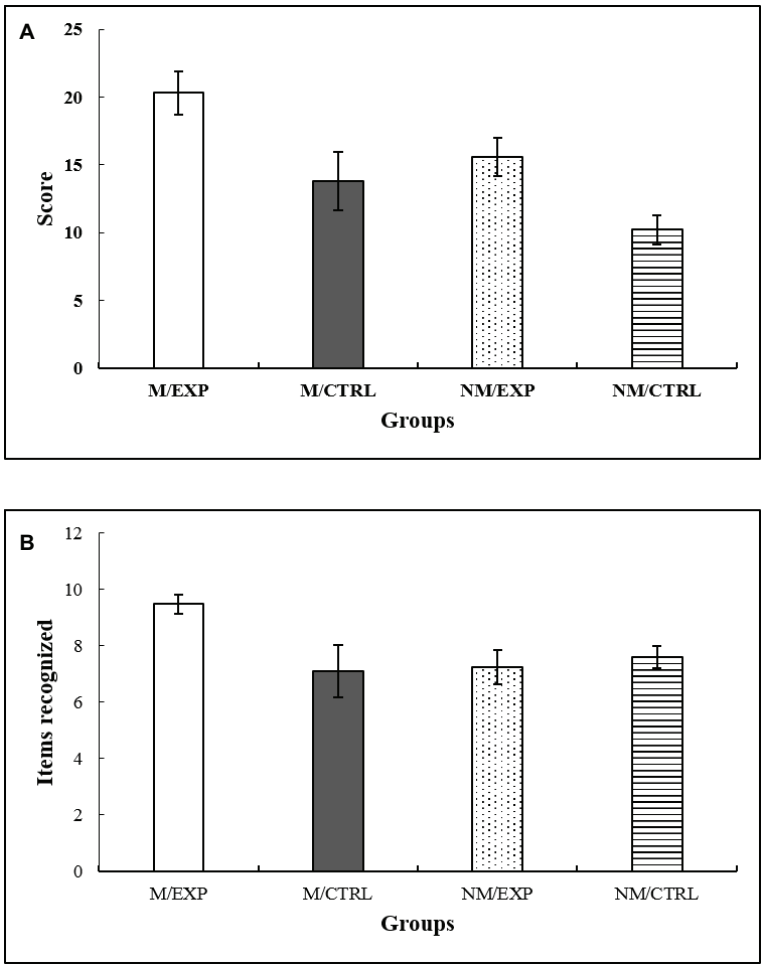

FIGURE 4 | (A) Immediate free recall. Score obtained according to the accuracy and location of the figure drawn from memory. **(B)** Immediate recognition. Number of items recognized in a set of 24 items. M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group. Vertical lines represent standard errors of the mean.

(Faul et al., 2007). They were recruited from different senior cultural centers through online announcements. The participant exclusion criteria were the same as those used in Study 1. Each participant signed a written informed consent form and completed a questionnaire where socio-demographic and musical expertise information was requested. The procedure was approved by the University of Buenos Aires Ethics Committee.

Measures

General Cognitive State Evaluation

This evaluation was conducted in the same way as in Study 1.

Emotional Memory Evaluation

The material for the emotional memory task consisted of thirty-six pictures selected from the International Affective Pictures System (IAPS; Lang et al., 1995). Twenty-four pictures were emotionally arousing (12 with a positive valence and 12 with a negative valence) and 12 were non-arousing, neutral images. Following guidelines from previous works (Cahill et al., 2003), we selected the pictures, which covered a wide range of arousal (from 2.95 to 6.36) and valence (from 1.97 to 4.93) in line with the manual by Lang et al. (1995).

Instrumental Setting

The setting was the same as the one used in Study 1.

Musical Interventions

The musical interventions were the same as the ones used in Study 1.

Experimental Design

Because there were two interventions (EXP vs. CTRL) and the participants had different levels of musical expertise (M and NM), a 2(Intervention) × 2(Training) experimental design was run, with four groups with the following number of subjects: (1) M/EXP: musicians' improvisation group (n = 13); (2) M/CTRL: musicians' imitation group (n = 12);

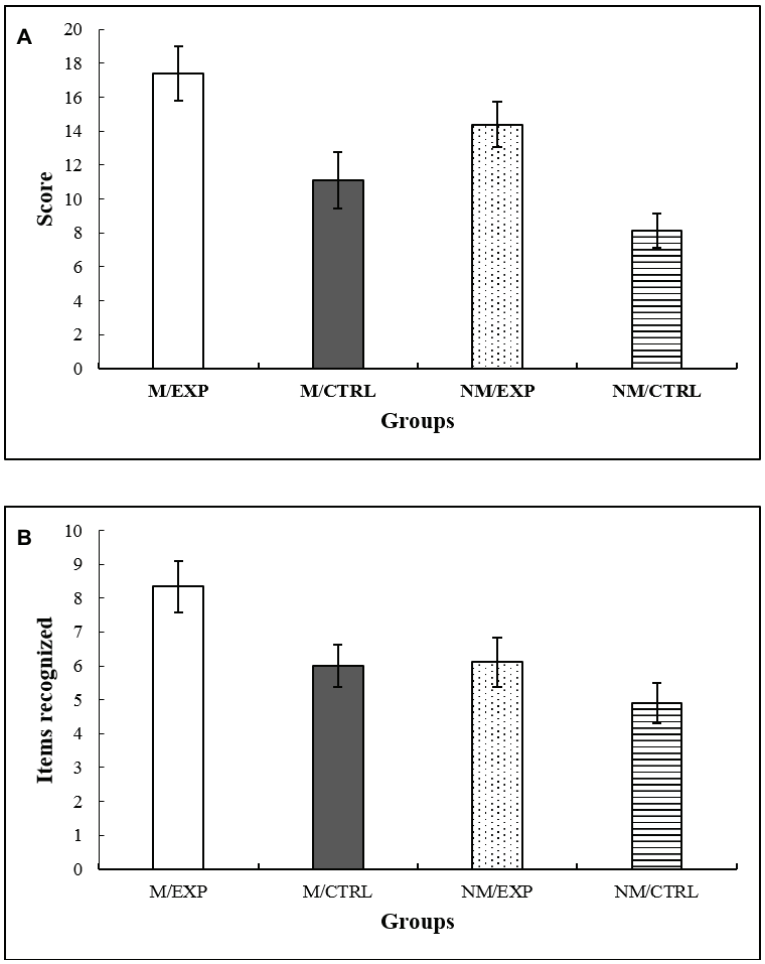

FIGURE 5 | (A) Deferred free recall. Score obtained according to the accuracy and location of the figure drawn from memory after 7 days. **(B)** Deferred recognition. Number of components recognized in a set of 24 figures, after 7 days. M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group. Vertical lines represent standard errors of the mean.

(3) NM/EXP: non-musician' improvisation group (n = 18); and (4) NM/CTRL: non-musicians´ imitation group (n = 20). Participants were randomly and blindly assigned to the different groups, and they were always tested in groups, with a minimum of four and a maximum of 10 participants in order for the researchers to control the involvement of each participant in the music performance.

Procedure

This study was also divided into two sessions with a one-week intersession interval. The first session consisted of four immediately consecutive phases. The first phase was identical to the one used in Study 1.

In the second phase (acquisition phase, about 7 min), the participants watched the 36 selected pictures for 7 s each. The pictures were presented in random order except for the first and last locations in the series, which had to meet the condition of being a neutral picture (Cahill et al., 2003). Simultaneously, the participants were asked to rate on a 0–10 scale "how emotional" or "activating" they felt each image was (from 0 = not arousing at all to 10 = highly arousing). This behavioral task (*Arousal* task) was included in order to (1) ensure that the participants paid attention to each image; (2) validate the selection of IAPS images for this research context, and (3) compare the emotional impact of the images between M-NM groups prior to the musical intervention.

The third phase (intervention) was identical to the one employed in Study 1. Soon afterwards, in the fourth phase (test phase, about 11 min), a two-task test was run. The participants were asked to describe in one word or short phrase as many pictures as they could remember (*Immediate Free Recall* task). Next, they observed the 36 original pictures mixed with 36 new pictures in a random order and they had to mark on a sheet of paper if they had seen the image before or not (*Immediate Recognition* task).

The second session (11 min) was held a week later, when the two-task test was run again (*Deferred Free Recall* task and *Deferred Recognition* task; see **Figure 2** for a schematic design of the procedure).

Data Analysis

Age, years of formal education, and years of musical education were analyzed independently *via* univariate analysis of variance (ANOVA), where *Intervention* (improvisation vs. imitation) and *Training* (musicians vs. non-musicians) were the between-factors.

Because musicians had more years of education than non-musicians (data shown in Results), arousal, recall, and recognition (immediate and deferred) were independently analyzed *via* repeated measures (RM) ANCOVA with *Intervention* (improvisation vs. reproduction) and *Training* (musicians vs. non-musicians) as the between-factors, *Picture* (neutral, positive, and negative) as the RM, and *Education* as the co-variable.

Post hoc least-significant difference (LSD) pairwise comparisons were conducted to analyze significant interactions. The partial Eta square (η_p^2) was utilized to estimate effect size. The alpha value was set at 0.05, and the SPSS software package was used to compute descriptive and inferential statistics.

RESULTS

Socio-Demographic Characteristics and General Cognitive State

The final sample was composed of 52 participants, because 11 evaluations were discarded due to cognitive impairment or depression; the final number of participants per group was as follows: (1) M/EXP = 12; (2) M/CTRL = 10; (3) NM/EXP = 15; and (4) NM/CTRL = 15. The general cognitive state information (MMSE and GDS) is depicted in **Table 2**.

Regarding socio-demographic information (**Table 2**), there were no differences between groups related to age $p > 0.05$. Nonetheless, there were differences depending on the educational level related to the Training factor $F(1, 44) = 5.79$, $p = 0.02$ $\eta_p^2 = 0.116$. The musicians had a higher academic level than the non-musicians, and therefore, this variable was considered a co-variable in the statistical analyses that were performed for memory. There were differences in musical level related to the Training factor $F(1, 45) = 29.53$, $p < 0.0001$, $\eta_p^2 = 0.39$, as expected, since we selected musicians and non-musicians for the samples. The average year of musical experience in the musicians' group was 16.05 ± 3.43 years. Non-musicians had an average musical experience of 0.53 ± 0.23 years.

Arousal

Arousal was the first dependent variable analyzed. Participants watched neutral, positive, and negative images, and simultaneously rated, from 0 to 10, how arousing the pictures were for them. The emotional pictures were rated as more activating than the neutral ones, and the rating of neutral images was affected by Training and Intervention (**Figure 6**). These impressions were corroborated by the statistical analysis, since the ANCOVA yielded a main effect of Picture $F(2, 86) = 12.817$, $p < 0.0001$, $\eta_p^2 = 0.230$, while the corresponding *post hoc* indicated that the emotional images were considered more activating than the neutral ones. Besides, the effect of the Picture × Intervention interaction was significant $F(1, 43) = 5.23$, $p = 0.027$, $\eta_p^2 = 0.108$, and the triple interaction Picture × Intervention × Training was also significant $F(2, 86) = 4.27$, $p = 0.017$, $\eta_p^2 = 0.09$. The analysis of the triple interaction indicated that the M/EXP group rated the neutral images as more activating than did the M/CTRL group, while the opposite pattern was observed in non-musicians since the NM/CTRL group rated the neutral images as more activating than did the NM/EXP group. In addition, the NM/CTRL group rated the neutral images as more activating than did the M/CTRL group.

Immediate Measures

After participants were exposed to the intervention (imitation or improvisation), they were asked to recall as many pictures as they could. The ANCOVA indicated a significant effect of Intervention $F(1, 43) = 6.93$, $p = 0.012$, $\eta_p^2 = 0.139$, where the *post hoc* showed that the improvisation group remembered more images than the imitation group. Also, the double interaction Picture × Intervention achieved significance $F(2, 86) = 5.22$, $p = 0.007$, $\eta_p^2 = 0.108$. The *post hoc* indicated that the improvisation group remembered more negative images than the imitation group. The results are depicted in **Figure 7**.

After the free recall, the participants observed the 36 original pictures randomly intermixed with 36 new ones. They had to discriminate the new images from the old ones. The ANCOVA indicated no significant differences in Training, Picture, or Intervention, or any of their interactions, $p > 0.05$ (data not shown).

Deferred Measures

The test of free recall and recognition tasks was repeated a week later. **Figure 8A** illustrates the results of the free recall task. The ANCOVA indicated a main effect of Intervention

TABLE 2 | Socio-demographic and general cognitive state data.

Groups	Age	Education	Musical educ.	MMSE	GDS
M/EXP	73 ± 2.32	14.36 ± 0.93	15.36 ± 3.84	28.78 ± 0.43	4.71 ± 0.7
M/CTRL	73 ± 3.28	17.25 ± 2.15	17 ± 6.63	27.67 ± 0.9	4.14 ± 1.06
NM/EXP	71.8 ± 1.72	12.93 ± 1.06	1.07 ± 0.41	28.94 ± 0.25	4.76 ± 0.58
NM/CTRL	71.47 ± 1.59	13.07 ± 0.65	0 ± 0	28.73 ± 0.21	3.47 ± 0.71

Media ± standard deviation, for age, years of education, years of musical education, MMSE, and GDS. M/EXP, musicians' improvisation group; M/CTRL, musicians' imitation group; NM/EXP, non-musicians' improvisation group; and NM/CTRL, non-musicians' imitation group.

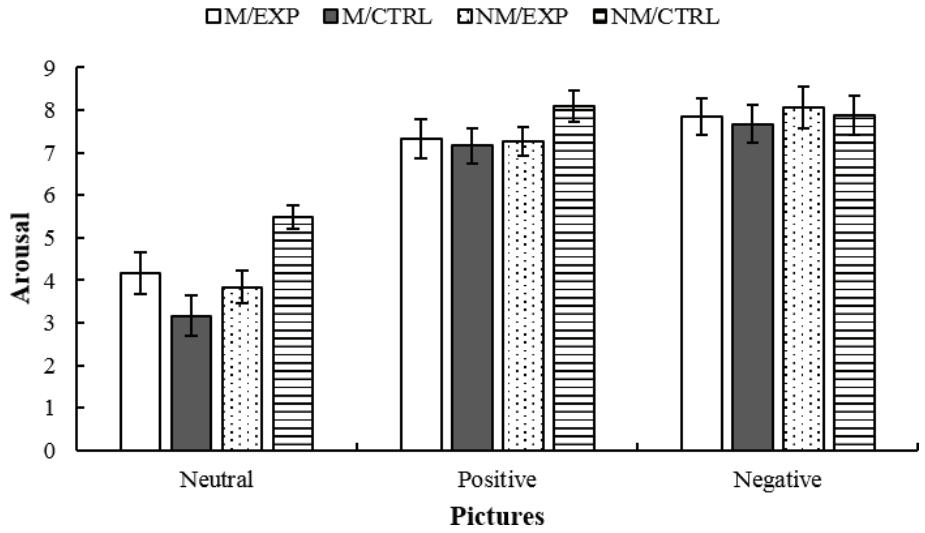

FIGURE 6 | Assessment of neutral, positive, and negative images on a scale from 0 to 10 (0 = non-arousing at all. 10: The highest level of arousal elicited from the participant). M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group. Vertical lines represent standard errors of the mean.

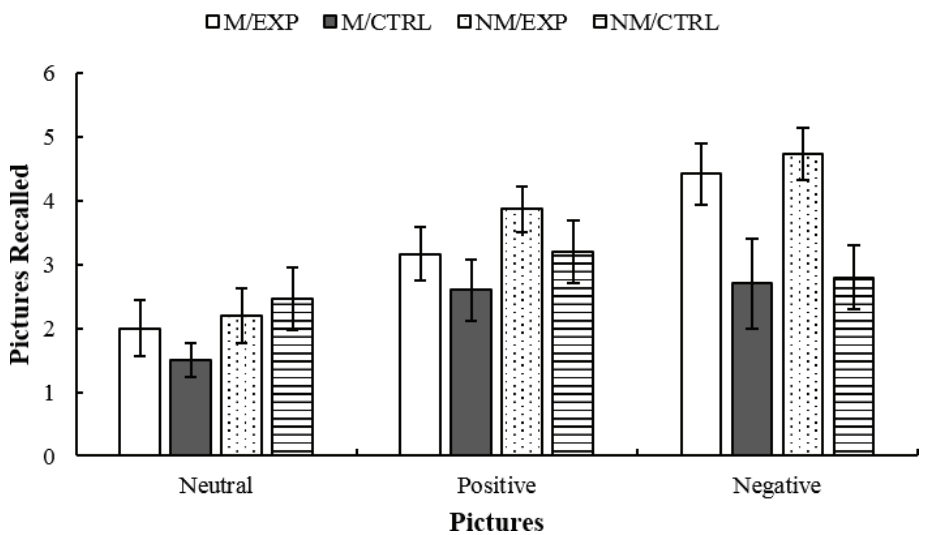

FIGURE 7 | Immediate free recall. Mean number of neutral, positive, and negative pictures that groups could remember after the treatment. M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group. Vertical lines represent standard errors of the mean.

$F(1, 43) = 18.27$, $p < 0.0001$, $\eta_p^2 = 0.29$, the *post hoc* showed that the improvisation groups remembered more images than the imitation groups. The double interaction Picture × Intervention also achieved significance $F(2, 86) = 5.59$, $p < 0.005$, $\eta_p^2 = 0.115$, and the corresponding *post hoc* indicated that for positive and negative images the improvisation groups remembered more images than the imitation groups.

To evaluate recognition, the 36 target pictures were mixed with 36 new pictures and participants had to indicate whether the images were new or old (**Figure 8B**). False recognitions were subtracted from the total recognition score (from each of the pictures). The ANCOVA showed a significant main effect of Intervention $F(1, 43) = 9.76$, $p = 0.003$, $\eta_p^2 = 0.185$, where the improvisation groups recognized more images than the imitation groups. In addition, there was a main effect of Picture $F(2, 86) = 3.17$, $p = 0.047$, $\eta_p^2 = 0.069$, and the *post hoc* indicated that the neutral pictures had a better recognition score than the positive and negative ones and also that the positive images were better recognized than the negative ones. Finally, the double interaction Picture × Intervention achieved significance $F(2, 86) = 3.29$, $p = 0.042$, $\eta_p^2 = 0.071$. This interaction indicated that in the three

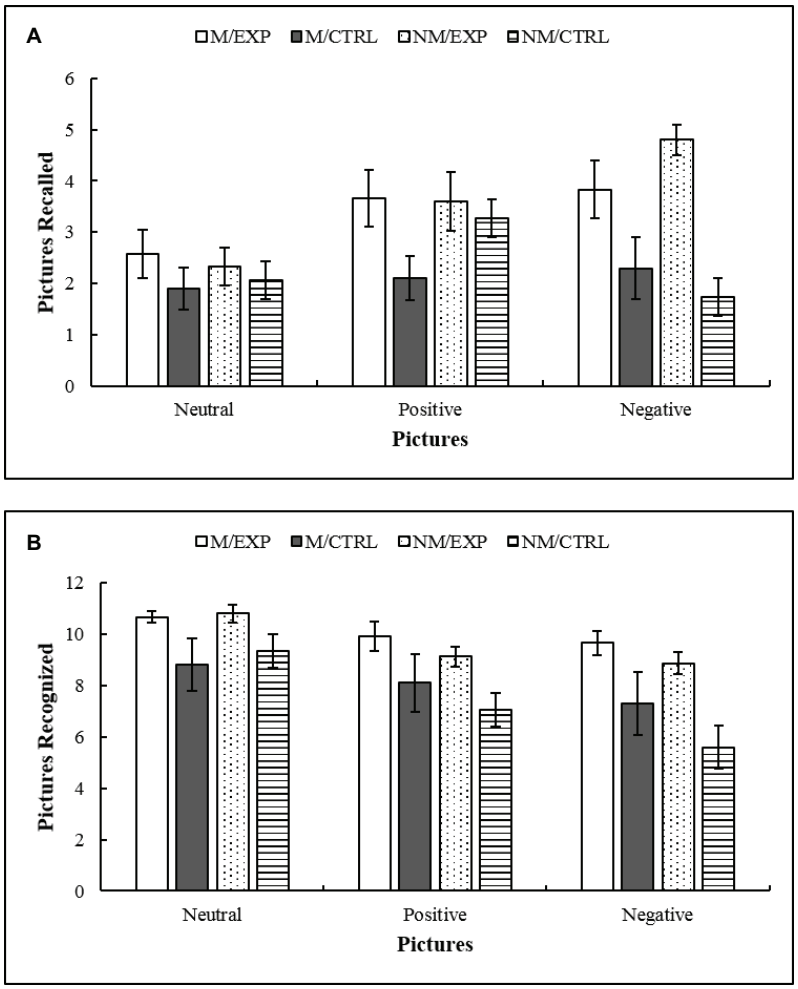

FIGURE 8 | (A) Deferred free recall. Mean number of neutral, positive, and negative pictures that groups could remember after a week, between treatment and testing. **(B)** Deferred recognition. Number of neutral, positive, and negative pictures that participants could recognize as previously seen from a pool of 72 images. M/EXP: musicians' improvisation group; M/CTRL: musicians' imitation group; NM/EXP: non-musicians' improvisation group; and NM/CTRL: non-musicians' imitation group. Vertical lines represent standard errors of the mean.

types of images, the improvisation group had a better recognition score than the imitation groups.

DISCUSSION

The goal of this work was to evaluate if a musical intervention could improve neutral or emotional memory in older adults with or without formal musical knowledge. Our control group was not a passive one; instead, it participated in a group musical activity, allowing us to detect specific parameters in each type of intervention that could explain the possible benefits of improvisation. The main results indicated that musical improvisation enhanced memory especially when the information to be consolidated was emotional, indicating that the intervention is more linked to the emotional content than to the neutral one. In addition, musicians performed better than non-musicians. In the following paragraphs, each of the findings is explained in detail.

In both studies, the improvisation groups had a better mnemonic performance than the imitation groups. Nonetheless, this effect was higher in Study 2, where memory with emotional content was evaluated. The improvisation groups performed better at their immediate and deferred free recall and also at their deferred recognition than the imitation groups. Furthermore, in the immediate free recall, the negative images were better remembered; in the deferred free recall, both positive and negative images; and in the deferred recognition, the three types of images were better recognized. In other words, over time, the information had a better consolidation and the participants remembered or recognized more information. By contrast, in the complex figure, better performance was achieved in the improvisation condition only for the deferred free recall. These results would indicate that there was an interaction between musical improvisation and visual memory, and the greatest effect was found for the emotion-laden information.

A possible explanation for these findings is that during the experience of musical improvisation a melody and a rhythm are spontaneously created, integrating the emotional with the different cognitive levels (Bruscia, 1998, 1999). In this musical technique, all the body is used to express intentions, emotions, and memories. For this reason, musical improvisation is defined as a special self-expression technique (Gilboa et al., 2006; Punkanen, 2011; Godman, 2012; McPherson et al., 2014). Besides, it has been shown that sound is a potent elicitor of emotions and that musical experiences activate specific pathways in several brain areas associated with emotional content, such as the cingulate and insular cortices, hypothalamus, hippocampus, amygdala, and prefrontal cortex (Boso et al., 2006; Koelsch, 2012, 2014). A study conducted by Koelsch et al. (2018) demonstrated that the auditory cortex, activated during the musical perception, hosts regions that are influential within networks underlying the affective processing of auditory information. The emotional state induced by the musical improvisation may have enhanced the emotions produced by the affective pictures, thus strengthening the memory process. Some studies indicate that music, because of the emotionality state that it generates (Koelsch, 2012), will work as an enhancer of visual elements loaded with emotion (Logeswaran and Bhattacharya, 2009; Kamiyama et al., 2013), causing a synergy between both emotional states. In the first study, this synergic effect between the emotion aroused by the improvisation and the emotion aroused by the task was not observed, probably because the stimuli lacked emotional content.

Musical improvisation, as opposed to imitation where a pattern is replicated, is characterized by the presence of creative elements. This characteristic would indicate that it is not the music itself that modulates memory, since in the imitation condition, participants also perceive and produce musical components but rather the creation of a novel musical product in groups. In future studies, a creative non-musical group could be added to address this topic. Besides, spontaneous improvisation, as opposed to the performance of learned sequences (as in the imitation), is characterized by an extensive deactivation of the medial dorsolateral prefrontal cortex and lateral orbital regions with a focal activation of the medial prefrontal cortex (Limb and Braun, 2008). In addition, there is a relation between musical improvisation and autobiographic memories, since independently of the level of complexity used in the improvisation, the prefrontal and medial temporal cortices are activated, and these areas are involved in memory (Limb and Braun, 2008).

Imitation could interfere with memory. When there are restrictions, especially attentional ones where the participant is asked to replicate, repeat a pattern, adjust to it in intensity, and synchronize, this intervention could diminish cognitive resources and lead to mnemonic deterioration (Miendlarzewska et al., 2013). This is relevant since most musical activities designed for older adults are repetitive (the typical case is the choir, where the participant has to memorize his or her part, pay attention to the tuning, rhythm, etc.). Even though these activities reinforce musical contents *per se*, they are less efficient when the goal is to improve cognitive skills such as memory.

In the first study, an effect of musicianship was found, which is in line with previous studies about the effect of musical training on visual memory (Hanna-Pladdy and MacKay, 2011). Musicians outperformed non-musicians in immediate free recall and recognition and in deferred recognition. A plausible explanation for the better performance of musicians is that there are structural and functional brain differences between musicians and non-musicians (Zatorre, 1998; Gaser and Schlaug, 2003; Lotze et al., 2003; Bermúdez and Zatorre, 2005; Zatorre et al., 2007; Justel and Diaz Abrahan, 2012; Barrett et al., 2013; Strait and Kraus, 2014; Schlaug, 2015; Herrero and Carriedo, 2018; Li et al., 2018). Becoming a skilled musician requires extensive training, and the type of learning involved entails the development of several abilities (e.g., perception, cognitive control, memory, motor skills, among others). The abilities developed by musicians induce connections and interactions between several brain areas. The brain structural differences between musicians and non-musicians were found to involve the enlargement or thickening of numerous areas in people with musical training. Some of these differences were associated with the anteromedial portion of Heschl's gyrus, the corpus callosum, the *planum temporale*, and with changes in gray matter that implied a greater plasticity (Luders et al., 2004; Bermúdez et al., 2009; Anaya et al., 2016).

At the same time, the structural differences are accompanied by functional and behavioral divergences in several domains (Herrero and Carriedo, 2018). Depending on the extent of the effect of musical training, the near transfer label is used when the cognitive functions affected by training are those related closely with music, such as the recognition of melodic contour or intervallic sequences (Fujioka et al., 2004). While musical training could transfer cognitive advantages that go beyond musical areas, if the functional change is observed in non-musical skills such as language (Schlaug et al., 2005), mathematical reasoning (Vaughn, 2000), or attentional functions (Wang et al., 2015), the process is named far transfer. In the present work, we contribute evidence to the far-transfer literature, since the benefits for musicians were observed in a cognitive skill not strictly related to musical training.

The fact that we found no differences in terms of musical training in the second study could be associated with non-musicians benefiting from the information with emotional content, and accordingly the greater effect was observed in the intervention factor (improvisation vs. imitation). Because, in the first study, the emotional components were not present, the prevailing factor was musicianship (training). Therefore, the emotionality effect associated with the intervention (improvisation) could have shadowed the training factor effects in the second study.

Nonetheless, it is not necessary to be a professional musician and have lifetime experience in music to benefit from musical training. Some studies indicated that only 1 week of stimulation in musical perception and production resulted in functional changes in the participants (Bangert and Altenmüller, 2003). Besides, it has been demonstrated that older adults who began their musical training in old age had benefits in several cognitive domains (Bugos et al., 2007). Thus, focal musical interventions (such as the one proposed in the present work) as well as short- and long-term interventions induced a benefit in the cognitive functions of older age participants.

Studies about the effect of music in visual memory are scarce. As far as we know, no research has so far focused on memory with emotional content, and it is in this topic that the novelty of our study lies. Besides, the relation between musical experience and neutral visual memory has been the topic of few studies, with conflicting results. Fauvel et al. (2014) found no enhancement of neutral memory in older adults. However, in agreement with our results, Hanna-Pladdy and Mackay (2011) found an improvement in the visual memory of musicians compared to non-musicians. Notably, as far as learning and evaluation of memory are concerned, there are different tests to evaluate this cognitive function, and it is precisely this issue that differentiates the mentioned studies. The methodologies used for measuring memory could have resulted in the divergences found in the studies.

The limitations of our study involve the inclusion criterion to be considered a musician. The criterion was to have more than 5 years of musical training, and although the participants were asked what musical instrument they played, they were not asked whether they were currently active, how many hours a week they devoted to musical training, or how old they were when they started learning music. These questions will be included in future research. In addition, although we found differences regarding the educational level, this variable was used as a co-variable in the statistical analyses so as not to bias the results. Another limitation of our studies is the sampling. In both studies, more than half of the samples were women, and it is possible that the effects might vary across genders, given that some studies show female participants to be more receptive to emotional cues (Andreano et al., 2008; Nielsen et al., 2011, 2013; Felmingham et al., 2012). We intend to improve this point in future research.

A key challenge for successful aging is to discover cognitive treatments or interventions that have the ability to integrate multiple neural systems that alleviate or prevent age-related cognitive decline (Bugos et al., 2007). Making music is the optimal cognitive intervention that includes multimodal sensorimotor integration, creation of novel elements, motivation, and difficulty. It is relevant to highlight the difference between improvisation and imitation, since the standard musical activities for older adults involve repetitive tasks with no novelty component. By endorsing the advantages of improvisation, group activities could be designed for the purpose of creating something musically novel in a context of social interaction. In addition, improvisation being a social practice, it increases the adherence to the treatment diminishing the dropout rates. Besides, this musical intervention had the benefit that it was pleasant, a motivational factor for the participant to perform this kind of activity, as opposed to other types of training. As a result, despite being a focal intervention, it could be presented in a regular schedule, since the core component is the creation of something musical and always novel. As music improvisation modulates memory, music treatment may provide a simple, safe, and effective method of preventing the potentially harmful physiological concomitants of memory impairment, with great potential for clinical application.

ETHICS STATEMENT

The participants of the studies gave voluntary written consent to take part in the studies without obtaining any type of remuneration and according to the requirements of The Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

VDA and NJ contributed to the conception and design of the studies. VDA conducted the studies. VDA and NJ contributed to data analysis. VDA, FS, and NJ participated in the writing of the paper and interpretation of the data. FS and NJ supervised and integrated the information.

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Diazepam Reduces Escape and Increases Closed-Arms Exploration in Gerbils After 5 min in the Elevated Plus-Maze

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Despite the wide implementation of the elevated plus-maze (EPM) test to assess anxiety-related behaviors in rodents, the interpretation of these measures in gerbils has received limited attention. Here, male gerbils were treated with vehicle or diazepam, followed by a 20-min EPM session. EPM data were subjected to minute-by-minute, 5-min bins and factor analyses. During the first 5-min, gerbils avoided the closed arms in favor of the open arms and diazepam increased open-arms entries; furthermore, a single factor (escape behavior) explained all the analyzed measures. Only after 5-min, gerbils reduced open-arms exploration and three independent factors emerged for each subsequent 5-min bin. These findings suggest that EPM data from gerbils should be analyzed in at least two 5-min bins. Measures from the standard 5-min session seem to be related to an escape response from the EPM through the open arms. Once habituated, measures from the second 5-min bin seem to be related to a conflictive situation: keep trying to escape unsuccessfully (due to open-arms height) or seek protection in the closed arms (unsafe places). Diazepam seems to reduce this conflict by mitigating the escape response (Factor 1 – Anxiety) and increasing closed-arms approach (Factor 2) and risk assessment (Factor 3). Unlike mice and rats, a decrease in open-arms exploration and an increase in risk assessment could be interpreted as an anxiolytic-like effect in gerbils.

Keywords: elevated plus-maze, mongolian gerbil (*Meriones unguiculatus*), anxiety-related behavior, diazepam, factor analysis, ethopharmacology, escape

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INTRODUCTION

The elevated plus-maze (EPM) is a widely used test to evaluate anxiety-related behaviors and to detect anxiolytic properties of compounds in rodents (Carobrez and Bertoglio, 2005; Pawlak et al., 2012). Validated for rats and mice (Pellow et al., 1985; Lister, 1987), the test is based on the natural tendency of these rodents to avoid open spaces in favor of protected areas (Pellow et al., 1985; Lister, 1987; Rodgers et al., 1997; Pawlak et al., 2012). Rats and mice exposed to a 5-min session in the EPM spent more time in the closed arms of the apparatus, and the previous administration of anxiolytic compounds such as benzodiazepines increases open-arms exploration (for review see Carobrez and Bertoglio, 2005). Thus, the increase of entries and time spent in the open arms have been interpreted as a reduction of anxiety-related behaviors (Pellow et al., 1985; Lister, 1987; Rodgers et al., 1997; Pawlak et al., 2012).

The Mongolian gerbil has become a popular animal model used in research areas from gastric and neurological disease to animal cognition (for a review see Hurtado-Parrado et al., 2015). In contrast to rats and mice, gerbils are monogamous and diurnal/crepuscular species (Romero-Morales et al., 2018; Hurtado-Parrado et al., 2019). Furthermore, the fact that their NK1 receptors are closer in homology to the human NK1 receptor (Griffante et al., 2006; Leffler et al., 2009), makes this species a promising model for the study of anxiety disorder (Frick et al., 2015).

The validation of the EPM with female gerbils showed similar results to those found in rats and mice – i.e., gerbils avoided the open arms and prior treatment with diazepam produced increased exploration of those open areas (Varty et al., 2002a). However, male gerbils exhibited high open-arms exploration, despite exposure to a long session of 20-min to the EPM (Rico et al., 2016), and explored the open arms more than rats did (Wang et al., 2018).

Despite the wide implementation of the EPM to assess anxiety-related behaviors in rodents, the interpretation of these measures in gerbils has received limited attention. For instance, differences between gerbils and other rodents in species-specific defense reactions related to exploration of open and closed areas have been not tested.

Gerbils are social rodents commonly found in desert habitats, which live in subterranean nesting burrows interconnected by tunnels (Ågren et al., 1989). Both aerial and ground threats elicit defensive responses of gerbils located on the surface (Ågren et al., 1989; Kotler et al., 1993). Ellard (1996) analyzed these defensive responses using an open field in which gerbils were exposed to six presentations of overhead visual stimuli that resembled an aerial predator. Whereas first presentation of the aerial threat triggered a fleeing response, repeated presentation of this stimulus attenuated this response. Ellard (1996) interpreted this effect as the gerbils' habituation to the repeated association of a threatening stimulus with an increasingly familiar context. In addition, Ellard (1996) proposed that such attenuation of fleeing is the most adaptive response in a situation in which no shelter is available. When gerbils had access to a safe refuge (an attached enclosure in the open field with a solid roof), they spent extended periods inside of it.

The results of the light/dark test suggest that a dark area does not necessarily represent a safe refuge for gerbils. While rats of the vehicle group exposed to light/dark test spent almost the entire session in the dark compartment (98%; Chaouloff et al., 1997), gerbils spent less than half of the session in the dark area (Bridges and Starkey, 2004; Bradley et al., 2011). In addition, gerbils spent more time in the central area of an open field when compared with rats (Wang et al., 2018). These results suggest that in laboratory conditions, gerbils prefer protected areas that resemble the burrows in which they live in natural environments (enclosure with a solid roof) but not dark areas without roof, such as the closed arms of the EPM.

Research with other experimental preparations has also shown differences between gerbils and other rodents in terms of their defensive behavior. In fear conditioning and avoidance tests with shocks, rats freeze considerably more than gerbils, which results in better performance of rats in inhibitory tasks such as step-down and step-through avoidance. Conversely, gerbils show better adjustment to shuttle and lever-press avoidance, which entail active responses, including movement across the instrument (Ashe and McCain, 1972; Galvani et al., 1975; Powell et al., 1978; Crawford et al., 1981; Hurtado-Parrado et al., 2017).

Novelty and stress in the form of exposure to an strange and unprotected environment is one of the most effective means of triggering seizures in gerbils, whereas habituation reduce seizure frequency (Kaplan, 1975; Ludvig et al., 1991; Bertorelli et al., 1995). The fact that gerbils often exhibit spontaneous seizures in the EPM (Bridges and Starkey, 2004; Starkey et al., 2007; Rico et al., 2016) suggests that exposure to this instrument forces naïve gerbils to explore a novel environment that entails a conflicting situation in which closed arms do not provide a safe area, whereas open arms could offer an escape route.

Considering the documented defense reactions of intact gerbils to novelty and open/closed areas, the present study aimed to determine the effect of the benzodiazepine diazepam (DZP) on the spatiotemporal behavioral patterns of male gerbils exposed to an extended 20-min EPM session.

MATERIALS AND METHODS

Subjects

Forty-two outbred male 10-weeks old Mongolian gerbils (50–70 g; *Meriones unguiculatus*), obtained from the National Institute of Health at Bogotá-Colombia, were used in this study. Animals were housed in groups of 2–3 in polycarbonate cages (42 × 20 × 20 cm) which contained dust free wood shaving bedding, and were kept in an animal room under a 12 h light/dark cycle (lights on at 08:00 h) with water and standard rodent pellets available *ad libitum*. The room temperature was maintained at 23°C with 55% relative humidity. Animal handling was limited to home cage-cleaning time and the animals were not habituated to a novel environment before the first test session. All experimental procedures were performed in accordance with the United States National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by our Institutional Animal Care and Use Committee (CICUAL-KL/COM43-2016).

Apparatus

An EPM similar to that previously described elsewhere (Starkey and Bridges, 2010; Rico et al., 2016) was used in this study. The apparatus consisted of two open arms (40 cm × 8 cm) at right angles with two closed arms of the same size, with 30-cm high black Plexiglas walls. Arms emerged from a central platform (8 cm × 8 cm). The floor of the plus-maze was made of black smooth Plexiglas and the entire apparatus was elevated 50-cm from the ground. A raised Plexiglas edge (0.5-cm) surrounded the open arms to prevent gerbils from falling. The level of illumination of the test room was adjusted to 30 lux measured at the central area of the plus-maze.

Drugs

The benzodiazepine DZP (0.5 mg/kg; Roche, São Paulo) was dissolved in physiological saline (NaCl 0.9%; Vehicle). Thirty minutes before placing the gerbil in the EPM, DZP or Vehicle was administrated intraperitoneally (i.p.) in a volume of 1 ml/kg. Drug doses and administration protocol were based on those used in similar studies using gerbils (Varty et al., 2002a; Bradley et al., 2007a,b, 2011).

Behavioral and Scoring Procedures

In rats, the aversion to the open arms seems to be influenced by procedural factors, such as the time of the day at which testing occurs (Griebel et al., 1993). In gerbils, testing in the EPM has been performed between 8:00 and 16:00 h (Bridges and Starkey, 2004; Starkey and Bridges, 2010). Accordingly, our experimental sessions were carried out during the light phase (08:00–13:00 h). In order to characterize the spatiotemporal behavioral pattern during the session and to obtain behavioral dimensions that emerge from factor analysis, the sample of control group was expanded. Thirty minutes prior to testing, each animal was removed from the home cage, weighed and injected intraperitoneally with DZP (0.5 mg/kg; $n = 9$) or with Vehicle ($n = 33$). Then, gerbils were placed in the central area of the EPM facing one of the open arms, and could freely explore the instrument for 20-min. At the end of each session, the maze was cleaned with a 10% ethanol solution and dried with a cloth. All behavioral tests were recorded with a video camera placed above the EPM and connected to a digital video recorder in an adjacent room. A trained observer, blind for treatment, analyzed the videos (intra-observer agreement >90%). Behavioral measures were scored using the ethological free software X-PloRat (Tejada et al., 2017). The frequency of entries and the time spent into the open and the closed arms, the number of crossed squares into each arm and the frequency of stretching attend posture (SAP; a movement where gerbil leans forward with a flattened back followed by retraction to original position), were analyzed. An entry into an arm or into a square (8 cm × 8 cm) within an arm was scored after all four paws of the gerbil entered it. When an animal jump from the EPM, it was replaced on the central area as soon as possible facing one of the open arms, and additional time proportional to the time the subject was off the maze was added to the session. In these cases, video scoring was paused until the animal was repositioned in the EPM.

Data Analysis

Gerbils occasionally exhibit seizures when exposed to the EPM, often followed by a period of immobility (Bridges and Starkey, 2004; Rico et al., 2016). Six animals from the control group that displayed seizures were excluded from the analyses.

In order to characterize the spatiotemporal behavioral pattern across an extended EPM session, data from 33 animals of the vehicle group were analyzed minute-by-minute and 5-min bins. Then, a factor analysis was performed for each of the 5-min bins to capture the behavioral dimensions that emerge across different segments analyzed with this session. Finally, the effects of DZP on the behavioral response of gerbils during the session were analyzed according to the factors that were identified. Behavioral parameters from vehicle group were expressed as mean ± SEM and submitted to Friedman repeated measures (RM) analysis of variance (ANOVA) on ranks. The factor analysis was performed by principal-component followed by an orthogonal Varimax rotation. Factors with eigenvalues greater than 1.0 and loadings greater than 0.7 were kept. The measures analyzed with this procedure were the percentage of time spent in the open arms and in the central area; the percentage of entries in the open-arms and the closed-arms entries; the distance traveled in the closed-arms and the frequency of SAP in the central area. Data from the effects of DZP were expressed as mean ± SEM and submitted to Friedman RM-ANOVA on ranks for intra-group analysis. For each minute or for each 5-min bin, comparisons between the DZP and vehicle group were made by means of Mann-Whitney rank sum test. Whenever necessary, the SNK *post hoc* test were used. In all cases, the significance level was set at $p < 0.05$.

RESULTS

Vehicle Group

Results of temporal analysis on vehicle group data are summarized in **Figure 1** and **Table 1**. Eleven animals jumped from the open arms of the EPM during the first minute of exposure to the apparatus. As described in method section, subjects were replaced in the central area and session time was adjusted. For the percentage of time spent and percentage of entries in the open and closed arms, RM-ANOVA on ranks showed differences between the intervals in which the session was divided (min-by-min and 5-min bins; **Table 1**).

Post hoc minute-by-minute tests indicate that animals spent more time in the open arms during the first 3 min of the session, as compared to the segment between the 11th and 19th minute (**Figure 1A**). Similarly, the number of entries to the open arms was higher during the first 3 min than during minute 7 through 19 (**Figure 1B**). *Post hoc* minute-by-minute tests also showed an opposite pattern compared to the closed arms; namely, less time spent and fewer numbers of entries during the first 3 min of the session as compared to later segments. A further analysis based on 5-min segments showed significant differences between the first bin and the rest of the segments for both measures (time spent and entries) and both types of arms (open and closed) – See **Figures 1D,E**. RM-ANOVA on ranks also showed differences in the distance that the gerbils ran in the EPM across different moments of the session (**Table 1**). *Post hoc* minute-by-minute tests showed that the gerbils reduced the locomotion in the open arms during the last 11 min of the session as compared to the segment between the 1th and 5th minute (**Figure 1C**). *Post hoc* tests also indicated that across the de 5-min bins, gerbils' locomotion gradually decreased (**Figure 1F**).

Factor Analysis

Factor analysis results are summarized in **Table 2**. For each of the four 5-min bins of the session, a factor analysis was performed. Behavioral parameters related to open and closed arms exploration, as well as time spent and stretching

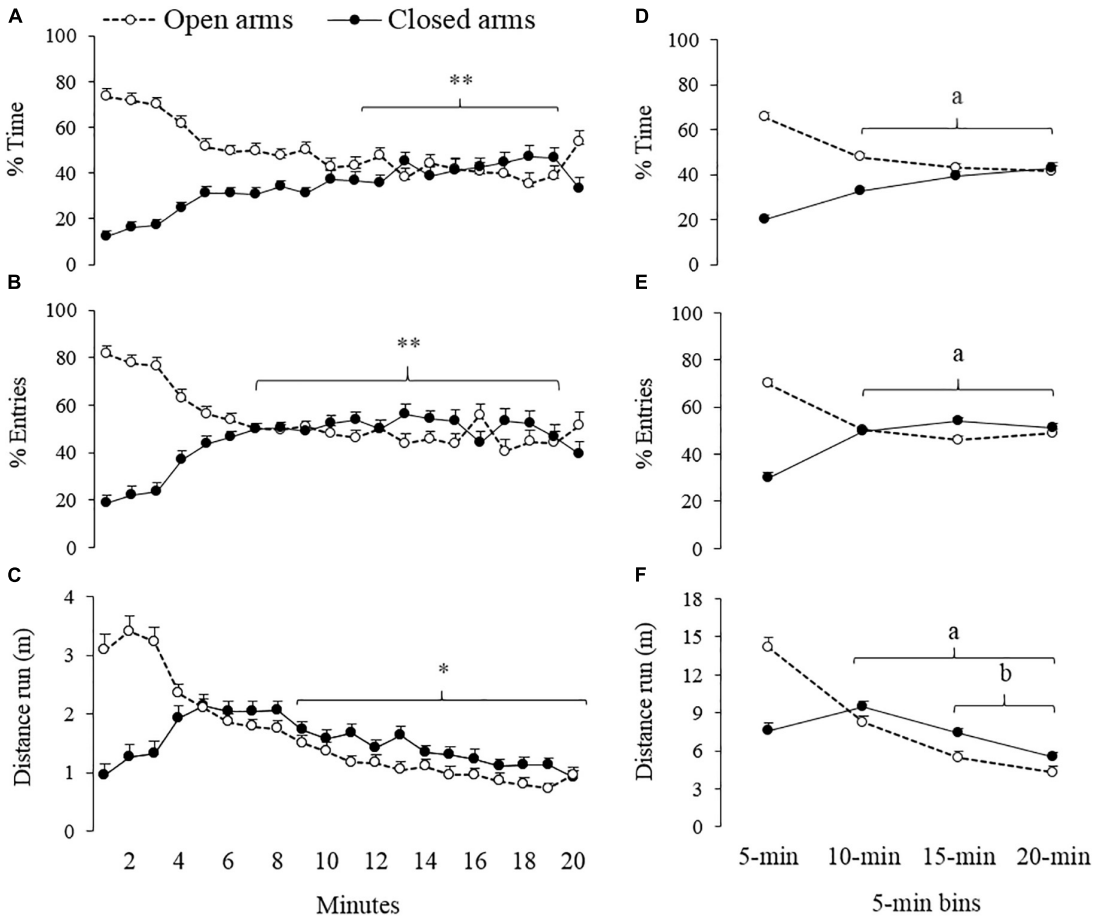

FIGURE 1 | Spatio-temporal analyses of exploratory behavior of gerbils (n = 33) exposed to 20-min session in the EPM. Left panels **(A–C)** show minute-by-minute session data and right panels **(D–F)** the same data in 5-min bins. **different from 1 to 3-min for open and closed arms, *different from 1 to 5-min for open arms, [a]different from 0 to 5-min for open and closed arms, [b]different from 6 to 10-min for open and closed arms ($p < 0.05$).

attended posture (SAP) in the central area of the maze were processed using principal-component analysis. After an orthogonal Varimax rotation, a single factor representing the 80.9% of the variance emerged for the first 5-min of the session. For that single factor, open-arms exploration measures were negatively correlated with closed-arms and central area exploration. For the following three bins, three factors emerged explaining 88.3, 86.1, and 85% of the variance, respectively. A similar pattern of factor solution was observed for the last three 5-min bins of the session. One factor grouped the open-arms exploration measures, while the other two factors grouped measures related to closed-arms exploration and central area activity (**Table 2**).

Effect of DZP

The effects of the anxiolytic compound DZP on the behavioral response of gerbils are summarized in **Figures 2–4** and **Table 1** (Vehicle vs. DZP). RM-ANOVA indicated differences between the bins into which the session was divided for the open arms (percentage of time and entries), the closed-arms (entries and distance run) and the central-area activity-related measures (percentage time and SAP; **Table 1**). *Post hoc* tests of minute-by-minute data showed that starting in the fourth minute of the session, gerbils treated with vehicle and DZP significantly reduced open-arms exploration in terms of percentage of time and entries (**Figures 2A,C**, respectively).

Similarly, a 5-min bin analysis showed significantly lower time allocation and entries to the open-arm during the last three bins as compared to the first bin (see **Figures 2B,D**). *Post hoc* tests also showed that compared to the first 5-min, gerbils treated with DZP increased both closed-arms exploration and central-area activity during the last 15 min of the session (**Figures 3**, **4**). A Mann-Whitney test was used to compare vehicle and DZP groups. It showed that during the second minute and the first 5-min bin, DZP-treated gerbils increased significantly the percentage of entries to the open arms ($p < 0.05$) compared to vehicle group (**Figures 2C,D**). In addition, during the first 5-min bin DZP also increased SAP frequency ($p < 0.05$). A Mann-Whitney test for the last 15-min of the session, indicated that DZP-treated gerbils reduced significantly ($p < 0.05$) time allocation and entries to the open-arms, as compared to vehicle group (**Figure 2**). Lastly, DZP significantly increased ($p < 0.05$) gerbils' entries and distance run

TABLE 1 | Summary of statistical parameters from minute by minute and 5-min bins analysis of the session time in the EPM.

Behaviors	Min-by-min		5-min bins	
	$\chi^2_{(19)}$	*p*	$\chi^2_{(3)}$	*p*
Vehicle group (Figure 1)				
% Time (s)				
Open arms	124.45	< 0.001	43.36	< 0.001
Closed arms	113.97	< 0.001	53.76	< 0.001
% Entries				
Open arms	132.54	< 0.001	43.46	< 0.001
Closed arms	121.22	< 0.001	43.46	< 0.001
Distance run (m)				
Open arms	315.91	< 0.001	73.11	< 0.001
Closed arms	104.05	< 0.001	75.56	< 0.001
Vehicle vs. diazepam (Figures 2–4)				
% Open-arms time (s)				
Vehicle	124.45	< 0.001	43.36	< 0.001
Diazepam	77.97	< 0.001	16.60	< 0.001
% Open-arms entries				
Vehicle	132.54	< 0.001	43.46	< 0.001
Diazepam	86.43	< 0.001	17.13	< 0.001
Closed-arms entries				
Vehicle	177.47	< 0.001	48.06	< 0.001
Diazepam	95.24	< 0.001	16.06	0.001
Closed-arms distance run				
Vehicle	104.05	< 0.001	37.66	< 0.001
Diazepam	63.42	< 0.001	11.40	0.001
Central area SAP				
Vehicle	30.49	0.046	12.453	0.006
Diazepam	36.76	0.008	14.733	0.002
% Central area time (s)				
Vehicle	64.65	< 0.001	22.16	< 0.001
Diazepam	75.99	< 0.001	17.13	< 0.001

in the closed arms, and time spent and SAP frequency in the central area (**Figures 3**, **4**).

DISCUSSION

In the present study, we analyzed the effect of the anxiolytic DZP on the defensive behavior of gerbils exposed to a 20-min EPM session. In addition, data of the vehicle group (n = 33) were analyzed minute-by-minute and in 5-min bins to characterize the spatiotemporal behavioral pattern across the session. Lastly, a factor analysis was conducted to determine the behavioral dimensions related to the EPM test.

Gerbils in the vehicle group showed a different pattern of defensive responses during two stages of the session: avoidance of the closed arms and preference for the open arms during the first 5-min, followed by homogeneous exploration of open and closed areas during the remaining of the session. Animals treated with DZP showed increased locomotion, risk assessment, and closed-arms exploration.

This is the first temporal analysis of the effects of DZP on the defensive behavior of gerbils exposed to an extended 20-min EPM session. The results of the first 5-min of the session are not consistent with observations in other rodent species – i.e., rats and mice exposed to a standard 5-min EPM session avoided the open arms, and anxiolytic treatment lead to increased exploration of those areas (for a review see Carobrez and Bertoglio, 2005; Pawlak et al., 2012). To interpret our results, species-specific differences in defensive reactions between gerbils and other rodents were considered. We propose that exposure to the EPM activates the gerbil's defensive system, in preparation for aversive events such as the potential attack of an aerial or ground predator. It appears that gerbils alternate defensive responses when confronting a new environment, such as the EPM. During the first 5 min of the session, gerbils primarily show escape behavior throughout the open arms; in fact, despite the use of a raised edge surrounding the open arms to prevent gerbils from falling, one third of the control animals escaped from the EPM jumping from these areas. Similar to gerbils, Holmes et al. (2000) reported that three of fourteen male wild mice also jumped from the plus-maze when placed on the apparatus. When confronting imminent predation in a new environment, that entails open and partially protected areas (uncovered closed arms), it seems that the most adaptive defensive response that gerbils could display is identifying and pursuing escape routes from the instrument – i.e., fleeing via the open arms. Conversely, closed arms do not seem to be a good option as they do not represent safe refuge and actually obstruct escape due to the walls.

This approach could explain the behavioral patterning of the gerbils during the first 5 min of the session – i.e., avoidance of the closed arms and preference for the open arms and it is consistent with observations under other controlled situations. For instance, during encounters with snakes in a maze, gerbils showed overall more exploratory behavior than rats, and identified the exits and safe or dangerous places of the apparatus within the first minutes of the session (Guimarães-Costa et al., 2007). Moreover, when confronted simultaneously with aerial and ground threats, gerbils reduced the use of protected areas in favor of the open sections of an outdoor aviary (Kotler et al., 1992).

Though this gerbils' tendency to avoid the closed arms and prefer the open areas of the EPM is consistent with the results obtained in other studies (Bradley et al., 2007a,b; Starkey and Bridges, 2010; Rico et al., 2016), Varty et al. (2002a,b) found that female gerbils of a control group avoided open-arms and preferred closed arms. A possible explanation for this discrepancy may be related to the characteristics of the instrument that was utilized. Whereas in Varty et al's study the walls of the closed arms were clear to allow for constant illumination in all parts of the maze (Varty et al., 2002a,b), we used an apparatus similar to those described elsewhere in which the closed arms were surrounded by black walls (Bradley et al., 2007a,b; Starkey and Bridges, 2010; Rico et al., 2016). It seems that the use of dark instead of clear walls in the closed arms affect the defensive response of gerbils in the EPM.

Having elapsed 5 min of the session, gerbils reduced exploration and the escape response via the open arms. It is possible that during this period of habituation animals learn

TABLE 2 | Orthogonal factor loadings obtained from control group gerbils for the first 5-min and the following 5-min of the session in the elevated plus-maze test.

Behaviors	First 5 min	6 to 10 min		
	Factor 1 Escape-related behaviors	Factor 1 Anxiety-related behaviors	Factor 2 Approach to the closed arms	Factor 3 Risk-assessment behaviors
% Open arms time (s)	−0.97	0.79		
% Open arms entries	−0.93	0.91		
Closed arms entries	0.94		0.87	
Closed arms distance run (m)	0.94		0.91	
% Central area time (s)	0.82			0.87
Central area SAP	0.79			0.90
% of variance	80.90	27.06	27.99	33.28

Principal-component analysis was followed by an orthogonal Varimax rotation. Factors with eigenvalues greater than 1.0 and loadings greater than 0.7 were kept. Minus signs (−) indicate the direction of the particular loading. A single factor representing the 80.9% of the variance emerged for the first 5-min of the session. For that single factor, open-arms exploration measures were negatively correlated with closed-arms and central area exploration. After a 5-min period (6–10 min), the EPM test measured three independent defensive behaviors, namely anxiety (% of time and entries to the open arms), approach to the closed arms (entries and distance traveled), and risk assessment (% of time in the central area and SAP).

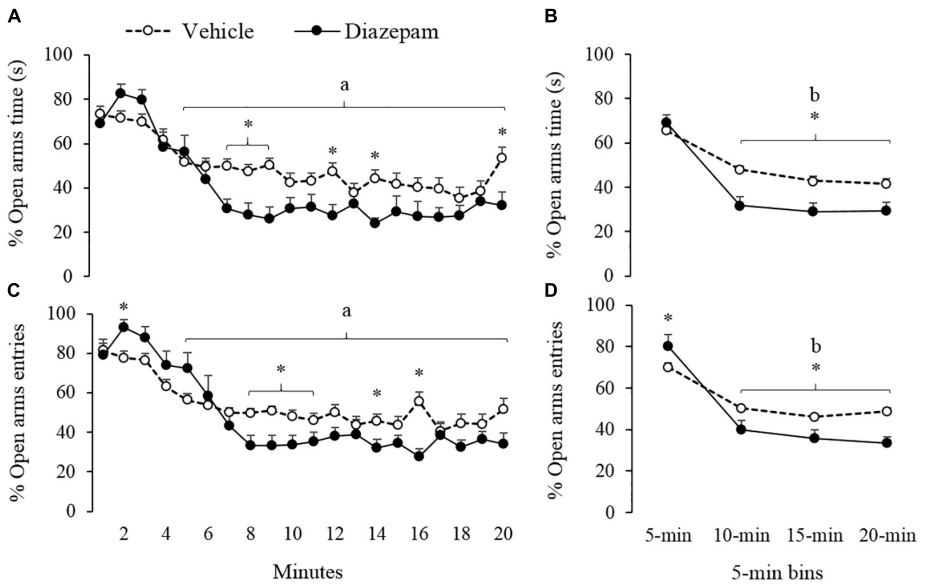

FIGURE 2 | Effect of DZP on escape-related behaviors of gerbils exposed to 20-min session in the EPM – i.e., percentage of time and percentage of entries. The left panels showed minute-by-minute data (Panel **A**, **C**) and right panels show data in 5-min bins (Panel **B**, **D**). After 5-min of exposure to the EPM, DZP reduced escape-related behaviors. [a]different from minute 1 to 4 for vehicle and DZP group, [b]different from 5-min bin for vehicle and DZP group, *different from vehicle group ($p < 0.05$).

that, due to the height of the open arms (50 cm), the escape response is not functional. Accordingly, gerbils start to use the closed arms for refuge, without fully abandoning the escape attempts through the open arms. This interpretation would explain the homogeneous pattern of exploration of open and closed arms observed after the fifth minute of the session, which is consistent with the habituation of the escape response reported by Ellard (1996).

Factor Analysis and Effect of DZP During the First 5 min of the Session

This is the first study to report a factor analysis of a 5-min EPM session for gerbils. Whereas behavioral measures in rats and mice (Wall and Messier, 2001) tend to group into at least three independent factors related to anxiety (exploration of open arms), locomotion (exploration of closed arms), and decision making/risk assessment (time in the central area and SAP), in our study a single factor emerged. The factor solution observed in gerbils suggests that during the first 5 min of the session, the EPM test is measuring a single defensive behavior characterized by a negative relationship between exploration of open arms (percentage and number of entries) and closed-arms (number of entries and distance traveled), and time in the central area and SAP. Similar to the escape response described by Ellard (1996), during the first 5 min of the session gerbils showed vigorous locomotion in the open arms, which included bursts

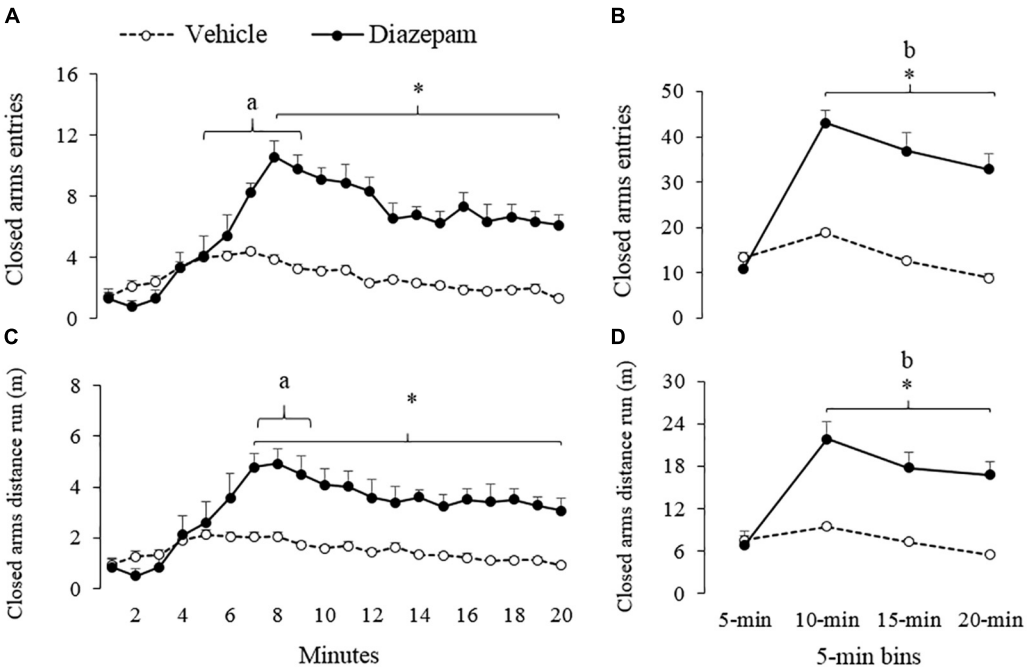

FIGURE 3 | Effect of DZP on the approach to the closed arms of gerbils exposed to 20-min session in the EPM – i.e., percentage of entries and distance run (m). The left panels show minute-by-minute data (Panel **A**, **C**) and right panels show data in 5-min bins (Panel **B**, **D**). After 5-min of exposure to the EPM, DZP increased the approach to the closed arms of the maze. [a]different from minute 1 to 4 for vehicle and DZP group, [b]different from 5-min bin for DZP group, *different from vehicle group ($p < 0.05$).

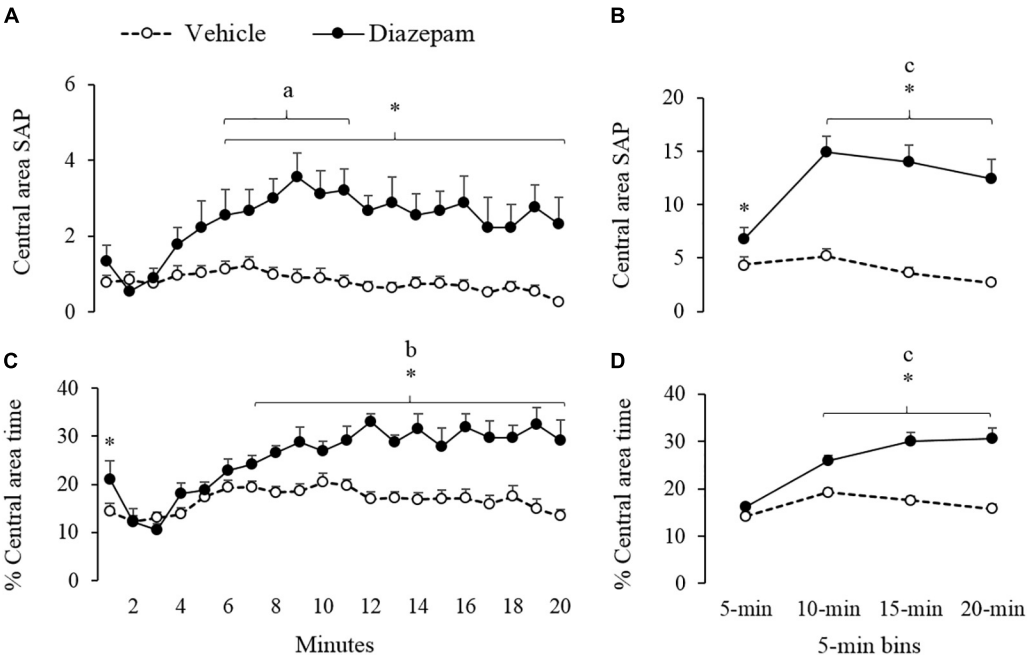

FIGURE 4 | Effect of DZP on risk-assessment behaviors of gerbils exposed to 20-min session in the EPM – i.e., SAP and percentage of time. The left panels show minute-by-minute data (Panel **A**, **C**) and right panels show data in 5-min bins (Panel **B**, **D**). After 5-min of exposure to the EPM, DZP increased the risk-assessment behaviors. [a]different from the 2nd minute for DZP group, [b]different from the 2 to 5 min for DZP group, [c]different from 5-min bin for DZP group, *different from vehicle group ($p < 0.05$).

of high-speed running in the absence of risk-assessment and visits to the closed arms. Accordingly, it seems that during the standard 5-min session the measures obtained in the EPM are mostly related to the escape behavior and not to anxiety-related responses.

Animals in the vehicle group exhibit a high percentage of entries to the open arms (69.9%), which was increased by the DZP treatment (80.3%). Our findings suggest that DZP facilitates the escape response through the open arms during the first 5 min of the session. Though Bradley et al. (2007a) also reported an increase in the percentage of entries to the open arms in DZP-treated gerbils, Varty et al. (2002a) reported that the same anxiolytic increased the time spent but not the number of entries to the open arms. Again, these inconsistencies with Varty et al's results may be related to the characteristics of the EPM that was utilized (clear closed arms) and the sex of the gerbils (females).

Factor Analysis and Effect of DZP After the First 5 min of the Session

Factor analysis of the subsequent 5-min bins of the session revealed three independent factors that grouped measures of open- and closed-arms exploration, and activity in the central area, respectively. These findings suggest that after a 5-min exploration period, the EPM test measures three different behavioral dimensions. According to our interpretation, once habituated to the instrument, gerbils may face a conflict between finding shelter in the closed arms – notwithstanding these areas do not represent an entirely safe refuge – or continue trying to escape via the open arms. We propose that the identified factors possibly correspond to three independent defensive behaviors, namely anxiety (% of time and entries to the open arms), approach to the closed arms (entries and distance traveled), and risk assessment (% of time in the central area and SAP). During the last three 5-min bins of the session, gerbils treated with DZP reduced their time spent and entries to the open arms, while increased their exploration of closed-arms, the time in the central area, and incidence of SAP. These effects of DZP on the defensive behavior of gerbils are different to those reported for mice and rats (for a review see Carobrez and Bertoglio, 2005).

It has been proposed that the EPM is a conflict test in which a novel situation produces both approach (open-arms) and unconditioned avoidance (closed arms), interpreted as curiosity and caution, respectively (Handley and McBlane, 1993; Ohl, 2005). In this context, anxiolytic compounds such as DZP can shift the balance of this conflict from avoidance toward approach (Gray and McNaughton, 2000/2003). Although further tests of our interpretations are needed, we propose that the conflict produced by the presentations of open and closed areas appears later in the session, once gerbils have habituated to the EPM. This conflict would consist on the tendency to continuous attempts to escape via the open arms and finding shelter in the closed arms. Accordingly, DZP would rapidly reduce the escape response through the open arms, while increasing approximations to the closed arms and risk-assessment that precedes the visits to the open arms.

Our results also indicate that after 5 min in the EPM, DZP-treated gerbils increase their locomotion in the closed arms. Apparently, this effect of DZP is related to the gerbils' defensive response to novelty; it has been shown that gerbils' reactivity to novel environments interferes with the effect of diverse compounds, included DZP (Babcock et al., 2002; Okano et al., 2005; Prinssen et al., 2006). Moreover, gerbils treated with DZP increase locomotion in new environments, but not in familiar settings (Prinssen et al., 2006).

Our results indicate that male *naïve* gerbils require sessions of at least 10 min in the EPM in order to properly measure anxiety-related behavior in this model. Data from this session should be analyzed in two 5-min bins; during the first 5 min (standard-session length), the obtained measures seem to be related to a vigorous escape response via the open arms triggered by the novel environment. Once habituated to the instrument, the escape response decreases, and approximations to the closed arms increase. Gerbils seem to face a conflicting situation between continue attempting to escape via the open arms or find shelter in the closed arms, notwithstanding these areas do not offer entirely safe refuge as they are not covered. This conflict appears to be reduced by DZP treatment, which produces a reduction in the escape behavior and increases both approximations to the closed arms and risk assessment. Accordingly, anxiety-related measures obtained in the EPM for gerbils could be only observed after the 5-min habituation period. Unlike in rats and mice, decrements in exploration of open-arms and increments in risk-assessment could be interpreted in gerbils as an anxiolytic effect. Further studies are necessary to test the role of procedural factors such as the effect of DZP in EPM-experienced gerbils, with or without prior habituation to the experimental room or the experimenter (manipulation).

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

AUTHOR CONTRIBUTIONS

JR and ML developed the study concept and design. LM-T performed the data collection. JR and LM-T performed the data analysis. JR, ML, and CH-P contributed to the interpretation of the data and provided important critical revisions. CH-P contributed to style of language in English writing. All the authors approved the final version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of Nodal Distance on Conditioned Stimulus Valences Across Time

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A meaningless symbol that repeatedly co-occurs with emotionally salient faces (US) can transform into a valenced symbol (CS). US-to-CS valence transformations have been observed for CS that have been directly (US→CS0) and indirectly (US→CS0→CS1→CS2) linked with face US. The structure of a US→CS0→CS1→CS2 series may be conceptualized in terms of "nodal distance," where CS0, CS1, and CS2 are 0, 1, and 2 nodes from the US respectively. Increasing nodal distance between an evaluated CS and its linked US can reduce magnitude of observed CS valence transformations. We explored currently whether nodal distance can influence CS valence *extinction*, which describes reductions in CS valence following repeated exposures to CS without any accompanying US. In our study, faces with happy/neutral/sad expressions (US) were directly linked with nonsense words (US→CS0). The directly linked CS0 was concurrently linked with other words (CS0→CS1, CS1→CS2). Subjects evaluated all stimuli before and after conditioning, then continued to provide CS evaluations twice a week for 6 weeks. Bayesian factors provided credible evidence for the transformation and extinction of CS valences that were 0 and 1 nodes from US (all BF_{10}'s > 100). The variability across post-conditioning CS evaluations provides indirect evidence for context-sensitive/propositional and structural/associative operations during CS evaluations.

Keywords: extinction, valence transformation, learning theory, classical conditioning, emotion

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INTRODUCTION

Meaningless symbols can become emotionally salient following correlations with emotion-eliciting events (US) under controlled conditions (Staats and Staats, 1957; Mowrer, 1960; Osgood, 1980). For example, correlating nonsense words with pictures of happy faces (US) can transform the former into a positively valenced Conditioned Stimulus, or CS (Amd et al., 2018). CS valence transformations are not limited to CS that directly appeared with US only; in natural language, it is more often the case that words acquire valence following contextually mediated relations with other, valenced CS (Staats, 1961; Dymond and Rehfeldt, 2000; Tonneau, 2004; Amd et al., 2018). To see how, imagine the CS from the above example (call it CS0) was linked with a second term (call it CS1) in a context that promotes functionally equivalent stimulus relations/links (Tonneau, 2001). Establishing

CS0-CS1 and CS0-US links can transform both CS0 and CS1 valences, despite CS1 having never directly co-occurring with US (Tonneau, 2004). In the latter case, the "indirect" relation between CS1 and US is mediated both by context and any intervening CS (Mowrer, 1960; Osgood, 1980; Fields and Verhave, 1987). The structure of US-CS relations can be characterized by "nodal distance" (Fields et al., 1990). Suppose a CS is paired directly with a US; call this relation US-CS0. Next, suppose our CS is paired with another nonsense word; call this CS0-CS1. To describe how "far" each CS is from its linked US, we say that CS0 and CS1 are 0 and 1 nodes from the US, respectively (e.g., Amd et al., 2018). Observing valence transformations for CS1 is an instance of indirect, 1-node transformation. The investigation of nodal distance effects may help us understand how derived representations relate to one other across psychological space to produce direct and indirect valence transformations (Fields and Verhave, 1987; Fields et al., 1990; Fields, 2016).

Indirect valence transformations have long fascinated psychologists interested in symbolic behavior since (most) linguistic symbols are thought to become salient through similar transformations (Mowrer, 1954; Osgood and Tzeng, 1990; Dickins and Dickins, 2001; more recently, see investigations in "relational frame theory"—Dymond and Roche, 2013; Amd and Roche, 2015). We know that when CS and US categories consist of nonsense words and emotional faces respectively, valence transformations appear limited by nodal distance. Specifically, valences for CS that are two or more nodes away from their face US may not significantly transform (Bortoloti and de Rose, 2009; Amd et al., 2018; Silveira et al., 2018). A recent study by Amd et al. (2018) illustrates this point.

In that study, subjects were trained along US→CS0, CS0→CS1, and CS1→CS2 relations, producing CS that were 0, 1, and 2 nodes away from emotional face US respectively (**Figure 1**). All stimuli were evaluated before and after conditioning on visual-analogue scales (VASs) corresponding to emotional dimensions of valence and arousal (**Figure 2**). Those authors reported significant valence transformations for CS0 and CS1 and marginal effects for CS2, which was 2 nodes from the US (p. 12). Those findings raised the question as to whether nodal distance may influence the *extinction* of CS valences (Baeyens et al., 1988; Baeyens et al., 1995). Briefly, "extinction" describes how repeated presentations of a CS alone in a context where it had previously been associated with a US may cause the CS valences to trend toward their pre-conditioning state. We replicated Amd et al. (2018) procedure for establishing three 5-member stimulus sets that varied along valence (positive/happy, neutral, negative/sad). We expand on Amd's work by having subjects evaluate CS intermittently for approximately

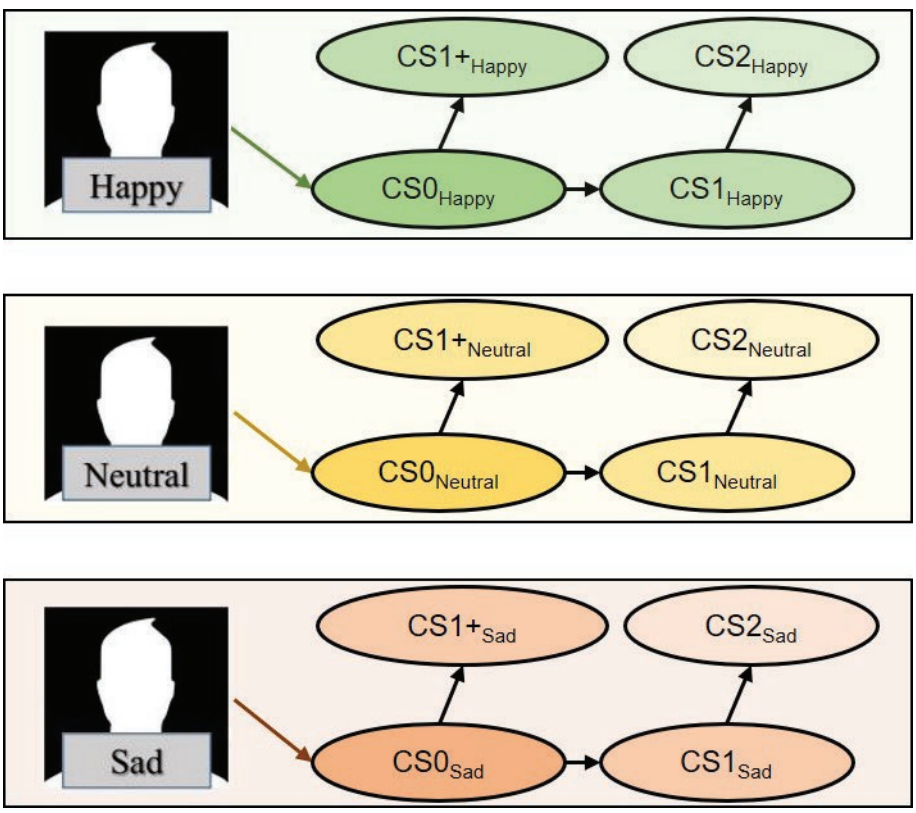

FIGURE 1 | Subjects were presented with US-CS and CS-CS sequences designed to establish stimulus sets with CS that were 0, 1, and 2 nodes apart from their respective USs. Stimuli linked together during training are connected by arrows. For instance, faces were exclusively linked with CS0. CS0 was linked equally with CS1 and CS1+. Finally, CS1 was linked with CS2.

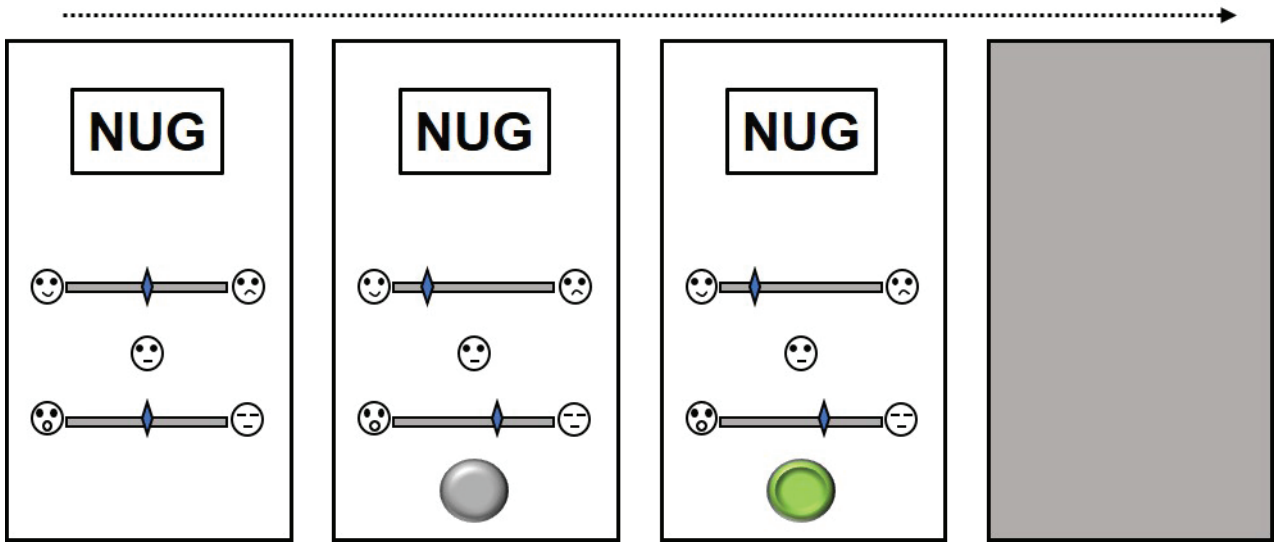

FIGURE 2 | Subjects evaluated CS and distractors (represented by the meaningless word "NUG") using valence and arousal sliders scored between 0 (sad/calm) and 100 (happy/aroused). Each trial commenced with both sliders set at 50 (Neutral). Subjects were required to move both sliders before a gray button would appear near the bottom of the screen. Pressing the button turned it green, produced a jittered blank interval followed by the next evaluation trial.

2 months to determine how CS were evaluated across time. Reductions in CS valence would indicate extinction.

Before describing our study further, it is worth noting that in human evaluative learning, the prediction that repeated exposures to nonreinforced CS will cause their valences to extinguish has not always been met (Hofmann et al., 2010). In a representative study by Hermans et al. (2002), pictures of human faces (CS) were linked with aversive electric shock (US) for human subjects. After face→shock (CS→US) acquisition trials, some subjects immediately evaluated CS. Other subjects first underwent a block of extinction trials, where CS appeared without accompanying shocks, and were then asked to evaluate CS. Despite the latter group undergoing extinction, Hermans reported no significant group differences between CS evaluations. The retention of CS valence following CS-alone trials was interpreted to mean that CS valences were "resistant" to extinction. Those authors also collected US expectancy ratings, where subjects' responding was found to cohere with learning histories. Specifically, US expectancy was high following CS→US (acquisition) trials, and low following CS-alone (extinction) trials. This disassociation between US expectancy (high/low) and CS valence (high/high) has been proposed as evidence for the dual operation of propositional and associative processes during CS valence transformations (Bar-Anan and Moran, 2017; Gawronski and Bodenhausen, 2018; McLaren et al., 2018).

According to a dual-process view, US expectancy ratings correlate with the relational information provided by the contextualized (propositional) relationship between the CS and US (e.g., CS *predicts* US, CS *does not predict* US, CS *is different from* US). On the other hand, without a context constraining evaluation strategies, collected CS valences may (or not) resemble their linked US, independent of relational information available (Jones and McLaren, 1999; Sweldens et al., 2014). Some supporting evidence comes from Moran and Bar-Anan (2013), who conditioned images of cartoons (CS) with positively/negatively valenced sounds/words (US) for their subjects. A key manipulation was the sequence of CS-US presentations, where some CS anticipated US onset (CS→US) while other CS correlated with US offset (US→CS). Following conditioning, subjects evaluated CS using explicit and implicit evaluation measures. Across explicit evaluations, subject performances corresponded with the relational information provided—for example, CS which signaled the offset of negative US were explicitly preferred over CS which signaled the offset of positive US. When looking across implicit evaluations however, CS linked with negative US were always evaluated as negative, regardless of whether they had signaled US onset/offset during conditioning. The latter implies the operation of an associative process by which the "mere" correlation of a CS with a valenced US would suffice *ipso facto* to transform CS valences, particularly if the capacity to engage in propositional deliberation is dampened (Bar-Anan and Moran, 2017). The disassociation between explicit and implicit CS evaluations provides further evidence of dual processes underlying CS valence evaluations (McLaren et al., 2014, 2018; Sweldens et al., 2014).

The hypothesis that dual processes contribute toward the CS valence transformation and extinction was recently challenged by Aust et al. (2018), who suspected that the expectancy-valence disassociation may be an artifact of contextually cued judgment strategies (rules). Aust et al. reasoned that acquisition (CS-US) and extinction (CS-alone) contexts cue different rules for evaluating CS valence and US expectancy based on the learning histories associated with the respective contexts. Those authors tested this hypothesis by having subjects undergo acquisition and extinction in separate contexts. After conditioning,

different groups provided CS valence and US expectancy ratings in acquisition or extinction contexts. Evaluations were also collected across contexts that combined features of both acquisition and extinction (called an "integrated" context), as well as novel contexts. Subjects who evaluated CS valence and US expectancy in the acquisition context (associated with CS→US) produced high valence and expectancy values. Alternatively, subjects who evaluated CS in the extinction context (associated with CS-alone) produced low valence and expectancies, supporting those authors' predictions. Alternatively, if US expectancies were collected in an acquisition context and CS valence in the alternate extinction context (or vice versa), the familiar expectancy-valence disassociation re-appeared (Aust et al., 2018, p. 27). Specifically, valence was high (resistant to extinction) when subjects evaluated CS in acquisition contexts; valence was low (extinction) when CS were evaluated in extinction contexts. CS valences in novel and integrated contexts fell in between. Aust reasoned that the latter ratings were consequated by an integration of learning histories associated with acquisition and extinction contexts. While this could explain CS evaluations in the integrated context, the generalizability of this explanation to CS valences collected in novel contexts is less convincing. In any case, the results reported by Aust et al. (2018) suggest that expectancy-valence disassociations in earlier studies may have been driven by different, context-sensitive judgment strategies for evaluating CS valences and US expectancies. We considered Aust's findings during the design of the present study (described below), where all CS evaluations were collected in a context that did not resemble the learning context.

In our study, subjects underwent a relational learning (conditioning) task for establishing three 5-member series structured along US→CS0, CS0→CS1, CS0→CS1+, and CS1→CS2, where US consisted of happy/neutral/sad faces (**Figure 1**). Before and immediately after conditioning, subjects evaluated stimuli along valence and arousal VASs (**Figure 2**). Subjects continued evaluating CS twice a week for 6 weeks. Earlier proof-of-concept studies revealed that varying the interval between CS evaluations reduced the likelihood of control by temporal cues, as subjects could not predict which days they would be probed for CS evaluations (e.g., Matute et al., 2011). After the final evaluation, subjects returned to the lab to complete a set of card-sorting tasks that were unrelated to the present investigation. Three hypotheses were currently investigated. First, we predicted that the valences of CS both directly and indirectly linked to USs would transform following conditioning. Second, the magnitude of the CS valences will inversely relate to increasing nodal distance from US (Amd et al., 2018). Finally, post-conditioning CS valences trending toward their pre-conditioning values would suggest extinction.

MATERIALS AND METHODS

Subjects

Fourteen college students (12 females, 18–24 years) participated in the present study. Subjects were informed they would be participating in a study on "emotional memory" and provided no additional details until the end of their participation. All subjects provided written and informed consent prior to participating. Our study corresponds with the principles described in the Declaration of Helsinki. All procedures reported were approved by the ethics committee on human research at the Federal University of Sao Carlos. All subjects received R$50.00 (USD15.50) for participation. None of the subjects were familiar with the task before the procedure.

Materials

Fourteen trigrams were randomly assigned to be CS (12) or distractors (2) for each subject. Specifically, our program randomly selected 12 items from an array of 14 trigrams provided as input for each subject. No two subjects viewed the exact same 12 trigrams as CS. Distractors appeared only during the stimulus evaluation task. Nine emotional faces (three happy, three neutral, three sad) from the Karolinska face database (Goeleven et al., 2008) were used as US. During pre-training (see Procedure), we used nine images of real objects (apple, banana, melon, car, bus, motorbike, hammer, wrench, pliers). All materials were the same as those reported in Amd et al. (2018). All evaluations were collected on a touch screen application developed on LiveCode (v. 6.7) platform and installed on subjects' smartphones for the duration of the study (**Figure 2**). The conditioning task was designed and implemented on E-Prime 2 (Schneider et al., 2012) on a Windows laptop with a 43-cm high-definition screen. All data analyses were conducted on the freely available JASP software (JASP, 2018). All subjects underwent conditioning in quiet temperature-controlled rooms in the Laboratory of Human Behavior Studies (in Portuguese—LECH) at the Federal University of Sao Carlos. The locations for subsequent stimulus evaluations were contingent on the subjects' whereabouts during the time of ratings.

Procedure

Baseline Evaluation

After receiving verbal and written consent, the experimenters installed the evaluation task on subjects' smartphones. The task commenced with an instruction screen and a button labeled START. Pressing this would begin the trial sequence illustrated in **Figure 2**. On each trial, a CS/distractor would appear in the screen center with two VASs underneath. Subjects had to move each slider, one anchored by sad and happy faces (representing valence) and the other by calm and excited faces (representing arousal). Slider positions were scored from 1 (sad/calm) to 100 (happy/excited). Moving both sliders produced a gray button near the bottom of the screen. Pressing this produced the next trial. A blank screen, jittered between 0.5–1.5 seconds, seperated evaluation trials. We jittered our interval to prevent temporal conditioning artifacts (Balsam, 1984). CS presentation sequences were randomized for each iteration of the evaluation task. A black screen with a button labeled FINISH would appear after all evaluations were complete. Pressing this emailed the stimulus evaluations to a secure server

and terminated the application. After collection of these baseline evaluations, the subject commenced the conditioning phase of the experiment on a laptop.

Conditioning

Subjects underwent 60 pre-training trials followed by 180 conditioning trials using the procedure described in Amd et al. (2018). During pre-training, subjects viewed naturally congruent stimulus sequences (e.g., melon→banana, apple→banana) followed by a card-sorting test where they had to pair congruent stimuli together (e.g., "apple" with "melon"). The pre-training phase was designed to help subjects understand task demands (Amd and Roche, 2017). During conditioning, subjects viewed face→word (US→CS0) and word→word (CS0→CS1, CS0→CS1+, CS1→CS2) sequences across 180 trials in randomized order. Three faces from each US category (happy/neutral/sad) were correlated with a single CS0 5 times across 15 trials. We used multiple US since single exemplar US-to-CS mappings are insufficient for transforming CS valences (Gawronski et al., 2017, Experiment 1; also see Dube et al., 1992). CS0 was linked with CS1 over 15 trials, CS0 with CS1+ over 15 trials, and CS1 with CS2 over 15 trials. CS1 and CS1+ were both 1 node away from the US, where CS1+ functioned as an end-term. This allowed us to check whether end-terms (CS1+) are differentially evaluated relative to intermediate terms (CS1) during extinction, since no differences are expected during acquisition (Amd et al., 2018). After conditioning, all subjects produced card pairs from a deck containing all CS0 and CS2 stimuli as a test for contingency awareness for an unrelated investigation. As contingency awareness may not be critical for CS valence transformations (Amd and Baillet, 2019) and the present investigation was not concerned with sorting/transitivity performances anyway (but see Amd and Roche, 2017; Amd et al., 2018), no further mention of sorting performances are provided.

Post-evaluations

All subjects immediately evaluated stimuli after conditioning. Afterward, subjects would receive a message stating "*um lembrete* (a reminder)" between 3 PM and 4 PM twice a week for the next 2 months. The message functioned to cue completion of the evaluation task installed on subjects' smartphones. All post-conditioning CS evaluations were collected within 1–3 h of sending the prompt. We varied the day on which CS evaluations were collected to reduce temporal discriminative control (Savastano and Miller, 1998). The collection of 13 additional evaluations marked the end of the current experiment.

Data Analyses

For all subjects, CS evaluations were collected over 14 time points (t1*t2*t3…t14), where baseline/pre-conditioning evaluations were collected at t1. CS were parsed by nodal distance (CS0*CS1*CS1 + *CS2). CS evaluations collected between time points t1 and t2 were contrasted with Bayesian paired *t* tests, where significant effects would inform whether CS valences transformed. We next calculated difference scores between t1 and t2 for all CS and subjected the data to three analyses of variance (ANOVAs), one per valence condition (happy, neutral, sad) with nodal distance (4) as the independent factor. Significant effects would highlight whether nodal distance modulated valence transformation for that condition. To measure extinction, we ran three 4*13 mixed ANOVAs for each valence condition (happy, neutral, sad) with nodal distance (4) and post-conditioning time (13) as independent and repeated factors respectively. Significant interactions were followed with tests for simple main effects and Bonferroni-corrected *post hoc* contrasts. Prospective power analyses with GPower 3 (Faul et al., 2007) recommended sample sizes for a large effect (f = 0.5), with alpha and power set at p = 0.05 and 1–β = 95% respectively. Assuming nonsphericity across all time points (epsilon = 1), the recommended sample for a 4*13 ANOVA was 12 participants and a critical F (F_{Crit}) of 1.54. Assuming sphericity was violated at all time points, the nonsphericity correction would be (1/(1–13)) = − 0.08 (p. 181). In that case, the recommended sample size with all remaining parameters would be 32, F_{Crit} = 2.73. As we did not know our sampling distribution *a priori*, we chose an intermediate epsilon of 0.5, which then yielded a recommended sample size of 12 subjects with F_{Crit} = 1.82.We complemented our ANOVAs with Bayes factors (BFs) estimated to a precision of ±2% and computed using a standard Cauchy prior of width 0.707 and a scaling parameter of 0.5 for fixed effects (Rouder et al., 2012). BFs evaluate the quality of evidence underlying an observed effect relative to the non-effect predicted by the null hypothesis for that same data set (Kruschke, 2013). We used BF_{10} here to qualify the evidence for any statistically significant effects (e.g., 3 > BF_{10} > 1 = weak; 10 > BF_{10} > 3 = moderate; 30 > BF_{10} > 10 = strong; BF_{10} > 30 = very strong). All reported *F* values were Greenhouse–Geisser corrected when appropriate.

RESULTS

US-to-CS Valence Transformations

We found no convincing evidence for significant transformation effects across any of the CS arousal ratings (all p's > 0.05; all BF_{10}'s < 2). Subsequent analyses are reported for CS valences only (**Figure 3**). Contrasting evaluations between pre- and post-conditioning time points (time 1 vs. 2—**Figure 3**) revealed significant effects for $CS0_{Happy}$ (t = 8.2, p < 0.001, d = 2.18, BF_{10} > 1,000) and $CS0_{Sad}$ (t = 20.9, p < 0.001, d = 5.59, BF_{10} > 1,000), i.e., for CS directly linked with happy and sad faces. Contrasts for CS associated with neutral faces were not significant (p > 0.05). For CS indirectly linked to US, significant effects appeared for $CS1_{Happy}$ (t = 5.2, p < 0.001, d = 1.39, BF_{10} = 177); for $CS1+_{Happy}$ (t = 3.9, p = 0.002, d = 1.03, BF_{10} = 22); for $CS1_{Sad}$ (t = 7.3, p < 0.001, d = 1.96, BF_{10} > 1,000); and for $CS1+_{Sad}$ (t = 7.5, p = 0.002, d = 1.06, BF_{10} = 26). The differences for $CS2_{Happy}$ (p = 0.015, d = 0.75) and $CS2_{Sad}$ (p = 0.037, d = 0.62) were statistically significant, but not credible (all BF_{10}'s ≤ 4.1). Significant ANOVAs across CS in

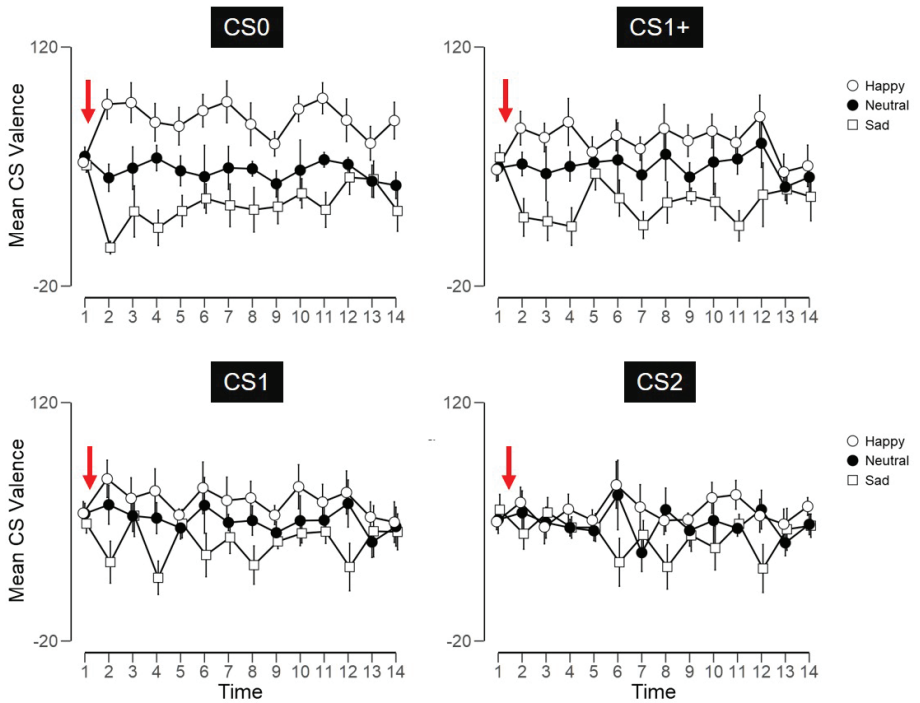

FIGURE 3 | CS valences were collected across 14 time points (*x*-axis) and were scored from 1/sad to 100/happy (*y*-axis). Conditioning took place between times 1 and 2 (red arrow). All subsequent evaluations were spaced 2–3 days apart. Error bars indicate 95% confidence intervals.

the happy (F = 4.68, p = 0.006, η_p^2 = 0.21, BF_{10} = 8) and sad (F = 9.09, p < 0.001, η_p^2 = 0.34, BF_{10} = 396) conditions illustrate an inverse relation between increasing nodal distance and CS valence magnitude.

Effects of Nodal Distance on Conditioned Stimulus Extinction

4*13 ANOVAs for each valence condition (happy, neutral, sad) produced significant effects of time appeared for all conditions (all p's < 0.001, all BF_{10}'s > 1,000). Nodal distances interacted significantly with time for happy (F = 1.59, p = 0.017, η_p^2 = 0.08, BF_{10} > 1,000) and sad (F = 6.45, p < 0.001, η_p^2 = 0.11, BF_{10} > 1,000), but not neutral (p = 0.672) conditions. Correspondingly, significant nodal distance effects appeared for CS linked with happy (F = 23.7, p < 0.001, η_p^2 = 0.58) and sad (F = 13.8, p < 0.001, η_p^2 = 0.44), but not neutral (p = 0.118) faces. *Post hoc* tests across negative/sad CS revealed significant (p < 0.005) differences between CS0 and CS1, and between CS1 and CS1+. Across positive/happy CS, *post hoc* tests revealed CS0 to be significantly (p < 0.001) greater than all remaining CS (CS1, CS1+, CS2). No other contrast reached significance (all p's > 0.05).

DISCUSSION

Subjects underwent a conditioning task where nonsense words (CS) were concurrently linked with happy/neutral/sad faces (US→CS0) and other nonsense words (CS0→CS1, CS0→CS1+, CS1→CS2). Subjects evaluated CS before and after conditioning, then provided 13 additional CS evaluations over 6 weeks. Comparison of CS valences before and after conditioning confirmed earlier reports by Amd et al. (2018), who reported significant valence transformations for CS that were 0 and 1 nodes removed from happy and sad US. Analysis of the variances across post-conditioning evaluations provided evidence for positive and negative CS valences trending toward pre-conditioning values, moderated by nodal distance.

Our investigation was not designed to compare dual-process and single-process accounts, but they raise some questions regarding the account provided by Aust et al. (2018). According to those authors, CS extinction measurements reflect the "integrative summaries of the learning history" (p. 28) which, during the present study, constituted of acquisition trials only. Our findings partially support Aust's claim, in that the first collection of CS evaluations immediately after conditioning suggested that subjects were integrating valence information following acquisition (time 2, **Figure 3**). The issue arises when trying to account for the significant variability across remaining evaluations, particularly for CS indirectly linked to US (time 3 onward, **Figure 3**). If evaluations are consequated by judgment strategies/rules bound to specific contexts, how would evaluating the same CS in the same context evoke variable ratings? Alternatively, if subjects attribute qualifiers to familiar CS and these determine CS evaluations (as per

De Houwer, 2018), variability across CS valence should have stabilized across time, whereas our observations indicate the exact opposite. On balance, an unpublished pilot study preceding the reported work showed subjects who evaluated CS daily (instead of every 2–3 days, as was the case here) repeated the values provided during the first post-conditioning evaluation across all remaining evaluations. Those subjects were shown to accurately recall CS-US contingencies from acquisition after all post-conditioning evaluations were complete. That study indicated CS evaluations can reflect integrative summaries of earlier learning histories, *pro* Aust et al., albeit with the caveat that acquisition and extinction learning histories can be subjectively delineated post-conditioning.

In contrast, subjects in the current study could not recollect which CS and US "went together" during an informal post-experiment interview. While there is some evidence that CS valences may transform without explicit knowledge of CS-US contingencies (e.g., Hütter et al., 2012), we cannot answer whether this was presently the case since we do not know if/when CS-US contingency knowledge was "forgotten" post-conditioning. Relatedly, although our evaluation context differed from the learning context, we could not control the context where subjects provided the ratings, nor their subjective state at the moment of the ratings. The confluence of contextual and subjective factors may have contributed to the variability observed across post-conditioning evaluations. A future work could address both issues by (1) interspersing CS evaluations with tests of contingency awareness and (2) collecting post-conditioning evaluations in standardized contexts. The first manipulation will determine whether retention of acquisition contingencies influences post-conditioning variability in CS valence (e.g., extinction). The second manipulation would mitigate the influence of interpersonal contextual differences.

An additional point involves nodal distance effects reported here and in earlier works (e.g., Bortoloti and De Rose, 2009; Amd et al., 2018). A central feature of those studies was the demonstration of untrained transitive relations. For example, imagine we train a competent subject along the propositions "*A* goes with *B*" and "*B* goes with *C*." If our hypothetical subject can derive "*A* goes with *C*" or "*C* goes with *A*" without additional training, one could assume *A*, *B*, and *C* are functionally equivalent (Tonneau, 2001). Now imagine that *A* consists of happy faces while *B* and *C* are meaningless words, as was the case presently. All else remaining equal, why would *B* and *C* evoke non-equivalent valences? These effects can be accommodated by validation processes operating alongside spreading activations across contextually specified mediators (e.g., Bar-Anan and Moran, 2017; Gawronski and Bodenhausen, 2018; then see Berlyne, 1965). Is the explanation from a single-process perspective "better" (in terms of parsimony and falsifiability)? Deriving explanations through assumptions incorporating propositional processes exclusively can be parsimonious, but with the considerable cost that single-process models are "virtually immune to falsification" (De Houwer, 2018, p. 14). It may be more fruitful to hypothesize structural/associative and context-sensitive, propositional processes interact to produce CS evaluations (Berlyne, 1965). We already have evidence of bottom-up valence effects during time windows too early to attribute to propositional reasoning processes (Amd et al., 2013; Bayer et al., 2017; Amd and Baillet, 2019). To be fair, advocating for dual processes requires a satisfactory explanation of how propositions arise from, as well as interact with, structures of associative S-S links, for which we have no satisfactory response presently (De Houwer, 2018; although see Gawronski and Bodenhausen, 2018). Yet, associative models can already account for the emergence of transitive relations following direct training of linear stimulus relations without requiring formal representations of relational qualifiers (Tovar and Westermann, 2017). A valuable next step could be to determine whether CS valence transformations can be modeled without formal representations of relational qualifiers.

We conclude by noting some limitations of the present work. First, some may criticize our exclusion of implicit CS evaluation and US expectancy measures, both of which are common across investigations of CS valence extinction (e.g., Hu et al., 2017). We had no reason to include US expectancy measures as task demands across evaluation and acquisition contexts were established *a priori* as different. No CS-US sequences appeared during post-conditioning evaluations, hence there was no reason to expect US in the first place. We excluded implicit CS evaluation measures for three reasons. First, we were concerned with poor reliability when repeated assessments are made with implicit tests (Buchner and Wippich, 2000). Second, merely identifying a CS (such as during some implicit evaluation task) can cause subjects to derive CS-valence links unrelated to explicitly provided CS-US information (cf., the misattribution effect—Jones et al., 2009). This means that *any* (explicit/implicit) measure of CS valence could potentiate the derivation of CS valences unrelated to task demands (Jones et al., 2009; Hahn and Gawronski, 2018). For fairness, the same argument applies to CS evaluations reported here since we did not assess whether subjects misattributed valences to CS. A future replication could nevertheless incorporate implicit evaluation measures to determine whether repeated evaluations potentiate CS valence misattributions that manifest across explicit evaluations. A second criticism can be raised regarding the limited power afforded by our sample size (n = 14). While other research should replicate our procedure with larger samples, the incorporation of Bayesian factors alongside our frequentist analyses implies our depicted effects as highly credible. It is worth asking whether the present effects would be replicated with symbolic CS incorporating US across various modalities (e.g., visual/olfactory/auditory). Some important work in this area has been conducted by relational frame theorists, who have demonstrated the transformation of CS response properties beyond valence (e.g., Dymond and Rehfeldt, 2000; Dymond and Roche, 2013). Observing extinction, or a lack thereof, of other response properties would highlight the extent to which the present effects may be universal to symbolic conditioning investigations, and relational categorization in

general (cf., Goldwater and Schalk, 2016). It could also be the case that our reported effects are limited to preparations involving emotional faces and meaningless words. Answering these questions will clarify the confluence of structural and propositional processes during CS transformation and extinction.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the university ethical review committee for human research. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Federal University of Sao Carlos ethical review committee for research on human subjects.

AUTHOR CONTRIBUTIONS

MA designed the study, prepared the manuscript, and analyzed the data. MAO and DAP performed the data collection. AM and JR assisted with manuscript preparation.

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Emotion Regulation and Attitudes Toward Conflict in Colombia: Effects of Reappraisal Training on Negative Emotions and Support for Conciliatory and Aggressive Statements

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Control of negative emotions (e.g., anger and fear) by political cues perpetuate intractable conflict by mobilizing public support for aggressive actions. Halperin et al. (2013) found that reappraisal – an adaptive form of emotion regulation – decreased negative emotions triggered by anger-inducing information related to the Israeli–Palestinian conflict, and increased support for conciliatory statements. We tested these effects in the context of the conflict between the Colombian government and the Fuerzas Armadas Revolucionarias de Colombia-Ejército del Pueblo (FARC-EP). Reappraisal training reduced negative emotions produced by a presentation that illustrated FARC's violent actions, and increased support for conciliatory statements (with overall moderate effect magnitudes). We also found that negative emotions mediated the effects of reappraisal on the support for aggressive and conciliatory statements. These findings indicate a high degree of generality of the phenomena, especially considering the differences between the Israeli–Palestinian conflict and the Colombian conflict. Our findings also show promise for replicating these effects on other types of intergroup conflicts and guiding effective public policy.

Keywords: emotion regulation, cognitive reappraisal, conciliation, aggression, peace, intractable conflict, Colombia, FARC-EP

INTRODUCTION

Violent intractable conflicts occupy a central social and political position in the affected societies and require immense material and psychological investment (Kriesberg, 1993; Bar-Tal, 1998; Gross et al., 2013). Individuals living in areas of intractable conflict frequently experience high intensity negative emotions, such as anger, fear, despair, and hatred, irrespective of how directly

the events inherent to those conflicts affect them (Halperin, 2014; Rosler et al., 2015). The grief over the loss of known or unknown combatants or civilian casualties, fear from being hurt or having a friend or relative hurt, and despair over failed attempts to achieve peace are the type of intense emotions experienced by members of societies affected by intractable conflict. In this context, political cues gain considerable control over these negative emotions, which in turn, seem to perpetuate the continuation of conflicts by mobilizing public support for aggressive actions, and hindering progress toward conflict resolution (Gross et al., 2013; Halperin et al., 2013; Halperin, 2014).

If negative emotions related to conflict limit the attempts toward a conflict's peaceful resolution, it seems plausible that efforts to decrease such emotions could result in important outcomes. Research that has extended the field of emotion regulation (Gross, 2013, 2014) to the study of conflict resolution has provided evidence in support of this notion (Gross et al., 2013; Halperin et al., 2013; Halperin, 2014; Halperin and Pliskin, 2015; Rosler et al., 2015; Čehajić-Clancy et al., 2016; Gutentag et al., 2016).

Emotion Regulation and Intractable Conflict: The Effects of Cognitive Reappraisal

Emotion regulation has been defined as the processes by which individuals influence the intensity and valence of their emotions, when they have them, and how they are expressed (Gross, 2014). Individuals implement a wide range of emotion regulation strategies during their day-to-day aversive interactions, including distraction, rumination, wishful thinking, and social sharing (Páez et al., 2013). However, they are not equally adaptive in terms of effectiveness to decrease subjective distress and/or physiological arousal, while maintaining the individual's ability to pursue meaningful short- and long-term personal and interpersonal goals (Gross, 2014). Cognitive reappraisal is an emotion regulation strategy aimed at reducing or increasing emotional responses by reinterpreting the meaning of the triggering situation (Gross, 2002). This reappraisal process therefore entails detecting and assessing the significance of the environment for wellbeing (Frijda, 2009) - e.g., perceiving the positive aspects of the triggering event, changing its personal relevance, or imagining better or worse outcomes. Over the last decade, mounting research has shown that reappraisal importantly improves physical and psychological well-being (Gross and John, 2003; Webb et al., 2012; Gross, 2014; Hu et al., 2014; Picó-Pérez et al., 2017). However, only recently it has been suggested that it also has a positive role in larger scale phenomena, such as intractable conflicts.

In the context of the Israeli–Palestinian conflict, Halperin and Gross (2011) found a positive correlation between the implementation of reappraisal and support for providing humanitarian aid to Palestinian citizens by Israeli citizens. More recently, direct evidence of the causal role of emotion regulation on changes to political attitudes in intractable conflicts was obtained. Halperin et al. (2013) tested if reappraisal training could reduce negative intergroup emotions and, in doing so, could decrease support for aggressive actions and increase conciliatory attitudes toward conflict-related events. Israeli participants (college students) first received either reappraisal training or were assigned to a control condition, which in both cases was followed by a presentation of anger-inducing information related to the Israeli–Palestinian conflict (texts, pictures, and music). After the presentation, the participants indicated the extent to which they felt positive and negative emotions, together with their degree of support for different statements reflecting conciliatory policies (e.g., "Regardless of the security situation, Israel needs to transfer food, and medication to Gaza residents") and aggressive policies (e.g., "If the Israeli Defense Forces detects a terrorist in a building full of civilians, Israel should bomb the building even if most of the civilians will most likely be killed") toward Palestinians in the Gaza strip. Halperin et al. (2013) found that (a) participants in the reappraisal condition reported lower levels of negative emotions (e.g., anger) compared with control participants; (b) participants in the reappraisal condition expressed more support for conciliatory statements and less support for aggressive statements than participants in the control condition; (c) negative emotions were positively associated with support for aggressive statements and negatively associated with support for conciliatory statements; and (d) negative emotions, specially anger, mediated the effect of reappraisal on support for conciliatory and aggressive statements. In our research, we aimed to reproduce these findings in the Colombian context.

The present study was conceived by the time major efforts to end the more than five-decade Colombian conflict between the government and the FARC-EP (Revolutionary Armed Forces of Colombia – Army of the People) were being implemented. Also, with consideration of the potential extension of the findings reported by Halperin et al. (2013) regarding the role of emotion regulation on changing attitudes toward conciliatory and aggressive policies in intractable conflicts.

The Colombian Government-FARC Conflict and the Peace Accord

Fuerzas Armadas Revolucionarias de Colombia (FARC) was officially founded in 1964. It is one of the multiple expressions of agrarian struggles that emerged in Colombian rural areas during the 1930s, and the bipartisan-fueled violence that erupted during the 1950s. FARC shared with other guerrillas the influence of the ideological and political climate of the Cold War and the Cuban Revolution (Thomson, 2011; Centro Nacional de Memoria Histórica, 2013).

FARC proclaimed in the 1980s that their goal was to overthrow the Colombian government. During the same decade, other armed actors emerged, including right-wing paramilitary groups, which had ties to the army, political figures, drug cartels, and large landowners. By the end of the decade, the conflict escalated, including a dramatic upsurge in violent actions affecting the civilian population (e.g., terrorist acts, kidnapping, massacres, landmines, forced recruitment, forced displacement, disappearances, and selective executions; Abello-Llanos et al., 2009; Grajales, 2011).

Drug trafficking became a major funding source for both paramilitary groups and the FARC during the following decades, which resulted in their strengthening. The Colombian state forces (police and army) also grew substantially as response to the consolidation of the other actors. The end result was a modernized and exacerbated conflict, which further affected the civilian population – including frequent human rights' violations. The estimated number of victims surpassed six million (Human Rights Watch, 2015; Unidad para las Victimas, 2017), including millions of internally displaced and more than 200,000 deaths (near 80% civilians; Historical Memory Group, 2016).

The first step toward the termination of the Government-FARC conflict was the signing of the "General Accord for the Termination of the Conflict and the Construction of a Stable and Lasting Peace" on August 26, 2012 in Havana, Cuba. After 4 years of negotiations, the parties signed on June 23, 2016 the 6-point accord on the end of the conflict, which included agreements related to an integral rural reform, political participation, a bilateral and definite ceasefire and cessation of hostilities, security guarantees for human-rights defenders and social movements, a solution to the problem of illicit drugs, victims' reparation and compensation, and mechanisms for implementation and verification of the accord (Colombian Government, 2016a; Amnesty International, 2017).

In addition, the parties agreed on a national referendum (plebiscite) as a mechanism for ratification of the signed peace accord, which took place on October 2, 2016. With a 37.4% turnout, 50.2% of Colombians voted against it, and 49.8% voted in favor (Registraduria Nacional del Estado Civil, 2016). This unexpected result obligated the Colombian government and FARC to revise the agreement, this time with the participation of the opposition. The amended version of the agreement was approved by the Colombian Senate and the House of Representatives on November 29 and 30, 2016 (Colombian Government, 2016b).

Emotional factors had an important role in the difficulties that the peace process encountered – especially the results of the national referendum – which continue to be key to the success of ongoing processes of forgiveness and reconciliation (López-López et al., 2013, 2018; Cortés et al., 2015). The success of the controversial campaign ran by the opposition party (Centro Democrático) against the accord supported this notion. Reports indicate that one of its main strategies was triggering negative emotions in the voters, such as anger and fear, by distorting some of the contents of the accord regarding impunity for the FARC members and the negative economic and political implications of accepting the accord (El Espectador, 2016; El País, 2016).

Overview of the Study

The present study aimed to test whether the effects of cognitive reappraisal training reported by Halperin et al. (2013) could be reproduced in the context of the Colombian government-FARC conflict and the efforts of these actors to end the conflict. The experiment was conducted during the week before the national referendum on the acceptance of the peace agreement took place (October 2, 2016). The methodology of Halperin et al.'s (2013) study was adapted to systematically replicate, as close as possible, the conditions of their experiments (e.g., selection of relevant images and texts for the anger-inducing presentation, measurement of negative emotions, and selection of the conciliatory and aggressive statements toward FARC). Analogous to Halperin et al.'s (2013) study, college students received cognitive reappraisal training, or a control condition, followed by a presentation of anger-inducing information related to FARC's violent actions during the conflict. Immediately after the presentation, the participants indicated the extent to which they felt positive and negative emotions. They were also asked to rate their degree of support for different conciliatory or aggressive statements toward FARC, which were prepared by taking excerpts from the peace accord already signed between the involved parts (to be voted on the national referendum; Colombian Government, 2016a) or quotes of public statements made by political opponents to that peace agreement (published in national newspapers). Based on Halperin et al.'s (2013) findings, we predicted that reappraisal training would have resulted in (a) lower levels of negative emotions triggered by anger-inducing information related to FARC's violent actions, and (b) more support for conciliatory statements and less for aggressive statements. In addition, we expected that (c) negative emotions, including anger, would mediate the effect of reappraisal on support for conciliatory and aggressive statements.

MATERIALS AND METHODS

Participants

One-hundred eight college students voluntarily participated in the study (69 women and 39 men, M_{age} = 20.3 years; SD_{age} = 1.79), which were recruited from the following undergraduate psychology programs in Bogotá (Colombia): Konrad Lorenz Fundación Universitaria (n = 24), Universidad San Buenaventura (n = 24), Pontificia Universidad Javeriana (n = 22), and Corporación Universitaria Minuto de Dios – Soacha (n = 38). These institutions were selected to capture the wide range of socioeconomic statuses that characterize the Colombian population. Students received a refreshment at the end of the study in return for their participation.

This study was carried out in accordance with the ethical guidelines and recommendations of the American Psychological Association – APA (2002) and the Colombian Board of Psychologists (2016). All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Research and Ethics Committee at Konrad Lorenz Fundación Universitaria.

Procedure

Students were invited to the laboratory to participate in a study related to human emotion. Initially, they completed a trauma inventory (Bremner et al., 2007) aimed at screening their direct experience with the events that were portrayed in subsequent phases of the experiment (e.g., they were asked if they had witnessed a murder or violent acts). As per recommendation of the ethics committee that oversaw the study, participants who reported any of the traumatic experiences listed in the inventory

did not continue to further stages of the experiment (n = 31, different from the 108 participants of the study). The different phases of the experiment are described below, which were applied in a single session (approximately 15 min). The participants were individually tested.

Reappraisal Training

Students were randomly assigned to a reappraisal-training condition (n = 54) or a control condition (n = 54). The protocol for the reappraisal intervention replicated that reported by Halperin et al. (2013), who adapted it from that implemented by Richards and Gross (2000). Each participant was exposed to his/her corresponding condition individually. Using a Microsoft PowerPoint®presentation (available on request) projected in a computer screen, the participant observed five anger-inducing pictures related to the Colombian conflict (used from an ongoing project aimed at validating the emotional properties of conflict-related images), and was asked to "respond to them like scientists, objectively and analytically trying to think about them in a cold and detached manner" (Halperin et al., 2013, p. 2). The experimenter explained how to use the reappraisal technique in the presence of the first image, and the participant was asked to apply the technique in the presence of four additional pictures. During all the trials, the experimenter ensured that the participant applied the technique appropriately. Participants in the control condition were exposed to the same set of pictures, but they were only asked to respond to them naturally.

Presentation of Anger-Inducing Information

After the reappraisal or control manipulation, all participants watched a 4-min PowerPoint presentation that portrayed FARC's violent acts (e.g., displacement, forced disappearance, and sexual violence – presentation available on request) with pictures, text, and music. This presentation was adapted to resemble, as close as possible, the presentation implemented by Halperin et al. (2013) – e.g., number of pictures, music, and length and content of the texts. Prior to the projection of the presentation, participants in the reappraisal condition were asked to apply the technique that they had learned in the previous phase, whereas those in the control group were reminded to respond naturally.

Negative/Positive Emotion Assessment

After the anger-inducing presentation, the participants indicated the degree to which they felt positive (interested, optimistic, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and negative (angry, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid) emotions using the Positive and Negative Affect Schedule (PANAS – Watson et al., 1988 – adapted to Spanish by Dufey and Fernández, 2012; $\alpha_{positive}$ = 0.78; $\alpha_{negative}$ = 0.87). Participants indicated to what extent they presently felt each emotion toward FARC using a Likert-style rating scale that ranged from 1 = "*very slightly or not at all*" to 5 = "*Extremely.*" Specific scores for each emotion and total scores for negative and positive emotions were analyzed. The sum of the scores on the corresponding individual emotions were used to produce *PANAS Negative* and *PANAS Positive scores* for each participant; accordingly, scores in each of these two measures could range between 10 and 50.

Support for Conciliatory and Aggressive Statements

In what was presented as a separate study on attitudes toward the Colombian conflict, the participants indicated their support of three statements reflecting conciliatory statements, and another three items reflecting aggressive statements. Conciliatory statements consisted of excerpts copied verbatim from the peace accord signed between FARC and the Colombian government (Colombian Government, 2016a), which was to be voted on a national referendum days after the experiment took place (e.g., "Reincorporation of the FARC forces to the civil life will be an integral and sustainable process, exceptional and transitory, that will take into account the interests of the community and FARC, including their members and their families, and which will be oriented toward the regional strengthening of the social tissue"). Aggressive statements consisted of verbatim public statements made by political opponents of that peace agreement (e.g., statements in national newspapers – "Although FARC announced 1 year ago a unilateral cease fire, they have not stopped dealing drugs or extorting. These are some of the criminal activities that they have never recognized, which affect the public order and the community" – Interview with Senator E. Macias by Diario del Huila, 2016). The Likert-style rating scale for the conciliatory and aggressive statements ranged from 1 = *highly oppose*, to 6 = *very much in favor*. Factor analysis revealed that there were indeed two factors, one measuring conciliatory statements ($\alpha_{conciliatory}$ = 0.65), and another measuring aggressive statements ($\alpha_{aggressive}$ = 0.68). See complete list of aggressive and conciliatory statements on **Appendix 1** (see **Supplementary Materials**). Participants also completed the 33-item Marlowe–Crowne Social Desirability Scale (Crowne and Marlowe, 1960 – adapted to Spanish by Ferrando and Chico, 2000).

Data Analyses

Halperin et al. (2013) tested the effects of reappraisal on overall negative and positive emotion scores (PANAS positive and PANAS negative) and scores on specific emotions (e.g., anger). Accordingly, we conducted one-tailed independent-samples t tests to assess directional effects of reappraisal on *PANAS negative* and *PANAS positive* and on each of the ten positive and ten negative emotions (e.g., based on Halperin et al.'s (2013) findings we expected significant decrements in negative emotions resulting from reappraisal training).

Also following Halperin et al.'s (2013) approach and findings, we (a) assessed whether reappraisal increased support for conciliatory statements and reduced support for aggressive statements using directional t tests; and (b) tested the mediating effects of negative emotions on support for aggressive and conciliatory statements using PROCESS procedure for SPSS with model 4 and 5000 bootstrap resamples (Hayes, 2013).

The potential effect of reappraisal on the scores of the Marlowe–Crowne Social Desirability Scale were assessed with a two-tailed independent-samples t test. Cohen-s'd effect-size calculations were conducted (when applicable).

RESULTS

Effects of Reappraisal Training on Negative Emotions and Conciliatory and Aggressive Statements

A comparison of the overall negative emotions scores (PANAS negative) indicated that participants exposed to reappraisal training prior to the presentation of the anger-inducing information reported less intense negative emotions (M = 19.76, SD = 7,24) than participants in the control condition (M = 23.46, SD = 8,46), $t(106) = 2.445$, $p = 0.008$, Cohen's $d = 0.47$. No significant differences in the overall scores of positive emotions (PANAS positive) were found between reappraisal (M = 24,81, SD = 6.80) and control (M = 24.31, SD = 5.87) groups; $t(106) = 0.409$, $p = 0.342$.

Similar to Halperin et al.'s (2013), we tested the effects of reappraisal on each individual positive and negative emotion using one-tailed independent-samples t tests. As shown in **Table 1**, reappraisal significantly reduced scores on anger, distress, fear, irritability, and uneasiness, with moderate-to-high effect magnitudes (Gignac and Szodorai, 2016), whereas it had no significant effect on participants' reports of nervousness, shame, guiltiness, and hostility (ps > 0.115) or in any of the positive emotions assessed (ps > 0.145).

Participants in the reappraisal condition expressed more support for conciliatory statements (M = 4.88, SD = 0.93) when compared with participants in the control group (M = 4.56, SD = 0.96), $t(106) = 1.735$, $p = 0.043$, $d = 0.33$. The obtained Cohen's d indicated a moderate effect of reappraisal on increasing support for conciliatory statements.

No significant differences between control (M = 3.42, SD = 1.14) and reappraisal groups (M = 3.36, SD = 1.32) were found regarding support for aggressive statements, $t(106) = 0.234$, $p = 0.407$. No interactions between condition and gender on support for conciliatory and aggressive statements were observed.

Negative Emotions as Mediators of the Effect of Reappraisal on Support for Aggressive and Conciliatory Statements

Halperin et al. (2013) found that global score on negative emotions (i.e., PANAS Negative) and anger mediated the effect of reappraisal on support for conciliatory and aggressive statements. Here we tested whether these measures had the same mediating role. In addition, based on the reports that the campaign against the peace accord between government and FARC was successful by triggering fear in the population (El Espectador, 2016; El País, 2016), we assessed if this emotion had a mediating effect between reappraisal and support for conciliatory and aggressive statements.

All procedures were conducted using Model 4 of the SPSS macro PROCESS (Hayes, 2013) and 5,000 bootstrap resamples. We followed Hayes and Rockwood's (2016) approach to determine whether a predicted variable M mediated the effect of an independent variable (X) on a dependent variable (Y). To that aim, we estimated the indirect effect of X (e.g., reappraisal) on Y (e.g., support for aggressive statements) operating through M (e.g., anger), and conducted an inference about that effect using a 95% bootstrap confidence interval (CI). A resulting CI that was entirely above or below zero (i.e., did not include zero) was considered evidence of a mediation effect, whereas a CI that included zero indicated no mediated effect of X on Y through M. Though in this approach the significance of the effects of X on M (e.g., reappraisal on anger) and M on Y (e.g., anger on support for aggressive statements) is not relevant in establishing mediation, as recommended by Hayes and Rockwood (2016) we included information on these effects to supplement the analysis – e.g., checking the consistency of the signs of the X-M and M-Y relationships with the predicted indirect effect.

The Mediating Effect of Negative Emotions on Support for Aggressive Statements

The prediction that negative emotions mediated the relationship between reappraisal and support for aggressive statements was confirmed with the observation that the indirect effect of reappraisal on support for aggressive statements through negative emotions was significant [95% CI did not include zero; (−0.354, −0.001)]. The signs of the associations between reappraisal and negative emotions (negative; $B = -3.72$, $p = 0.016$), and between negative emotions and support for aggressive statements (positive, $B = 0.03$, $p = 0.05$) were consistent with the predicted mediation effect.

We tested whether fear mediated the relationship between reappraisal and support for aggressive statements. The test of the indirect effect of reappraisal on support for aggressive statements through fear was significant [95% CI did not include zero; (−0.333, −0.002)], which confirmed that fear mediated the

TABLE 1 | One-tailed independent-samples t tests and effect sizes (Cohen's d) for scores on specific negative emotions of participants in the reappraisal training (RT) and control condition (CC).

Angry	Upset	Fearful	Irritable	Uneasy	Scared
M_{RT} = 2.31	M_{RT} = 2.00	M_{RT} = 1.89	M_{RT} = 1.78	M_{RT} = 2.37	M_{RT} = 1.96
M_{CC} = 2.76	M_{CC} = 2.50	M_{CC} = 2.31	M_{CC} = 2.30	M_{CC} = 2.89	M_{CC} = 2.43
SD_{RT} = 1.24	SD_{RT} = 0.99	SD_{RT} = 1.08	SD_{RT} = 0.90	SD_{RT} = 1.39	SD_{RT} = 1.21
SD_{CC} = 1.24	SD_{CC} = 1.26	SD_{CC} = 1.23	SD_{CC} = 1.19	SD_{CC} = 1.25	SD_{CC} = 1.34
t = 1.859	t = 2.298	t = 1.919	t = 2.547	t = 2.034	t = 1.882
df = 106	df = 106	df = 106	df = 106	df = 106	df = 106
p = 0.033	p = 0.012	p = 0.029	p = 0.006	p = 0.022	p = 0.031
d = 0.36	d = 0.44	d = 0.37	d = 0.49	d = 0.39	d = 0.36

effects of reappraisal on support for aggressive statements. The signs of the associations between reappraisal and fear (negative, $B = -0.43$, $p = 0.058$), and between fear and support for aggressive statements (positive, $B = 0.26$, $p = 0.011$) were consistent with the predicted mediation effect.

Following Halperin et al. (2013), we tested whether anger mediated the relationship between reappraisal and support for negative statement. We found no significant indirect effect of reappraisal on support for aggressive statements through anger [95% CI did include zero; (−0.181, 0.056)].

The Mediating Effect of Negative Emotions on Support for Conciliatory Statements

The test for the indirect effect of reappraisal on support for conciliatory statements through negative emotions was significant [95% CI did not include zero; (0.01, 0.20)]; thus, results indicated that negative emotions do mediate the relationship between reappraisal and support for conciliatory statements. The signs of the associations between reappraisal and participants' negative emotions (negative, $B = -3.72$, $p = 0.016$), and between negative emotions and support for conciliatory statements (negative, $B = -1.69$, $p = 0.094$) were consistent with the predicted mediation effect.

Regarding fear, the test for an indirect effect of reappraisal on support for conciliatory statements through fear was significant [95% CI did not include zero; (0.001, 0.229)], thus indicating that fear mediated the effects of reappraisal on support for conciliatory statements. The signs of the associations between reappraisal and fear (negative, $B = -0.43$, $p = 0.058$), and between fear and support for conciliatory statements (negative, $B = -0.18$, $p = 0.015$) were consistent with the predicted mediation effect.

Similar to Halperin et al. (2013), we tested whether anger mediated the relationship between reappraisal and support for conciliatory statements. We found that the indirect effect of reappraisal on support for conciliatory statements through anger was significant [95% CI did not include zero; (0.002, 0.189)], thus confirming the predicted mediating role of anger on the relationship between reappraisal and support for conciliatory statements. The signs of the associations between reappraisal and anger (negative, $B = -0.44$, $p = 0.066$), and between anger and support for conciliatory statements (negative, $B = -0.13$, $p = 0.057$) were consisted with the predicted mediation effect.

Social Desirability

A two-tailed independent-samples t test on the Marlowe–Crowne Social Desirability Scale scores (Crowne and Marlowe, 1960 – adapted to Spanish by Ferrando and Chico, 2000) indicated no significant differences between reappraisal ($M = 17.94$, $SD = 5.40$) and control groups ($M = 17.54$, $SD = 5.25$), $t(106) = 0.397$, $p = 0.692$. The fact that these scores were between the average reported in Spanish (Ferrando and Chico, 2000) and Mexican (Lara-Cantú, 1990) populations ($M_{\text{Spanish}} = 15.83$, $SD = 5.15$; $M_{\text{Mexican}} = 19.10$, $SD = 5.57$) indicated that participants in the present study showed an average degree of concern for the social desirability of their responses.

DISCUSSION

The present study tested whether the effects of reappraisal training reported by Halperin et al. (2013) could be reproduced in the context of the armed conflict between the Colombian government and FARC. Reappraisal was expected to decrease the intensity of negative emotions evoked by anger-inducing information related to FARC's violent actions, and produce more support for conciliatory statements and less support for aggressive statements. Also, we expected that negative emotions would mediate the relationship between reappraisal and support for conciliatory and aggressive statements.

Reappraisal training reduced the levels of negative emotions – anger, irritability, fear, and uneasiness – reported by the participants, with moderate-to-high effect magnitudes. It also significantly increased participants' support for conciliatory statements related to the peace accord signed between FARC and the Colombian government (Colombian Government, 2016a), which was voted on a national referendum few days after the present experiment took place (e.g., acceptance of FARC's members' reincorporation to civil life). Also aligned with the findings reported by Halperin et al. (2013), our results indicated that negative emotions mediated the effects of reappraisal on the participants' support for aggressive and conciliatory statements.

These findings add to those of previous studies (Gross et al., 2013; Halperin et al., 2013; Halperin, 2014; Rosler et al., 2015; Čehajić-Clancy et al., 2016; Gutentag et al., 2016) that support the notion that negative emotional experiences related to intractable conflicts limit attempts toward peaceful resolution of these conflicts (Gross et al., 2013; Halperin, 2014; Halperin and Pliskin, 2015). The replication of most of the effects observed in previous studies related to the Israeli–Palestinian conflict (Halperin and Gross, 2011; Halperin et al., 2013) suggest a high degree of generality of these phenomena, especially considering the differences between the conflicts approached, e.g., the Colombian conflict is internal, has entailed both state and non-state violence, several actors have been involved (other guerrillas, paramilitary groups, and drug cartels), and diverse types of violence were implemented on the civilian population, including terrorist acts, kidnapping, massacres, landmines, forced recruitment, forced displacement, disappearances, and selective executions (Historical Memory Group, 2016; Unidad para las Victimas, 2017). This aspect shows promise for further research that not only continue testing the generality of these effects on other types of intergroup conflicts across the world, but also attempt their application to generate socially relevant behavioral change (e.g., voting, donating, or volunteering). Also, related studies could explore the specific pathways through which these effects manifest depending on contextual factors of the conflict – e.g., why fear, and not anger, showed more evidence of mediating the relationship between reappraisal and support for conciliatory and aggressive statements in the Colombian context.

Limitations and Further Research

The fact that we could only obtain evidence of an indirect effect of reappraisal on aggressive statements against FARC may relate

to the characteristics of the texts that were presented to the participants—i.e., excerpts from the peace accord signed between FARC and Colombian government and aggressive statements from national newspapers. For instance, Correa et al. (2018) found that linguistic aspects of the Colombian government-FARC peace accord made its legibility very low, which adds to the more general finding that legal and political texts are not easily comprehended by lay persons (Charrow and Charrow, 1979; Jones et al., 2012; Mandic et al., 2012). Pilot testing of the different statements used in the present study was not possible due to the close date established for the referendum on the signed peace accord (less than 2 months), which may have resulted in the moderate levels of internal consistency observed for both sets of statements ($\alpha_{conciliatory} = 0.65$; $\alpha_{aggressive} = 0.68$). It is recommended that further research addresses these issues, including securing the legibility of the textual stimuli prior to their presentation to the participants (e.g., using data mining to identify the ideal or especially difficult fragments in a given text—Correa et al., 2018—and conducting pilot tests).

Another potential factor that may explain the limited effect of reappraisal on the participants' attitudes toward the aggressive statements, is the fact that support for these ideas was overall low (scores typically ranged between 2 and 3 in the 6-pt Likert scale, irrespective of group assignment). This floor effect may have resulted from having chosen participants from a target population that was reported to be in favor of the peace accord (urban young adults; Caracol Radio, 2016), and thus against actions that may have compromised it. In addition, different to Halperin et al.'s (2013) experiment, which had a clearly identifiable outgroup, in the present study some participants may not have perceived FARC as an outgroup. Accordingly, further research could test the effects of reappraisal on populations with a different relationship to the conflict and its actors —e.g., victims and members of the armed forces—and thus other attitudes toward the peace process.

The assessment of changes in emotional states is an aspect that needs to be considered for future research. Same as in Halperin et al.'s (2013) study, participants' emotions were only measured after receiving the cognitive reappraisal training (or control instructions) and the anger-inducing information. This limited the assessment of the effect of reappraisal, as participants' emotional state at baseline was unknown. Though the strategy of randomly assigning participants to the reappraisal condition provided some degree of control over this potential confound, future studies could implement a pre and post intervention measurement.

Regarding the limited mediational role of anger, which contrasted with Halperin et al.'s (2013) findings, it seems plausible that this outcome relates to the overall low emotional level reported by the participants (below theoretical mean). This may have resulted from exclusion of participants that experienced traumatic events and/or the fact that Colombian young urban population characterizes for absence of strong emotions related to conflict-related information. Ongoing research in our lab is precisely testing the latter notion (Hurtado-Parrado et al., unpublished).

Lastly, the results of the national referendum on the peace accord (50.02% of the votes were against it) indicated a highly polarized public opinion across the Colombian population. This outcome suggests other lines of research that could entail (a) testing whether the effects reported in the present study could be reproduced in the population that voted against the accord, and (b) analyzing the contents and effects of the controversial campaign ran by Colombian opposition party (Centro Democrático) against the accord, which apparently was successful by triggering negative emotions in the voters (e.g., by distorting some of the contents of the accord regarding impunity for the FARC members and the negative economic and political implications of accepting the accord – El Espectador, 2016; El País, 2016). Exploring the role of fear in this context seems particularly relevant, since it was seemingly triggered by the opposition campaign and our data indicates a mediating role for supporting conciliatory and aggressive statements.

CONCLUSION

Our findings add to those of previous research in showing the potential that resides in efforts to constructively cope with negative emotions that emerge during intractable conflicts. Future research should not only address the limitations of the present study, but also extend it by testing the long-term effects of the reappraisal intervention and its potential influence on actual political behavior (e.g., voting, donating money, or volunteering) and guiding public policy on areas affected by conflict. This is specially relevant in places in which efforts toward conflict resolution are currently being undertaken—as is the Colombian case.

Lastly, the positive effects of cognitive reappraisal training show promise to enhance the processes entailed in the development of peace cultures (Bar-Tal, 2000). This is particularly the case for the Colombian context, in which the much-needed processes of forgiveness and reconciliation seem heavily affected by negative emotions (e.g., feelings of hatred or distrust associated to a construed image of an enemy – Alzate et al., 2009; López-López et al., 2013, 2018; Cortés et al., 2015).

ETHICS STATEMENT

This study was carried out in accordance with the ethical guidelines and recommendations of the American Psychological Association – APA (2002) and the Colombian Board of Psychologists (2016). All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Research and Ethics Committee at Konrad Lorenz Fundación Universitaria.

AUTHOR CONTRIBUTIONS

CH-P, MS-P, MEH, AM, DG-V, JC, WL-L, and KH contributed to the conception, design, and implementation

of the study. CH-P, MS-P, AM, DG-V, LV, and AC-C conducted the field work and organized the database. CH-P, MEH, and JR performed the statistical analysis. CH-P wrote the first draft of the manuscript. MEH, MS-P, WL-L revised and extended the manuscript. All authors contributed to manuscript revision, and read and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.00908/full#supplementary-material

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Emotional Faces in Symbolic Relations: A Happiness Superiority Effect Involving the Equivalence Paradigm

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The stimulus equivalence paradigm presented operational criteria to identify symbolic functions in observable behaviors. When humans match dissimilar stimuli (e.g., words to pictures), equivalence relations between those stimuli are likely to be demonstrated through behavioral tests derived from the logical properties of reflexivity, symmetry, and transitivity. If these properties are confirmed, one can say that those stimuli are members of an equivalence class in which each member is substitutable for the others. A number of studies, which have established equivalence classes comprised of arbitrary stimuli and pictures of faces expressing emotions, have found that valences of the faces affect the relatedness of equivalent stimuli. Importantly, several studies reported stronger relational strength in equivalence classes containing happy faces than in equivalence classes containing angry faces. The processes that may account for this higher degree of *relatability* of happy faces are not yet known. The current study investigated the dynamics of the symbolic relational responding involving facial expressions of different emotions by means of the Implicit Relational Assessment Procedure (IRAP). Participants were 186 undergraduate students who were taught to establish two equivalence classes, each comprising pictures of faces expressing either happiness (for one class) or a negative emotion (for another class), and meaningless words. The IRAP effect was taken as an index for the relational strength established between equivalent stimuli in the different equivalence classes. The dynamics of arbitrary relational responding in the course of the four IRAP trial types revealed that the participants exhibited a stronger IRAP effect in trials involving the happy faces and a weaker IRAP effect in trials involving the negative faces. These findings indicate that the happy faces had higher impact on the symbolic relational responding than the negative faces. The potential role played by the orienting function of happy vs. negative faces is discussed. By considering other studies that also reported a happiness superiority effect in other contexts, we present converging evidence for the prioritization of positive affect in emotional, categorical, and symbolic processing.

Keywords: symbolic behavior, stimulus equivalence, implicit relational assessment procedure, facial expressions, happiness superiority effect

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INTRODUCTION

Humans seem to adapt to the environment in ways that are intrinsically symbolic, flexible, and generative (Barnes-Holmes et al., 2016). Despite the apparent agreement on this human feature, difficulties arise when one tries to distinguish between truly symbolic behaviors and non-symbolic interactions (Deacon, 1997). Sidman (1971) was pioneer in developing a behavioral analysis of derived relations (cf. Critchfield et al., 2018; see also Sidman and Tailby, 1982; Sidman, 1994). The Sidmanian paradigm of stimulus equivalence proposed that derived equivalence relations provide a basic functional account for the establishment of symbolic meaning.

Sidman and Tailby (1982) used the mathematical definition of an equivalence relation to provide operational criteria determining whether a relation between stimuli established in the lab is a relation of equivalence. For instance, a relation r between stimuli A and B (ArB), and between B and C (BrC), may be established by different procedures, such as matching-to-sample (e.g., Sidman, 1971), stimulus pairing (Leader et al., 1996), or a go/no-go procedure (Debert et al., 2007). If the relation r is an equivalence relation, training ArB and BrC should generate derived relations indicative of transitivity (ArC), symmetry (BrA and CrB), and reflexivity (ArA, BrB, and CrC), as well as combined symmetry and transitivity (CrA). These behavioral indicators of the logical properties of reflexivity, symmetry, and transitivity are used to infer that A, B, and C constitute a class of equivalent stimuli. Sidman (1994) stated that the mutual substitutability implied in the equivalence paradigm specifies "one way that symbols do become established as such, one way that words can come to "mean" what they "stand for" (p. 563).

Subsequent theoretical and empirical work has shown that other relational aspects need to be considered for a more complete account of derived relational responding (e.g., Hayes et al., 2001). Nevertheless, research on stimulus equivalence has enabled researchers to create artificial symbols in the laboratory. These artificial symbols can substitute for their referents, acquiring their psychological functions in a process that has been called symbolic generalization (e.g., Dymond et al., 2015; Bennett et al., 2015), or transfer of stimulus functions (e.g., de Rose et al., 1988; Gatch and Osborne, 1989; Barnes and Keenan, 1993; Dougher et al., 1994; Dymond and Barnes, 1994; Perez et al., 2017). The "symbolic status" of these artificial symbols has been demonstrated by several methods, such as lexical decision tasks (Barnes-Holmes et al., 2005; Bortoloti and de Rose, 2011a), semantic differential ratings (Bortoloti and de Rose, 2009), the Implicit Association Test (O'Toole et al., 2007), the Implicit Relational Assessment Procedure (IRAP) (Bortoloti and de Rose, 2012), semantic false memories (Guinther and Dougher, 2014), and Event-Related Potentials (Barnes-Holmes et al., 2005; Haimson et al., 2009; Bortoloti et al., 2014; Tabullo et al., 2015).

Different studies have shown that the relational strength (or relatedness) of members of equivalence classes vary as a function of several experimental parameters, such as the nodal distance between them (Fields et al., 1995; Bortoloti and de Rose, 2009), the simultaneous or delayed MTS employed in relational training (Bortoloti and de Rose, 2009, 2011b, 2012), and the amount of baseline training (Bortoloti et al., 2013), as well as the time elapsing between training and testing sessions (Silveira et al., 2016). Therefore, the paradoxical fact that stimuli regarded as equivalent may differ in relational strength has been pointed out by some investigators (e.g., Bortoloti and de Rose, 2011b; Doran and Fields, 2012).

Recent research has shown that the inclusion of a pre-experimental meaningful stimulus in an equivalence training influences likelihood of class formation (Fields et al., 2012; Fields and Arntzen, 2018) and also influences the relational strength within the class (Bortoloti and de Rose, 2012). A number of studies that established equivalence classes comprised of arbitrary stimuli and pictures of faces expressing emotions have found that valences of the faces affect the relatedness of equivalent stimuli. Bortoloti and colleagues (e.g., Bortoloti and de Rose, 2009, 2011b, 2012; Bortoloti et al., 2013) reported stronger relational strength in equivalence classes containing happy faces than in equivalence classes containing angry faces; Silveira et al. (2016) reported stronger stability in equivalence classes containing happy faces. None of these experiments was originally designed to compare the relational strength determined by facial expressions with different valences, but all of them showed what could be described as a *happiness superiority effect*. Processes that may account for this higher degree of *relatability* of happy faces are not yet known.

The current study sought to investigate the consistence of the happiness superiority effect with a larger sample of participants, and also tried to account for the impact of different types of facial stimuli on the symbolic relational responding induced in laboratory. The experimental design involved the application of the IRAP (Barnes-Holmes et al., 2010) to analyze the dynamics of the relational responding involving emotional faces and pseudo-words after equivalence training had simulated symbolic relations between these stimuli.

The IRAP has typically been used to measure brief and immediate relational responding that the participants have learned throughout their history of social interactions. It is assumed that the faster the response, the stronger is the participant's attitude toward the relation presented on the screen. As hypothesized for other *implicit measures* (Cummins et al., 2018), the IRAP allows researchers to determine the existence and strength of relations between stimuli.

The IRAP involves the simultaneous presentation of a *label*, a *target*, and two *relational terms*. The experimenter can work with various labels and targets that alternate along successive trials. The participant is required to respond by pressing a key that relates label and target in a predefined way along blocks of trials that sometimes cohere and sometimes do not cohere with the presumed learning history of the participant. In general, experimenters arbitrarily set the relations in the *consistent condition* blocks of trials as the ones to which participants are expected to respond faster than to the ones in the *inconsistent condition* blocks of trials, depending on coherence with presumed history of social interactions. The difference between response latencies in the consistent and inconsistent tasks is called *IRAP effect*. Specifically, a difference score, based on the response latencies divided by the pooled standard deviation of response

times across the consistent and inconsistent blocks, is used to infer the biases regarding the relation specified on the screen.

The IRAP has been used largely as a type of psychometric instrument for the measurement of implicit cognition (e.g., Hughes and Barnes-Holmes, 2011; Carpenter et al., 2012; Rabelo et al., 2014). More recently, several studies have demonstrated that the IRAP is also useful for exploring and analyzing the dynamics of arbitrary relational responding (e.g., Oliver, 2014; Finn et al., 2016; Maloney and Barnes-Holmes, 2016; Finn et al., 2018). Oliver (2014), for instance, asserted that features such as *coherence* to the history of reinforcement, *complexity* of the stimulus relationship, and the level of the participant's experience with the stimulus relationship (*derivation*) presented on the screen might all influence response latency in IRAP trials. Consistent with this view, Finn et al. (2018) proposed that interactions between the function of the stimuli, the relationship between them, and the response options presented on the screen might account for different patterns of IRAP performances. In this sense, if an IRAP trial component has, for instance, a stronger orienting function, this feature will influence the dynamics of the arbitrarily applicable relational responding in the course of the IRAP trials. By considering the functions and interactions between the IRAP elements proposed by Finn et al. (2018), it would be possible to capture the strength of a given pattern of relational responding *in flight* (cf. Barnes-Holmes et al., 2016).

The aim of the present study was to capture the relative strength of experimentally induced relational responding by means of the IRAP. Participants were 186 undergraduate students who were submitted to two experimental phases. The first phase established two classes of equivalent stimuli involving pseudo-words and pictures of human faces expressing emotions. One of the classes was comprised of pictures of faces expressing happiness and the other was comprised of faces expressing a negative emotion, which was sadness for Subgroup 1, fear for Subgroup 2, disgust for Subgroup 3, and anger for Subgroup 4. In Phase 2, pseudo-words and faces were, respectively presented as *label* and *target* in IRAP tasks. The IRAP effect was taken as an index for the relational strength established between equivalent stimuli in the different equivalence classes. We had predicted that, if positive and negative emotional faces differently influenced the strength of experimentally simulated symbolic relations, this differential effect could be indexed by means of the IRAP effect. Part of the participants from each subgroup were submitted to the IRAP immediately after the relational training, and part were submitted to the IRAP one week later, in order to investigate the stability of the equivalence relations over time.

MATERIALS AND METHODS

Participants

Participants were 186 undergraduates (59 males), students in a Brazilian university. Their native language was Portuguese, and they were not familiar with stimulus equivalence, IRAP, or related phenomena, concepts, and procedures.

All procedures performed in this study were in accordance with the ethical standards of the Brazilian National Health Council. The protocol was approved by the Federal University of Minas Gerais ethical committee. Participants were informed through a disclosure statement, provided to them at the beginning of the study, that they would serve as participants in an experimental simulation of symbolic relations. Participants were informed that their completion of the study was part of their training as experimental researchers since they would use similar procedures later in the semester in their own experiments, but they could quit their participation at any time. Information on all known risks and benefits of the study as well as confidentiality procedures was provided.

Equipment, Setting, and Stimuli

Sessions were conducted collectively, with 10–20 participants, in a 7-m × 12-m laboratory facility equipped with 32 standard desktop computers. Each participant worked alone on a single computer. These computers were equipped with software for the matching-to-sample procedure and also with the IRAP software.[1] Each matching-to-sample trial displayed five white windows (6 cm × 6 cm) on a gray screen, one at the center and one near each of the screen's corners; participants responded by moving the computer's mouse to position a cursor on a window and then clicking the mouse's button. Each IRAP trial displayed two stimuli and two response options on a white screen; participants responded by pressing two keys on the computer's keyboard.

Figure 1 presents the stimuli employed in the experiment. Set A was comprised of eight pictures: four happy faces (A1) and four non-happy faces (A2) that could express sadness, fear, disgust, or anger, depending on the participant's subgroup. Sets B, C, and D were comprised of two nonsense words each. Each participant was submitted to a relational training to generate two equivalence classes, one comprised of pictures of happy faces and nonsense words and the other comprised of pictures of one type of non-happy faces and other nonsense words.

The pictures were extracted from the *Pictures of Facial Affect*© CD-ROM, purchased from Paul Ekman's website.[2] Pictures of human faces depicting valid expressions of happiness, anger, disgust, fear, surprise, and sadness (Ekman and Friesen, 1976) are available in this CD-ROM.

Procedure

Phase 1: Establishment of Equivalence Classes

Each matching-to-sample trial began with the presentation of the sample stimulus in the central window. A click on this window displayed two comparison stimuli, in two of the peripheral windows. The two other peripheral windows remained blank, and the sample remained in the central window. A click on the window containing the stimulus designated as correct produced a display of stars moving on the computer screen. Incorrect responses blackened the screen for 3 s. The consequence for a correct or an incorrect response ended the trial, and the next trial began after a 2-s inter-trial interval.

Participants learned the conditional discrimination AB first, with a block of 24 AB trials in which samples A1 and A2 were

[1] https://osf.io/kg2q8/

[2] www.paulekman.com

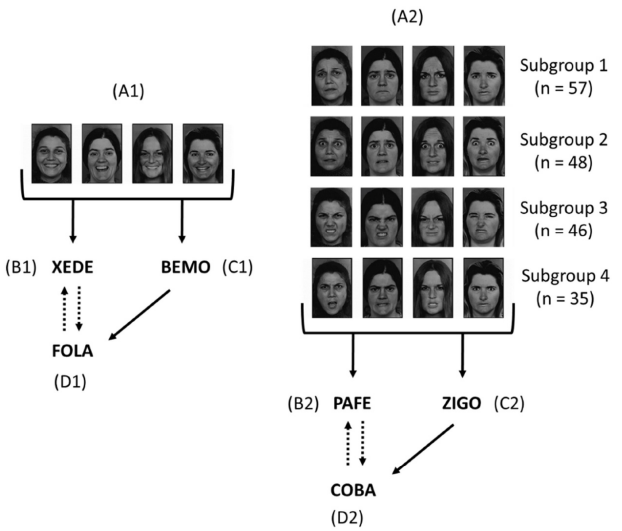

FIGURE 1 | Diagram of the relational training employed in Phase 1. Solid arrows indicate relations directly taught; dashed arrows indicate derived relations tested in equivalence probes (see text for details). Stimulus set A is comprised of pictures of faces expressing emotions, and sets B, C, and D are comprised of meaningless pseudo-words. A1 is comprised of four happy faces and A2 of four non-happy faces, which expressed sadness for participants from subgroup 1, fear for participants from subgroup 2, disgust for participants from subgroup 3, and anger for participants from subgroup 4. Note that pseudo-words related to happy and non-happy faces were counterbalanced among participants.

presented 12 times each in a randomized sequence. Sample A1 could be any one of the happy faces and sample A2 could be any one of the non-happy faces assigned for the participant (with expression of either sadness, fear, disgust, or anger). The positions of the comparison stimuli were determined according to a randomized sequence. In the first eight trials of this block a written prompt appeared on the screen. The Portuguese equivalent of the phrase "When this is here" appeared above the sample, and the equivalent of "Choose this" appeared above the correct comparison. These eight trials were followed by 16 trials without these prompts. If the learning criterion (correct choices in all 24 trials) was not achieved, the block was repeated. AB teaching ended when this criterion was attained, and then teaching of the AC relation began, with a similar procedure. When the participant made correct choices in all AC trials, CD training started, with a similar procedure. Each of these blocks – AB, AC, and CD – could be repeated for a maximum of three times. If the participant did not achieve the criterion in three presentations of a block, she or he was dismissed.

The next block verified maintenance of the cumulative baseline (AB, AC, and CD) and mixed 12 trials of each of these conditional relations, comprising, therefore, 36 trials in a randomized sequence. This block, with a different trial sequence, was repeated for a maximum of three times until the participant made no more than one incorrect selection.

When this criterion was achieved, the Portuguese equivalent of the message *The computer will no longer signal if your choices are correct or wrong* was displayed on the screen, and the cumulative baseline block (12 mixed trials of each conditional discrimination – AB, AC, and CD – totaling 36 trials) was repeated without differential consequences for correct and incorrect responses, until the participant made no more than one error. If the participant made incorrect choices in more than five trials, he or she returned to the cumulative baseline with differential consequences.

Equivalence probes

Two blocks of 16 probe trials without differential consequences tested equivalence-class formation. The first block evaluated the emergence of the BD derived relation. It was followed by the cumulative baseline block without differential consequences. Finally, the second probe block tested emergent conditional discrimination DB. These emergent conditional discriminations logically imply that trained conditional relations have the properties of symmetry and transitivity. Reflexivity is often assumed without tests in recent equivalence research. In addition, this arrangement permitted us to conduct the tests without the joint presentation of faces and words, which would be target and samples, respectively, in IRAP trials (see below). **Figure 1** shows a schematic representation of the trained and tested relations in this phase.

In sum, participants were taught to establish two 4-member equivalence classes including both meaningful and arbitrary stimuli. The meaningful stimuli, designated as A1 and A2, were not individual stimuli; rather, each was comprised of four pictures of faces, with each face belonging to a different person. The common feature of the faces in each category was the emotional expression, which was a happy expression in A1 and a negative expression in A2 (see **Figure 1**). Different pictures were used to ensure that abstract stimuli would be equivalent to a particular emotional expression and not to idiosyncratic features of a particular face.

The next phase was designed to be performed primarily by participants who made no more than three errors in the two equivalence probe blocks. These participants met the criterion used to conclude that they formed the intended equivalence classes (i.e., one equivalence class containing the happy expression and three nonsense words, and another equivalence class containing one type of non-happy expression and three nonsense words). Some participants who did not achieve the equivalence criterion were also submitted to Phase 2, in order to compare IRAP data from participants that succeeded or not in equivalence-class formation.

Phase 2: The Implicit Relational Assessment Procedure (IRAP)

About 40% of the participants who demonstrated formation of equivalence classes were submitted to the IRAP immediately after the equivalence probes. The remaining 60% were submitted to the IRAP seven days after the equivalence probes. Participants who did not achieve the equivalence criterion were submitted to the IRAP seven days after the equivalence probes.

The IRAP trials were divided into "consistent" and "inconsistent" blocks. Appendix A presents the instructions

FIGURE 2 | Schematic illustration of the four trial types presented during the IRAP blocks.

for participants. On each IRAP trial, a sample, a target, and two response options were displayed on the computer screen. A sample word – either stimulus D1 (FOLA) or stimulus D2 (COBA) – was presented on the top of the screen; a single picture target – a happy or a non-happy face – appeared at the center; and the response options – V (for true) and F (for false) – were displayed at the two bottom corners of the screen (V on the left and F on the right[3]). All stimuli remained visible until the participant pressed one of the response keys. The task consisted of choosing one of these options by pressing either the "d" or the "k" key, corresponding to V (true) or F (false), respectively. The choice of the option considered correct removed all stimuli from the screen and, after 400 ms, the next trial was presented. The choice of the option considered incorrect produced a red X in the middle of the screen (immediately below the target picture). The next trial was presented only after the participant pressed the correct key.

All participants were presented with blocks of 24 trials – at least two practice blocks and six test blocks. The practice blocks were repeated until at least 80% correct choices had been made consecutively in one consistent and one inconsistent block. Within each block, the target stimulus could be either a happy face or a non-happy face, in a randomized sequence, with the restriction that the target was happy in 50% of the trials and non-happy in the other 50%. The happy face could be one of the four pictures in this category, in a randomized order, so that each of the specific happy faces appeared four times. The same applied to the non-happy faces. In the consistent blocks, trials that presented FOLA as sample and a happy face as target, and trials that presented COBA as sample and a non-happy face as target, both demanded the choice of the option V, whereas F was the correct choice in trials that presented FOLA as sample and a non-happy face as target, and in trials that displayed COBA as sample and a happy face as target. Incorrect choices caused a red X to be presented below the face, and the participant had to make the correct selection in order to advance to the next trial. In the inconsistent blocks, the opposite responses were required. **Figure 2** illustrates the four different trial types in this part of the experiment.

After completion of all practice blocks and the six test blocks, a written message indicated the end of the experiment. The participant was thanked, debriefed, and any questions about the experiment were answered.

Data Analysis

The most important IRAP data is the "response latency", defined as the time in milliseconds (ms) that elapses between the beginning of the trial and the correct response by the participant. In line with contemporary research involving measurement of implicit bias, IRAP latency data were transformed into "D scores", which minimized the impact of factors such as age, motor skills, and/or cognitive ability of participants (Greenwald et al., 2003). In this study, the transformation was achieved by means of an adapted version of the D algorithm developed by Greenwald et al. (2003) for the Implicit Association Test. This adaptation of the D algorithm for the IRAP is called D-IRAP. The transformation of latency data into D-IRAP scores allowed us to infer differences between conditions aside from contamination, which comes from individual differences associated with extraneous factors.

All latency data were processed by the D-IRAP algorithm, available in the IRAP software. The algorithm processed the data as follows: (1) latencies obtained in training trials were discarded, and only latencies from tests blocks were used; (2) latencies above 10,000 ms were excluded from the analyses; (3) participants who presented more than 10% of test-block trials with latencies less than 300 ms were excluded from the study; (4) standard deviations for the four trial types were computed: four for the response latencies from Test Blocks 1 and 2, four from Test Blocks 3 and 4, and four more from Test Blocks 5 and 6 – a total of 12 standard deviations; (5) twenty-four mean latencies were calculated, one for each trial type in each test block; (6) difference scores were calculated for each of the four trial types, for each pair of test blocks, by subtracting the mean latency of the consistent block from the mean latency of the corresponding inconsistent

[3]The most common Portuguese words for true and false are "verdadeiro" and "falso", with initials V and F, respectively.

block; (7) each difference score was divided by its corresponding standard deviation calculated in step 4, generating one D-IRAP score for each trial type for each pair of test blocks: 12 D-IRAP scores in total; (8) four trial-type D-IRAP scores were calculated by averaging the scores for each trial type across the three pairs of test blocks; (9) a final D-IRAP score (overall D-IRAP) was produced by averaging the 12 trial-type D-IRAP scores from step 7 (Timko et al., 2010).

Finally, latencies for the consistent blocks were subtracted from latencies for the inconsistent blocks. Thus, positive D-IRAP scores indicate that the participants responded faster in the consistent blocks; negative D-IRAP scores indicate that participants responded faster in the inconsistent blocks. A higher D-IRAP score indicates a larger difference in response latencies between consistent and inconsistent trials.

RESULTS

One-hundred-thirty-five participants (72.6% of total) showed formation of equivalence classes (Phase 1) and attained criteria for the IRAP (Phase 2). Fifty-two of them completed the IRAP right after the equivalence training and the other 83 completed the IRAP seven days after the equivalence training. **Table 1** presents these numbers distributed by subgroup of participants.

Twenty-four participants who did not achieve the equivalence criteria (12.9%) completed the IRAP correctly seven days after the relational training. The remaining 27 participants (14.5%) did not produce IRAP scores (they either did not reach the IRAP criteria in the practice block or did not return to the experiment seven days later) and will not be considered in the following analysis.

Overall D-IRAPs

The overall D-IRAP scores produced by participants who established the equivalence classes were positive and significantly different from zero [Overall D-IRAP$_{\text{right after}}$: Mean = 0.13, SD = 0.19, $t(51) = 4.94$, $p < 0.0001$; Overall D-IRAP$_{7\ \text{days}}$: Mean = 0.11, SD = 0.22, $t(82) = 4.41$, $p < 0.0001$]. These results indicate that, in general, participants who established equivalence classes responded faster in the consistent than in the inconsistent IRAP conditions. On the other hand, participants who did not establish the experimental classes produced a mean overall D-IRAP that was not significantly different from zero [Overall D-IRAP$_{\text{no equivalence}}$: Mean = 0.08, SD = 0.29; $t(23) = 1.42$, $p = 0.17$]. This result indicates that, in general, these participants took similar times to respond under the consistent and inconsistent IRAP conditions.

A one-way ANOVA showed a significant effect for the difference between the mean overall scores presented above [$F(2,156) = 8.23$, $p = 0.0004$]. To extend the interpretation of this result, a Tukey-Kramer multiple-comparison test was conducted and the results of this *post hoc* test are presented in **Table 2**.

A significant difference was observed between the overall mean D-IRAP scores produced by participants who established equivalence classes and by participants who did not establish these classes. There was no significant difference between the overall D-IRAP scores produced immediately after class establishment and the scores produced seven days later.

Overall D-IRAPs From Different Subgroups

Out of the 135 participants with equivalence-consistent performances, 45 established classes involving happy and sad faces, 30 established classes involving happy and fearful faces, 34 established classes involving happy and disgusted faces, and 26 established classes involving happy and angry faces (see **Table 1**). The mean overall D-IRAPs from these subgroups were all positive and significantly different from zero, as shown in **Table 3**.

A one-way ANOVA showed that these mean general scores were not significantly different from each other [$F(3,131) = 1.12$, $p = 0.34$], indicating that the type of negative emotional expression had no significant differential impact on the magnitudes of D-scores. Therefore, the different non-happy faces will be henceforth referred to collectively as "negative expressions".

D-IRAPs for Different Trial Types

The mean overall D-IRAP scores produced by the 135 participants appear to have been more significantly impacted by

TABLE 1 | Number of participants in each subgroup who achieved both equivalence and IRAP criteria.

Subgroup	Completed the IRAP		Total
	Right after	7 days later	
(1) Happy and sad faces	17	28	45 (33.3%)
(2) Happy and fearful faces	11	19	30 (22.2%)
(3) Happy and disgusted faces	11	23	34 (25.2%)
(4) Happy and angry faces	13	13	26 (19.3%)
Total	52	83	135
	(38.5%)	(61.5%)	(100%)

TABLE 2 | Results from the Tukey–Kramer Multiple Comparisons Test.

Comparison	Mean difference	*Q*	*p*-value
No equivalence vs. Right after	−0.217	5.428	<0.001
No equivalence vs. 7 days	−0.196	5.228	<0.001
Right after vs. 7 days	0.021	0.723 ns	>0.05

If the value of q is greater than 3.352, then the P-value is less than 0.05. ns = non-significant.

TABLE 3 | One-sample *t*-tests calculated for the mean overall D-IRAPs generated by the participants of each subgroup.

Subgroup	Overall D-IRAP	*SD*	*t*- and *p*-values
Happy and sad faces	0.110	0.21	$t(44) = 3.53$, $p < 0.001$
Happy and fearful faces	0.180	0.19	$t(29) = 5.16$, $p < 0.0001$
Happy and disgusted faces	0.081	0.23	$t(33) = 2.04$, $p < 0.05$
Happy and angry faces	0.117	0.23	$t(25) = 2.56$, $p < 0.01$

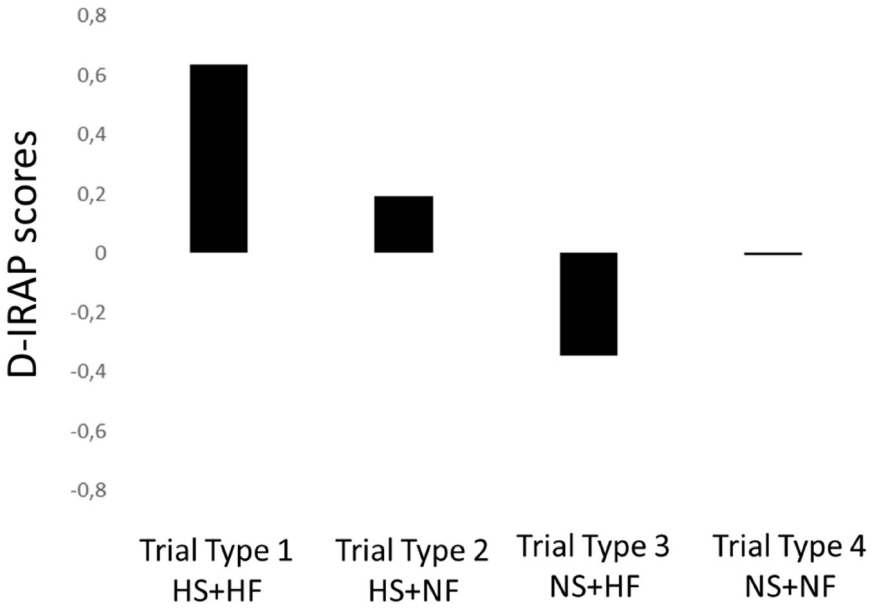

FIGURE 3 | D-IRAP scores for each trial type extracted from the performances of the 135 participants who achieved both the equivalence and the IRAP criteria. HS stands for happy symbol, HF for happy face, NS for negative symbol, and NF for negative face.

the participants' performance in trials involving happy faces and their symbol (equivalent word) than by trials involving negative expressions and their symbol, as shown in **Figure 3**.

Participants were significantly faster to respond in the consistent blocks of trial types 1 and 2, slower in the consistent blocks of trial type 3, and did not show significant differences between the times to respond in the consistent and inconsistent blocks of the trial type 4. **Table 4** presents the mean D-IRAPs scores for each trial type confronted with a value of zero.

A repeated measures ANOVA showed a significant effect for the difference between the mean D-scores that participants produced for the IRAP trial types [$F(3,134) = 132.75$, $p < 0.0001$]. To extend the interpretation of this result, a Tukey-Kramer multiple-comparison test was conducted and the results of this *post hoc* test are presented in **Table 5**. Significant differences were observed between all the mean D-IRAP scores produced by participants who established equivalence classes.

Time Elapse From Equivalence Tests and IRAP

Time between equivalence tests and IRAP had a marginally significant effect on the modulation of D-IRAP for trial type 1 [Type $1_{\text{right after}}$: Mean = 0.72, SD = 0.35; Type $1_{7\ \text{days}}$: Mean = 0.58, SD = 0.46; $t(133) = 1.83$, $p = 0.069$], as depicted in **Figure 4**.

Time elapsing had no significant impact on the D-IRAPs extracted from trial type 2 [Type $2_{\text{right after}}$: Mean = 0.22, SD = 0.41; Type $2_{7\ \text{days}}$: Mean = 0.18, SD = 0.34; $t(133) = 0.58$, $p = 0.56$], trial type 3 [Type $3_{\text{right after}}$: Mean = −0.34, SD = 0.50; Type $3_{7\ \text{days}}$: Mean = −0.35, SD = 0.47; $t(133) = 0.09$, $p = 0.93$], and trial type 4 [Type $4_{\text{right after}}$: Mean = −0.06, SD = 0.39; Type $4_{7\ \text{days}}$: Mean = 0.03, SD = 0.38; $t(133) = 1.43$, $p = 0.15$].

Note: The data supporting the conclusions of this manuscript will be made available upon request to the first author.

DISCUSSION

The IRAP has typically been employed to measure the strength of derived relational responding in socially loaded contexts. The present study was different in the sense that it attempted to

TABLE 4 | One-sample *t*-tests calculated for the mean D-IRAP scores from the four types of trials.

Trial type	D-IRAP	*SD*	*t*- and *p*-values
1: happy symbol + happy face	0.64	0.42	t (134) = 17,44, $p < 0.00001$
2: happy symbol + negative face	0.19	0.37	t (134) = 6.04, $p < 0.0001$
3: negative symbol + happy face	−0.34	0.48	t (134) = 8.39, $p < 0.0001$
4: negative symbol + negative face	−0.005	0.39	t (134) = 0.16, $p > 0.05$

TABLE 5 | Results from the Tukey–Kramer Multiple Comparisons Test considering the four IRAP trial types.

Comparison	Mean difference	*Q*	*p*-value
Type 1 vs. Type 2	0.442	12.427	<0.001
Type 1 vs. Type 3	0.982	27.593	<0.001
Type 1 vs. Type 4	0.641	18.002	<0.001
Type 2 vs. Type 3	0.540	15.167	<0.001
Type 2 vs. Type 4	0.198	5.575	<0.001
Type 3 vs. Type 4	−0.341	9.592	<0.001

If the value of q is greater than 3.65, then the P-value is less than 0.05.

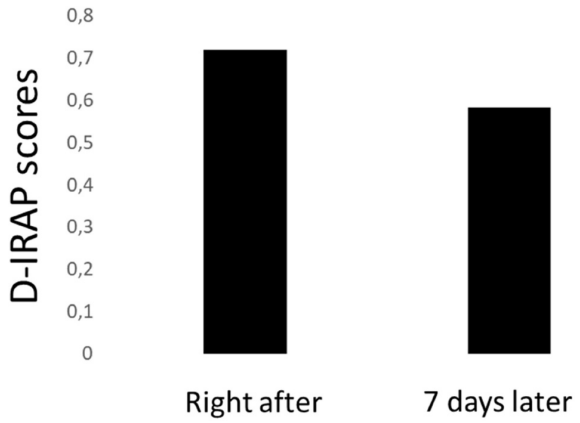

FIGURE 4 | D-IRAP scores for trials involving the simultaneous presentation of the happy symbol and the happy face (trial type 1) produced right after and seven days after relational training and tests.

measure stimulus relations established in the laboratory after nonsense words were made equivalent to happy facial expressions and different types of negative expressions. When responding to the IRAP, differences in response latencies between *consistent* and *inconsistent* relations reveal behavioral biases frequently attributed to the learning history of the participants. The current study demonstrates that the IRAP effect is also sensitive to the nature of the stimuli presented through the trials and to experimentally induced, derived relations. Moreover, this study is consistent with the claim that the participant's performance is multi-determined by the stimulus functions interacting with stimulus relations during the course of the IRAP trials (see Finn et al., 2018). In this section, we discuss how functions of certain stimuli may have even more impact on the direction and magnitude of the IRAP effect than the relational coherence previously learned by the participant.

The most important data presented in this article are the D-scores for the four different trial-types, which showed combinations among faces expressing happy or negative emotions and pseudo-words indirectly related to these faces. Only trial-types 1 and 2 yielded positive scores (i.e., faster responses in the consistent blocks, which required the participants to choose *true* when faced with the combination happy symbol–happy expression and *false* when faced with happy symbol–negative expression, respectively). D-IRAP scores for the trial-type 1 were significantly larger than the scores for the trial-type 2. For trial-type 3 (negative symbol–happy face = *false* in the consistent blocks), D-IRAP scores were negative, and scores were close (on average, statistically equal) to zero for trial-type 4 (negative symbol–negative face = *true*). The negative D-IRAP in trial-type 3 was particularly intriguing because it means that participants were faster to respond *true* than *false* for the combination of negative symbol and happy face; it suggests that the nature of the stimuli displayed in such a trial might have had more influence on the participants' performance than the relational coherence derived from their previous experimental learning histories. In the following paragraphs, we attempt to explain these results and explore their implications.

Once the IRAP is a procedure that requires the participant to respond accurately and quickly, differences in recognition of any stimuli present in the experiment may contribute to stronger or weaker effects. In this sense, the happy faces used in the current study were probably easier to detect and to recognize than the negative faces, in both the equivalence training and the IRAP trials. This hypothesis is consistent with studies that report a *happiness superiority effect* in visual search paradigms (Becker S. et al., 2011; Craig et al., 2014; Lee and Kim, 2017) as well as in categorization processes (Leppänen and Hietanen, 2003). Using visual search paradigms, a broad range of studies suggests that happy expressions may be easier to detect than negative expressions (Juth et al., 2005; Calvo and Nummenmaa, 2008; Becker D. V. et al., 2011), at least when faces from the Pictures of Facial Affect database (Ekman and Friesen, 1976) are used (Tottenham et al., 2009). We have already reproduced this happiness superiority effect in our laboratory by measuring, in a visual search paradigm, the response latency and the number of ocular saccades needed to identify happy and angry targets as a function of the orienting function of these targets (Pinto, 2018). The happy face advantage in categorizing processes refers to the verification that happy faces are categorized faster as *happy* than, for example, angry faces are categorized as *angry* (Leppänen and Hietanen, 2003).[4] Taking that into account, and consistent with the claim that emotionally salient stimuli can influence how attention is allocated (Fenske and Raymond, 2006), we assume that the orienting function of the happy faces might have played a critical role in the IRAP effects that can be inferred from the D-IRAP scores.

The role of the experimental stimuli that we chose to present as the response options in the IRAP trials also requires contemplation. It seems plausible to consider that the orienting function of the response option *true* may have been stronger than the orienting function of *false* (*true* may have a stronger orienting function since it frequently serves as a confirmatory, favorable, or positive response in natural language). Indeed, previous research (e.g., O'Shea et al., 2016) has shown that participants may find it easier to respond *true* to positive stimuli than to press *false*, if these are the IRAP response options. Based on the current results, it is possible to suggest that a behavioral bias to relate happy faces and *true* have emerged in the course of the IRAP trial types that presented such a combination (i.e., trial-types 1 and 3). This way, this conceivable behavioral bias may have affected the participants' responses when happy and *true* were presented, by facilitating pressing the *true* key frequently faster.

Taking the above into account, we assume that relevant properties of the experimental stimuli, combined with the equivalence relations previously established, allow for an interpretation of the observed results. Once the pseudo-words FOLA and COBA were indirectly related to the faces through equivalence, probably some of the functions of the faces

[4]Early experiments of the happy-face advantage described it as a general phenomenon. More recently, however, differences in the size of the happy-face advantage across pictures differing in ethnicity (Hugenberg, 2005) or gender (Hugenberg and Sczesny, 2006) have been investigated (Refer to discussion below).

expressing emotions were transferred, and this effect was also critical for the results observed. When the *positive* pseudo-word was presented with a happy face (trial-type 1), the relational coherence between these equivalent stimuli may have been added to the hypothesized bias to relate the happy faces and *true*, resulting in a very robust positive D-IRAP score (i.e., faster responses in the consistent blocks). The bias to relate happy faces and *true* seems to have overcome the effect of the relational training on IRAP performances. In trial-type 3, when the *negative* pseudo-word was presented with the happy face, participants responded faster to the *true* relative to the *false* option, yielding thus a negative D-IRAP score (i.e., faster responses in the inconsistent relative to the consistent blocks). It could be that the negative pseudo-word had not acquired properties of the negative faces robustly during the relational training, and this may have contributed to diminishing the control of this stimulus. Notwithstanding this conjecture, it is possible that the mean negative score registered for the trial-type 3 would have been even more negative in the absence of the relational training. Additional research is needed to investigate such a possibility; in the absence of a baseline measure, it is not possible at this time to determine the precise impact that the relational training had on the IRAP performances. Further investigation on the role of the response options in studies such as the current one is also necessary. For instance, if we employed *similar* and *different* instead of *true* and *false*, would we get different results?

For the trial-types that presented the faces expressing negative emotions (trial-types 2 and 4), the effects were different. The positive D-IRAP score for trial-type 2, which presented the positive pseudo-word and the negative faces, indicates that participants performed faster in rejecting this relation (i.e., by responding *false* in the consistent blocks) than in confirming this relation (by responding *true* in the inconsistent blocks). If the positive pseudo-word acquired functions of the happy faces to some degree, we should expect a competition between the tendency to respond to *true* (based on the functions transferred from the happy expressions) and the tendency to respond to *false* (since the relation between label and target do not cohere with the experimental learning history). In such a case, coherence with the experimental history seems to have prevailed, since pressings of the *false* key were frequently faster. Considering the trial type 4, an analysis of performances indicates a probable indifference of the participants to the equivalence relation between the negative pseudo-word and the negative faces. The mean D-IRAP score produced for this trial type was statistically equal to zero, which means similar latencies to respond *true* for the relation between the negative pseudo-word and the negative expressions in consistent blocks and to respond *false* to the same relation in inconsistent blocks. This pattern suggests that the relational coherence between the negative pseudo-word and the negative faces was weak for the participants, even though the establishment of this derived relation during the equivalence probes was demonstrated. These results indicate that the relatively small number of trials in relational training may have established a relational coherence that was probably stronger in the happy class and weaker in the negative class, a finding that is consistent with previous work by our research group (e.g., Bortoloti and de Rose, 2009, 2011b, 2012; Bortoloti et al., 2013). Apparently, the relational strength increases with the number of trials in relational training, as evidenced by Bortoloti et al. (2013). Future research may systematically investigate how experimental parameters that favor the establishment of stronger equivalence relations would influence the D-IRAP scores.

On balance, the happy expressions seem to have influenced the IRAP scores more than the negative expressions. The influence was even stronger when the relation between the happy expression, presented as target, cohered with the happy pseudo-word, presented as label. Additional investigation should elucidate whether such an influence in this sort of experiment may be modulated by variables such as stimuli gender and ethnicity. Several studies have demonstrated that attributes such as sex, ethnicity, and age can influence the perception of the emotion displayed by the face and its orienting function (e.g., Hugenberg and Sczesny, 2006; Becker et al., 2007; Aguado et al., 2009; Hess et al., 2009; Lipp et al., 2015). As an illustration, when both female and male faces expressing anger and happiness are categorized, a happy-categorization advantage is often observed for female faces, whereas for male faces the happiness advantage is typically attenuated or even reversed (Hugenberg and Sczesny, 2006; Becker et al., 2007; Bijlstra et al., 2010). Furthermore, the critical role of stimulus selection for the observation of the happiness superiority orienting function in visual search has been often highlighted (Savage et al., 2016). Considering that the pictures selected for this study came from a single database (Ekman and Friesen, 1976) and only white female posers were presented to the participants, further investigation is necessary to assess the impact of different facial attributes on the symbolic relational responding.

Stability of Equivalence Classes Including Happy Faces

In this study, the time elapsing between the equivalence tests and application of the IRAP marginally decreased the mean score for the IRAP trial-type 1, involving the happy symbol and the happy expression, but this trial type still dominated over the other trial types. This finding seems consistent with those reported by Silveira et al. (2016), in a study that investigated the stability of equivalence relations and transfer of functions over time. The authors showed that an equivalence class that included happy faces and arbitrary forms proved to be more stable than an equivalence class that included angry faces and arbitrary forms. Specifically, the number of participants who maintained the happy class was nearly double the number of those who maintained the angry class in a test conducted 30 days after the original relational training. The happiness superiority effect reported by Silveira et al. (2016) is consistent with the cognitive literature that claims a special place for happy faces in long-term memory processes (e.g., D'Argembeau and Van der Linden, 2007, 2011). In a typical old/new paradigm, happy faces are often more accurately identified, compared to angry, fearful, neutral, surprised, and disgusted faces. Some authors even predicted that the more positive the valence of an expression shown by a face,

the better the recognition of this face in long-term memory tasks (D'Argembeau et al., 2003). This means that the best performance is predicted for happy expressions, followed by neutral ones, which may in turn be superior to negative expressions. The results reported here and by Silveira et al. (2016) are consistent with this predictive hypothesis.

CONCLUSION

Participants of the current study produced positive overall D-IRAP scores, but considering the variability of outcomes observed for each trial type (one very strong and positive, one strong and negative, one mild and positive, and one close to zero), any conclusion based solely on these overall scores would not be reliable. In this sense, we believe that the interpretation of the individual trial types provided a more accurate explanation for the behavior observed during the experiment. Broadly speaking, the D-IRAP scores were more influenced by the happy expressions than by the negative expressions. This difference is consistent with a *happiness superiority effect*, as described earlier. Further experiments can present more evidence of the critical role of stimulus selection for both equivalence and IRAP studies.

ETHICS STATEMENT

All procedures performed in this study were in accordance with the ethical standards of the Brazilian National Health Council. The protocol was approved by the Federal University of Minas Gerais ethical committee. Participants were informed through a disclosure statement, provided to them at the beginning of the study, that they would serve as participants in an experimental simulation of symbolic relations. Participants were informed that their completion of the study was part of their training as experimental researchers since they would use similar procedures along the semester in their own experiments, but they could quit their participation at any time. Information on all known risks and benefits of the study as well as confidentiality procedures was provided.

AUTHOR CONTRIBUTIONS

RB and JdR contributed conception and design of the study. RB and RdA collected data and organized the database. RB performed the statistical analysis and wrote the first draft of the manuscript. JdA contributed to discussion of the results. All authors contributed to manuscript revision, read, and approved the submitted version.

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BRIEF RESEARCH REPORT
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Tonic Immobility in PTSD: Exacerbation of Emotional Cardiac Defense Response

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Among defensive behaviors, tonic immobility (TI) is considered the last defensive resort when life is at extreme risk. Post-Traumatic Stress Disorder (PTSD) is the main psychiatric consequence resulting from exposure to traumatic events. Increasing evidence indicate an association between peritraumatic tonic immobilility and severity of PTSD. Cardiac defense response, a reactivity to perceived danger or threat, has been studied by recording heart rate changes that follows the presentation of an unpredictable intense auditory aversive stimulus. The aim of this study was to investigate potential distinctiveness in cardiac defense response among PTSD patients who presented – compared to those that did not – TI reaction in the laboratory setting. Patients (N = 17) completed the TI questionnaire for signs of immobility elicited by passive listening to their autobiographical trauma script. After a while, they were exposed to an intense white noise, while electrocardiogram was recorded. The heart rate during the 80 s after the noise, subtracted from baseline, was analyzed. Higher reports of TI to the trauma script were associated with stronger and sustained heart rate accelerations after the noise. The effects on cardiac defense response add to increasing evidence that some PTSD patients are prone to repeated re-experiences of TI, which may implicate in a potentially distinct pathophysiology and even a new PTSD subtype.

Keywords: tonic immobility, cardiac defense response, post-traumatic stress disorder, PTSD, humans, heart rate

INTRODUCTION

Post-Traumatic Stress Disorder (PTSD) is the main psychiatric consequence resulting from exposure to traumatic events. It is a frequent, chronic, debilitating, treatment refractory condition (American Psychiatric Association [APA], 2013).

Tonic immobility (TI) is an involuntary reflexive reaction triggered by the perception of inescapable danger, characterized by reversible profound motor inhibition and relative unresponsiveness to external stimuli (Ratner, 1967). Systematic studies with TI scales based on retrospective reports, which were further corroborated with objective biological recordings (see Volchan et al., 2017); pointed to the presence of TI in humans. Although the precise underlining subtracts of human TI has yet to be identified, biological indicators using posturography and

electrocardiography recordings were successfully reported, namely reduced body sway, tachycardia and low heart rate variability (Volchan et al., 2011). Importantly, experimental induction of TI using script-driven symptoms provocation was more evident in patients with PTSD than in trauma-exposed controls (Volchan et al., 2011, 2017). For retrospective reports, in a large epidemiological sample, greater TI scores were found to be associated with PTSD (Kalaf et al., 2015). Other studies observed that retrospective reports of peritraumatic TI are correlated with the severity of PTSD symptoms (Fiszman et al., 2008; Rocha-Rego et al., 2009; Lima et al., 2010; Portugal et al., 2012; Maia et al., 2015; Kleine et al., 2018).

Cardiac defense response can be defined as the cardiac reactivity to perceived danger or threat. Recording heart rate changes and the presentation of an unpredictable intense auditory aversive stimulus, studies showed two accelerative-decelerative components: a brief acceleration reaching maximum amplitude around second 2-3 followed by a fast deceleration and then a more sustained acceleration followed by a final deceleration, the whole pattern lasting up to 80 s (Vila et al., 2003; Vila et al., 2007). Under a pre-existing aversive emotional state – people with generalized anxiety disorder, chronic worry, or with specific phobia having visualized the phobic object prior to the intense auditory stimulus – the response pattern changes to a single sustained accelerative response without the initial deceleration (Vila et al., 2007; Delgado et al., 2009). Schalinski et al. (2013) investigated the cardiac defense response in women war refugees. Those with PTSD showed sustained accelerative response pattern without the initial deceleration, while those without PTSD showed the initial acceleration-deceleration followed by a reduced second acceleration-deceleration.

Our aim was to investigate potential distinctiveness in cardiac defense response among PTSD patients who presented – compared to those that did not – TI reaction after re-experiencing the trauma in the laboratory setting. Based on our previous observations of robust tachycardia under scrip-driven paradigm (Volchan et al., 2011), we hypothesized that subsequent exposure to an intense aversive sound would evoke, in PTSD patients reporting TI during script-driven symptoms provocation, a response pattern characterized by sustained acceleration significantly different from the response evoked by PTSD patients not reporting TI. The latter would show the initial acceleration-deceleration followed by a reduced second acceleration-deceleration.

MATERIALS AND METHODS

Participants

Victims of urban violence diagnosed with PTSD (7 men, 10 women) participated in the study. They were under treatment with selective-serotonin-reuptake-inhibitors and in the waiting list for cognitive-behavior-therapy in the Outpatient University Clinic specialized in PTSD. Psychiatric diagnoses were obtained using the DSM- IV Axis I (First et al., 1997; Del-Ben et al., 2001). Exclusion criteria were psychotic disorders, severe personality disorders, use of Haloperidol, and significant cognitive impairment. For more information about the sample, see **Table 1**.

The study was approved by the local Ethics Institutional Review Board and participants provided written informed consent.

Psychometric Assessment

Based on their index trauma, participants completed the PTSD Checklist-Civilian Version (PCL-C for DSM IV) (Weathers et al., 1993; Berger et al., 2004).

Based on TI Scale (TIS-C: Forsyth et al., 2000), we used a four-item measure of motor aspects of TI which has been validated

TABLE 1 | Socio-demographic, trauma-related and clinical characteristics of the sample (n = 17).

Age	
Mean (SD) (in years)	41.1 (6.43)
Marital status	
Married	62.5%
Single	25%
Divorced	6.3%
Living maritally	6.3%
Occupation	
White collar	37.5%
Blue collar	62.5%
Educational level	
College graduate	18.8%
Some college	12.5%
High school graduate	18.8%
High school dropout	18.8%
Elementary school graduate	18.8%
Elementary school dropout	12.5%
Type of traumatic event	
Armed robbery	31.3%
Traffic accidents	18.8%
Shooting	18.8%
Rape	12.5%
Being kidnapped	12.5%
Other	6.3%
Childhood abuse	
Sexual	6.3%
Physical	6.3%
PCL-C	
Mean score (SD)	66.8 (9.94)
BDI	
Mean score (SD)	30.3 (12.1)
Current psychiatric comorbidities	
Mean number (SD)	4.0 (1.59)
Major depressive disorder	87.5%
Obsessive-compulsive disorder	75%
Panic disorder and/or agoraphobia	37.5%
Social anxiety disorder	37.5%
Specific phobia	37.5%
Substance use disorder	18.8%

PCL-C, post-traumatic stress disorder checklist. BDI, beck depression inventory.

(Lima et al., 2010) and employed in previous studies (Rocha-Rego et al., 2009; Volchan et al., 2011; Portugal et al., 2012): (i) froze or paralyzed; (ii) unable to move; (iii) unable to call out or scream, and (iv) unable to escape. Total scores range from zero to 24. Cronbach alpha for this sample was 0.88.

Stimulus

The auditory stimulus was an intense white noise of 105 dB with 500 ms duration and instantaneous rise time, delivered binaurally throughout earphones (Eartone). Given the fast habituation of the cardiac defense response (Ramírez et al., 2010), the stimulus was presented just once.

Data Acquisition

Two PC-compatible computers controlled auditory stimulus presentation and data acquisition of the electrocardiographic parameters, running Presentation (Neurobehavioral Systems) and Acknowledge (BIOPAC Systems Inc.) softwares. Electrocardiographic recordings were collected at a sampling frequency of 1000 Hz through an electrocardiograph ECG100C module coupled to the MP150 system (BIOPAC Systems Inc.).

Procedures

A psychiatrist accompanied the patient throughout the experimental session and debriefing. Participants had electrocardiogram electrodes attached on the chest at lead II and wore earphones. Posturography was recorded while participant stood on a force platform and, after a 60s-neutral playback, they listened to a 60s-audio-play narrating his/her personal traumatic script, Then, participant was seated and the psychiatrist applied the TI questionnaire to assess signs of immobility elicited by the trauma script (for more details and cardiac and postural results, see Volchan et al., 2011). After relaxing for 25 min, they were exposed to the auditory stimulus, while electrocardiogram was recorded.

Data Reduction

An off-line peak detection algorithm (derivative plus threshold) was used to estimate R-wave fiducial points, after which the series was screened by hand and corrected for artifacts. R–R intervals were converted to heart rate each second using weighted averages during the 80 s following stimulus onset and expressed as differential score subtracting the weighted average of the 15 s prior to stimulus onset. Data processing followed the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) concerning the measuring of heart rate instead of heart period when using time units (second-by-second) versus organismic units (beat-by-beat), accompanied by weighted averages versus arithmetic averages within the time window (each second). Previous studies used to further reduce the 80 second-by-second heart rate values to 10 intervals (Vila et al., 2007) or 12 intervals (Schalinski et al., 2013). Given that current statistical software allows the analysis of long time series, as repeated measures, the present study maintained the 80 heart rate values, without further reduction, in order to fully describe the response pattern.

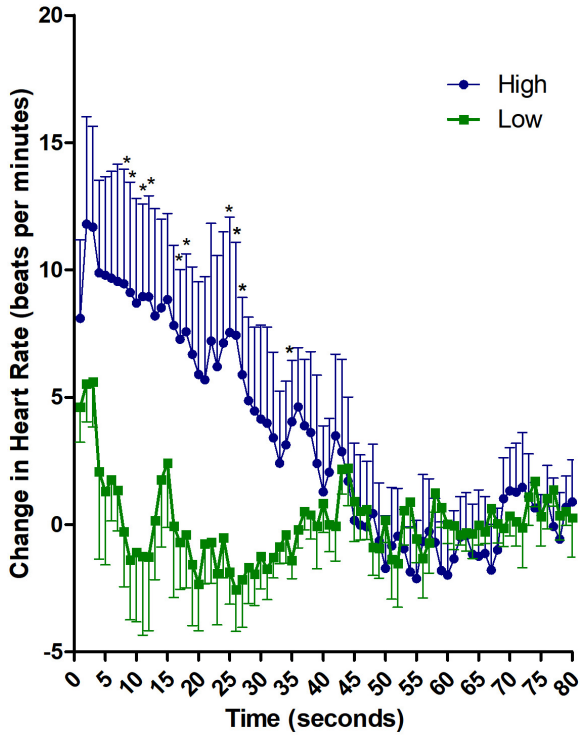

FIGURE 1 | Descriptive analysis of cardiac defense response in HIGH and LOW tonic immobility patients. Heart rate changes along 80 s after the noise stimulus, subtracted from mean baseline. *$p \leq 0.05$ in seconds 8, 9, 11, 12, 17, 18, 25, 26, 27, and 35.

Statistical Analyses

Sum scores in the four motor questions of the TI scale were calculated for each participant. Those with scores higher than the sample's median were assigned to the "HIGH" group and those with scores equal or lower than the median, to the "LOW" group. We used Student's *t*-test for the comparisons of TI scale, PCL scale and Baseline Heart Rate immediately before the delivery of the sounds between groups. A 2 x 80 ANOVA with one between group factor (Group) and one repeated measures factor (Time) was employed to compare the heart rate data between groups. The Greenhouse-Geisser correction was applied to control for sphericity violation in the repeated measures factor.

Pearson's correlation analyses were also performed between heart rate and TI scores for the whole sample.

The results are reported with the original degrees of freedom, the corrected *p* values, and the effect sizes. The threshold for significance was set at $p \leq 0.05$.

RESULTS

Psychometric Assessment

Based on scores in the four motor questions of TI elicited by listening to the personal trauma script, 8 participants (4 women) were ascribed to the "HIGH" and 9 (6 women) to the

"LOW" group. Scores differed significantly between the groups (HIGH: 17.9 ± 5.57; LOW: 3.1 ± 3.18; $t_{(15)} = 6.82$, $p < 0.01$). Comparison of PTSD symptoms, based in scores on the PCL-C scale, between "HIGH" and "LOW" groups were not significant (HIGH: 61.1 ± 9.23; LOW: 54.7 ± 13.66; $t_{(15)} = 1.13$, $p = 0.27$).

Heart Rate

Baseline heart rate before the delivery of the sound did not differ between "HIGH" and "LOW" groups (HIGH: 80.3 ± 16.7; LOW: 71.2 ± 15.3; $t_{(15)} = 1.18$, $p = 0.26$). Descriptive analysis of heart rate (subtracted from baseline), after the exposure to the aversive noise, showed a larger and longer accelerative response in the HIGH than in the LOW TI group (**Figure 1**). The peak of the acceleration occurs at second 3 after stimulus presentation. The LOW TI group presented a fast decrease afterward reaching decelerative values after second 7, whereas the HIGH TI group showed a sustained acceleration that lasted until second 45.

The 2 x 80 ANOVA revealed significant effects of Time [$F_{(79,1185)} = 4.66$, $p < 0.01$; $\eta p^2 = 0.237$] and Group x Time [$F_{(79,1185)} = 3.78$, $p < 0.01$; $\eta p^2 = 0.201$]. Follow-up analysis of the interaction revealed significant differences between HIGH and LOW immobility groups in the first 30 s after stimulus onset [$F_{(1,15)} = 4.44$, $p = 0.05$; $\eta p^2 = 0.228$]. Average increase in heart rate in the first 30 s was 7.88 ± 10.1 for HIGH group and 0.03 ± 4.5 for LOW group. When the heart rate was analyzed second-by-second, significant differences between the groups appeared in seconds 8, 9, 11, 12, 17, 18, 25, 26, 27, and 35 ($p \leq 0.05$; Cohen' $d \geq 1.0$ for all comparisons). Further, correlation analyses between heart rate and TI scores was significant for average heart rate in the first 30 s ($r = 0.491$; $p = 0.04$) and for seconds 6, 8, 9, 10, 11, 12, 17, 20, 25, 26 and 35 ($r \geq 0.49$; $p \leq 0.05$ for all comparisons).

DISCUSSION

This is the first study to investigate the modulation of the cardiac defense response in PTSD patients after the induction of a TI-like reaction in a laboratory setting. PTSD patients who reported high levels of immobility after listening to their autobiographical trauma exhibited, upon exposure to the unexpected and intense auditory stimulus, a more pronounced and sustained accelerative cardiac defense response than patients who reported low levels or did not report at all signs of immobility. Comparisons of PTSD symptoms scores between those with high and low scores of TI did not reach statistical significance, which favors TI as a main factor differentiating sustained versus transient tachycardia in response to the aversive sound.

Recent research has been looking for the relationship between defensive reflex responses and psychopathology, investigating if different patterns of responsiveness can be associated with clinical conditions (McTeague and Lang, 2012). Contributing with the RDoC initiative, Lang et al. (2016) suggests a complex and dynamic modulation of defensive reactivity across the anxiety disorder spectrum by physiological responses.

Schauer and Elbert (2010) proposed stages of defensive reactions in which TI, accompanied by the highest sympathetic activation, is at the peak of the fright stage. Alves et al. (2014) showed in victims of urban violence that high scores on peritraumatic TI were associated with tachycardia during exposure to trauma-relevant pictures. Volchan et al. (2011) showed that reports of TI after trauma-script listening were inversely correlated with amplitude of body sway and with heart rate variability, and directly correlated with heart rate; specially for the urban-violence victims with PTSD (Volchan et al., 2017). The authors concluded that peritraumatic TI was experienced in the laboratory setting and was accompanied by tachycardia and lowering of vagal tone.

In the present study, PTSD patients displaying peritraumatic TI after exposure to trauma-script presented a pattern of cardiac defense response with more accelerative and sustained cardiac response indicating that they are more prone to cardiac sympathetic activation in response to varied aversive intense stimuli accompanied by reduced influence of the parasympathetic system.

This distinctiveness adds to a growing literature concerning peritraumatic TI symptoms which might indicate a specific PTSD subtype responding differently to pharmacological treatment (Fiszman et al., 2008; Lima et al., 2010) and deserving the development of alternative interventions based on a potentially distinct pathophysiology. Still, the present sample was small and PTSD patients were under medication. Large scale randomized controlled trials are necessary to corroborate the promising findings.

ETHICS STATEMENT

The study was approved by the Ethics Institutional Review Board of the Institute of Psychiatry (Federal University of Rio de Janeiro). Written informed consent was provided by all participants, in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

CN, EV, VR-R, IF, GdS, MM, WB, and JM developed the study concept and the study design. CN, GdS, WB, and ML conducted the experimental sessions. CN, JM, JA, JV, GdS, and EV performed data analysis. GdS, CN, VR-R, and EV drafted the manuscript. IF, WB, MM, ML, and JV provided important critical revisions. All the authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Emotional Influences on Cognitive Flexibility Depend on Individual Differences: A Combined Micro-Phenomenological and Psychophysiological Study

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Imagine a scenario where you are cooking and suddenly, the contents of the pot start to come out, and the oven bell rings. You would have to stop what you are doing and start responding to the changing demands, switching between different objects, operations and mental sets. This ability is known as cognitive flexibility. Now, add to this scenario a strong emotional atmosphere that invades you as you spontaneously recall a difficult situation you had that morning. How would you behave? Recent studies suggest that emotional states do modulate cognitive flexibility, but these findings are still controversial. Moreover, there is a lack of evidence regarding the underlying brain processes. The purpose of the present study was, therefore, to examine such interaction while monitoring changes in ongoing cortical activity using EEG. In order to answer this question, we used two musical stimuli to induce emotional states (positive/high arousal/open stance and negative/high arousal/closed stance). Twenty-nine participants performed two blocks of the Madrid Card Sorting Task in a neutral silence condition and then four blocks while listening to the counterbalanced musical stimuli. To explore this interaction, we used a combination of first-person (micro-phenomenological interview) and third-person (behavior and EEG) approaches. Our results show that compared to the positive stimuli and silence condition, negative stimuli decrease reaction times (RTs) for the shift signal. Our data show that the valance of the first emotional block is determinant in the RTs of the subsequent blocks. Additionally, the analysis of the micro-phenomenological interview and the integration of first- and third-person data show that the emotional disposition generated by the music could facilitate task performance for some participants or hamper it for others, independently of its emotional valence. When the emotional disposition hampered task execution, RTs were slower, and the P300 potential showed a reduced amplitude compared to the

facilitated condition. These findings show that the interaction between emotion and cognitive flexibility is more complex than previously thought and points to a new way of understanding the underlying mechanisms by incorporating an in-depth analysis of individual subjective experience.

Keywords: cognitive flexibility, emotions, music, micro-phenomenological interview, P300, neurophenomenology

INTRODUCTION

To successfully navigate everyday life, we must continuously balance the need to fulfill a myriad of internal motivations with the capacity to react to a changing environment. As we confront the world, we choose many of the things we do, think or act upon, but we must also react to unexpected changes, adapting our behavior and, not infrequently, changing plans. In many such situations, adaptive behavior is, from a cognitive perspective, the result of being able to inhibit an ongoing action and effectively reorient our resources to deal with whatever the novel situation turns out to be (Diamond, 2013).

The skill that allows us to change between stimuli, operations, and mental sets is known as cognitive flexibility (Lin et al., 2013). It is usually studied through the use of task-switching paradigms: participants must adopt a certain mental set in order to accomplish a given task, but then must change it in response to a cue or an instruction, in order to continue to succeed. Most tasks used for studying cognitive flexibility are variations of the classical sorting card task (Berg, 1948; Grant and Berg, 1948; Kiesel et al., 2010; Doebel and Zelazo, 2015). Here, participants must sort a sequence of cards according to one of many possible characteristics (color, for example). Mid-way through the task, and upon being instructed, participants must start sorting the cards according to a different rule (e.g., number). Trials in which the rule must be changed (i.e., switch trials) take longer to solve than those in which the rule must be repeated, a quantity known as switch cost. Errors too are more likely in switch trials than in repetition trials.

Card sorting tasks have been extensively studied from behavioral and neuropsychological testing perspectives (Dehaene and Changeux, 1991; Barceló, 2003; Doebel and Zelazo, 2015; see Nyhus and Barceló, 2009 for an overview). From the point of view of underlying brain activity (Barceló and Rubia, 1998; Barceló et al., 2000; Barceló, 2003; Adrover-Roig and Barceló, 2010; Gajewski and Falkenstein, 2017), switch trials are accompanied by an event-related potential around 300 ms after the onset of a cue (also known as P3), a positivity that is absent in repetition trials. Some authors have reported this P3 as being the result of two different components, a frontally distributed potential that appears at 300–400 ms and is called P3a and a latter and posteriorly distributed potential called P3b. Traditionally, P3a activity has been related to novelty (Schroger and Wolff, 1998; Friedman et al., 2001), whereas P3b to context updating (Donchin and Coles, 1988, 1998). However, not all studies have named this potential consistently, sometimes referring to it as a "cue-locked positivity" (Karayanidis et al., 2010) or a switch-related positivity (Rushworth et al., 2002, 2005; Miniussi et al., 2005; Li et al., 2012).

Yet, in daily life, our choices, our changes of mind and the many ways we deal with the different challenges we face, do not occur in a vacuum. We encounter the world as living, sentient beings and as such, we do so from a point of view that always carries with it an emotional tone and a bodily disposition (Cosmelli and Thompson, 2010). This embodied encounter is not an added ingredient to a cognitive agent, but a condition of possibility of any meaningful interaction in the world (Damasio, 1994; Colombetti and Thompson, 2008; Colombetti, 2010). In other words, cognition and emotion are deeply intertwined and shape together our behavior, a fact that has received substantial empirical support from several branches of psychology and cognitive and affective neuroscience (Pessoa, 2008; Storbeck and Clore, 2008; Todd and Anderson, 2011; Todd et al., 2012a). It is therefore all the more surprising that how emotions affect cognitive flexibility, such a core skill for adapted behavior, has received little attention.

Despite the existence of a number of studies dealing with the effects of emotional arousal on a diversity of cognitive processes ranging from perception to decision making (Mather and Sutherland, 2011; Todd et al., 2012b), there are only a few studies that deal with the specific interaction between cognitive flexibility and emotions. Demanet et al. (2011) showed that the level of arousal of an affective stimulus can produce a change in switch costs, while the valence dimension seems to affect global task performance. On the contrary, Lin et al. (2013) showed that positive affect promotes cognitive flexibility. In addition, there is evidence that points to the effects of permanent emotional moods, such as anxiety, on cognitive flexibility. Compared to high levels of anxiety, low levels of anxiety seem to promote cognitive flexibility (Ansari et al., 2008; Derakshan et al., 2009). It is unclear where these differences are coming from. Among other possibilities, it could be that the presentation of an emotional stimulus is insufficient to generate a true internal change (or a transient emotional state) that can affect task-switching performance. Moreover, to our knowledge, there is still no evidence showing how specific emotional states could modulate the neural dynamics of task switching processes.

In this study, we were interested in elucidating if and how emotional states can affect cognitive flexibility as measured by a task switching paradigm, and how this would be observed in terms of the underlying brain activity. Taking into account the contradictory results that have been reported, we also aimed at developing a more in-depth approach to the emotional experience, based on a systematic investigation into the first-person experience that participants had during the task. We did this because evoking a specific emotional response can be highly dependent on the individual, the inducing stimulus and/or the experimental conditions (Gabrielsson, 2002; Lutz et al., 2002).

As mentioned above, it could be that some of the inconsistencies in previous studies stem from the fact that not all participants experience emotional inductions in the same way. Also, participants could be merely recognizing emotions but not experiencing them as such, which would amount to a different type of intervention.

To address these questions, we used a version of the card sorting task that has been adapted for psychophysiological recordings (Barceló, 2003), and paired it with an emotional induction based on musical stimuli. Usually, musical stimuli used to induce emotions are fragments of highly stereotyped popular songs that, by virtue of them being widely known, can easily be related to personal episodes that bias emotional states (see for example the music used in Stephens et al., 2010). To avoid this effect on the emotional induction (i.e., how I should feel *versus* how I actually feel), the stimuli used in this study were novel for all participants. All musical stimuli were designed and validated to induce two specific emotional dispositions: positive valence, high arousal, and open stance or negative valence, high arousal and closed stance (Vásquez-Rosati, 2017). The "stance" dimension refers to the postural/bodily disposition that is associated with a given emotion: while an open stance is associated with the feeling of an overall extension of the body and an availability to stimulation, a closed stance is associated with a feeling of inward retraction of the limbs and protection from stimulation (Kim and Andre, 2008). To assess these dimensions, we used a modified version of the Self Assessment Mannequin scale (SAM; Bradley and Lang, 1994; Vásquez-Rosati, 2017). In addition to the standard arousal and valence dimensions, the modified SAM scale evaluates the bodily disposition evoked by the musical stimulus to assess the open/closed stance dimension.

In order to ensure a consistent emotional experience throughout the entire block, we presented acoustic and visual stimulation during an emotional induction phase before the task (de Gelder and Vroomen, 2000; Baumgartner et al., 2006; Spreckelmeyer et al., 2006; Logeswaran and Bhattacharya, 2009). Then, to aid in sustaining of the emotional disposition without interfering, only the musical stimulus was maintained while the participant undertook the task. Finally, we used a micro-phenomenological interview (Petitmengin and Bitbol, 2009; Petitmengin and Lachaux, 2013) to gather in-depth experiential, first-person information about the emotional experience and its relation with task performance. As we hope to demonstrate, this approach can provide a much richer account of how emotional disposition can affect cognitive flexibility, complementing and extending the traditional psychophysiological approach.

MATERIALS AND METHODS

Participants

Twenty-six participants (12 females; mean age 28.3 ± 5.2 years, range 20–41 years) took part in the study. They had normal or corrected-to-normal visual acuity and no history of neurological or psychiatric disorder. Three of them had musical and instrument studies and two of them had only instrument studies. Twenty-one participants reported listening to music while doing other tasks. All participants enjoyed listening to music. The study reviewed and approved by the Ethics Committee of the Escuela de Psicología, from the Pontificia Universidad Católica de Chile. All participants read and signed an informed consent form before their participation in the actual experiment.

Stimuli

Musical Stimuli

In a previous study, in collaboration with a musician and composer, we designed two musical stimuli to induce opposite emotional states: negative valence, high arousal, closed stance (stimulus A); and positive valence, high arousal, open stance (stimulus B). These were validated through self-report questionnaires and first-person descriptions (Vásquez-Rosati, 2017). The "white" noise used in this experiment was the same used by Stephens et al. (2010) and Nyklicek et al. (1997) (70 dB intensity). Both music and white noise clips were presented with headphones from a 16-bit and 44,100 Hz stereo file during the entire block. The sound of the music was kept between 50–90 dB, but adjusted subjectively for each participant before the beginning of the experimental session, in order to ensure a comfortable and constant level throughout the experiment.

Affective Images

We selected 92 images from the International Affective Picture System (IAPS; Lang et al., 2008), 46 affective images of positive valence and high arousal (mean valence = 7.19 ± 0.55; mean arousal = 5.94 ± 0.75) and 46 affective images of negative valence and high arousal (mean valence = 2.53 ± 0.07; mean arousal = 6.20 ± 0.65). Each set of affective images was separated into two groups of 23 images, maintaining the same level of affective valence and arousal between them, according to the database score. The images were presented twice each, for a duration ranging from 3 to 5 s randomly. Therefore, each induction totaled approximately 3 min.

Madrid Card Sorting Task (MCST)

We used the MCST developed by Barceló (2003), which is a computer version of the Wisconsin Card Sorting Test (WCST) adapted to the study of concurrent Event-Related Potentials (ERPs). The MCST consists of 24 cards from the original set of WCST. Each card can be unequivocally matched with one of the four key cards according to one of the dimensions of the stimulus (color, shape or number). These 24 cards are used repeatedly in the 137 pseudo-randomized trials, in 18-series arrays. The classification rule is unknown to the subject and changes randomly from one series to the next. In each series array, the participant has two trials to find the new rule (shif1, shift2). Once the new rule is identified, a variable number of repetition trials must be completed (rep1, rep2, rep3, rep4 – last). Participants sat one meter away from the computer screen and stimuli were adjusted in size to reproduce the viewing conditions of the original experiment (4° horizontally and 3.5° vertically; Barceló, 2003). Presentation Neurobehavioral System® software was used to present the entire task. The task was applied identically to the original except for feedbacks: auditory feedbacks were replaced by visuals feedbacks ("+" stay feedback, "o" shift

feedback y "x" error feedback) in order not to interfere with the ongoing music. Participants responded using both hands and a four-button keypad.

Procedure and Measurements

Procedure

Once in the laboratory, participants signed the informed consent form and completed a personal information questionnaire to obtain demographic data and whether or not the participant had any formal musical studies. Once the EEG electrodes were installed, the participant sat in a comfortable chair in front of the screen where the task would be presented and listened to the instructions of the experiment. First, a short practice block of the task was presented to ensure that the participant had understood the instructions. The practice block was followed by two silent blocks (without music: MCST_S). Following the blocks without music, the blocks with music were presented interleaved and counterbalanced among participants (ABAB or BABA, whereby blocks are named according to the musical clip presented throughout it). Blocks with music started with a white noise, which had a variable duration between 50 and 60 s. During this stage, participants were instructed to clear their mind of thoughts, emotions, sensations, etc. Then, to induce the specific emotional state before the task-switching block itself the music and the presentation of IAPS images began. During this stage, that lasted approximately 3 min; participants were instructed to listen to the music while freely exploring the images, submerging and recognizing the sensations that appeared throughout their presentation. After the sequence of images was completed, participants pressed a button to start the actual task while the music continued in the background. After completing each task-switching block (12 min max), participants were asked to evaluate through the SAM self-report questionnaire how they felt during the execution of the task. At the end of the experiment, electrodes were removed and the micro-phenomenological interview was carried out. Finally, participants received a debriefing of the experiment.

EEG Measurements

EEG was recorded using the BioSemi® ActiveTwo amplifier system from 32 scalp sites (10–20 system) with Ag/AgCl electrodes mounted on an elastic cap (Klem et al., 1999; Stern et al., 2001). Eight additional electrodes were attached to left and right mastoids, the two outer canthi of both eyes (HEOG), and infraorbital and supraorbital regions of the eye (VEOG), while a Lead II configuration on the chest was used to measure the EKG. All signals were digitalized with a sampling rate of 1024 Hz and 24 bit A/D conversion. Off-line, EEG and EOG were processed as follow: first, the data was segmented in 3 s epochs (1 s before and 2 s after card presentation) then each epoch was re-referenced. Trials that presented blinks or between 300 ms before and 600 ms after the presentation of the stimulus were eliminated. To identify and remove ocular artifacts we used an Independent Component Analysis (ICA) (Jung et al., 2000a,b). On average, we removed 1.46 (±0.81) components out of a total of 32. The resulting signals were then bandpass-filtered between 0.1–25 Hz (phase shift-free Butterworth filter; 24 dB/octave slope). After baseline correction (−500 to 0 ms), the average ERP of the artifact-free trials was calculated for each subject at each recording site. A grand average across subjects was then obtained for the two conditions of interest: shift1 (change rule, novelty) and rep2 (maintain rule, repetition). We use rep2 instead of rep1 trials because when the participant has to use a second attempt to find the rule (shift2), that shift trial will most likely be correct and thus somewhat ambiguous regarding novelty vs repetition. Accordingly, the following repetition (rep2) is the first time that the participant unambiguously repeats the rule.

Micro-Phenomenological Interview

The micro-phenomenological interview is a method that allows the description of a singular experience in detail, and has its historical roots in the *explicitation interview* (Vermersch, 1999, 2004; Petitmengin, 2014). The particularity of this approach is that, through open questions, it focuses on actions or processes involved in the lived experience from an embodied perspective, in order to reveal implicit aspects of it. Then, the analytical approach works across two axes: the diachronic (the temporal development of experience) and synchronic (the configuration of the experiential space) dimensions of experience (Vermersch, 1994; Petitmengin, 2006). This approach has been used to gather descriptions of emotional and bodily experience (Valenzuela-Moguillansky, 2013; Depraz and Archives, 2017; Vásquez-Rosati, 2017), seizure anticipation in epilepsy patients and its correlates with neural activity (Petitmengin et al., 2007) and meditative experience (Ataria, 2015; Petitmengin, 2017; Przyrembel and Singer, 2018).

The micro-phenomenological interview involves two main steps: the interview itself and its analysis. In the first, the interviewer guides the interviewee to the evocation of a specific situation, and from this state, he helps the description of this particular experience. During this process, the interviewer must have special care not to induce any kind of specific response. To this end, the interviewer uses "content-empty" or "structure-driven" questioning (Petitmengin and Bitbol, 2009). During the description of the experience the diachronic dimension is explored with temporal questions as "How did you start?" "What happened after/before that?" To explore the synchronic dimension, the interviewer reorients the attention of the interviewee toward the structural characteristics of a particular moment of experience using questions as "How do you see that?," "How do you feel that?." The interview concludes when no new information is provided by the interviewee.

The analysis is an iterative process aimed at revealing invariants in the diachronic and synchronic structures of experience. To do so, first, an individual analysis is made for each interview. To obtain the individual diachronic structure, it is necessary to transcribe the interview, select useful information (actions and processes), organize information according to the temporal development of experience and find the temporal categories or phases of experience. The individual synchronic structure can be mapped out with the information from each phase of experience or with the initially selected information. In order to find experiential categories, iterative questioning and abstraction operations are

used. These results can be represented as dynamic lines or as a diagram (Valenzuela-Moguillansky and Vásquez-Rosati, 2019). Once this process is completed for all interviews, it is possible to build the generic structure of the experience. In the generic diachronic structure, individual structures are aligned according to external or internal referents (which depend on the objective of the study) to find temporal invariants. Then, the generic synchronic structure of each generic phase of experience is generated as the individual synchronic analysis (Valenzuela-Moguillansky and Vásquez-Rosati, 2019).

Results can be validated by their internal phenomenological consistency or external phenomenological consistency. In the first case, validation is obtained by the confirmation of the hypothetical structure gathered from the descriptions through the iterative questioning process. In the second case, validation is obtained by the detection of neural configurations according to phenomenological clusters (neurophenomenology) and/or inter-subjective confirmation (Petitmengin and Bitbol, 2009; Høffding and Martiny, 2015; Petitmengin et al., 2018).

In this study, 21 participants were interviewed after the experimental procedure. We were especially interested in the analysis of the synchronic dimension of the descriptions, in order to understand how the music and the subsequent emotional disposition might affect task performance. We then used this synchronic generic structure of experience to cluster and reanalyze the behavioral and electrophysiological data. As a result, we used two groups for the electrophysiological analyses (seven participants per group) and four groups for the behavioral analyses (nine, ten, two, and eight participants, respectively).

Behavioral and Electrophysiological Data Analysis

Mean reaction times (RTs) and ERP were obtained from efficiently completed MCST series only (Barceló, 2003). RT outlier's values were defined as those beyond two standard deviations above the average of the trial type and were removed from each participant's data. Mean RTs were subjected to a multivariate analysis of variance (MANOVA) design with Block Order (1–6) × Condition (S, A and B) × Trial (shift1, shift2, rep1, rep2, rep3, rep4, rep5, and last) as repeated-measure factors.

From the total of twenty-six subjects, six EEG recordings were excluded from the analysis due to noisy signals, leaving a total sample of twenty subjects for the analysis. Taking into account the lack of prior results regarding the effects of emotional induction on brain activity during task-switching, and the challenges inherent to the use of first-person experiential categories to guide the neurophysiological analysis, we chose to focus on well-known ERP components. Thus, area below the curve ERP amplitudes, for feedback-locked averages, was computed in the 490–540 ms window post-stimulus onset, for the P300 component. Additionally, we performed a non-parametric cluster-based permutation test between conditions (S, A, and B) which effectively controls for the family-wise error rate (FWER; Maris and Oostenveld, 2007). All *post hoc* tests of simple effects were performed using one-way ANOVA for multiple group comparisons or *t*-test for two group comparisons.

RESULTS

Emotional Disposition According to SAM Self-Report Questionnaire

Participants reported how they felt after each block of the experiment using a bi-dimensional scale of arousal and valence (Bradley and Lang, 1994). There were significant differences between the silent condition and the first exposition to A music (SA) in arousal (mean Silent: 4.36 (±2.5), mean SA: 7.2 (±2.2); $p = 0.047$) and valence (mean Silent: 5.8 (±1.4), mean SA: 2 (±0.7); $p = 0.0001$). In contrast, no differences were observed between the silent condition and the first exposition to B music (SB) in arousal (mean Silent: 4.36 (±2.5), mean SB: 5.3 (±3.3); $p = 0.87$) or valence (mean Silent: 5.8 (±1.4), mean SB: 6.5 (±4.1); $p = 0.73$). When comparing SA versus SB we found significant differences in valence (mean SA: 2 (±0.7), mean SB: 6.5 (±4.1) $p = 0.004$) but not in arousal (mean SA: 7.2 (±2.2), mean SB: 5.3 (±3.3); $p = 0.13$). These results show that participants felt similarly during the silent and music B conditions, while they felt less pleasure and more activation with music A, as compared to the silent condition (**Figure 1**). In the second emotional block, no significant differences were found between music conditions (arousal, mean AB: 5.4 (±0.9), mean BA: 6.5 (±3.7); $p = 0.89$; valence, mean AB: 6 (±1.6), mean BA: 4.3 (±2.2); $p = 0.06$).

Behavioral Results

We first compared RTs of all blocks of the task, independently of their emotional content. As expected, the RTs of all blocks were slower for shift trials and got faster throughout the repetition trials. We also found that RTs decreased as the experiment progressed, so that that the first block had slower RT in comparison to subsequent blocks ($F_{(5,149)} = 1.59$, $p = 0.012$; **Figure 2**). Detailed RT results for all trial types and conditions are available in the supplementary material. These differences were found for shift trials (shift1: $F_{(5,149)} = 2.7$, $p = 0.023$; shift2: $F_{(5,149)} = 3.49$, $p = 0.0051$) and for the first three repetition trials

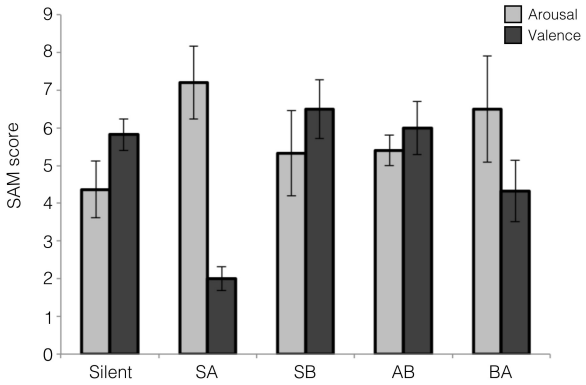

FIGURE 1 | Subjective evaluation of emotional experience with SAM scale. SA: A condition preceded by silent condition, SB: B condition anteceded by silent condition, AB: B condition anteceded by A condition, and BA: A condition anteceded by B condition. The error bars correspond to the standard error of the mean.

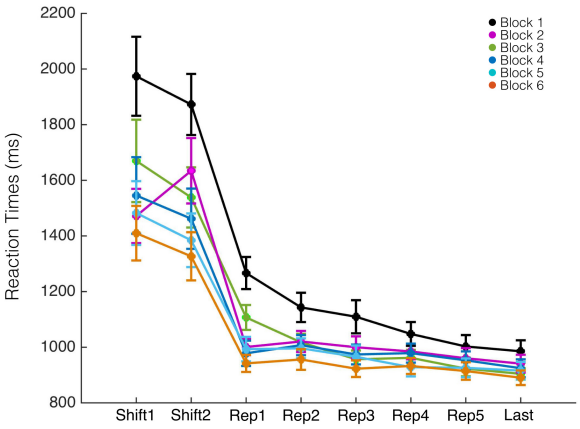

FIGURE 2 | Reaction times according to experimental block presentation. The first block (in black) showed longer RTs for switch trials (shift1, shift2) and repetition trials (rep1–rep3). Error bars show the standard error of the mean.

(rep1: $F_{(5,149)} = 7.75$, $p = 0.00001$; rep2: $F_{(5,149)} = 2.59$, $p = 0.028$ and rep3: $F_{(5,149)} = 2.52$, $p = 0.032$; rep4: $F_{(5,149)} = 1.74$, $p = 0.13$; rep5: $F_{(5,149)} = 0.92$, $p = 0.47$; Last: $F_{(5,149)} = 1.17$, $p = 0.33$). These results are consistent with a training effect, so that participants take more time to complete the first blocks (silent condition) than the last blocks.

Regarding the effect of the emotional disposition induced during the task on performance (**Figure 3A**), we found an interaction effect between block order and emotional condition ($F_{(3,145)} = 1.59$, $p = 0.039$): when emotional disposition A is induced in the first block (ABAB sequence), RTs were faster compared with blocks that started with emotional disposition B (BABA sequence; $F_{(1,24)} = 4.51$, $p = 0.042$). Interestingly, this effect is not only present in the first emotional block, but can also be observed in the subsequent blocks, independent of the musical condition (shift1: $F_{(7,96)} = 2.47$, $p = 0.022$; shift2: $F_{(7,96)} = 3.67$, $p = 0.0015$). These results suggest that the first emotional disposition determines performance in successive affective blocks as if the first emotional disposition that is induced prevails over time. Furthermore, when comparing the silent (S) condition with the first induced emotional condition, RTs were faster in emotional condition A when compared to those of condition S, but no differences were found between conditions B and S ($F_{(2,49)} = 3.89$, $p = 0.027$; S vs. A $p = 0.0096$; S vs. B $p = 0.36$).

Moreover, when condition A is anticipated by condition S (SA), shift1 has faster RTs than when A is anticipated by condition B (BA; $p = 0.0032$; **Figure 3B**). The opposite effect is observed

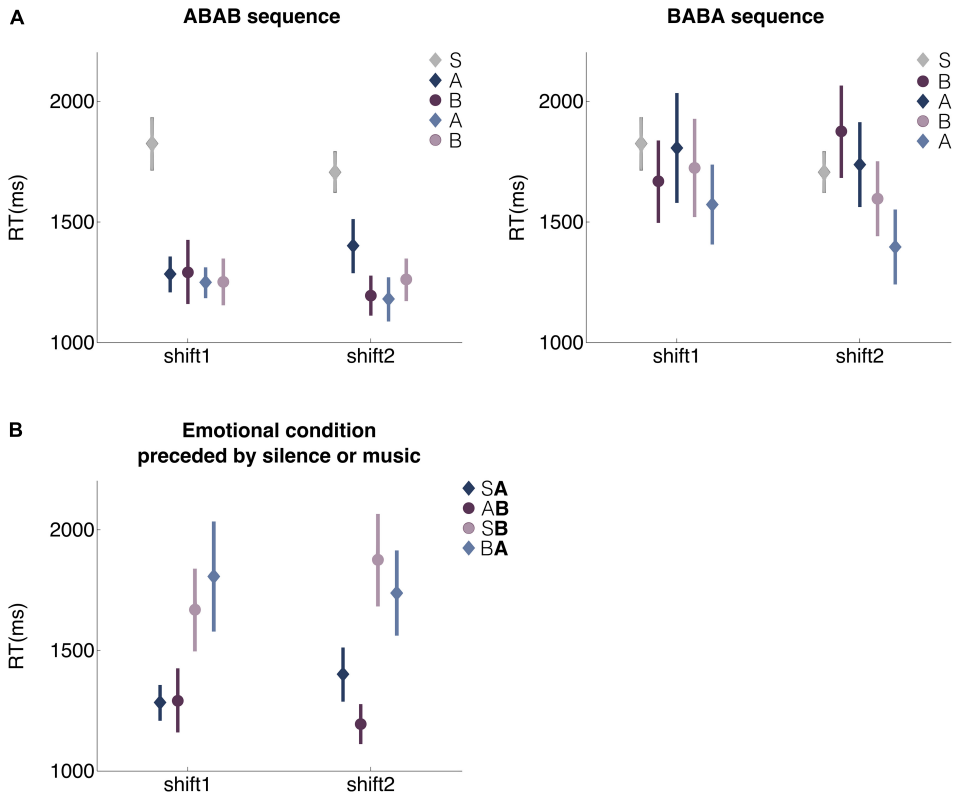

FIGURE 3 | Reaction times for emotional blocks. RTs of shift trials are shown. **(A)** ABAB and BABA sequence. S: second block of the silent condition. A: block with negative music, high activation, closed stance. B: block with positive music, high activation, open stance. Shift1 and shift2: first and second rule change attempt, respectively. **(B)** RT for emotional conditions. SA: A emotional condition preceded by silence condition. AB: B emotional condition preceded by A emotional condition. SB: B emotional condition preceded by silence condition. BA: A emotional condition preceded by B emotional condition. Error bars correspond to standard error of the mean.

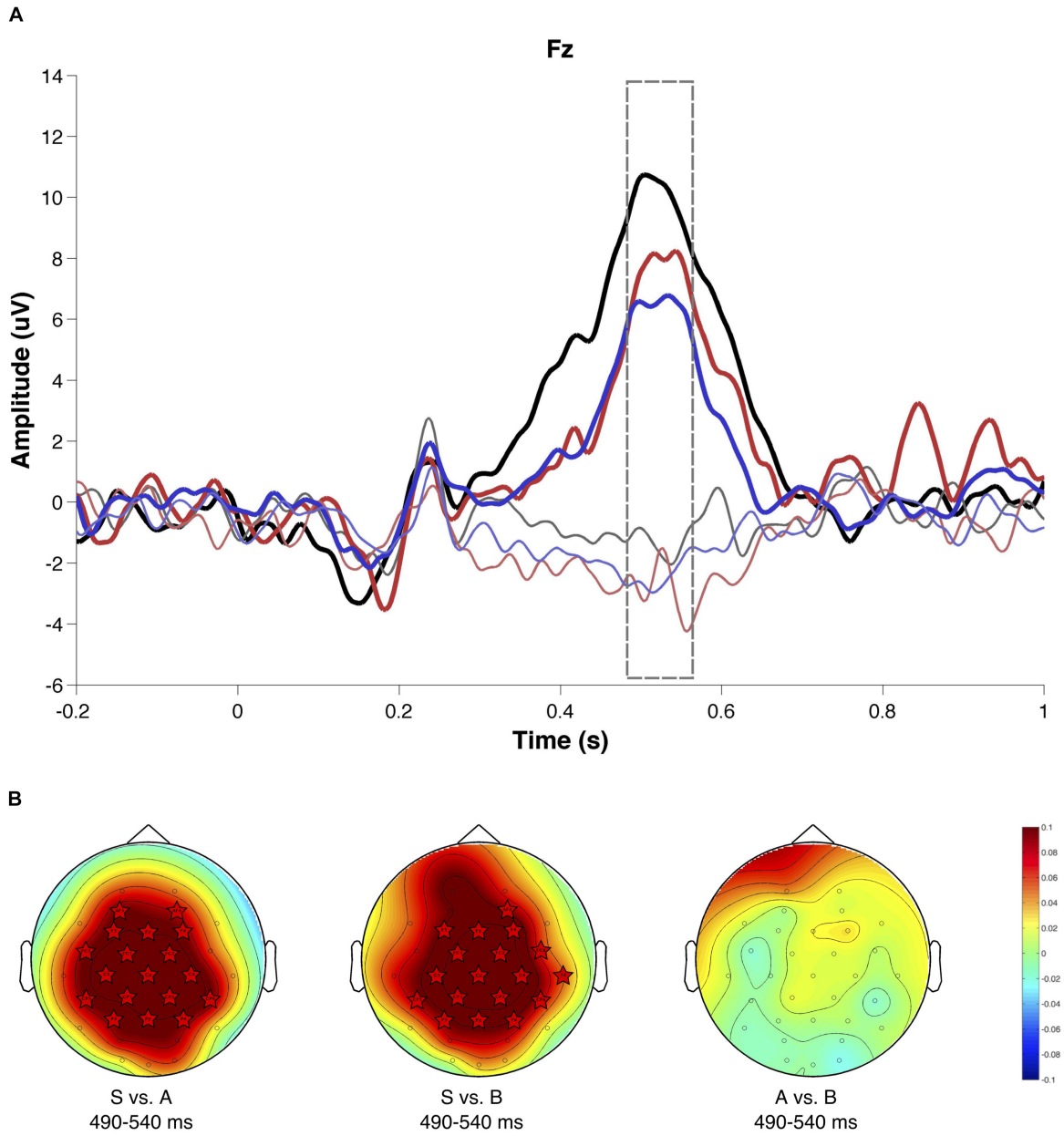

FIGURE 4 | Evoked brain activity associated with shift (Shift1) and repetition (Rep2, see text for details) trials. **(A)** The temporal window for ERP analysis of P300 area under the curve is shown dashed line. Silence condition is shown in black, A condition in red, and B in blue. The darker line shows the potential associated to shift feedback, while the soft line shows the potential to the second repetition of the correct rule. **(B)** Scalp amplitude difference maps for P300 (490–540 ms): stars show electrodes that present significant differences between conditions according to a non-parametric cluster-based permutation test.

for shift2 trials when condition B is anticipated by condition A (AB): RTs of block B were faster than those obtained when blocks B were anticipated by condition S (SB; $p = 0.0029$). These results show that affective valence A produces faster responses as compared to conditions S and B.

ERP Results

The grand-average ERP for feedback-locked events from shift1 and rep2 trials of the MCST series are shown in **Figure 4A**. The silent condition shows a P300 component (Fz electrode) evoked for shift1 trials (novelty) compared to repetition trials (rep2). P300 amplitude in Fz electrode is reduced in the emotional blocks, independent of their emotional valence (S vs. A $p = 0.0097$; S vs. B $p = 0.0025$; A vs. B $p = 0.58$). A cluster-based permutation test showed more sensors participating in the P300 significant cluster ($p < 0.02$), corresponding to the silent condition compared to the emotional condition, but not between emotional conditions (**Figure 4B**).

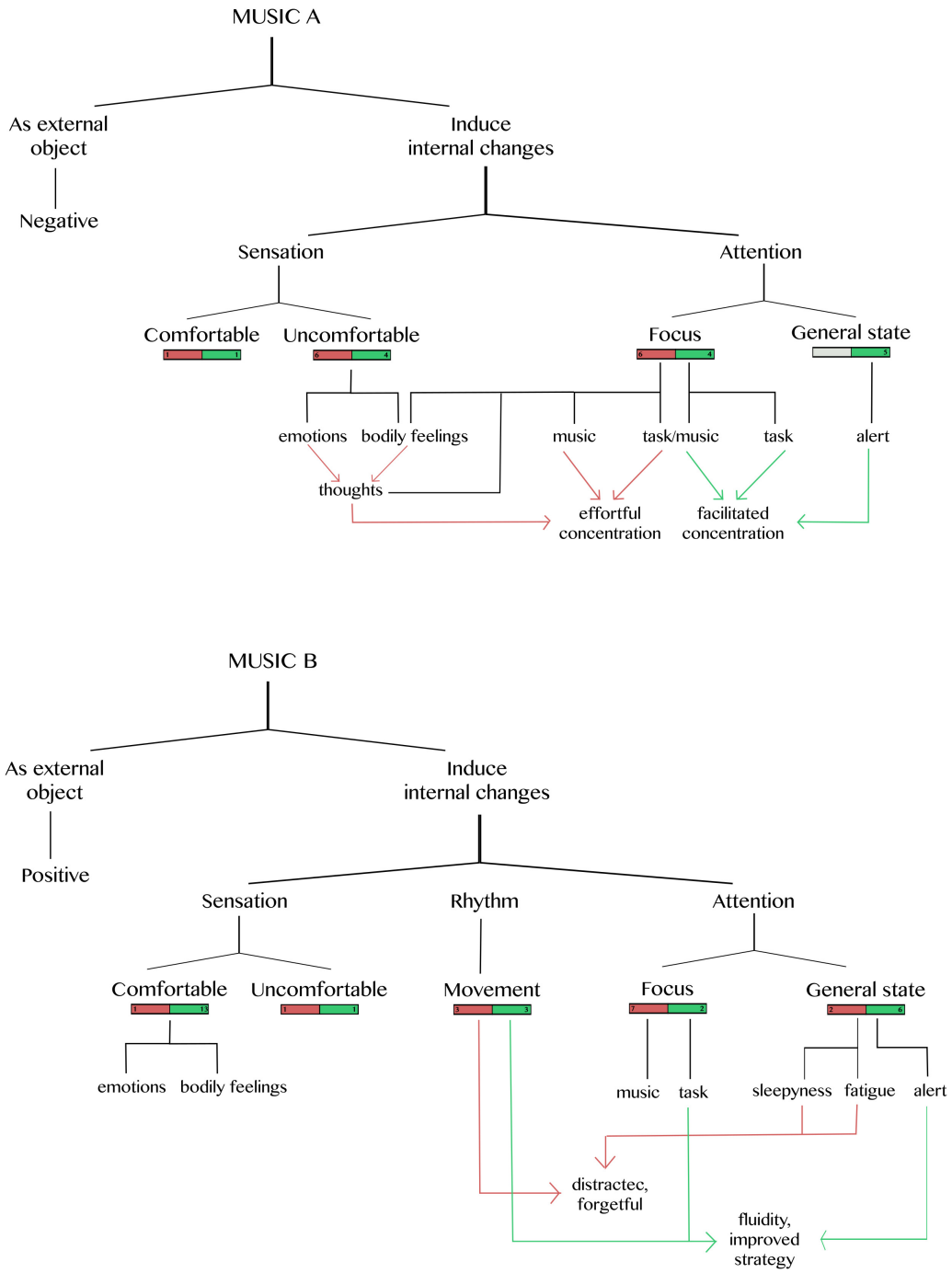

FIGURE 5 | Representative scheme of the generic structure of the experience of performing the task with background music. Experiential categories that emerged from the experience are shown: squares in red show the number of participants who felt hindered during task execution; green squares show the number of participants who felt facilitated during task execution. Arrows relate the experiential categories and the level of on task concentration of the participants. **Upper panel**: music A; **Lower panel**: music B.

Emotional Experience and Task Performance

Because we were interested in understanding how the emotional disposition induced by music might affect task performance, we considered only the synchronic information gathered through the description of the experience. The results of this analysis (**Figure 5**) describe in detail the experiential space generated by each music and which elements of it affect participants task performance, from their subjective point of view.

Music A

The first distinction that appears during the experience of doing the task with music is the difference between perceiving and feeling an emotion. In the first case, participants were capable of distinguishing emotional characteristics *in* the music (perceive), while in the second they were aware of internal changes produced *by* the music (feel). Most participants report both distinctions regarding the musical experience.

When music is perceived, a negative connotation was attributed to it. In particular, participants perceive the music as something unpleasant (n = 9), that gave the impression of something horrific (n = 5) and that stimulated aggression (n = 1).

On the other hand, when music-induced internal changes (n = 12). It was reflected specifically in sensations and in the attentional disposition. In the first case, participants described that they could feel comfortable (n = 2) and uncomfortable (n = 10) sensations. The comfortable sensations were activated and relaxed. The uncomfortable sensations were characterized by emotions such as fear (n = 2) and hate (n = 2) and/or by bodily sensations as shivering (n = 2), tension (n = 4), stress (n = 2) and anguish (n = 5). Four participants reported that they experienced this uncomfortable state as facilitating the execution of the task. This was congruent with their behavioral performance (see below). Uncomfortable bodily sensations in some cases (6) promoted negative thoughts (finish quickly, do not want to listen to music), which interfered with the execution of the task.

Regarding changes in attentional disposition, five participants described that music A led them to a general state of being alert; all of them felt that this state facilitated the execution of the task. Also, participants reported that they could have specific attentional focuses: on the task (n = 2, facilitated), between music and task (n = 6), their sensations (n = 2, not facilitated) and their thoughts (n = 2, not facilitated).

When participants felt that the emotional disposition generated by the music hindered their task performance, their reports agree in that they had to do an additional effort to concentrate on the task. To do so, they repeated internally the rule which they were selecting, while trying to disengage from the music by focusing their attention on the figures on the screen and reminding themselves to do it efficiently and quickly. Conversely, when the music facilitated the task, participants described that they were more concentrated, the rule was present to their mind or was easier to recall, and they were more easily disengaged from the music.

Music B

As in the description of the experience with music A, the distinction between perceiving and feeling an emotion also appears with music B. Participants perceive this music as pleasurable (n = 2), happy or joyful (n = 3) and ludic (n = 1). On the other hand, when music generates internal changes (n = 17), this was reflected in sensations (n = 12), attentional disposition (n = 6) and the appearance of an inner rhythm (n = 6).

Sensations were marked by a pleasant general feeling, with the appearance of emotions as happiness and joyfulness (n = 5) and bodily sensations of relaxation and tranquility (n = 9). Mostly, these pleasant sensations facilitated the execution of the task. Only two participants had uncomfortable sensations that in one case facilitated while in the other hindered the execution of the task.

On the attentional dimension, music B brought participants to an activated state of alert (n = 6). Participants related this state to the experience of doing the task more easily. Only one participant felt sleepy and another experienced fatigue, which in both cases were related to the experience of higher difficulty in performing the task.

The inner rhythm was described as recognizable, consonant and familiar melodies in music that generate an internal rhythm that accompanies the execution of the task, either by following the rhythm with their feet or by gently tapping the keypad. This could either facilitate task execution (n = 3) or act as a distracting element that made it more difficult (n = 3). When this emotional disposition facilitated the task (n = 6), participants described that the music awakened them and that they had less interior dialogue (n = 2); their attention was on the task, making it more fluent and improving the strategy used to resolve it (n = 4). On the contrary, when participants felt hampered doing the task (n = 9), they described that their attention was directed to the music (n = 7) and that they were more forgetful and distracted (n = 3). Participants realized that it was difficult for them to do the task because they made more mistakes (n = 3), forgot the rule (n = 1), or were not attentive to the feedback that indicated rule changes (n = 2).

Behavioral and Electrophysiological Results According to Micro-Phenomenological Invariants

Behavioral Results

We classified participants according to their first-person experience in four groups: facilitated by music A (fA), facilitated by music B (fB), hindered by music A (hA) and hindered by music B (hB). As **Figure 6** shows, significant differences were found between fB and hB. Shift trials show faster reaction times where participants felt facilitated by music B (shift1: $F_{(1,34)} = 11.43$, $p = 0.0018$; shift2: $F_{(1,34)} = 6.93$, $p = 0.01265$). This effect was not significant between fA and hA conditions (shift1: $F_{(1,33)} = 2.27$, $p = 0.14$; shift2: $F_{(1,33)} = 1.23$, $p = 0.28$).

Electrophysiological Results

Because of the need for a large number of trials for calculating the ERPs, we classified participants in two groups: facilitated or hindered by music, independently of the music's *a priori* emotional intrinsic characteristics. We found that when music hindered task execution, P300 amplitude was attenuated as compared to the facilitated condition ($p = 0.00041$; **Figure 7A**). The non-parametric cluster-based permutation test (**Figure 7B**) confirmed this significant difference ($p < 0.02$), and the spatial distribution of the activity over frontal and left electrodes.

DISCUSSION

In this study, we have tackled the question of whether emotional dispositions can affect cognitive flexibility in a commonly used card sorting task. In addition to the traditional approach based on behavioral results and self-report questionnaires,

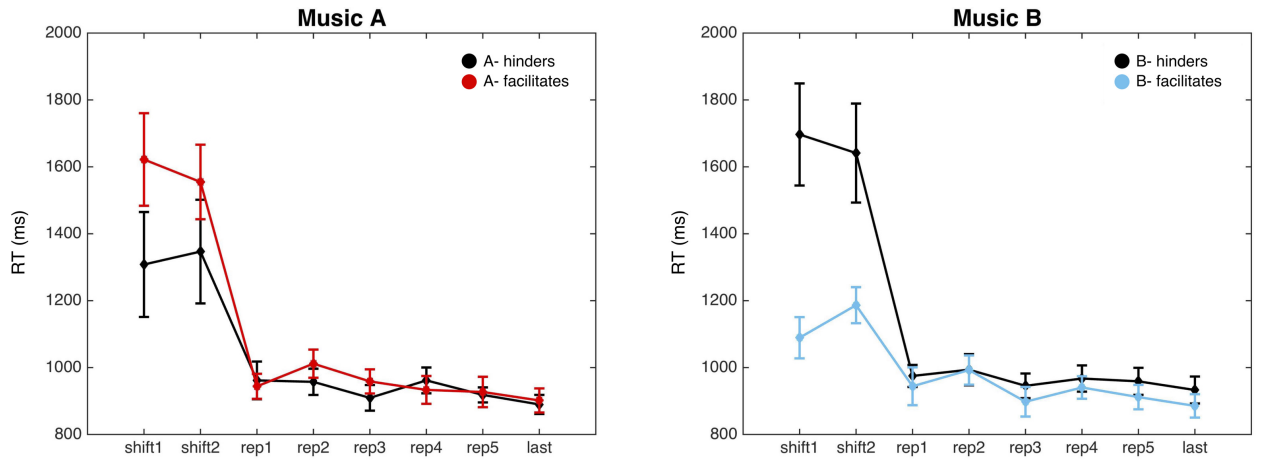

FIGURE 6 | RTs analyzed based on subjective experience. The **left panel** shows the RTs when music A facilitates or hinders the execution of the task. The **right panel** shows the RTs when music B facilitates or hinders the execution of the task. The *X*-axis shows the sequence of trial types and the *Y*-axis shows the time in milliseconds that it takes for the participants to respond. Error bars present the standard error of the mean.

we have included in-depth first-person descriptions obtained through a micro-phenomenological interview and used the ensuing categories to orient the analysis of the behavioral and electrophysiological data. Among other results that we discuss below, we consistently found that the intrinsic (expected) valence of the emotional stimuli is not determinant in its effect on the person or their task performance: participants could either feel facilitated or hampered by the emotional disposition generated by the same musical stimulus, depending on how they experienced the situation, affecting both their task performance and the underlying neuronal correlates.

THIRD-PERSON ANALYSIS

Behavioral Results

Regarding our behavioral results, we found a training effect during the experimental session that was evident despite the existence of a formal training period before the task itself. This effect has not been shown in previous research (Barceló, 2003). We also replicated previous findings, which show faster reaction times for repetition trials compared with switch trials (see for example Barceló, 2003).

We did find an emotional effect on the task: music A was associated with overall faster RTs in comparison to B and S conditions. Interestingly, this effect persists in time so that the RTs of the sequence that started with music A (ABAB) were faster when compared to a sequence starting with music B (BABA). An important conclusion from these results is that emotional states induced in experimental conditions can have long-lasting effects, an issue that should be taken into account when designing experiments that assess the effects of emotional induction (in contrast to emotion recognition) on other cognitive or behavioral processes.

Participants' reported comparable ratings in both the arousal and valence dimensions for the silent condition and music B (medium-high values for valence and arousal). They differed however in terms of the feelings generated by music A, which were characterized by high arousal and low valence. In this sense, these results suggest that the emotional disposition promoted by music A would be facilitating for cognitive flexibility. It is worth noting that it has been proposed that negative emotions allow a better focus on the task (Andrews and Thomson, 2009). Indeed, negative emotions and their association with problematic environments have sometimes been shown to facilitate precautions and controlled responses (e.g., Bless et al., 1996 in Andrews and Thomson, 2009).

In line with our results, Rowe et al. (2007) hypothesized that positive emotional states relax inhibitory control, resulting in a widened scope of attention. However, because music B promoted a state which was similar to the silence condition, our understanding of the effects of positive emotional states on cognitive flexibility remains limited. Previous studies have shown that positive affect induced by emotional images enhanced task-switching abilities on a set-shifting task, whereas negative affect did not (Dreisbach and Goschke, 2004). This improved switching ability came at the cost of increased distractibility, which is believed to result from poorer task maintenance in working memory during positive affect (Dreisbach, 2006).

Finally, in relation to the duration of the emotional effect, some authors have argued that in order to induce an emotional mood, the stimulus must have a minimum duration of approximately 7 min, and that the effect should last from 5 to 40 min (Västfjäll, 2002) to 3 h or more, in some cases (Oatley et al., 2006). In agreement with this, we induced an emotional state in our participants that was highly dependent on the first emotional condition, and that apparently interfered with posterior mood induction. As stated above, this information is relevant for future research as the duration of the emotional stimuli and the emotional induction could be decisive in terms of experimental design and, in particular, in terms of the number of sessions of the study.

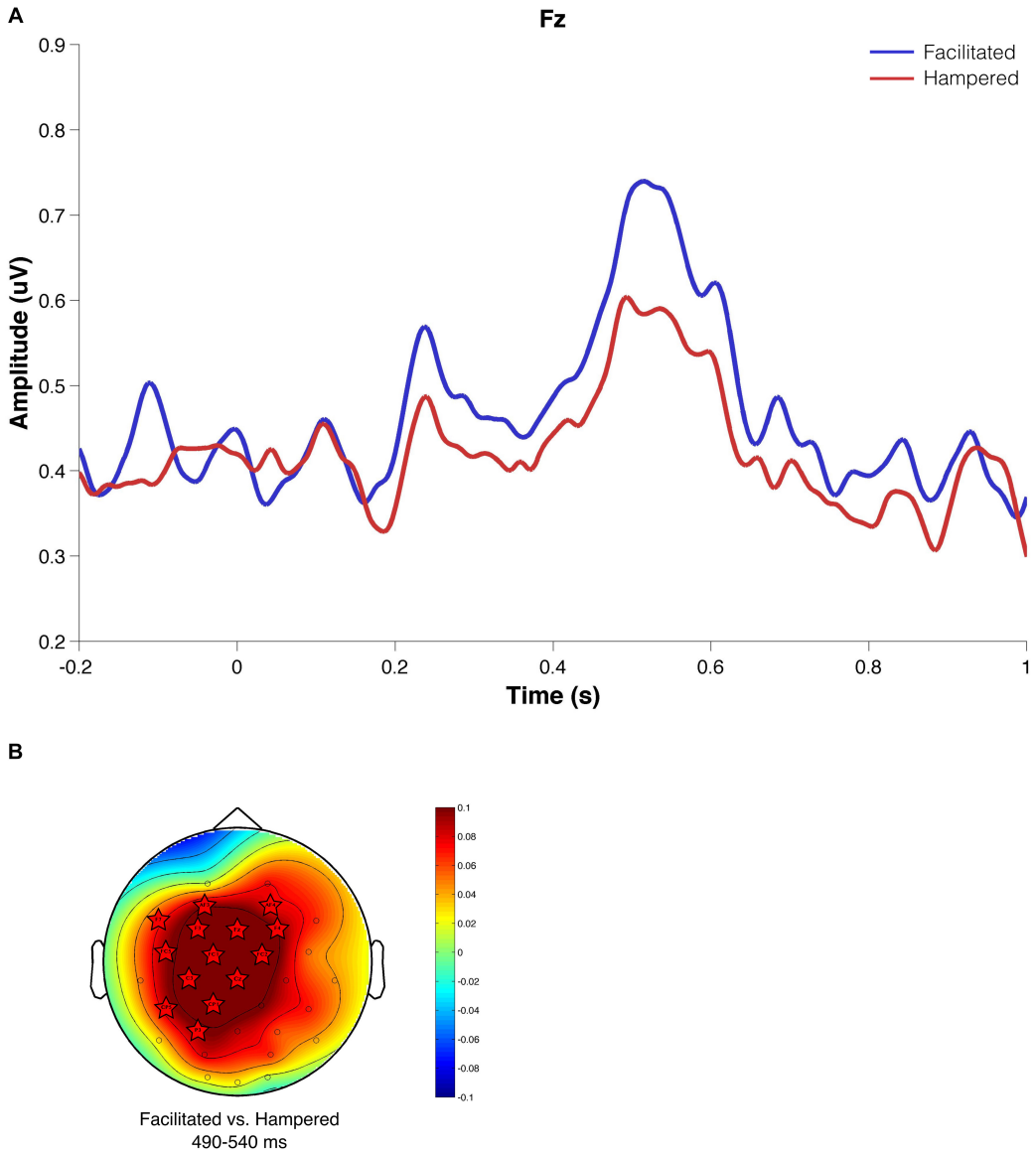

FIGURE 7 | Evoked brain activity based on subjective experience. **(A)** Evoked activity to the first shift signal. The facilitated condition is shown in blue and the hampered condition in red. **(B)** Scalp amplitude difference map for the P300 time window (490–540 ms). Stars show electrodes that present significant differences between conditions according to a non-parametric cluster-based permutation test.

Electrophysiological Results

As expected, the shift signal evoked a P300 between 490–540 ms in all conditions. An increase in P300 amplitude is associated with the change of a mental set (Barceló, 2003; Periáñez and Barceló, 2009). In our study, P300 was larger in the silence condition compared to both emotional conditions, but no significant differences were found between emotional conditions. As in Barceló's (2003) study, the P300 presented a frontal distribution for rule change trials, which then disappeared for rule repetition trials. Several authors have interpreted the P300 as a neural signature of the cognitive control mechanisms required for mental set reconfiguration during perspective changes (Kieffaber and Hetrick, 2005; Nicholson et al., 2005; Kopp et al., 2006). In the context-update hypothesis (Donchin, 1981; Donchin and Coles, 1988 in San Martín, 2012) the P300 is an index of brain activity underlying the revision of a mental model of the current task. This revision is induced by a stimulus that updates the model, being the amplitude of the P300 proportional to the amount of cognitive resources used during the revision of the model. Since music produces a decrease in the amplitude of the P300, it is possible that cognitive resources are used partly by the task and partly by the musical stimulus, regardless of its intrinsic emotional characteristic. Another way to explain these results is that music produces an increase in

workload, thus producing a decrease in the P300 component, as reported by Hasegawa et al. (2004).

COMBINED FIRST AND THIRD-PERSON RESULTS

Self-report questionnaires are commonly used to assess participants' subjective experience in experimental paradigms. However, and despite the wealth of information that they can provide under well-controlled conditions, they remain limited when it comes to obtaining a more nuanced view of the experience, one that is less constrained by the experimenters' expectations or previous knowledge (Colombetti and Thompson, 2008; Colombetti, 2013). Consequently, the interpretation of the subjective experience is difficult and does not allow to explain, comprehend and interpret the observed behavioral and physiological information that accompanies the experience.

To address this limitation, we used the micro-phenomenological interview to guide participants in their description of this particular experimental situation. As described in the methods section, this approach has been used in a wide range of contexts that require a more in-depth description of a specific experience, while allowing for an open exploration of the participant's phenomenology (Petitmengin et al., 2007; Valenzuela-Moguillansky, 2013; Ataria, 2015; Depraz and Archives, 2017; Petitmengin, 2017; Vásquez-Rosati, 2017; Przyrembel and Singer, 2018). The analysis of the interview revealed that the different musical stimuli could be perceived as an external object or could induce internal changes in participants. This result supports the proposal of Gabrielsson (2002) in which "perceive" or "feel" an emotion are different processes in response to music. In the current study, we found a positive correspondence between presented and perceived emotions. Music A was perceived as carrying a negative emotion and music B as carrying a positive one. However, when the music was felt, we found positive and negative relationships for both emotional stimuli: both music A and music B produced comfortable and uncomfortable sensations.

Interestingly, independently of the emotional valence of the stimulus, participants could feel hindered or facilitated by the music. This information allowed us to clustered participants according to their experience and then re-analyze behavioral and electrophysiological data. We found significant differences between facilitated and hindered conditions only when music B was played. When the music hindered performance according to the participant's description, it promoted distractibility and RTs were slower than when the music accompanied the execution of the task by promoting concentration. According to the descriptions of subjective experience, the attentional focus of participants while doing the task with music B could be either on the music or on the task, something that is coincident with the behavioral findings. On the contrary, differences in music A were not so clear. Here, the generic structure of experience showed three possible attentional focuses (music, task/music, and task), which could explain the similitude in the RTs of both conditions.

Brain activity was also grouped according to phenomenological information. Given the low number of trials, however, it was necessary to group all those participants who felt facilitated or hindered in the task, independently of the emotional valence of the musical stimulus. These results showed that when the music hindered the task, the evoked potential P300 decreased its amplitude. As previously reported, the amplitude of P300 is associated with cognitive resources used in task updating (Donchin, 1981; Donchin and Coles, 1988) and workload (Hasegawa et al., 2004). Therefore, the decreased amplitude of the P300 may reflect fewer cognitive resources, associated with the feeling of being hampered in performing the task with music. This may also be accompanied by an increase in workload, given the participants' constant struggle to bring attention back to the task.

In general, our results reveal individual differences in the relationship between emotional states and cognitive functions. Reaction times and cortical activity results show that not all individuals reacted equally to a stimulus or emotional state when executing a task. Emotional experience is unique to each individual, and the way music alters our mood is in most cases predictable. However, this does not hold when are faced with changing demands in our environment. Faced with situations of change of perspective, the emotional expressiveness of music is not necessarily in line with the emotional disposition required to accomplish a goal and can, therefore, affect it substantially.

The first- and third-person information dialogue in the study of emotional states is a relatively new field that needs to be explored with subtlety. As shown in this study, each emotional experience is unique and so are its electrophysiological correlates. Both types of information require methodologies that allow us to reveal the underlying generic structure, and thus be able to achieve a higher explanatory level of the relationship between psychological and physiological processes.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics Committee of the School of Psychology, from the Pontificia Universidad Católica de Chile. The protocol was reviewed and approved by this Committee. All participants read and signed an informed consent form prior to their participation, in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AV-R contributed to the experimental design, performed the experiments and the interviews, analyzed the behavioral and interview data, contributed to the interpretation of results, and wrote and edited the manuscript. RM-S performed the EEG analysis, and contributed to the writing of the manuscript and critical review of the results. VL contributed to the experimental design, and the critical review and interpretation of the results. DC contributed to the experimental design, and interpretation of the results, and wrote, edited, and critically reviewed the manuscript.

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Regulation During the Second Year: Executive Function and Emotion Regulation Links to Joint Attention, Temperament, and Social Vulnerability in a Latin American Sample

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Although a growing body of work has established developing regulatory abilities during the second year of life, more work is needed to better understand factors that influence this emerging control. The purpose of the present study was to examine regulation capacities in executive functions (i.e., EF or cognitive control) and emotion regulation (i.e., ER or control focused on modulating negative and sustaining positive emotions) in a Latin American sample, with a focus on how joint attention, social vulnerability, and temperament contribute to performance. Sixty Latin American dyads of mothers and children aged 18 to 24 months completed several EF tasks, a Still-Face Paradigm (SFP) to examine ER (Weinberg et al., 2008), and the Early Social Communication Scale to measure joint attention (Mundy et al., 2003). Parents completed the Early Childhood Behavior Questionnaire Very Short Form to measure temperament (ECBQ-VS, Putnam et al., 2010) and the Social Economic Level Scale (SES) from INDEC (2000). Results revealed the typical responses expected for toddlers of this age in these EF tasks and in the SFP. Also, we found associations between EF and ER and between non-verbal communication related to monitoring infants' attention to objects (i.e., responding to joint attention) and initiation of pointing (e.g., pointing and showing of an object while the child alternates his gaze to an adult) with EF. Regarding social factors, family differences and type of housing contribute to regulation. For temperament, effortful control was associated with both regulatory capacities. Finally, only age predicted EF. These results suggest that many patterns regarding the development of these abilities are duplicated in the first months of life in a Latin American sample while further highlighting the importance of considering how the environment and the individual characteristics of infants may associate to these regulatory abilities, which is particularly relevant to developing public policies to promote their optimal development.

Keywords: executive functions, emotion regulation, joint attention, social vulnerability, temperament, still-face paradigm

INTRODUCTION

EF and ER in the Second Year of Life

Executive functions (i.e., EF or cognitive control) and emotion regulation (i.e., ER or management of emotions) play a fundamental role during child development, contributing to and predicting the development of cognitive abilities, academic achievements, and developmental disorders (Cunningham and Zelazo, 2016; Costa et al., 2017).

The importance of these abilities likely stems from the influence they have on the regulatory capacities. Work during preschool and school age demonstrates that a host of social and individual difference factors relate to these abilities (e.g., Diamond, 2006; Zelazo and Carlson, 2012; Frick et al., 2017). The current research is often limited to primarily Caucasian samples of mid to high socioeconomic status from North America and Europe. Recent work has called for studies of regulation earlier in life to better understand their emergence and the early factors that influence the development of these foundational skills (e.g., Miller and Marcovitch, 2015; Devine et al., 2019).

Although the toddler years have been identified by some as a particularly understudied age in cognitive development (e.g., Hughes and Ensor, 2007). Several frameworks suggest that the development of cognitive control begins early. For example, theoretical EF frameworks suggest the psychological processes involved in the conscious control of thought and action emerge within the second year alongside the emergence of symbols and language. That may aid children in forming task-relevant representations to guide behavior (e.g., Zelazo, 2004; Marcovitch and Zelazo, 2009).

Despite the fact that several researchers have suggested that EF eventually develops to include component abilities such as cognitive flexibility (e.g., changing focus and adapting it to changes in the environment while ignoring the distractors), working memory (e.g., remembering and following directions), and inhibitory control (e.g., stopping impulsive behavior in pursuit of one based on reflection) (Diamond, 2013), other frameworks have highlighted the unity and diversity of EF. These theories suggest that all EF tasks may require something common (i.e., termed a common EF) related to the ability to form and maintain task-relevant information used to guide behavior (Miyake and Friedman, 2012). The proposal of a unitary EF actually aligns well with developmental models showing that unitary models may best explain EF at young ages (Wiebe et al., 2010). This is possibly because developing symbolic abilities can be used to form and maintain information important to a goal. It may underlie an emerging unitary ability in EF (Miller and Marcovitch, 2015).

Empirically, EFs are notoriously difficult to evaluate before the first 3 years. It is necessary to manipulate and sustain meta representations that guide behavior toward the established objective (Zelazo, 2004; Jacques and Marcovitch, 2010). Representational skills in early childhood are still developing. Perhaps the mostly widely measured task thought to tap into early EF is the A-not-B task, in which children have demonstrated the ability to overcome a prepotent motor response, to search for a hidden object at a previously rewarded location and execute search at the correct location over increasing delays with age (e.g., Diamond, 2006, 2013).

However, only recently has research focused on studying early EF with a battery of tasks capable of examining relations between tasks and individual differences. The few studies that have used this approach with infants from 1 to 2 years have shown little consistency and stability in EF skills related to the consolidated ability to guide behaviors through meta representations (Wiebe et al., 2010; Gandolfi et al., 2014; Johansson et al., 2015; Miller and Marcovitch, 2015; Devine et al., 2019). Thus, this has led some researchers to hypothesize that EF may be emerging during the second year of life with component abilities that are not well-integrated yet (Garon et al., 2008; Devine et al., 2019) or not fully developed. This may be supported by emerging representational abilities (e.g., Miller and Marcovitch, 2015).

Unlike EF, ER (i.e., control related specifically to emotion and involves monitoring, evaluating, and modifying emotional reactions to accomplish goals; Thompson, 1994) has a history of early measurement. For example, early measures have often been studied in infants through the Still-Face Paradigm (SFP), which consists of a mother–infant interaction at three phases: (I) mother–infant free interaction in which the dyads are free to engage in a typical interaction with each other, (II) Still-Face period in which the mother stops interacting with the infant and observes them with a "poker" face, and (III) a second mother–infant interaction, in which the mother goes back to free interaction.

Each phase lasts between 90 and 120 s (Stack and Muir, 1990; Mesman et al., 2009) with the critical response focusing on how children regulate emotion, when mothers stop interacting with children in phase II. The typical responses in phase II consist of a decrease in positive affect of the infant (e.g., smiles) and an increase in negative affect (e.g., crying) and aversion to gaze (e.g., avoid looking at the mother) compared to phase I. Further, a carryover effect is also typically observed into phase III, where the type of response produced in the Still-Face phase is continued in phase III, even when the mother re-engages in interaction (Tronick et al., 2005; Gunning et al., 2013).

These trends have been widely observed across cultures (e.g., Ecuador and in ethnic groups belonging to China and Africa; Segal et al., 1995; Kisilevsky et al., 1998; Handal et al., 2017). Examining the critical response to mother's lack of attention in phase II is indicative of ER, and some have suggested that ER is especially important to study, because learning to modulate negative emotions and sustain positive ones is critical for cognitive development (Kogan and Carter, 1996).

Some researchers have noted an overlap between EF and ER. On the one hand, ER occurs in the service of solving problems and definitions of ER also encompass cognitive and behavioral processes (Carlson and Wang, 2007). On the other hand, to solve classic EF tasks, children need to regulate their emotional reactivity (Miyake et al., 2000; Zelazo and Cunningham, 2007). This has led some researchers to integrate the two constructs by suggesting that domain-general EF may be recruited in the regulation of emotion (Zelazo and Cunningham, 2007).

A distinction has been made between "cool" EF related to abstract problem solving and "hot" EF related to problem solving involving motivation and emotional influences (Zelazo and Carlson, 2012). As EF is typically required in conscious problem solving, when the problem to be solved is ER, EF and ER may be synonymous and more in line with hot EF (e.g., Zelazo and Cunningham, 2007). There is some evidence to support this approach (see Zelazo and Cunningham, 2007) and work with preschoolers demonstrates that individual differences in EF are predictive of ER (Kieras et al., 2005).

However, work examining these relations before 3 years of age are scarce given limitations in studying early EF. Some studies showed that ER and emotion reactivity at 12 and 24 months predicted EFs at 48 and 60 months (Feldman, 2009; Ursache et al., 2013). However, no work to date examines how ER with the SFP may relate to early measures of EF in the second year.

Joint Attention Links to EF and ER in the Second Year of Life

As previously stated, a number of researchers suggest that representational abilities (e.g., the use of symbols) may be a cornerstone of consciously controlled behavior (e.g., Zelazo, 2004; Zelazo and Cunningham, 2007; Miller and Marcovitch, 2015). Measures of non-verbal communication are often studied during the first years of life when linguistic ability is limited. A significant development during the first months are often studied *via* measures of joint attention; defined as the ability to establish a triad relationship between the infant, an adult, and an object (Bruner, 1975; Tomasello and Farrar, 1986). This type of non-verbal communication is considered fundamental in forming the necessary basis for the subsequent development of linguistic capacity (Tomasello and Farrar, 1986).

Mundy and Gomes (1998) distinguished two types of joint attention: responding to joint attention (RJA, e.g., monitoring the attention of others to objects) and initiating joint attention (IJA, e.g., directing the adult's attention by pointing or showing). Both dissociate during development, with RJA as a more basic skill linked to a primitive attention system emerging at 6 months, and IJA involving higher levels of attention control emerging at 9 months.

Links between joint attention and ER using the SFP have been established in the first year of life (Weinberg et al., 2008; Mesman et al., 2009). For instance, researchers found that even the number of behaviors related to IJA and coordinated joint increased during the second phase; the SF effect (i.e., decrease in positive affect when mothers disengage) was only associated with RJA measure with the Early Social Communication Scales (Rochat and Striano, 1999; Yazbek and D'Entremont, 2006).

Many researchers (Tomasello and Farrar, 1986; Mundy and Gomes, 1998; Yazbek and D'Entremont, 2006) suggest that RJA is a type of joint attention that does not require volitional attention management system, driven by an understanding of an adult's intention toward an outside object (e.g., IJA and coordinated joint attention require children's consciously attempt to engage another). Thus, it is possible that ER as measured by the SFP is related to children's more passive response to a lack of joint attention bids in their environment, rather than anything more intentional during phase II. However, this work is limited to 1-year-old infants and more research is needed to extend the joint attention–EF relationship in the second year.

Regarding relation between joint attention and EF, there is more evidence that EF and vocabulary develop in parallel, influencing each other dynamically (Fuhs and Day, 2011; Bohlmann et al., 2015; Slot and Von Suchodoletz, 2018). However, the time, strength, and direction of these associations are still under debate and less studied in the early years of life. There is actually a lack of focus on the link between EF and joint attention in early development, likely due to the fact that the study of early EF itself is primarily based on samples of preschool children.

There are few studies that investigate their development in the first years of life (Gandolfi et al., 2014; Mulder et al., 2014; Miller and Marcovitch, 2015; Rodríguez et al., 2017). A few studies that do examine EF and joint attention in the second year find that they do relate in this early period (e.g., joint attention abilities show both concurrent and longitudinal links to EF from 14 to 24 months, Miller and Marcovitch, 2015).

This has been suggested to be linked to the absence and gradual emergence of unified EF, marking the first years as a transition period, during which a unified executive functioning (Wiebe et al., 2010; Miller and Marcovitch, 2015) involves sustained attention and the ability to inhibit external and internal stimuli. This must be developed first—and may be supported by representational abilities in communication—before the formation of more advanced EF abilities in cognitive flexibility and working memory (Marcovitch and Zelazo, 2009; Diamond, 2013). More work examining joint attention links to EF and ER during the second year will be helpful to substantiate theories stressing the importance of early representational abilities to regulatory ones.

Temperament Links to EF and ER

Another factor thought to influence the development of regulatory abilities are the individual differences children demonstrate in reactivity and self-regulation. They have a constitutional origin due to their genetic endowment, known as temperament (Carranza and González, 2003; Rothbart, 2007). Although the idea of stable individual differences linked to a genetic endowment is a distinguishing characteristic of temperament, it is also thought to be regulated by the environment and learning (Rothbart et al., 2000).

The temperamental styles described by Rothbart (1981) are divided into surgency (positive affect, level of activity, impulsiveness, risk taking), negative affect (fear, anger, sadness, irritability/discomfort), and effortful control (change of attention and focus, perceptual sensitivity, inhibitory control, and activation). Given that work in temperament focused on individual differences present early in development, there is a large body of work focused on infancy and the toddler years (e.g., Rothbart, 1981). However, it is also important to note that effortful control is proposed as later emerging abilities, driven by development in the executive attention network (Rothbart et al., 2000).

Numerous investigations have demonstrated that temperament impacts other measures of regulation during the first years of life (Rothbart and Ahadi, 1994; Rothbart et al., 1994; Lemelin et al., 2006). For example, temperament relates to EF (at preshcool years) and ER (at first year of life), as effortful control promotes the capacity of working memory and inhibitory control, and negative affect decreases it Freund (2018) and Lin et al. (2019). However, results examining temperament links are contradictory (e.g., effortful control or surgency does not always positively influence cognitive performance, Zhou et al., 2012; Yoo and Reeb-Sutherland, 2013; Frick et al., 2017; Lin et al., 2019) and are thus in need of further research.

Social Vulnerability Links to EF and ER

Finally, an important social factor in the development of regulation is social vulnerability, defined as a multidimensional variable that includes not only the economic income, but also the type of housing, caregiver's educational level, overcrowding, access to services, stimulation to education at home, and the presence of basic needs. Individuals faced with social vulnerability in Latin America often experience overcrowding, precarious housing, informal work, and incomplete secondary school.

There are a number of studies that have demonstrated an impact of socioeconomic status on EF and ER (Hackman et al., 2015; Lawson et al., 2018). For example, children with unsatisfied basic needs expressed more perseverations in the A-not-B and Tower of London compared to children with satisfied basic needs (Lipina et al., 2004). Also, maternal education and income predicted working memory and planning between age 2 and 3 (Hackman et al., 2015).

However, links between social vulnerability and EF have been only examined in 2-year-old children or older (Hackman et al., 2015; Lawson et al., 2018). Further, a number of these studies are primarily conducted in samples from North America and Europe. Finally, there are few studies examining the impact of SES on the Still-Face task as a measure of ER (Mesman et al., 2009).

The Present Study

In the present study, we examine regulatory abilities in EF and ER in a Latin American sample of mother–infant dyads during the second year, to address two research objectives: (1) How does EF and ER (as measured by the SFP) develop and relate during the second year? (2) How does joint attention, temperament, and social vulnerability relate to EF and ER?

There are also age and gender differences in cognitive development; we also examine these effects. Specifically, female infants tend to have better performance in different EF and ER tasks (Espy et al., 1999; Lipina et al., 2004). Further, performance across these tasks also typically improves across a short age range. For example, Wiebe et al. (2010) showed evidence that the number of perseverations in the A-not-B and Three Boxes task decreased from 15 and 20 months.

This work is novel in several regards. First, more work is needed to better understand the development of regulatory abilities during the second year, especially given the importance of early intervention to prevent difficulties in these areas (Campbell and Ramey, 1994; Burchinal et al., 2000; Arán-Filippeti and Richaud de Minzi, 2012). Second, a focus on a Latin American sample will corroborate and extend work examining EF and ER links to joint attention, temperament, and social vulnerability to a new sample.

Given that the number of poor people in Latin America reached 186 million in 2016 (30.7% of the population, CEPAL, 2017), it is important to understand how EF and ER develop not only in this novel context but also with regard to socioeconomic status as well. Although this study extends examination of EF and ER to a novel sample, we expected that relations between joint attention, temperament, and SES will align with past research in other populations and older samples.

More specifically, in line with representational theories of EF (Zelazo, 2004), joint attention—especially self-initiated gestures and following others' attention—should encourage stronger representations used to control thoughts, behavior, and emotions (Zelazo, 2004). Finally, in line with past work, toddlers from households with satisfied basic needs, lower levels of negative affect, and greater effortful control and surgency would have better performance across regulatory tasks in EF and ER.

MATERIALS AND METHODS

Participants

Participants consisted of 60 mother–infant dyads with children from 18 to 24 months, recruited from public and private maternity gardens and homes in the Autonomous City and Province of Buenos Aires, Argentina, sampled using non-probabilistic, intentional, and snowball methods. In the evaluated sample, 38 mothers were Argentine, 15 from Paraguay, 4 from Bolivia, 2 from Peru, and 1 from Ecuador. All the infants evaluated were Argentines.

To find infants with typical development, we screened the clinical histories of both the mother and the child. The selection of the sample followed strict criteria: Spanish as the primary language, normal vision and hearing, no evidence of serious illness, no family history of psychiatric illness, and no history of significant head injuries, seizures, neurological disease, and substance abuse or dependence. Infants did not show symptoms of acute disease and were born full term and with adequate height and weight for gestational age. Three infants were excluded from the final sample because they presented an atypical development (i.e., hearing loss, $n = 1$) and failed to complete the first session because of fussiness ($n = 2$).

Procedure

Infants were evaluated together with their mothers. Mothers were asked to keep the children in their lap and not to give any kind of help or cues during evaluation. If the children interacted with the caregivers, they were asked to respond in a natural way and direct attention back to the experimenter, so that the evaluations could be continued. Behaviors were videotaped and timed using a Sony HD HDR-CX160® video recorder and a chronometer Model CR202 of the Galileo Italy® line for timing.

Sixty infants were evaluated in EF and joint attention and 50 completed the SFP. The same male evaluator presented the tasks on a table set between the infant and evaluator in the same order to balance fatigue effects across the sample: (a) Object's spectacle task, (b) Book presentation task, (c) Following-gaze task, (d) A-not-B task with multiple locations, (e) Spatial reversal task, (f) Delay of gratification task, and (g) Face-to-Face Still-Face task. The administration of this battery of tasks took approximately 45 min. After the tasks were completed, mothers answered the SES and temperament measures. For all tasks, two trained observers coded the behaviors of the sessions independently.

Parent-Report Measures

Social Economic Level Scale (SES, INDEC, 2000)

The SES was used to estimate the family socioeconomic level and classify the participants in the standard cutoffs of Unsatisfied Basic Needs (UBN) or Satisfied Basic Needs (SBN) (INDEC, 2000). This scale defined social vulnerability as a multidimensional variable, including the following: (1) paternal and maternal educational level (between 0 and 12 points according to the level of schooling reached), (2) occupational level (between 0 and 12 points according to the type of activity and level of autonomy), (3) housing characteristics (between 0 and 12 points according to the type of household, the type of materials, and access to drinking water), and (4) overcrowding (between 0 and 9 points according to the number of people per room). The maximum total score of the scale was 45 points (total SES).

Children were classified as UBN if one of the following criteria were met: they lived in a precarious settlement ("shantytown"), the house had no bathroom, the house had no access to mains water, it was overcrowded (three or more people per room), elementary school-aged children in the household were not attending school, or the parents in the house did not have a primary school education. The descriptive data for the sample split by socioeconomic status measured *via* the Social Economic Level Scale can be seen in **Table 1**.

Early Childhood Behavior Questionnaire Very Short Form (Putnam et al., 2010)

The Early Childhood Behavior Questionnaire Very Short Form (ECBQ-VS) was used to assess temperament in children aged 18 to 36 months, which assesses the emotional behavior of children from the point of view of caregivers. Behavior was classified following a Likert scale of eight points: (1) Never, (2) Almost never, (3) Less than half the time, (4) Approximately half the time, (5) More than half the time, (6) Almost always, (7) Always, and (8) It did not happen.

This test consisted of 36 items that formed three subscales in surgency, negative affect, and effortful control. The effortful control scale evaluated the ability to inhibit or suppress dominant responses. Surgency was related to positive emotion, rapid approach to potential rewards, and high activity level. Finally, negative affect included predisposition to fear, anxiety, sadness, frustration, and discomfort. The Cronbach's alphas were 0.61 for surgency, 0.65 for negative affect and 0.62 for effortful control. The coefficients are a little lower than those obtained by Putnam et al. (2010) on six samples of children 18–36 months of age. This could be due to the sample size and age (18–24 months) of the present study.

TABLE 1 | Composition of the sample by SES and gender.

Groups	Gender		Total
	Male	Female	
UBN	18	17	35
SBN	8	17	25
Total	26	34	60

UBN, unsatisfied basic needs; SBN, satisfied basic needs.

Executive Function Measures

A-Not-B Task With Multiple Hiding Locations (Miller and Marcovitch, 2015)

A box with five holes (9.5 cm in diameter, 7 cm deep) used as hiding places were embedded in a wooden box (43 cm long × 56 cm wide × 7 cm high). The holes were arranged in a semicircle configuration, so that each hiding place was 16 cm from the point where the box would be placed in front of the children to search. Each hiding place was covered by a blue felt that sealed and opened with a Velcro in the middle to reveal the contents of the hiding place. Two white poster boards of 56 cm × 43 cm were also used. The toys presented to children consisted of three small dolls (about 6 cm high). During the familiarization phase, children chose between these three dolls and watched as it was placed in the center hiding location (with the other holes covered by one of the poster boards). Children were then asked to retrieve the doll to become familiar with the instrument and goal of retrieving the hidden object. In the A-trial phase, the toy was hidden in location A in view of the children. A 10-s delay was imposed where the experimenter counted to 10 aloud and all the holes were covered. After the delay, children were asked to search for the object. This procedure for the A-trials was repeated until children found the object three times at location A. Next, children were presented with the B-trials in which the object was moved to a new location, location B. Children were asked to search until they found the object at location B twice.

The hiding locations for the object were counterbalanced and that the center location was not used as a hiding position because it was used during training and children often demonstrate a midline bias to search in the center. Location B was always placed on the opposite side of the midline of location A. Children were considered to search in a location when they broke the Velcro of one of the locations. On B-trials, perseverations (i.e., continued searching in location A) and whether children successfully completed the task (i.e., search correctly twice in B) were measured. This task measured cognitive flexibility (change focus and adapt it to different displacements while ignoring the distractors), working memory (remembering and following directions), and inhibitory control (stopping impulsive behavior in pursuit of one based on reflection).

Spatial Reversal Task (Espy et al., 1999)

Two plastic yellow cylinder containers 10 cm in height and 12 cm in diameter were used. They were placed on a blue fabric 30 cm long × 20 cm wide. At the start of each session, the object was hidden under one of the containers (container A) and children were asked to look for the object. Unlike the A-not-B task, children did not observe the hiding of the toy (Pennington and Ozonoff, 1996), as hiding occurred behind a cardboard screen. This was repeated until children found the object four times at container A. Next, the experimenter moved the toy to a new position, container B, and children were asked to continue search until they found the object at location B twice. The number of perseverations (i.e., searches back to container A once the object was moved to container B) and whether children successfully completed the task (i.e., search correctly twice in container B) were measured. This task measured cognitive flexibility (change focus and adapt it to different displacements while ignoring the distractors), working memory (remembering and following directions), and inhibitory control (stopping impulsive behavior in pursuit of one based on reflection to find the object).

Snack Delay Task (Kochanska et al., 1998)

A 7-cm-tall yellow bell, a 22-cm-diameter red shallow plastic dish, and a transparent plastic container 14 cm high and 10 cm in diameter were used in this task. Cookies that were sweet with chocolate and vanilla flavor filling of the Mini Oreo® brand of approximately 3 g each were also used. To begin the task, a cookie was placed on a plate and a transparent plastic container was placed on top of it. The experimenter told children that: "you can eat the cookie when the bell rings, you have to wait." There was a total of three trials, varying in time between 10, 20, and 30 s, respectively. The experimenter measured the average number of trials that children ate the cookie and whether children successfully completed the task (i.e., the children waited until the bell ring to eat the cookie in the three trials). This task measured inhibitory control (stopping impulsive behavior of eating and wait for the bell).

Executive Function Reliability Coding

For reliability, the primary coder recorded the measured variables for each EF tasks on all the videos. A second coder recorded the measured variables from 12 randomly selected videos (20% of total). Interrater reliability for continuous variables (intraclass correlation) was significant at the 0.005 level or below and was greater than 0.93 for all EF measures. Reliability for all categorical measures (Kappa) was greater than 0.97.

Emotion Regulation

Still-Face Task (Weinberg et al., 2008)

To assess ER, we used an adaptation of the Still-Face task for children, which, unlike infant measures, is done on the floor and with a series of standardized toys (Weinberg et al., 2008). A children's play carpet was placed on the floor 120 cm long × 90 cm wide, and three toys were placed on it: a multi-colored ball (20 cm high), a puppet (30 cm high), and a dog plush toy (25 cm high). The toys were kept constant and mothers were told that they could not use other toys external to those presented. The task consisted of three 90-s videotaped phases. In the first phase, the mother had to play freely with children with the provided set of toys. After 90 s, the experimenter moved on to phase II by prompting the mother with a slight sound to cue her to stop playing and observe with a neutral face. It was explained to the mothers that the neutral face involved looking at the child with a poker face and avoiding all contact. In the last phase, the experimenter cued the mother again to resume the phase of free play. The total test lasted 4′30″.

Coding was completed for all three episodes in accordance with the Child and Caregiver Mutual Regulation (CCMR) scoring system (Weinberg et al., 2003). Child affect was measured based on facial expressions (smiles, frowning, etc.) and vocalizations with affective tone (crying, shouting with enthusiasm, etc.). These were divided into two types of affects:

(1) Positive: facial expressions of joy (for example, smile, laugh) and positive vocalizations with exuberance and enthusiasm.
(2) Negative: facial expressions of anger, sadness, fear, subdued/withdrawn, or perplexed/worried affection and negative vocalizations such as crying, complaining, frustrating, irritation, discomfort, or impatience.

In addition, the number of specific behaviors of the children were measured, which included verbal and non-verbal approaches: (a) verbal approach (call the mother by name or role), (b) physical approach (e.g., approaching the mother, touching, or hugging the mother), (c) show the mother a toy (e.g., approach toy or point to an object), (d) aggressive acts (e.g., shouting, throwing a toy, hitting the mother), (e) displacement (time the infant withdraws from the interaction with the mother, leaving the focus of the camera), (f) aversion (e.g., turning the back on the mother), and (g) self-comforting behaviors (e.g., sucking on a thumb or finger). These behaviors were coded according to the frequency (rate per phase), except the duration of the child's withdrawal from the mother. The behaviors were mutually exclusive. The camera was positioned on a tripod in front of the dyad to get optimal view of the infant face.

Emotion Regulation Reliability Coding

For reliability, a primary coder recorded the measured variables in the Still-Face Task for all the videos. A second coder recorded the measured affect from 10 randomly selected videos (20% of total). Reliability for continuous variables (intraclass correlation) were significant at the 0.005 level or below and were greater than 0.80 for all ER measures in the three phases.

Joint Attention Measures

Early Social Communication Scales (ESCS) (Mundy et al., 2003)

The skills of RJA (e.g., follow an adult's pointing), IJA (e.g., pointing and showing of an object while the child alternate his gaze to the evaluator or the caregiver), and initiation of behavior request (IBR; e.g., behaviors related to the request of an object initiated by the child) were evaluated through the following subscales of the ESCS.

(a) Object spectacle task. Four different objects were presented to children, consisting of a red plastic toy car, a balloon (which varied in color), a rubber toy (which whistles when squeezed), and a rope toy. Objects were presented on three occasions in time periods of 6 s. The toys were positioned out of the reach of the children, and the occurrences of IJA (e.g., child pointing to the object out of reach to initiate shared attention to the object), RJA (e.g., child responding to the experimenter's pointing to share attention), and IBR (e.g., child pointing or reaching to obtain the object) were measured. If children attempted to initiate joint attention with the experimenter, the experimenter provided them with a brief natural response (e.g., "I see!"). If they requested the toy by attempting to obtain it, the experimenter moved the toy within reach.

(b) Book presentation task. Children were presented with a book with different drawings and textures, called ¡*A comer! of the Tin Cat*® edition Guadal for 20 s. This children's book of 20 cm × 15 cm contained images of different foods and objects (banana, bib, cheese, milk, pear, biscuit, bread, orange, gelatin, and cutlery). Then, the experimenter pointed for 6 s on each page of the book and asked, "What do you see here?" IJA behavior was considered to occur when infants pointed to a picture. IBR was considered to occur if the infant requested to a page of the book by extending his/her arm toward an out of reach object.

(c) Gaze-following task. Four colorful posters were placed to the left, back left, back right, and right of the infant. There were four trials in this task in which the experimenter called children by name and pointed to each poster to determine whether children responded to joint attention bids (RJA). First, the poster on the right was pointed out, then the one on the left, and then the back right and back left. For each trial, the experimenter turned his entire torso, pointed to the poster with a slightly raised elbow off the table, looked at the poster, and said, e.g., "Did you see the doll?" We computed RJA if the child performed the behavior of following the signal, placing his eyes and head in the direction of the object indicated. Infants received credit for IJA behavior if they pointed to the poster to direct the experimenter's attention before he showed them the posters.

Joint Attention Reliability Coding

For reliability, the primary coder recorded instances of IJA, RJA, and IBR for all the videos. A second coder recorded instances of IJA, RJA, and IBR behaviors from 12 randomly selected videos (20% of total). Interrater reliability for continuous variables (intraclass correlation) were significant at the 0.005 level or below and were greater than 0.78 for all joint attention measures.

Analytic Strategy

To address our first research question focused on examining EF and ER during the second year of life, descriptive statistics are presented in **Tables 2**, **3**. To analyze ER, an analysis of variance (ANOVA) for the intrasubject comparison on the Still-Face Task was made. The application of Bonferroni method was used to compare each phase (I, II, and III). Then, we made a correlation of measures of ER that was presented in **Table 4**.

To address our second research question, a multiple linear regression was performed to assess the ability of the total scores of joint attention scales (RJA, IJA, and IBR), Total SES, Temperament, Gender, and Age to predict EF and ER, in order to determine to what extent the percentage of variance of the scores in this regulation abilities is attributable to these individual and social variables. Finally, Pearson *R* statistic was applied to evaluate the correlation between the quantitative scores of the SES scale (i.e., total education, housing, overcrowding, and occupation) and our temperament measures of interest (i.e., subscale scores for surgency, effortful control, and negative affect) with regulation abilities. In **Table 5**, we summarized the main correlations of the variables. In all analyses, the probability of a Type 1 error remained at 0.05.

TABLE 2 | Means and standard deviations of measured variables.

Measures	M (SD)	95% CI	Range	*n*
Executive function				
(1) Perseveration on A-not-B	3.60 (3.96)	[2.40, 4.55]	0–10	60
(2) Perseveration on SR	4.15 (3.43)	[3.02, 4.83]	0–10	60
(3) Eat snack (before sound)	1.28 (1.32)	[0.96, 1.64]	0–3	60
(4) Total EF tasks passed	2.02 (0.95)	[1.74, 2.30]	0–3	60
Joint attention				
(6) RJA	6.94 (2.66)	[6.22, 7.67]	0–9	60
(7) IBR	6.90 (4.59)	[5.77, 8.15]	0–17	60
(8) IJA	11.64 (7.32)	[9.66, 13.62]	0–31	60
Temperament				
(9) Surgency	5.60 (0.70)	[5.42, 5.78]	3.55–7	60
(10) Negative affect	3.37 (0.95)	[3.12, 3.61]	1.91–6.63	60
(11) Effortful control	4.90 (0.76)	[4.66, 5.06]	2.90–6.40	60
Social vulnerability				
(12) Total SES	29.26 (7.74)	[27.26, 31.26]	15–45	60

SR, spatial reversal; EFs, executive functions; RJA, responding to joint attention; IBR, initiation of behavior request; IJA, initiation of joint attention; SES, socioeconomic status.

RESULTS

EF and ER in the Second Year of Life

Executive Functions Task Performance

The means and standard deviations for all major variables are presented in **Table 2**.

Correlation between performance on the three EF tasks revealed that only perseverative behavior on the A-not-B and spatial reversal were correlated ($r = 0.53$, $p = 0.001$). Number of trials children were unable to delay on the Snack Delay was not related to perseveration on the A-not-B or spatial reversal, $r = 0.19$ and $r = 0.20$, respectively, p-values > 0.05. We created a composite score of EF with the average of the number of EF tasks passed.

Face-to-Face-Still Face (ER) Task Performance

Descriptive statistics for each still-face effect by phase is presented in **Table 3**. To examine still-face and carryover effects, multiple repeated-measures ANOVAs were conducted on each effect with phase as the within-subject variable. These tests indicated significant differences by phases (I, II,

TABLE 3 | Comparison of the behavior scores and the different phases in the Still-Face task among infants.

Still-face effect	Phase I	Phase II	Phase III	*F*	*p*	PI–PII	PII–PIII	PI–PIII
	M (SD)	M (SD)	M (SD)					
Positive	2.4 (1.9)	0.2 (0.4)	0.7 (0.9)	26.99	0.000	0.000	0.009	0.000
Negative	0.2 (0.1)	1.4 (1.3)	1.0 (1.2)	14.21	0.000	0.000	0.421	0.001
Show toy	2.5 (2.2)	1.5 (1.6)	1.2 (1.3)	12.33	0.001	0.105	0.812	0.004
Aggressive	0.4 (0.8)	0.7 (1.3)	1.0 (1.1)	4.55	0.095	0.229	1.00	0.065
Physical App.	1.3 (1.3)	2.9 (2.3)	0.9 (1.2)	12.20	0.000	0.006	0.000	0.865
Verbal App.	1.5 (1.5)	2.7 (3.1)	0.8 (1.2)	12.70	0.000	0.024	0.000	0.031
Displacement	11.4 (17.3)	26.3 (25.4)	19.4 (23.1)	9.46	0.000	0.001	0.136	0.067
Aversion	0.2 (0.4)	0.9 (0.8)	1.8 (1.6)	29.28	0.000	0.000	0.033	0.000
Self-comfort	0.2 (0.5)	0.6 (1.2)	0.4 (1.1)	1.89	0.159	0.203	1.00	0.560

The p-value of the compared Still-Face behaviors in each phase (I, II, and III) was reported. PI, phase I; PII, phase II; PIII, phase III; Physical app., physical approach; Verbal app., verbal approach.

TABLE 4 | Correlations among emotion regulation variables in the SFP (phase II).

Measures	1	2	3	4	5	6	7	8	9
(1) Positive	–	−0.02	−0.13	0.02	0.30*	0.07	0.16	0.08	−0.24
(2) Negative		–	−0.24	0.28	0.46**	0.26	0.11	0.45**	−0.21
(3) Show toy			–	−0.11	−0.22	0.06	0.12	−0.29	0.18
(4) Aggressive				–	−0.06	−0.02	0.22	0.04	−0.22
(5) Physical App.					–	0.51**	−0.37*	0.24	−0.07
(6) Verbal App.						–	−0.43**	0.24	−0.09
(7) Displacement							–	−0.05	−0.26
(8) Aversion								–	−0.16
(9) Self-comfort									–

*Pearson correlations are reported for all variables. App, approach. *p < 0.05; **p < 0.01.*

TABLE 5 | Correlations among composite measures of regulatory abilities, joint attention skills, SES subdimensions, temperamental styles, and age.

Measures	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) Executive functions	–	0.27*	0.31*	−0.05	0.30*	0.38**	0.26	0.23	0.33*	0.15	−0.12	−0.15	0.30*	0.28*
(2) Emotion regulation		–	−0.02	0.09	0.12	0.21	0.29*	0.12	0.17	0.20	−0.21	0.05	0.31*	0.01
(3) RJA			–	−0.01	0.37**	0.26*	−0.02	−0.05	0.37**	0.33**	−0.19	−0.17	0.22	0.32*
(4) IBR				–	0.06	0.48**	0.36**	0.36**	0.38**	0.17	0.20	−0.11	−0.03	0.28*
(5) IJA					–	0.33*	0.15	0.29*	0.33*	−0.01	−0.20	0.06	0.05	−0.06
(6) Total SES						–	0.79**	0.79**	0.74**	0.61**	−0.02	−0.28*	0.18	−0.05
(7) Parent's education							–	0.74**	0.29*	0.27*	0.09	−0.32*	0.03	−0.09
(8) Parent's occupancy								–	0.28*	0.21	0.16	−0.28*	0.17	−0.19
(9) Housing									–	0.55**	−0.04	−0.15	0.24	0.11
(10) Overcrowding										–	−0.02	0.01	−0.07	−0.14
(11) Surgency											–	−0.04	−0.05	0.02
(12) Negative affect												–	−0.31*	0.11
(13) Effortful control													–	0.01
(14) Age (in months)														–

*RJA, responding to joint attention; IBR, initiation of behavior request; IJA, initiation of joint attention; SES, socioeconomic status. Pearson correlations are reported for all variables. *p < 0.05; **p < 0.01.*

and III) in the variables of positive affect, negative affect, avoidance, displacement, verbal approach, physical approach, and show toys. No statistically significant differences were found according to the phases in the behaviors self-comfort and aggressive acts.

Still-face effects

Post hoc analysis using Bonferroni was conducted to examine still-face effects (i.e., a change in behavior when mother's stopped interacting in phase II). Results revealed a significant increase in negative affect, displacement, aversion to gaze, physical approach,

and verbal approach from phase I to phase II (Still-Face). In addition, a significant decrease in positive affect was found.

Carryover effects

In addition, *post hoc* analysis was also conducted to examine carryover effects. Results revealed a significant increase in positive affect and aversion to gaze from phase II to phase III. We also found a significant decrease in verbal and physical approach. Finally, we also examined changes from phase I (baseline) to phase III. We found a significant increase in the negative affect, avoidance behaviors, and displacement. There was also a significant decrease in positive affect, show toy, and verbal approach. These results indicate the typical response to the SFP: from phase I to II, there is a decrease in positive affect, and an increase in negative affect and the number of interactions, which continues into phase III. **Table 3** summarizes these findings.

Relations between still-face effects

Correlation between Still-Face variables during phase II was also analyzed (**Table 4**). Physical and verbal approach tended to increase and were highly correlated during this phase. As expected, negative affect correlated with the aversion to the gaze. The composite, aggregated score of ER was calculated as the mean of the standardized scores on negative, physical approach, and aversion to gaze during phase II, because these are some of the most important variables in this paradigm and are correlated with each other.

EF and ER Links to Joint Attention, Temperament, and SES

In **Table 5**, we summarize the correlations between EF, ER, joint attention, temperament, and SES.

Correlations Between EF and ER

We examined the correlation between our two composite measures of EF and ER. Results demonstrated a positive correlation between both measures of regulation (r = 0.27, p = 0.038), suggesting that the ability to execute cognitive control (EF) is related to the ability to regulate emotions (ER) during the second year.

Correlations Between EF and Joint Attention, SES Scale Scores, Temperament, and Age

For joint attention, RJA (r = 0.31, p = 0.038) and IJA (r = 0.30, p = 0.044) correlated with EF, with a higher amount of this joint attention behaviors increasing the performance on EF tasks. For SES, composite measures of EF correlated with Total SES (r = 0.38, p = 0.009) and Total Housing (r = 0.33, p = 0.030), suggesting that higher socioeconomic status and the quality of the home were associated with better EF. For temperament, composite EF was only correlated with effortful control (r = 0.30, p = 0.037), demonstrating that children with better effortful control performed better on EF tasks. Finally, for age, a positive correlation was found (r = 0.28, p = 0.046), with older infants having better performance in EF tasks.

Concurrent Predictors of EF

Multiple regression analysis was conducted, examining whether joint attention abilities (IJA, RJA, and IBR), Total SES, Temperament, Age, and Gender predicted composite measures of EF. For EF, the overall model was significant, indicating and explaining 36% of the variability in EF composite scores (F = 2.23, p = 0.047, R^2 = 0.361). Total SES significantly related to EF (β = 0.419, p = 0.045), indicating that as the SES increased, EF abilities across all tasks tended to improve. Age was also significantly related to EF (β = 0.310, p = 0.049), indicating older children tended to have higher scores on composite EF. Finally, only RJA predicted composite scores of EF (β = 0.250 p = 0.047), indicating that as the capacity of children to follow adults' pointing and gaze increased, EF abilities across all trials tended to improve.

Correlations Between ER and Joint Attention, SES Scale Scores, Temperament, and Age

Composite measures of ER correlated with parent's education (r = 0.29, p = 0.039), suggesting that parent's characteristics are associated with ER behavior in the second year. For temperament, composite measures of ER correlated with effortful control (r = 0.31, p = 0.019), demonstrating that effortful control increases were related to greater ER.

Concurrent Predictors of ER

Multiple regression analysis was conducted examining whether joint attention abilities (IJA, RJA, and IBR), Total SES, Temperament, Age, and Gender predicted composite measures of ER. For this variable, the overall model was not significant ($p > 0.05$). Only effortful control was significantly related to ER (β = 0.319, p = 0.036), indicating that effortful control was positively associated with ER.

DISCUSSION

The present study aimed to examine abilities in regulation during the less studied second year. Results suggested that children from Latin America demonstrate similar patterns of EF and ER performance during the second year. In EF, children appear to demonstrate significant development across this short developmental period. Although children demonstrate individual differences, EF abilities are not well-correlated yet at this age. For ER, the patterns of response in the SFP match those of other countries, as children demonstrate difficulties in ER, in response to a lack of parent attention. Importantly, these abilities in EF and ER also appear to be related in the second year of life. Finally, many of the concurrent predictors of regulation important in older samples (i.e., temperament, and SES) also appear to hold importance during this early developmental period. These results add to the growing body of work suggesting that regulatory abilities are showing significant development during the second year and are already showing links to environmental (e.g., SES) and individual (e.g., temperamental) factors.

Relations Between Regulation Abilities

Overall performance in both regulation tasks aligned with expectations based on previous work (Weinberg et al., 2008; Miller and Marcovitch, 2015). The typical response to the SFP was observed. These relationships were similar to other works using this paradigm (Mesman et al., 2009). Namely, from phase I to II, there was a decrease in positive affect and an increase in negative affect and the number of interactions—which continues into phase III. This work extends the results of the SFP to a Latin American sample. With regard to EF task performance, results on the search tasks were similar to past research (Espy et al., 1999; Marcovitch and Zelazo, 1999). Although the toddler years are often perceived as a time of struggle with regard to regulation, the majority of the infants solved our EF tasks. The task that proved to be difficult in the present study was the Snack Delay, as few children understood it. This is because of the strongest verbal component as it required the ability to understand vocabulary and the infant is exposed to a highly appetitive reward (Espy et al., 1999). Performance on the Snack Delay task was low in general compared to past work (Kochanska et al., 1998; Kim et al., 2013). There was sufficient variability in performance and no evidence for floor or ceiling effects.

A novel component to our study was the demonstrated link between EF and ER as measured by their response to the SFP. This association could be due to the fact that there is an emotional component that accompanies the development of the EF. More specifically, one of the tasks administered (i.e., the Snack Delay task) is hypothesized to measure more hot EF skills related to regulating more emotional responses directly related to the ER (Zelazo and Carlson, 2012). However, it is also important to note that researchers have also proposed that domain general abilities in EF are likely important to ER (Zelazo and Cunningham, 2007). In this sense, the other two more "cool" tasks likely measure the more domain general cognitive aspect of EF, but for its resolution in this age range, the ability to regulate emotional states is necessary (although to a lesser extent) (Carlson and Wang, 2007). This is some of the earliest evidence to suggest an EF–ER relationship during the second year as measured with the SFP, which is scarce in this age range (due to methodological issues).

Links to Socioeconomic Status

Environmental variables seemed to play a large role in regulation abilities in our present sample. Infants who came from environments with social vulnerability had less mature performances in EF and ER capacities. This is in line with previous studies suggesting that vulnerable contexts may impact infant regulation *via* adverse effects on brain development. For example, vulnerable environments are associated with higher levels of cortisol in children, which affects the cerebral and cognitive functioning of EF and ER tasks that demand the use of the prefrontal cortex (Arán-Filippeti and Richaud de Minzi, 2012; Doom and Gunnar, 2013). In addition, children from vulnerable contexts are exposed to different environmental toxic agents such as air pollution, less healthy lifestyles, and lower levels of nutrition (which regulate neural development from the prenatal stage, Lipina et al., 2013; Ngure et al., 2014; Kim et al., 2018). Finally, poverty environments impact educational levels and the type of education of the mother and father. This translates to differences in parental styles and levels of sensitivity to interaction—skills that would be associated to EF and ER (Conway et al., 2018; Lawson et al., 2018).

More specifically, the level of education of the caregivers and their type of housing were also associated with these regulation abilities in this age range. It should be noted that the correlation coefficients were between 0.30 and 0.35, comparable or larger than those found in other researches in early childhood (Sohr-Preston et al., 2013; Hughes et al., 2015; Lawson et al., 2018). This suggests that SES explained a portion of the variance in these regulatory behaviors. These skills could be linked to one component of the SES and not others; therefore, more research is required.

However, these results demonstrate the importance of distal context in development. The characteristics of the environment, the type of tasks performed by caregivers, the type of housing, and the educational level would promote cognitive development by emphasizing child stimulation already in this age range. For example, vulnerable environments often bring contexts of stress and violence related to lower levels of effortful control and maternal sensitivity—this has been shown to directly impact child regulation (Vargas-Rubilar and Arán-Filippetti, 2014). The links between lower SES and less mature ER and EF in the present sample align with these studies and extend these links to a younger novel Latin American sample.

Links to Individual Factors: Joint Attention, Temperament, Gender, and Age

With regard to joint attention, only RJA predicted EF composite measure. This could be due to the fact that a cohesive ability in EF (i.e., consistent positive performance across several EF tasks indicating performance driven by a cohesive cognitive control rather than individual task demands) is hypothesized to emerge in this age range. More specifically, previous studies have shown little consistency and stability in EF skills related to the consolidated ability to guide behaviors through meta representations (Zelazo, 2004; Wiebe et al., 2010; Miller and Marcovitch, 2015). Thus, less mature performance in EF may lead to weak associations between joint attention, with only lower levels of RJA associated with EF. Otherwise said, in the absence of a cohesive ability to control behavior across multiple context, individual task performance may better relate to RJA, which measures children's sensitivity and response to social cues in the environment.

However, these results are aligned with previous work demonstrating that IJA is associated with the later emergence of a more cohesive EF (Miller and Marcovitch, 2015). RJA, IJA, and EF could be related in this cross-sectional sample since both imply the ability to sustain attention and adapt it to different environmental changes. This also mirrors previous research showing concurrent RJA–EF relationships at 14 and 18 months (Miller and Marcovitch, 2015).

As for temperament, there were correlations between effortful control and the regulatory abilities with coefficients of 0.30, like other researches in this age range (Kochanska et al., 2000; Frick et al., 2017). Effortful control is associated with decreasing impulsive responses, and the maintenance of attention, skills necessary for the correct performance of regulation abilities (Rothbart et al., 2000). This could be because effortful control promotes the capacity of working memory and inhibitory control (Liew, 2012). However, in contrast to other researches (Mundy and Jarrold, 2010; Kim et al., 2014) and similar to others (Zhou et al., 2012; Frick et al., 2017), no associations were found between negative affect and surgency with these regulatory capacities. This may be because temperament styles were not directly assessed but were obtained *via* parental report. More research is necessary to solve these inconsistent results.

Regarding age, associations were also obtained in EF, aligning with the proposal that there is significant development in this regulatory ability during the second year (e.g., Wiebe et al., 2010; Miller and Marcovitch, 2015). Although there were no age differences in ER performance, the absence of associations could be due to the fact that the responses to the SFP are fairly stable throughout the different ages, as the majority of studies have also found a lack of age effects (Mesman et al., 2009; McMahon and Newey, 2018). However, more research is needed using this paradigm at 2 years of age.

Limitations and Future Directions

Taken together, these results underline the importance of early childhood interventions on individual and environmental variables, and on the incipient relationship between behavioral and emotional components of regulation and EFs and joint attention skills at early stages of development. In addition, it sheds light on the effects of the SFP in a Latin American population, with an effect that is increasingly robust. This reinforces the findings of previous research already mentioned and gives a first approach to the subject in a Latin American population in this age range.

However, our study has several limitations. These include a relatively small sample size, obtained with a non-probabilistic method and limited to a single geographic location (Buenos Aires City and Province). Follow-up studies should aim to overcome these limitations by increasing the size and diversifying the sample. Ours was a cross-sectional study, with all the limitations—and benefits—that such approach affords, and it awaits future longitudinal studies to explore how regulation behaviors unfold within each individual child. These future studies will bring us closer to a better understanding of the role that infant socioeconomic status, non-verbal communication, and temperament play on the development of regulation on the first years of life. Future research should also focus on whether early intervention helps promote the development of these regulation abilities, and it does so disproportionately on those infants with largest socioeconomic need.

Further looking toward the future, our results point toward the potential of early childhood interventions on individual and environmental variables. Researchers might want to focus those interventions not merely on the individual child, but rather on the family unit, with the goal of promoting more effective parenting styles and creating environments more conducive to healthy development.

ETHICS STATEMENT

This study and the protocol were carried out and approved by the Ethics Committee of the University of Buenos Aires. The evaluator explained the tasks to the mothers before they gave written informed consent, both for their own participation and for their child's participation in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

LGG, SM, GC, AM, MD, and AE made substantial contributions to the conception, design, collection, and analysis of the data of the work.

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frontiers in Psychology

CONCEPTUAL ANALYSIS
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What Is Complex/Emotional About Emotional Complexity?

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Affective experiences can fluctuate, be combined, and fused, resulting in various phenomena labeled as being emotionally complex. Despite the lack of a common theoretical framework, several phenomena including mixed emotions, emodiversity, meta-emotions, awe, among several others, have been defined as being emotionally complex. In this conceptual analysis, I aim to integrate the diversity of emotional complexity by describing various phenomena associated with this construct. This integration offers a more comprehensive panorama of the current usage of the concept of emotional complexity compared to previous attempts to consolidate the field. Furthermore, this conceptual analysis intends to disentangle the emotional fingerprints of emotional complexity. In particular, I present evidence and arguments showing that complex emotions can be characterized as having specific facial expressions, appraisals, and functional significance. Finally, I suggest that it is possible to describe emotional complexity using concepts and properties from the complex systems theory. Concepts such as the hierarchical organization of the affect system and emergent self-organization are used to explain current evidence on emotional complexity. I explain that applying complex systems theory to emotional complexity is not only theoretically convenient, but that complex systems theory also serves to advance new forms to conceptualize the affect system. The current conceptual analysis can help to organize current research and theory in order to encourage new research endeavors in the field of emotional complexity and acknowledge the importance of emotional complexity in models of affect, for which I suggest some specific guidelines.

Keywords: emotional complexity, affect system, emotional features, complex systems, mixed emotions, meta-emotions, aesthetic emotions

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Emotions are sometimes more complex than the words we commonly use to express our feelings. Watching a loved one who is suffering acute pain pass away; being cheated while been a cheater; saying goodbye to friends when graduating. Life is full of occurrences when it is hard to communicate how we feel, but we can count these situations as wholly emotional.

In recent years, a burst but disperse number of research has emerged showing that our emotional life is complex (e.g., Lindquist and Barrett, 2010; Hay and Diehl, 2011; Grossman et al., 2016). Different phenomena including the co-activation of opposite emotions at the same time (e.g., mixed emotions) or the experience of feeling guilty for being happy observing others' misfortune (i.e., meta-emotions) are forms of emotional complexity.

However, some obscurity remains in the literature when trying to comprehend a consensual definition of emotional complexity (Lindquist and Barrett, 2010; Grühn et al., 2013; Grossman et al., 2016). Furthermore, it is not clear what unifies different emotional experiences under the umbrella of emotional complexity. Likewise, no theoretical contribution in the field has accounted for the emotional or complex features of emotional complexity.

Hence, in this conceptual analysis, I address three issues related to emotional complexity and its role in emotional experience: (1) the diversity and apparent disparate number of phenomena characterized as emotional complexity, (2) the emotional fingerprints of emotional complexity, and (3) the complex nature of emotional complexity phenomena. I will present evidence and arguments showing that a unified emotional complexity framework is feasible, but it needs clarification of the emotional and complex features of emotional complexity.

REVISITING THE CONCEPT OF EMOTIONAL COMPLEXITY

Recently, Grossman et al. (2016) (see also Hay and Diehl, 2011) identified two different standard definitions of emotional complexity, which have resulted in disparate operationalization and measurement of the construct. They noticed that emotional complexity is generally understood either as emotional differentiation and emotional interdependence. These broad conceptualizations cover the majority of the common usages of the concept of emotional complexity in emotion science.

In this section, I expand on Grossman et al.'s distinction, identifying several streams of research that characterize the current usage of the concept of emotional complexity in the literature. The goal is not to provide a new definition of emotional complexity. Instead, I aim to succinctly describe some of the various phenomena related to emotional complexity, trying to identify a common theme across most of these phenomena. **Figure 1** illustrates this diversity and organizes the broad definitions and some of the related streams of research.

Emotional Complexity as Emotional Differentiation

One standard definition of emotional complexity concerns emotional differentiation. Emotional differentiation implies discerning among many positive and negative emotions (Grossman et al., 2016). In the emotional differentiation conceptualization of emotional complexity, it is possible to distinguish two different streams of research. One stream of research has considered subtle distinctions within emotional concepts as a meaningful expression of emotional complexity (Barrett, 2004; Kang and Shaver, 2004). For example, emotional granularity is an individual difference associated with the ability to make finer distinctions and well-differentiated reports of emotional experience, demonstrated by weak correlations between emotional states of the same valence (Barrett, 2004). Links between specific reports of emotional states and the corresponding subjective experience are substantiated in evidence showing that language contributes to the perception of emotions (Lindquist et al., 2006; Gendron et al., 2012).

The second stream of research conceptualizes emotional complexity as experiencing a broad and diverse range of different emotions (Quoidbach et al., 2014). The degree to which people can experience a diverse and abundant set of emotional experiences is a form of emotional complexity. Emodiversity is a measure of the richness of emotional complexity and the proportionality of experiences about a wide number of emotions (Quoidbach et al., 2014). Therefore, emodiversity and emotional granularity are at opposite sides of a hypothetical differentiation continuum, ranging from making thin distinctions between different emotions (emotional granularity) to experiencing an abundant and diverse range of emotions (emodiversity).

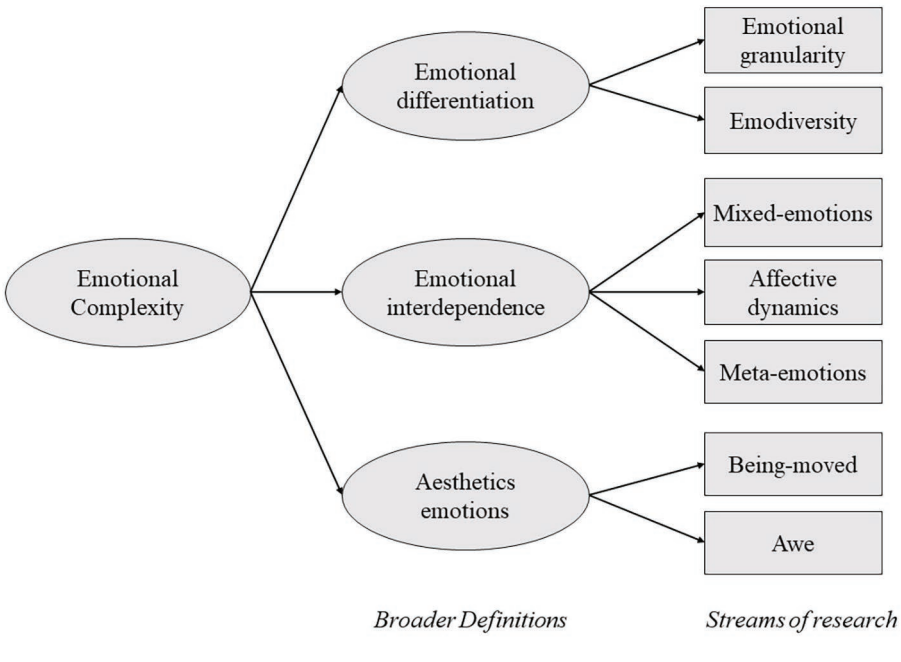

FIGURE 1 | Representation of the common broad definitions of emotional complexity and some streams of research associated with them.

Emotional Complexity as Emotional Interdependence

The other common definition understands emotional complexity as emotional interdependence (Grossman et al., 2016). Emotions mutually influence each other throughout an event, altering the intensity of subsequent affects, modifying the hedonic valence of ongoing experiences, coupling multiple emotional experiences as a consequence of similar appraisals, or changing the behaviors to be deployed at a given moment.

In the emotional interdependence conceptualization of emotional complexity, it is possible to distinguish three streams of research. First, interdependence can be conceptualized as the co-occurrence of emotional experiences of positive and negative valence (e.g., Larsen et al., 2001; Schimmack, 2001), such as in the experience of mixed emotions (e.g., happy-sad; fear-hope). Research has shown that students about to graduate experience more positive (happiness) and negative (sadness) emotions at the same time, compared to the same students surveyed at distant time points from graduation (Larsen et al., 2001; Hershfield et al., 2008).

Second, emotional interdependence is also defined as affective dynamics. Changes in emotion can result in diverse processes defined by the individuals' fluctuations in emotion (Davidson, 1998). For example, emotional variability or instability is defined by the intraindividual variability of emotions over time (Grühn et al., 2013). Emotional variability involves marked fluctuation between different emotions, such as feeling excited and, not very long after, feeling blue. In this dynamic, emotions change dramatically from one moment to another.

These two streams of research are consistent with the main findings of Grühn et al. (2013). Their results indicate that there are four, mostly independent, factors of emotional complexity organized in nine time-based indicators of emotional complexity studied during seven consecutive days. Two factors resemble co-occurrence and affective dynamics. Co-occurrence is accounted for by the covariation between positive and negative emotions, whereas the overall variation in affective reports represents affective dynamics. The remaining two factors (positive and negative differentiation) reflect the conceptualization of emotional complexity as emotional differentiation, in particular, how discrete and precise are the emotional experiences reported by the people, ranging between highly differentiated (i.e., granularity), and abundant, richly componential (i.e., emodiversity).

The third stream of research conceptualizes emotional complexity as meta-emotions, with one emotion prompting a secondary emotion (Gottman et al., 1996; Norman and Furnes, 2016). I integrate meta-emotion within the definition of emotional complexity as interdependence because there is a teleological cause between a pair of affective experiences, which is a form of interdependence. Experiencing a meta-emotion requires that one emotion (e.g., sadness) triggers a secondary emotion (e.g., anger). Fundamental in the understanding of meta-emotions as a complex emotional experience is that emotions can be hierarchically organized, forming finite layers of emotions. Whereas in the previous conceptualizations, emotions can precede, follow, or coincide with another emotional experience, in meta-emotions, emotions are aggregated on top of each other.

These three streams of research (mixed emotions, affective dynamics, and meta-emotions) have a similar feature; that is, different emotions establish different interrelations between them. In mixed emotions, two oppositely valenced emotions co-occur, resulting in the subjective experience of two emotions as occurring at the same time (Berrios et al., 2015b). Emotion dynamics are characterized by multiple idiosyncratic patterns of reciprocal associations between different emotions, distinguishing the ebb and flow of the everyday emotional experience (Davidson, 1998). Finally, in meta-emotions, it is possible to observe a causal relation between a pair of different emotions; there is one emotion that serves as an object to trigger a secondary emotion (Norman and Furnes, 2016).

Emotional Complexity as Aesthetic Emotions

Recent attempts to integrate the field of emotional complexity have not considered aesthetic emotions as a form of emotional complexity (e.g., Lindquist and Barrett, 2010; Grühn et al., 2013; Grossman et al., 2016). Aesthetic emotions are a group of affective experiences felt during aesthetic appreciation, including stimuli from nature (e.g., natural wonders) and human creation (e.g., painting or music), as well as emotional reactions that follow religious experiences or epiphanies (Keltner and Haidt, 2003; Gordon et al., 2016). These experiences are a form of emotional complexity, mostly because of the multiplicity of emotions involved and the difficulty to circumscribe them using single emotional words (Ortony et al., 1988; Keltner and Haidt, 2003).

Aesthetic emotions can be considered an independent category within this conceptualization of emotional complexity mostly because aesthetic emotions are a product of multiple emotions forming a synthesis. For example, research has shown that awe involves a mixture of surprise, pleasure, elevation, and astonishment (Keltner and Haidt, 2003).

Offering a complete characterization of the multiple streams of research on aesthetic emotions exceeds the goal of this section because many different emotional experiences have been studied. Thus, I introduce two complex emotional experiences commonly listed as aesthetic emotions: being-moved and awe.

Being-moved is a construct that is circumscribed to the arts and poetry, and only in recent years, it has been a subject of scientific exploration (Menninghaus et al., 2015). Menninghaus et al. (2015) have found that being-moved includes the emotional experiences of sadness and joy. Furthermore, they identified that critical life events, such as deaths and births, and significant relationship events (reunions) are the most common scenarios where this complex affective experience is triggered (Kuehnast et al., 2014). Finally, typical emotional appraisals observed when being-moved include high levels of compatibility with social norms and self-ideals. Frijda et al. (1989) also identified multiple cognitive appraisals when being-moved, including pleasantness, certainty, suddenness, importance, and other agency.

On the other hand, awe is a mixture of surprise, pleasure, elevation, and astonishment (Keltner and Haidt, 2003). Awe includes a feeling of wonder and amazement as a result of perceiving something vast that transcends our knowledge (Keltner and Haidt, 2003). In a recent study, Stellar et al. (2018) found that awe is preceded by appraisals of perception of vastness and need for accommodation (i.e., revise or create new mental schemas to account for paradoxical or unfamiliar information of the environment).

A Common Theme Across the Different Streams of Research

From the three previously sketched conceptualizations of emotional complexity, versatility emerges as a distinct feature of emotional complexity. Versatility refers to the flexibility of the affect system when one is experiencing complex emotions. Versatility relates to the idea that, in emotional complexity, emotions are felt in multiple ways allowing individuals to integrate complex information, producing new verbalizations to communicate genuine feelings. Thus, for example, versatility is observed in emodiversity where experiencing a wide variety of emotions indicates greater elasticity of the affect system. Likewise, when people combine, aggregate, or fluctuate between different emotions, they reveal the vast flexibility of the human affect system, which allows them to "feel mixed" when graduating from school or "feel angry for being sad" after a romantic disappointment.

The idea that versatility is fundamental in understanding complex emotions is not new. For example, the Evaluative Space Model (ESM; Cacioppo et al., 1999, 2004) contends that positive affect and negative affect exist in distinct biological structures, which allow the independent activation of positive and negative emotions. An organism that processes both positive and negative emotions in parallel is capable of displaying a broader set of behaviors (i.e., versatility) appropriate to the circumstances.

This stance is also shared by the communicative model of emotion (Oatley and Johnson-Laird, 1996). Oatley and Johnson-Laird (1996) proposed that individuals react to events by making multiple cognitive evaluations, which in turn, may elicit complex emotions, giving rise to facial expressions that combine more than one basic emotion (i.e., versatility). Thus, versatility is as a property of the affect system in which cognitive and affective components of emotional experience (e.g., appraisals, valence) are flexibly integrated.

WHAT IS EMOTIONAL IN EMOTIONAL COMPLEXITY?

Missing from previous efforts to conceptualize emotional complexity (i.e., Lindquist and Barrett, 2010; Grossman et al., 2016) is a closer examination of the emotional fingerprints of emotional complexity. Indeed, what evidence do we have concerning the emotional signatures that accompany emotional complexity? Therefore, the aim in this section is to show indications that emotional complexity encompasses emotional signatures present in well-established definitions of emotion.

Defining the concept of emotion has been the subject of extensive debate in the history of Psychology (Gendron, 2010; Pérez-Almonacid, 2019). However, currently, it is mostly accepted that the concept of emotion is a description of its dominant uses, which implies a certain fuzziness and over inclusivity (Dixon, 2012). According to Mulligan and Scherer (2012), the minimum conditions that define an emotion are that: (1) emotions are directed toward an object; (2) emotions involve bodily changes that are felt; (3) emotions contain a subjective experience; (4) emotions are triggered by a particular evaluation of an external event, usually referred to as an appraisal; and (5) emotions have functional implications for individual and/or social life.

Emotions also produce consistent patterns of feelings over time that distinguish one individual from another (Gohm and Clore, 2000). Individual differences in emotions are states of feelings or moods that do not require an object (Clore et al., 1994). Emodiversity is an exemplar of individual differences in emotional complexity (Quoidbach et al., 2014).

The Emotional Expression of Emotional Complexity

Previous research permits to describe three emotional fingerprints that characterize complex emotional experiences. First, during an emotionally complex episode, the affect system displays greater versatility, which produces characteristic facial expressions and physical reactions. Although facial expressions are not a definitive hallmark of the presence of a particular emotion (e.g., Russell, 1994), these are certainly an important marker of emotional experience in the literature (Keltner et al., 2003). Emotional expression can merge more than one gesture (Ekman and O'Sullivan, 1991; Kreibig et al., 2013, 2015; Du et al., 2014), and these expressions are combinations of emotions that have been thought to lie at opposite ends of the dimension of valence (c.f., Russell, 1980), such as disgust and joy.

New evidence supports previous incidental findings demonstrating that it is possible to identify 21 different and consistent facial expressions (Du et al., 2014), many of which reflect combinations of basic emotions (e.g., happily disgusted). Du et al. (2014) named all these combinations "compound emotions." Pictures of 230 individuals' emotional expression were taken during the elicitation of six basic emotions and 15 compound emotions, using imagery and images from previously validated studies. Through a computational model that automatically detects the shape of different features of the face, they showed that compound emotions are different from, but consistent with, the six basic emotions used in the study.

Likewise, recent research has shown that mixed emotions reflect specific facial muscle activation patterns that cannot be described merely as components of each emotion separately (Kreibig et al., 2013, 2015). Finally, some research on aesthetic emotions has shown that goose bumps or chills generally accompany awe and being-moved; these chills are not triggered by the componential emotions of being-moved, namely joy or sadness, but only by the complex experience of being-moved (Wassiliwizky et al., 2015).

The Functional Significance and Appraisals of Emotional Complexity

It is also possible to suggest that complex emotions carry useful information that individuals use to appraise relevant events. According to some authors (Schwarz and Clore, 1983; Keltner and Haidt, 1999), emotions convey information that people use to interpret their current situation. Similarly, here, I propose that paradoxical events are typically those that elicit complex emotions. This assertion has found support in a number of recent studies showing that conflicting goals (e.g., wanting to finish your duties at the office, while at the same time wanting to get home earlier for a family dinner) typically yield the experience of mixed emotions (Berrios et al., 2015a, 2018a,b).

Menninghaus et al. (2015) also noted that events such as deaths, births, and reunions commonly elicit aesthetic emotions. Similarly, the experience of awe has been described as the need to accommodate new information either in the form of an active seeking of experiences that challenge current schemas (Shiota et al., 2006) or the level of uncertainty an individual experiences (Valdesolo and Graham, 2014). In all these cases, complex emotions are indicative of an event or information that is challenging, enigmatic, or disconcerting for one's current mental schemas.

Figure 2 shows a representation of the relationship between the degree of paradoxical information and the level of versatility manifested by the affect system when observing two different stimuli. The stimulus in the left down corner is very straightforward to understand; the degree of paradoxical information is almost zero. As a result, the affect system prompts fixed responses that lie within some form of positive affect. On the contrary, the stimulus in the upper right corner shows a paradoxical image. The photograph shows two men playing tennis on a biplane's wings. The affect system triggers random responses, out of the standard spectrum that governs common emotional reactions (e.g., positive, negative), and as a result, one may feel anxious, surprised, and curious to determine whether the image is real (it is!).

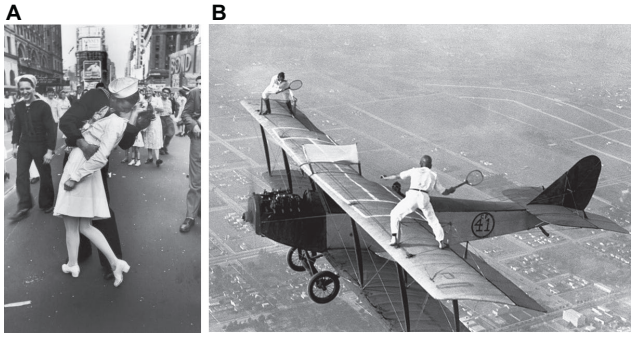

FIGURE 2 | Graphical representation of the relationship between the degree of paradoxical information and the degree of versatility observed as complex emotions emerge. **(A)** "V-J Day in Times Square" (Eisenstaedt, 1945; *with permission of Getty Images*). This is an iconic photograph; no complex emotions are expected from its appreciation. **(B)** "Daredevils Playing Tennis on a Biplane" (Bettmann, 1925; *with permission of Getty Images*). Gladys Roy and Ivan Unger playing tennis on the wings of a biplane above Los Angeles.

Finally, it is possible to assert that appraisals when experiencing complex emotions are also complex. Appraisals mean that the evaluation of the surrounding circumstances of an affective experience plays an important role in the elicitation and differentiation of emotions (Arnold, 1960; Ellsworth and Scherer, 2003). Appraisals are perceptions of external events which are not related to high cognitive processing (Moors et al., 2013). As stated by Ellsworth and Scherer (2003), the "appraisals process is a link between the organism and the situation that produces the emotion" (p. 574). Thus, to fully consider the claim that paradoxical information is a meaningful driver of emotional complexity, it is necessary to account for particular appraisal processes when experiencing complex emotions.

Some evidence suggests that appraisals can be flexibly combined. For example, Smith and Ellsworth (1987) showed that when different appraisals are combined, it is possible to observe emotional blends (e.g., hope, challenge, and fear). They examined the appraisals and emotional reactions of individuals when taking an exam, and results revealed that combinations of patterns of appraisals are common during these stressful situations, following the elicitation of emotional blends. These preliminary data are consistent with the theory stating that the confluence of multiple emotions can control actions because each emotion contributes with multiple appraisals and motives regulating behavior (Frijda et al., 2014).

Additionally, Menninghaus et al. (2015) showed that the complex emotion of being-moved includes high ratings for appraisals of compatibility with social norms and self-ideals, showing that complex emotions may have distinct appraisals that assist individuals in the evaluation of the affective situation. Finally, Keltner and Haidt (2003) noticed that the prototypical cognitive appraisals associated with awe are the perception of vastness or self-diminishment and the need to mentally attempt to accommodate this vastness into existing mental schemas.

In sum, complex emotions have patterns of facial expression and physical reactions that are exclusively accounted for by the complexity of these emotional experiences, rather than the simple aggregation of affective components observed when experiencing a single emotion. Furthermore, complex emotions can include appraisals that characterize certain complex emotions, whereas other complex emotional experiences may involve the confluence of several different appraisals. The evidence revised so far also permits the assertion that complex emotions are functionally meaningful in signaling the presence of paradoxical information that challenges, puzzles, or disconcerts an individual's current beliefs or mental schemas.

WHAT IS COMPLEX IN EMOTIONAL COMPLEXITY?

There is an unexploited opportunity to apply the concepts and methods from complexity science to better examine the complex nature of emotional complexity. Complex systems theory can be defined as "an interdisciplinary field of research that seeks

to explain how large numbers of relatively simple entities organize themselves, without the benefit of any central controller, into a collective whole that creates patterns, uses information, and, in some cases evolves and learns" (Mitchell, 2009: p. 4). Familiar exemplars of complex systems include the economies, and bee's colonies.

According to Mitchell, a system is complex when "large networks of components with no central control and simple rules of operation give rise to complex collective behavior, sophisticated information processing, and adaptation via learning or evolution" (Mitchell, 2009: p. 13). Paralleling these ideas, we can call complex emotions insofar as the single components of the affect system (i.e., emotional adjectives) interact forming patterns or categories that are integrated into systems which do not resemble the constituent emotions permitting adaptive functions. As previously shown, specific properties of the affective experience, including emotional expression, functional significance, and appraisals reveal dynamics that exceed the rules observed when experiencing single emotions.

In this section, I intend to apply some of the most relevant properties of complex systems theory (Mitchell, 2009) to the study of emotional complexity. I explain some of these attributes regarding their relevance for current research and theory of emotional complexity. The goal is to refine the concept of emotional complexity in order to facilitate future research endeavors, choosing some properties of complex systems theory that best reflect the current evidence.

Hierarchical Organization and the Emotional Lexicon

Spanish, English, or any other language, has a large number of terms that refer to emotions. Researchers usually simplify the structure of affect in order to explain the largest amount of variability using the smallest number of affective descriptors. These affective descriptors are used to account for the degree (or frequency) to which people experience a finite number of emotional adjectives. Language is essential in emotion research not only because it provides an essential research tool, but also because emotional words contribute to the subjective emotional experience itself (Barrett, 2004). In complex systems theory, languages and alphabets are considered forms of complex systems because multiple subsystems (e.g., words, codes) are tightly interrelated forming several new structures that actively communicate information (Simon, 1977).

One common property of complex systems is the hierarchical organization. According to Simon (1977), a complex system is characterized by different levels, systems and subsystems distributed following the interrelation among the elements. Simon (1977) also specifies that interactions among near elements are stronger compared to elements at a more considerable distance. In the science of emotion, the most common characterization of affect is the tree structure, where closer elements reveal stronger associations (see **Figure 3A**). The tree-shaped structure is the observable organization that emerges from traditional factorial analysis. Although complex in appearance, this structure ignores different associations at other levels of interaction.

Shaver et al. (1987), in *Study 1*, investigated the hierarchical structure of affect using cluster analysis. They determined that the lowest level corresponds to the emotional lexicon that describes the language of a community of native speakers (213 emotional adjectives surveyed). In the next upper level, two smaller sets of discrete emotions were described (love, joy, surprise, anger, sadness, and fear). These more or less correspond to the basic emotional adjectives found by theorists of basic emotions.

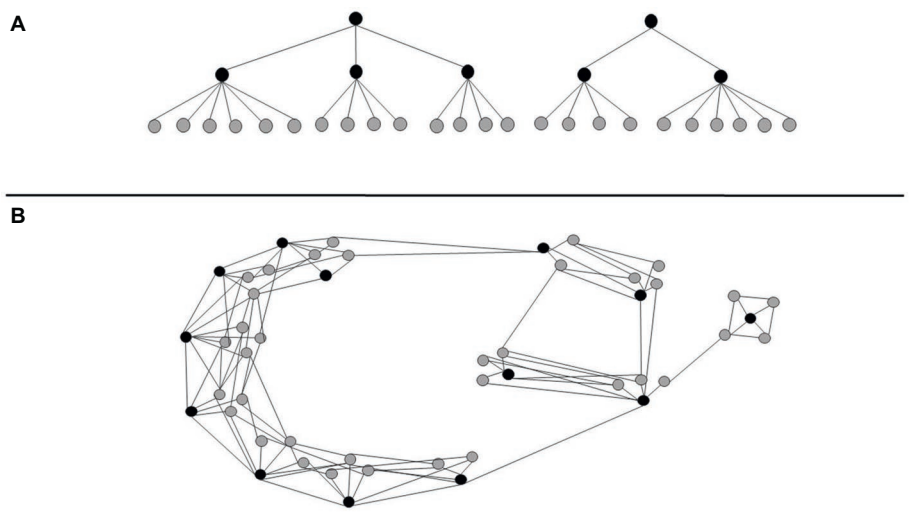

FIGURE 3 | (A) Representation of common factorial models, resembling a tree structure, where the numerous emotional words are summed within increasingly parsimonious categories, and **(B)** new findings from Cowen and Keltner (2017) showing that the structure of affect is more complex, including several emotional categories and abundant interrelations across categories.

The hierarchical structure described by Shaver et al. (1987) also distinguishes two broad characterizations of emotions as positive affect and negative affect at the top of the structure. Popular theories of affect, such as the circumplex model of affect (Russell, 1980), state that positive affect and negative affect represent opposite ends of a bipolar dimension of valence.

However, the emotional lexicon, when referring to most of the emotional complexity phenomena, seems to be more intricate. There are not many words to account for experiences such as mixed emotions, meta-emotions, or awe. Characterizations of affective life, either using basic emotions or overall dimensions of positive affect and negative affect, do not resemble the affect system when experiencing complex emotions. For example, **Table 1** shows different verbalizations of some complex emotional experiences in three languages (English, Spanish, and Portuguese). It is clear from the examples that these are genuine affective experience, but only some of them can be described using regular emotional words (e.g., happy, sad).

Simon (1977) contends that the observable associations in a system ignore the detailed structure at other levels of interaction. He exemplified this idea by explaining that the middle band of frequencies only determines the observable dynamics of the system (i.e., sounds we can hear). The structure of interactions in other subsystems is nearly independent or "nearly decomposable" at the next level (i.e., high or low frequencies; Simon, 1977). The affect system can be then also nearly decomposable. Emotional expressions such as mixed feelings or tears of joys are clearly out of the spectrum, but they are still genuine feelings, with identifiable emotional components.

Structures and (Non-)additive Models of Affect

Conventional representations of the affect system are different versions of factorial models, in which the abundant emotional lexicon is simplified to obtain more parsimonious descriptors of the emotional experience. These models can be categorized as *additive models of affect*. That is to say that, for example, emotional adjectives including happiness, joy, and excitement are grouped under the common name of positive emotions. On the other hand, emotional adjectives such as sadness, sorrow, and blue are classified as negative emotions. Following dimensional models of affect, all positive emotions and all negative emotions share the same affective valence (positive and negative, correspondingly).

TABLE 1 | Exemplars of common emotionally complex, linguistic expressions in English, Spanish, and Portuguese.

	English	Spanish	Portuguese
Co-activation of emotions	Mixed feelings	sentimientos encontrados	sentimentos misturados
	Tears of joy	lágrimas de alegría	lágrimas de felicidade
	Being-moved	conmovido	movido
Meta-emotion	Feeling guilty for feeling good	Sentirse culpable por alegrarse de la desgracia ajena	Sentindo-se culpado pela nossa alegria maliciosa
	I hate how much I love you	Odio cuanto te amo	Eu odeio o quanto eu te amo
	The joy of being sad	La alegría de estar triste	A alegria de estar triste

The structure of affect following additive principles is then limited to a small number of varieties of emotional experience. However, in order to account for complex emotional experiences, it is necessary that multiple interrelations across categories could represent emotional lexicon. In other words, to describe complex emotions, it is necessary to observe both a large number of distinct categories of emotional experience and strong networks across some of these categories. Thus, the classic factorial, tree structure needs to be subverted, giving place to new forms to represent the emotional experience.

Precisely, Cowen and Keltner (2017) investigated the taxonomy of emotional experience. In their study, 853 participants viewed a subsample of 30 film clips designed to elicit a wide variety of feelings (e.g., awe, disgust, melancholy). They also innovated in the mathematical framework using canonical correlation analysis in order to characterize the emotional experience as points within a semantic space, distributed along dimensions.

Their results showed that the semantic space is far more abundant than previously thought. Cowen and Keltner (2017) found 27 distinct varieties of emotional experience. This evidence elegantly coincides with the 21 different and consistent facial expressions found by Du et al. (2014). Evidence also showed that there are abundant interrelations between the 27 varieties of emotional experience, revealing continuous gradients between categories, rather than discrete, independent emotions or rigid dimensions of emotions. A simplified representation of the graphic map produced by Cowen and Keltner (2017) can be seen in **Figure 3B**.

These results suggest that it is reasonable to think that complex emotions are the result of new forms of organization of the affect system. For some *additive models of affect*, connections across categories are interpreted as measurement error, but here, I propose that connections across categories can construe subjectively meaningful emotional experiences, which we can accurately call complex emotions. In fact, recent research adds to this claim showing that emotional categories identified by subjects from five different cultures based on more than 2,000 speech samples can communicate at least 12 different categories of emotions, forming a structure connected by different blends of emotions (Cowen et al., 2019), which do not resemble the tree-shaped structure of the affect system.

Cowen and Keltner's findings concerning the observation of fuzzy boundaries across categories also suggest that complex emotions can be forms of *non-additive models of affect*, where the experience of certain complex emotions is a function of different relations among multiple categories of affective experience. Thus, for example, the experience of awe is not merely the result of the linear addition of surprise, astonishment, elevation, and pleasure, but a form of a more complex dynamic which results in an affective experience that is subjectively and physically distinct.

Emotional Complexity as an Emergent Self-Organization Phenomenon

Heylighen (1989) argues that emergence refers to properties of higher order structures that cannot be reduced to their constituents parts. Heylighen (1989) also adds that two critical characteristics of emergent dynamics are self-organization and the hierarchical or multilevel structure of the systems. Notions of hierarchical organization in complex systems and how this applies to emotional complexity have been already explained.

Self-organization, on the other hand, is understood as a spontaneous process of organization (Heylighen, 1989). Spontaneous means that multiple control systems reduce a state of chaos (i.e., disorganization) *via* continuous negative feedback loops among multiple systems (Guastello, 2002). An example of this is a flock of birds moving over the sky – they can form a qualitatively stable structure (i.e., shape) by multiple feedback loops among the individual animals; from an apparent state of chaos emerges a qualitatively stable structure that does not require cognizant awareness of the relationships between individuals and the collective.

Introducing the idea that emotional complexity can be understood as an emergent self-organization phenomenon departs from the fact that when experiencing complex emotions, the emotional lexicon is increasingly integrated, facilitating the emergence of *sui generis* emotional expressions. Thus, experiencing complex emotions may result in new verbalizations of emotions, uncommon in the emotional lexicon (e.g., mixed feelings), or the combination of multiple emotional adjectives into one single experience such as *awe*. Like in the case of the flock of birds, in emotional complexity, emotional words no longer reflect the current state of feelings, but they are mixed, combined, or intertwined in such a way that a new structure emerges, with identifiable emotional properties that are more than the single emotional words.

Emodiversity (Quoidbach et al., 2014) is an excellent example of complex emotions that follows principles of self-organization. Quidbach and colleagues sampled more than 300,000 observations and observed that people vary in terms of the number of emotional experiences they report on a given day. They found that the variety and relative abundance of the emotions people experience is an integral component that distinguishes the emotional experience between individuals. Emodiversity was also found to predict better physical and mental health. Quoidbach et al. (2014) used Shannon entropy equation to quantify the degree of emodiversity for each participant in the study. Shannon entropy quantifies the probability distribution of a set of elements (i.e., emotions; Ladyman et al., 2013). Therefore, emodiversity is a quantification of the chaos in a system, and the subsequent stability that can emerge as a result of the probability distribution of the components.

Other studies also suggest that complex emotions may be emergent self-organization phenomena. For example, Berrios et al. (2018a) showed that indices of mixed emotions are positively correlated both with positive and negative single emotions. These data reveal a discontinuity from the typical negative correlations observed between positive and negative emotions. Similarly, Kreibig et al. (2013, 2015) demonstrated that the experience of mixed emotions is generally accompanied by specific patterns of activation of facial muscles that combined oppositely valenced emotions (e.g., amused and disgust), and these patterns are different from those observed in single emotion expressions. Again, the whole is greater than the sum of the parts, suggesting that it is possible to interpret complex emotions as emergent self-organization phenomena.

CONCLUSIONS

Research and theory of emotional complexity have produced relevant data and innovative propositions that challenge traditional conceptualizations of the affect system. However, important tenets of the concept of emotional complexity itself have not been sufficiently addressed.

Previous efforts to theorize on emotional complexity have tried to bond a standard definition (Lindquist and Barrett, 2010), but they have not considered several recent phenomena studied (e.g., meta-emotions, emodiversity). Other scholars have tried to synthesize the diversity of definitions creating a conceptualization that takes into account specific features of emotional complexity (e.g., interrelations between emotions, differentiation, temporal dynamics of emotions; Grühn et al., 2013; Grossman et al., 2016). Still, there are phenomena such as meta-emotions and aesthetic emotions that have not been integrated into a unified framework, and no previous conceptualization of emotional complexity has explored the emotional and complex features of emotional complexity.

As a result, the goal of the present conceptual analysis was twofold. Firstly, I have tried to integrate the diversity of emotional complexity by describing the various phenomena associated with this construct. Secondly, I have disentangled the specific emotional components and complex features of emotional complexity. This conceptual analysis can, then, further a research agenda and facilitate future research initiatives and current studies using emotional constructs in situations where it is feasible to observe complex emotions.

Relevant available research was used to justify two main propositions. First, I sustain that emotional complexity entails some specific emotional signatures that are common in well-established definitions of emotion. Emotional complexity includes particular facial and physical reactions. These facial and physical reactions are better characterized by the complexity of the emotional experience, rather than by the single emotional constituents of a complex emotion. Furthermore, several studies point to the idea that emotional complexity is functional to situations involving paradoxical or puzzling information that individuals need to process. This claim anchors in the observation that several complex emotions include the confluence of multiple appraisals, which is also accounted for by the complex experience itself.

Second, so far, research and theory on emotional complexity have not sufficiently explained the complex nature of complex emotions. In order to commence to remedy this situation, I suggest that it is possible to describe emotional complexity using concepts and methods from the complex systems theory.

Specifically, emotional words are essential elements that can form different structures and organization systems. The simplest, observable subsystem is the traditional tree-shaped structure obtained using factorial analysis. This structure determines specific properties (e.g., valence) that accurately describe several of everyday emotional experiences.

However, as suggested by Simon (1977), other structures can be described, which not necessarily resemble the observable system. Thus, the same single emotional adjectives can be related in multiple forms, producing other subsystems. Recent evidence supports this idea showing that the emotional lexicon can produce 27 categories based on multiple interrelations (Cowen and Keltner, 2017) and that the structure of affect is connected by various emotional blends between categories (Cowen et al., 2019).

The organization of the affect system in the form suggested by Cowen and Keltner (2017) permits to sustain that several complex emotional experiences are feasible to be located in this new representation of affective life. Mixed emotions (i.e., the co-activation of a pair of oppositely valenced emotions); meta-emotions (i.e., the activation of two emotions, where one emotion is used as an object to experiencing a secondary emotion); and awe (i.e., the co-activation of more than one emotion, generally opposite in valence) are genuine feelings that subvert the common tree-shaped representation of affect. In all these examples (as well as in most of the phenomena commented in the present conceptual analysis), the complex emotional experience, as a whole, is greater than the constituents, single emotions.

Suggestions for Future Research on Emotional Complexity

Although the general consensus between specialists acknowledges the existence of emotional complexity as an overarching concept (e.g., Lindquist and Barrett, 2010), research is still scattered and not neatly integrated. One of the challenges for researchers interested in emotional complexity is to adhere to some basic conceptual tenets, susceptible to be tested in future research. The general expectation of this conceptual analysis is to contribute to the efforts to consolidate a unified field of study on emotional complexity, which can reinvigorate the interest of researchers in the intricacy of our emotional life.

In this regard, three suggestions can be offered. First, it is necessary to determine the differences or equivalence between complex emotions. For example, it has been suggested that mixed emotions may be a form of meta-emotions (Russell, 2017). Although these concepts are conceptually and experientially different (i.e., mixed emotions involve the co-activation of two oppositely valenced emotions, whereas meta-emotions involve an emotion used as a trigger of a secondary emotion), distinguishing between these phenomena may provide new tools to apply complex systems theory in the study of emotional complexity.

Future research should better justify the applicability of the emotional complexity phenomena in the context of specific research. For example, regarding the differentiation of mixed emotions and meta-emotions, it is vital to justify under which circumstance people are more probable to experience mixed emotions or meta-emotions (if they are different). Mixed emotions have mostly been observed in the context of goal conflict (Berrios et al., 2015a, 2018b), whereas meta-emotional experiences are more likely when people are paying more considerable attention to emotion (Bailen et al., 2018). These previous studies may suggest that mixed emotions are more instrumental experiences that respond to conflicting demands or expectations, whereas meta-emotions are more self-monitoring experiences that result from appraising our behavior. This type of distinction is still awaiting further research, but generally, researchers should devote more efforts when justifying the pertinence of the chosen complex emotional phenomenon.

The second suggestion derived from this conceptual analysis is a call for researchers to test for complex emotions when conducting studies involving situations or manipulations where it is feasible to observe some form of emotional complexity (i.e., situations or manipulations with increasing levels of paradoxical information). Although perhaps required in the near future, it is not currently necessary to implement sophisticated calculations to evaluate the influence of complex emotions on some outcomes. For example, when a researcher uses emotional adjectives to measure an affect-related construct, it would be feasible to examine the impact of some complex emotions, such as mixed emotions, if the situation or experimental manipulation involves conflict between goals (e.g., Berrios et al., 2015a).

Furthermore, introducing complex emotions as covariates is a highly recommended practice in future studies. The simplest form to include complex emotions is by testing the interaction between positive affect and negative affect in the model with covariates. If a significant effect is found or it turns out that the interaction acts as a confounder, this may suggest that a more complex feature of emotional experience is in place, which can stimulate the curiosity of researchers interested in the complexity of the emotional experience. Another form to test for complex emotions is to compute simple indices of mixed emotions, such as the minimum value (Schimmack, 2001). Finally, it could be possible to test for individual differences in emodiversity in the model, which can be implemented following the guidelines and code provided by Quoidbach et al. (2014).

Finally, in order to advance models based on the complex systems theory (Simon, 1977; Ladyman et al., 2013), new methods and techniques are needed. Important steps toward this direction have been taken by Cowen and colleagues (Cowen and Keltner, 2017; Cowen et al., 2019), who by implementing new mathematical techniques have shown new forms to represent our affective life, beyond the tree-shaped structure.

One alternative might be to describe interrelations among emotional adjectives as different forms of simplices. In algebraic topology, an n-simplex is a generalization of a geometric space to n dimensions (May, 1999). Simplices describe the structure of a group of elements, such that a simplex $n = 0$ is a single node, $n = 1$ is a straight line, and $n = 2$ is a triangle. Thus, for example, combinations of n-simplices without recursive paths might describe the structure of meta-emotions, where

the boundary of the paths is the difference between each pair of emotional adjectives. Here, one emotion must follow another one in an orderly sequence, similar to the experience of meta-emotions. Mixed emotions, on the other hand, could be represented as cycles, such that when the boundary of the paths of *n*-simplices equals zero, the structure is a cycle, involving recursive paths between emotional adjectives. The recursive paths reflect the interdependence of emotional adjectives of opposite valence, without a given order.

Similar propositions have been advanced by Heylighen (1989). He noted that the study of emergent self-organized systems could be represented using algebraic transformations of groups of relations, labeled as types of closures. Heylighen (1989) proposed four types of closure: recursive, cyclical, surjective (many-to-one), and inverse surjective (one-to-many). Moreover, the combination of them may result in several network topologies.

Overall, the application of complex systems theory to the study of emotional complexity is both intuitive and challenging. Emotional features identified in this conceptual analysis suggest that it is possible to distinguish complex emotions as experiences where the whole is greater than the sum of its parts. However, implementing new approaches and methods to explore the underlying structure of the affect system still need further research (although Cowen and Keltner, 2017). In any case, future research in emotional complexity may need to substitute classic models of affect, and start to explore the benefits of complexity when studying the emotional life. It is necessary to raise attention of researchers investigating emotional processes about the potential benefits of studying emotional complexity for gaining richer information of our current models of affect. Emotional complexity should not be considered as random variance without a fair examination guided by informed research and theory.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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So, It's Pricier Than Before, but Why? Price Increase Justifications Influence Risky Decision Making and Emotional Response

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In this paper, we investigated how justifications for price increases are associated with risky decision making and emotional responses. Across two studies with paired lottery choices and sequential decisions, we found that participants presented with a justification for price increases based on increasing demand decided to invest in a comparatively riskier asset more often than participants presented with a justification for price increases based on increasing tax or those presented with no justification at all. We also found that participants presented with justifications for price increases based on increasing demand also reported higher arousal and displayed higher galvanic skin response than people in the other two justification conditions. Together, these studies provide evidence that only the increasing demand condition underlying a price increase of a risky asset can influence the decision to buy and suggests that emotional activation has a crucial role in such a decision process.

Keywords: emotion, decision making, economic bubbles, framing, risk

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INTRODUCTION

Baijiu, a strong and pungent liquor, is the most popular distilled spirit in China. Suppose you've never had baijiu and you happen to read a news article mentioning how baijiu prices have been rapidly increasing in the last couple of months. The next day you are at a bar waiting for some friends and the bartender suggests you try their recently imported baijiu. Would you go ahead and buy a drink of the exotic alcoholic beverage?

Now consider two sets of reasons the article could mention to justify price increases. On the one hand, it could be that prices had been going up because baijiu had suddenly become a popular drink in the domestic market and many people were demanding it. On the other hand, it could be that the prices had been increasing because of recent import tariffs levied on the product in order to protect domestically produced spirits. In which situation do you think you are more likely to buy this drink? When prices go up because of demand or when the price is determined by the taxes set on it? Would you be indifferent to reasons and base your decision only on the price, taste, etc? The reason for the price increase of a product could potentially carry implicit information about the desirability of the product itself, for example through the social reputation of a decision maker (Schöbel et al., 2016). In this paper we examine the extent to which certain reasons for price increases influence a product's desirability and consequently how these reasons are linked to risky decision making.

We believe differences in price justifications might be responsible for the onset of phenomena like economic bubbles on an individual level. Economic bubbles are evidenced when the underlying assets or goods can be considered investments, that is, when they can be resold. As such, the increasing price of an acquired commercial asset that experiences continually increasing demand implies the possibility of realizing a profit when sold in the market. When an economic bubble expands, the justification for the price increase of the asset in question typically involves a component of increasing demand (Shiller, 2002). This usually triggers a positive emotion in the buyer driven by the prediction of higher gains, which further fuels the growth of the bubble. Is this justification alone generating a significant influence on the decisions that investors make? Specifically, does an explicit reason for the increase in the price of an asset, regardless of its soundness, affect risk aversion, and does the message conveyed in that reason influence decision making under risk?

During a bubble expansion, more people invest in the underlying asset and everyone has to invest comparatively larger amounts of money as the price of the asset increases. Assuming constant probabilities and risk parameters, such behavior entails a corresponding decrease in risk aversion as stake size increases. This stands in contrast to the evidence regarding greater relative risk aversion with higher stake sizes (Binswanger, 1981; Kachelmeier and Shehata, 1992; Holt and Laury, 2002) and seems to contradict the predictions of prospect theory in the gains domain (Kahneman and Tversky, 1979; Tversky and Kahneman, 1981). Many causes of the "irrational" decision to invest ever larger quantities of money in an expanding bubble have been proposed, including the effect of positive valence emotions (Akerlof and Shiller, 2010), the link between emotional arousal and risky decision making (Bechara et al., 1997; FeldmanHall et al., 2016), the relationship between emotional states and risky decision making (Schlösser et al., 2013; Stanton et al., 2014), as well as social herding models (Baddeley, 2010).

On the one hand, positive valence emotions have been hypothesized to promote peripheral information processing, leading individuals to rely more heavily on factors such as the source of the information compared to its content when making a decision (Petty and Cacioppo, 1986). Furthermore, it has been shown that inducing positive emotions leads to greater risky decision on gambling tasks in the lab (Yuen and Lee, 2003; Chou et al., 2007). Thus, it could be argued that betting that an asset, which has already seen an increase in its price, will continue to see its value grow could potentially result in arousal and positive valence emotions that lead investors to comparatively risky decision making. It has also been proposed that emotional arousal is linked to risky decision making (Loewenstein et al., 2001) with empirical evidence to support the notion that greater emotional arousal is tied to increased risky decision making in the lab (Figner et al., 2009). On the other hand, herd behavior has been identified as a major contributor of investment decision making in commodities markets, crowdfunding platforms, information security markets and stock markets (e.g., Demirer et al., 2015; Lei et al., 2017; Papapostolou et al., 2017; Vo and Phan, 2017; Shao et al., 2019). Herd behavior models suggest that people follow others and imitate group behavior rather than make individual investment decisions based on their own private information.

Within financial markets, such herd behavior generates instability and episodes of speculation that can transform into bubbles (Baddeley, 2010). If the reason for a price increase carries implicit information of the behavior of others, it could potentially trigger herd behavior. Moreover, it has been hypothesized that the underlying mechanism driving herd behavior is emotional in nature. More specifically, emotional contagion (Hatfield et al., 1993), has been postulated as a possible trigger of herd behavior of various social phenomena including economic bubbles (Kameda et al., 2015), and Baddeley (2010) has argued that herding in humans can be conceptualized as a proximate mechanism motivated and precipitated by emotional responses. Furthermore, within a neurobiological framework of value-based decision making, herd behavior may constitute a habit valuation system than competes with goal-directed valuation systems aimed at maximizing profit (Rangel et al., 2008) as in all real-world decisions. Additionally, the inherent risk and uncertainty involved in economic bubble contexts act as modulators of the habit and goal-directed valuation systems that ultimately play a vital role in financial decision making (Rangel et al., 2008).

However, the effect of the justification for the price increase has not been considered as a major determinant of risk preferences in an environment with progressively increasing prices (as in expanding bubbles or inflationary periods). Such a justification would act as a framing effect (Tversky and Kahneman, 1981). Moreover, even though the effect of emotions on economic behavior has been analyzed via physiological measurements (Crone et al., 2004; Smith and Dickhaut, 2005), the relationship between price increase justifications and emotions has not been investigated and research on how the reason for a price increase could affect economic decisions and influence emotional responses has not been conducted in an experimental setting. This issue has several economic applications; its implications for decision making during financial bubbles are discussed here.

This paper studies behavior in paired lottery choice experiments to test whether specific price increase justifications alter individuals' financial decisions under risk. Specifically, we are interested in whether changes in the justification for the increase in prices during a series of sequential decisions affect risk aversion and emotional response. We hypothesize that people who are told that prices are increasing because people are buying more of a given asset will take more risks than those who read that prices are increasing due to an alternative reason or are not given a reason for the price increase (Hypothesis 1), and people who read that prices are increasing because people are buying more of a given asset will have a greater emotional response than those who read that prices are increasing due to an alternative reason or are not given a reason for the price increase (Hypothesis 2). Raw data for both experiments is included in the **Supplementary Tables**. All materials, data sets and codes for analyses are available in the Open Science Framework Repository[1].

[1]https://osf.io/uexh5/

This study was carried out in accordance with the recommendations of the ethical committee of the Faculty of Social Sciences of the Universidad de los Andes, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

EXPERIMENT 1

Materials and Methods

A total of 162 students from several universities in Bogotá (Colombia) were recruited primarily through informal announcements such as Facebook, word of mouth and email. Mean age of participants was 23.2 (SD = 4.96) and 58% were female.

This study was conducted online over a period of 3 weeks using a Qualtrics interface. In the instructions, participants read that they would have to make a series of economic decisions over 10 rounds. They were also informed that one of the participants would be randomly selected and that this person would be paid the amount of money from one of their 10 rounds (also selected at random) by the researchers conducting the experiment (this same payment protocol was followed in Experiments 1 and 2). This setup was based on the paired lottery choice decisions task first designed by Holt and Laury (2002) to yield a measure of their risk preferences. Although the task was inspired by the original Holt and Laury risk preference task, the expected value of the products over the 10 rounds does not follow the same tendency observed in the original task (where the expected value of the risky option eventually surpassed that of the safe option) because the goal of this study was to isolate the effect of the price increase justification on risky decision making. Therefore, in this case, the task involved a decision between investing in product W which offered a lesser amount of money with complete certainty (100% probability) and investing in product X, which offered a substantially larger amount of money with less certainty (20% probability). At every round, the decision involved greater amounts of money than in the previous round for both products, such that the amounts at stake increased sequentially over the 10 rounds even though the probabilities remained constant (the payment matrix increased sequentially in an exponential manner to resemble price increase behavior in economic bubbles). Crucially, the safe option (product W) always offered a greater expected value that the risky option (product X) in each of the 10 rounds. Additionally, the amounts in question were considerable; by round 10 the potential gain of product X equaled almost half the minimum monthly wage in Colombia (which at the time of the study was about USD 220) and the sure gain of product W equaled about three times the minimum daily wage in Colombia (which at the time of the study was about USD 20). In all three conditions, participants received no feedback regarding the outcome of their investment decisions across all 10 rounds. Outcome information was purposefully withheld in order to avoid the potential influence of a win or a loss in any given round on posterior decision making. This allowed us to isolate the effect of the justification for the price increase on decision making.

Participants were randomly assigned to one of three groups; two treatment conditions and a control condition. Each of the treatment conditions consisted of a different justification regarding the reason for the price increase of product X as participants moved from round 1 to round 10. In the control condition no justification was provided. In all three conditions the question in round 1 was the same: "In which of these two products do you prefer to invest?" Likewise, in the instructions for all three conditions it was never stated that the participants would be playing with or against each other. Furthermore, the justifications between rounds in all three conditions included no explicit statements that could lead participants to believe that the information provided corresponded to other participant's behavior.

The first condition was the "Increasing Demand Justification" group. Participants assigned to this treatment (N = 52) were instructed to complete the series of 10 paired lottery choice decisions, but between round 1 and round 2 they read the following: "More people are buying product X. The demand for product X has increased, therefore, the prices for product X have increased for round 2." Between all other rounds, they read the following: "Even more people are buying product X. The demand for product X keeps increasing, therefore, the prices have increased for the next round." The purpose of this between-rounds frame was to provide a reason for the price increase based on the fact that more people buying the product was increasingly pushing demand upward and hence generating a price hike.

Participants assigned to the second condition (N = 53), "Increasing Tax Justification," completed the exact same paired lottery choices over the same 10 rounds with the same probability and amount parameters. However, instead of the increasing demand message between rounds, they read the following between round 1 and round 2: "The government has decided to impose a tax on product X. The cost of product X has risen, and the prices have therefore increased for round 2." Between all other rounds, they read the following: "The government has increased the tax on product X. The cost of product X keeps increasing, therefore, the prices have increased for the next round." The purpose of this between-rounds frame was to provide an alternative justification for a price increase that could be plausible but that, at the same time, did not involve an explicit rise in demand (as in the first treatment group) nor an implicit rise in demand (such as might be inferred by participants in the case of an inflation or a supply reduction).

Finally, in the control condition ("No Justification"), participants (N = 57) completed the lottery choice questions over the 10 rounds but were given neither the Increasing Demand nor Increasing Tax justifications between rounds. Nevertheless, exactly like in the other two conditions, the sequential increase in price over the 10 rounds was made explicit.

Once participants finished the 10 rounds, they were presented with a series of eight statements, randomly presented, and they had to report how much they agreed with each statement on a Likert scale within the same Qualtrics interface. These eight items constituted a self-report of emotional response experienced during the decisions they had just made. These statements were constructed based on the Risk as Feeling hypothesis (Loewenstein

et al., 2001), which posits that emotional reactions to situations that involve risk tend to diverge from the cognitive assessments of those risks and that, in such cases, emotional responses often drive behavior. The purpose of these items was to probe for self-reported emotional response at the time of decision making. They included statements explicitly associated with emotional states, but also with risky situations associated with positive emotions (Schonberg et al., 2011). The eight items closely followed the statements utilized by Figner et al. (2009) to assess self-reported decision strategies and self-reported decision-related emotional response in risky decision making. Specifically, participants answered how much they agreed with the following statements: "I made the decisions intuitively," "I made the decisions mathematically" (reverse coded), "I felt enthusiasm making the decisions," "I felt euphoria making the decisions," "The feeling while making the decisions was similar to driving a car at high speed," "The feeling while making the decisions was similar to gambling in a casino," "Decision making was a pleasant experience," "Decision making was difficult."

Results

To measure the degree of risk aversion we observed how many times each of the participants chose the risky product (product X) instead of the safe product (product W) over the 10 rounds. Each of these was considered a risky decision. Then the number of risky decisions made by all participants in all three groups was directly compared.

In **Table 1** and **Figure 1** we report the number of risky decisions (out of 10 possible decisions) that participants made under each of the treatment frames and the control group. Levene's test confirmed a slight violation of the homogeneity of variance assumption [$F(2,159) = 3.05$, $p = 0.05$] and we therefore report the Brown-Forsythe statistic for the variance analysis and performed *post hoc* comparisons.

The corresponding 3 (Condition: Increasing Demand, Increasing Tax and No Justification) X 2 (risky and not risky) ANOVA, estimated with the Brown Forsythe Correction, indicated a main effect of frame over the number of risky decisions $F(2,145.10) = 6.98$, $p = 0.001$, $\eta^2 = 0.08$. A *post hoc* contrast test (Bonferroni correction) indicated that adding a justification (Increasing Demand or Increasing Tax) significantly increased the number of risky decisions that participants made $t(144.68) = 3.05$, $p = 0.001$, $d = 0.46$. The Increasing Demand Justification significantly increased the number of risky decisions vs. the No Justification condition $t(91.92) = 3.69$, $p < 0.01$, $d = 0.61$, but there was no significant difference between the Increasing Tax and No justification conditions $t(91.92) = 1.3$, $p = 0.19$, $d = 0.28$. Crucially though, the Increasing Demand Justification frame increased the number of risky decisions compared with the Increasing Tax Justification $t(101.29) = 2.27$, $p = 0.02$, $d = 0.43$. Additionally, the linear tendency, which indicates that the frame affects the number of risky decisions is both significant and in the expected direction $F(1,159) = 7.01$, $p < 0.001$. A sensitivity analysis was run [with the software G*Power (Faul et al., 2007)] to identify the boundary conditions of our inferences. More precisely, we ran a sensitivity analysis which allows the user to determine the plausible effect size given an alpha level, sample size and desired power. This analysis revealed that assuming a power of 95%, with an alpha level of 5% and our sample size, our study has a minimum detectable effect of $f = 0.3$. We also computed the ν statistic as a way to estimate the accuracy of our estimates (Davis-Stober and Dana, 2014; Lakens and Evers, 2014) independently of the observed significant result. The ν statistic has also been suggested as an indication of the replicability of a finding (Davis-Stober and Dana, 2014), when considered as a measure of the stability of an estimator. Given that we estimated three parameters with the ANOVA model, with an observed r^2 of 0.08, this results in a $\nu = 0.73$. This statistic varies in a range from 0 to 1, where 1 indicates a high stability of the model parameters and an increased chance of replication.

In addition, we found a clear difference in the tendency to choose the risky product over the 10 rounds (**Figure 2**). In particular, in each of the 10 rounds, the absolute number of participants who selected the risky option in the Increasing Demand Justification frame was greater than in the Increasing Tax Justification and in the No Justification frames. Likewise, in the Increasing Demand Justification frame, the percentage of participants who selected the risky product increased constantly over 4 continuous rounds (between round 4 and round 8), one more round than in any of the bullish runs exhibited by participants in the other two frames.

To appropriately capture the dynamic nature of the decision-making process in this situation, we fitted a lagged dependent variable regression model so as to account for prior values of the dependent variable (e.g., choice in the previous round).[2] This model allows us to examine the predictive value of the condition, conditional on given knowledge of the previous choice (Finkel, 1995). As expected, prior choice was a good predictor of subsequent choices ($\beta = 0.47$, $p < 0.05$) and there was a significant difference between the increasing demand frame and the control condition ($\beta = 0.46$, $p < 0.05$) but not between the tax frame and the control condition ($\beta = 0.11$, $p = 0.41$). Given that participants only received information regarding the Justification for the price increase after round 1, the decisions participants made in the first round could not have been affected by the justifications (or lack thereof) for price increases. Therefore, we also fitted the model without the information from round 1 (i.e., taking into account only the decisions participants made in rounds 2–10) and, in this case, differences between experimental conditions and control conditions persist in the presence of the lagged variable.

TABLE 1 | Means, medians and standard deviations, for risky decisions.

Condition	Mean	Median	*N*	*SD*
Increasing demand	4.21	4	52	3.41
Increasing tax	2.77	1	53	3.05
No justification	2.09	1	57	2.46

SD indicates standard deviation.

[2]We are very grateful to the reviewers for this suggestion.

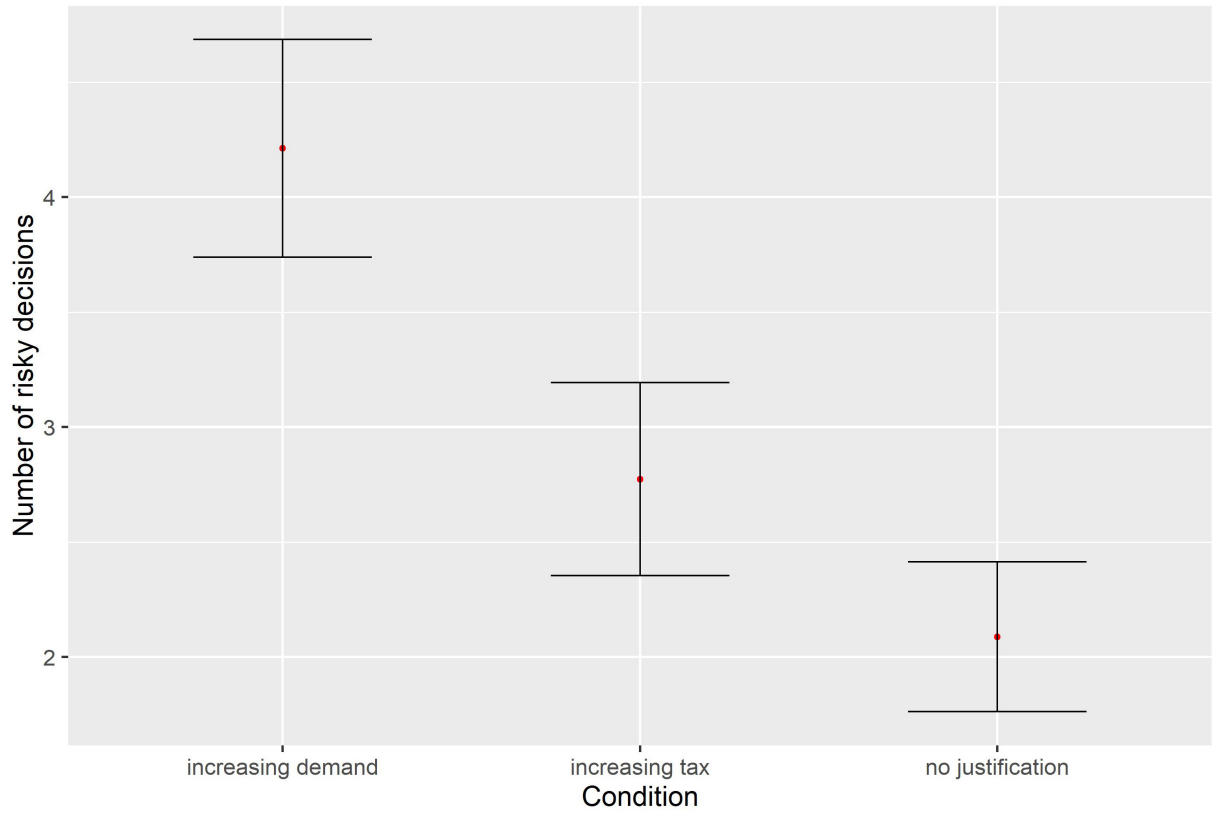

FIGURE 1 | Mean number of risky decisions per condition. Error bars represent standard errors.

The self-report emotion questions were analyzed in conjunction and an indicator of emotional response was constructed based on an average of the 8 questions (Cronbach's $\alpha = 0.66$, including our reverse coded item). As can be observed in **Figure 3**, the emotional response indicator reflected a tendency in the expected direction (i.e., greater emotional response for those participants who made the decisions under the Increasing Demand Justification group compared to other two frames) but the corresponding analysis of variance was not significant $F(2,159) = 1.69$, $p = 0.19$, $\eta^2 = 0.02$. We also performed an exploratory factor analysis on the self-report questions, to verify the assumption of a single underlying latent factor. This analysis suggested the presence of two factors, where one of them seems to tap more directly into emotional arousal and comprises only three of the eight questions (*enthusiasm*, *euphoria* and *feeling driving a fast car,* Cronbach's $\alpha = 0.73$). A one way ANOVA with this alternative indicator of emotional arousal is consistent with the previous analysis and fails to identify an effect of the Condition [$F(2,159) = 2.41$, $p = 0.09$, $\eta^2 = 0.03$], while exhibiting the same pattern of differences presented in **Figure 3**. Results with the average of the questions of the second factor, seemingly related to emotional valence, do not follow a similar pattern and result in a very different outcome when submitted to an ANOVA ($F < 1$).

However, we did find an important difference in the percentage of participants that made the decisions in both justification frames who reported increased emotional arousal (Increasing Demand = 54%, Increasing Tax = 51%), compared with the percentage of participants that made the decisions in the No Justification frame who reported increased emotional arousal (33%). Similarly, the percentage of participants that made the decisions in both justification frames reported feeling more of a pleasurable emotion (Increasing Demand = 40%, Increasing Tax = 45%) compared with the percentage of participants that made the decisions in the No Justification frame (32%). These differences do not reach statistical significance ($\chi 2 < 1$) but constitute a clear pattern suggesting a connection between risk and emotion.

Discussion

The results of experiment 1 suggest that information regarding the quantity of people buying a given asset seems to drive risky decision making more than information regarding taxation of that same asset. We observe this effect while keeping constant the probabilities and the expected value associated with the decisions. Moreover, given the results of study 1, it is reasonable to assume that people are also sensitive to the information itself, even limited impoverished information such as the one we used in this study. The information (the justification) seems to influence people's decisions to invest in a particular asset beyond the price and the perceived utility. As can be evidenced in **Figure 2**, there was a drop in risky decision making in the final two rounds for

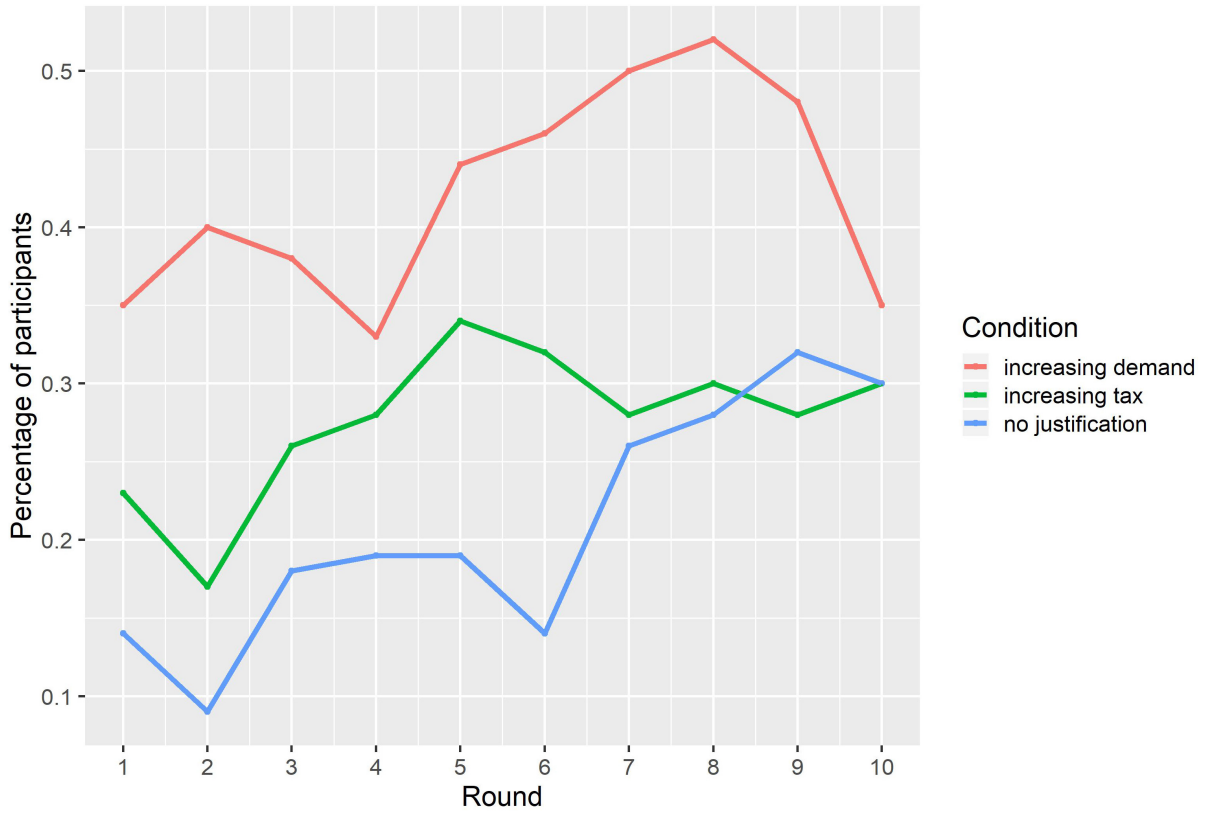

FIGURE 2 | Percentage of participants who selected the risky option in each round.

the Increasing Demand condition. Given that participants knew *a priori* that the task would run for a total of 10 rounds, it could be argued that this drop reflects an effort to compensate for the elevated risky decision making in earlier rounds compared to the other two conditions. Nevertheless, there is an observed risk seeking tendency in the three conditions as participants progressed through the 10 rounds (not including the last rounds of the treatment condition mentioned above). This tendency could be explained by an anticipated regret (Zeelenberg, 1999) for missing out on a large gain (i.e., from not choosing the risky option), especially given the fact that they received no sequential outcome feedback and would potentially realize the gains of only one out of their 10 choices. This characteristic of our experimental paradigm does not allow us to model how sequential time periods influence relative risk aversion as they would in a real-world economic bubble, but it does allow us to examine how the justifications for the price increases affect risk aversion.

In experiment 1, emotional response was measured with a set of questions aimed at measuring both emotional arousal and emotional response. Failure to detect differences in emotional response could have been caused by the inherent ambiguity of these questions. Namely, it is possible that the questions failed to describe the quality and quantity of the emotional response participants experienced. In order to tackle this issue in experiment 2, we used galvanic skin conductance as a proxy of emotional arousal (Figner and Murphy, 2010; Starcke et al., 2010) and the Self-Assessment Manikin (Bradley and Lang, 1994) for emotional valence.

EXPERIMENT 2

Materials and Methods

A total of 39 undergraduate students from a large private university in Colombia were recruited via word of mouth. Mean age of participants was 21.79 (SD = 3.03) and 59% were female. Sample size was determined based on the availability of lab resources and it is comparable with studies using similar techniques (Bechara et al., 1997; Krosch et al., 2012).

As in Study 1, participants were randomly assigned to each of the three same frame groups of the first study (Increasing Demand Justification, Increasing Tax Justification and No Justification). All participants were fully informed of the details of the procedure, which took place in the lab, and received a chocolate bar after signing a consent form as compensation for participating. Moreover, as in the first study, participants were informed that one of the participants from study 2 would be randomly selected and that this person would be paid the amount of money from one of their 10 rounds (also selected at random) by the researchers conducting the experiment. All participants made the same series of 10 decisions in a computer in the lab

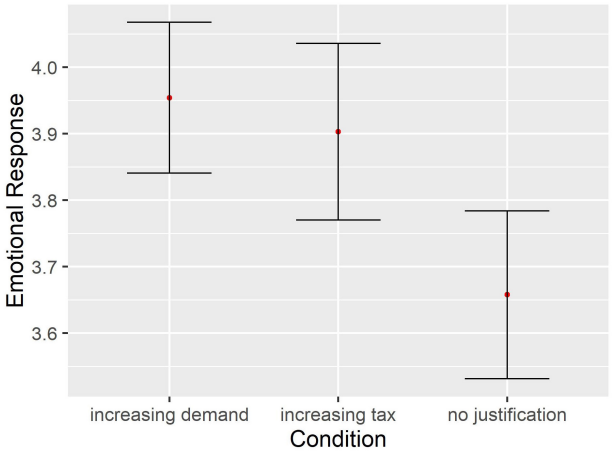

FIGURE 3 | Self-report of emotional response. Error bars represent standard errors.

while connected to two electrodes on their non-dominant hand. We decided to use Galvanic Skin Response (GSR) as a proxy of emotional arousal. GSR is used to study affective processes because the autonomic nervous system plays a significant role in emotion and motivation. Changes in electro-dermal activity and skin conductance are related to changes in eccrine sweating, which are in turn related to activity in the sympathetic branch of the autonomic nervous system. Accordingly, GSR measures have been used to study psychological processes related to sympathetic arousal in general (Figner and Murphy, 2010), and in tasks evaluating risky decision making in particular (Jenkinson et al., 2008; Figner et al., 2009).

We used the Biolink 1100 hardware and AcqKnowledge 1100 software to analyze galvanic skin conductance variations of participants. In order to establish a baseline skin conductance reading, all participants were first presented with the same relaxing landscape video for a period of 3 min and 30 s. Following the video, participants read the experiment's instructions and proceeded to make their decisions over the 10 rounds. Afterward, all participants selected a visual self-report query whose aim was to establish emotional valence. This query was constructed with Qualtrics based on the Self-Assessment Manikin first elaborated by Bradley and Lang (1994), which has been previously used in a study of a risk aversion in finance professionals (Cohn et al., 2015). Specifically, this question asked them to indicate their emotional state in a schematic representation of a face. The data obtained with this query was numerically coded in a scale from −2 (corresponding to the saddest looking face), −1, 0, 1, and 2 (corresponding to the happiest looking face).

Results

Decision data of this study follows the same trend of our findings in experiment 1. Participants who made the decisions in the Increasing Demand Justification condition made more risky decisions [M = 5.31, 95% CI (3.54,7.08)] than the participants who made the decisions with the Increasing Tax Justification condition [M = 2.77, 95% CI (1.78,3.76)] and more than participants who made the decisions with No Justification [M = 4.0, 95% CI (2.98, 5.02)]. The corresponding variance analysis indicated a main effect of frame over the number of risky decisions $F(2,36) = 4.46$, $p < 0.05$, $\eta^2 = 0.20$. However, a *post hoc* test only indicated a significant difference between the number of risky decisions made by participants in the Increasing Demand and Increasing Tax conditions but found no significant difference between the number of risky decisions of the Increasing Demand and the No Justification conditions. The corresponding visual self-report data of the subsequent question also seems to indicate a marginally greater positive valence emotion for those participants who made the decisions with the Increasing Demand Justification [M = 0.85, 95% CI (0.36, 1.33)] than for those participants who made the decisions with the Increasing Tax Justification [M = 0.69, 95% CI (0.12, 1.26)] and for those participants who made the decisions with No Justification [M = 0.69, 95% CI (0.24, 1.15)]. Likewise, these differences were not statistically analyzed given the reduced number of participants per condition (N = 13).

For the physiological analysis, the pre-established protocol package of the AcqKnowledge software was utilized in order to calculate dermo-galvanic skin response from the skin conductance raw data obtained with the electrodes. The magnitude of the dermo-galvanic skin conductance was determined by measuring the area under the skin conductance curve (Figner and Murphy, 2010). Every skin conductance session was divided in two phases: a baseline (established with the relaxing video previously mentioned) with a total duration of 210 s and a decision-making phase with an average duration of 200 s. In **Table 2** we report the area under the curve (μS/second) for each of the three frames in both phases.

Two mixed effects models (Bates et al., 2015) were fitted to analyze these data with phase (baseline, task) as a within-participants factor, condition (Increasing Demand, Increasing Tax, No Justification) as a between participants factor, and Dermo-Galvanic Skin Response (area under the curve standardized per second) as a dependent variable. The difference between the models was the inclusion of an interaction term between phase and condition. The inclusion of the interaction term resulted in a significant change [$\chi^2(2) = 11.65$, $p < 0.001$] and better fit (AIC = 1173 and 1165.3, respectively). The fixed coefficient for the interaction between Condition and Phase is significant [$F(2,36) = 6.27$, $p < 0.001$]. This last interaction effect can be visualized in **Figure 4** and clearly indicates that the Increasing Demand frame resulted in a higher arousal compared to the other conditions.

TABLE 2 | Dermo-galvanic skin response.

Frame	Baseline	*SD*	Task	*SD*	*N*
Increasing Demand	154.06	236.41	1083.59	562.67	13
Increasing Tax	272.82	266.01	728.03	465.77	13
No Justification	242.63	290.12	660.48	602.72	13

SD indicates standard deviation.

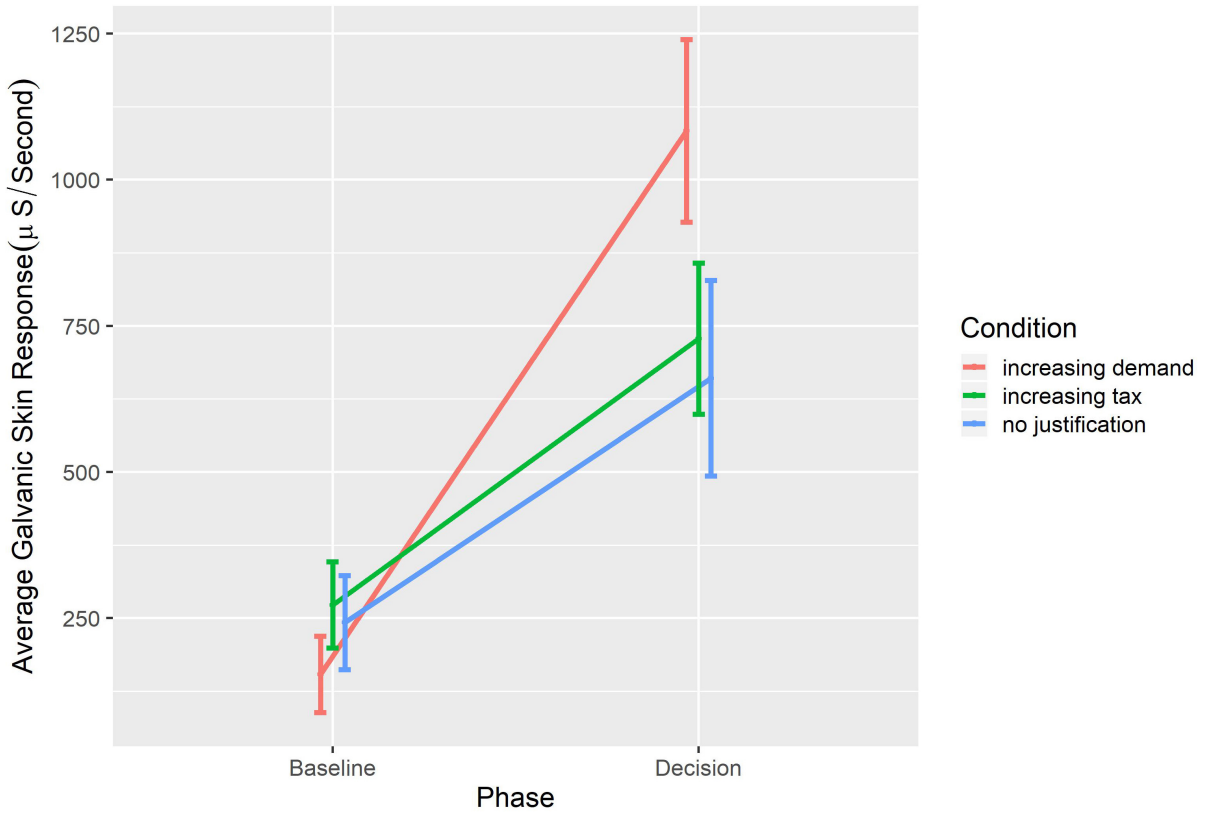

FIGURE 4 | Interaction effect of dermo-galvanic response in both phases for each of the three frames.

Independent ANOVA tests indicated no significant difference in the dermo-galvanic skin response during the baseline phase $F(2,36) = 0.71$, $p = 0.50$, $\eta^2 = 0.04$, nor during the decision phase $F(2,36) = 2.25$, $p = 0.12$, $\eta^2 = 0.11$. However, a paired t-test revealed a significant difference in the dermo-galvanic skin response between the baseline and the decisions across conditions $t(38) = -8.08$, $p < 0.001$, $d = 1.29$.[3] An analysis of variance performed on the galvanic response in the decision phase identified significant differences. Pairwise comparisons indicated that the increase in dermo-galvanic skin response for those participants who made the decisions with the Increasing Demand Justification ($M = 929.53$, $SD = 477.17$) was significantly larger than the increase in dermo-galvanic skin response exhibited by those participants who made the decisions in the Tax Increase Justification ($M = 455.21$, $SD = 383.47$) and larger than that exhibited by the participants who made the decisions with No Justification ($M = 417.85$, $SD = 362.56$), which can be visualized in **Figure 4**.

We fitted a linear model in order to predict the number of risky decisions based on the increase in dermo-galvanic skin response and condition. This model resulted in a significant model [$F(3,75) = 5.33$, $p < 0.001$, $r^2 = 27\%$] with a significant effect of the dermo-galvanic skin response ($t = 2.91$, $p < 0.01$). The number of risky decisions increased in 0.003 with every additional μS/second of dermo-galvanic skin response. There was no effect of condition (increasing demand, $t = -1.3$, $p = 0.21$; $t = 0.9$, $p = 0.37$), but there was an interaction between dermo-galvanic response and condition (see **Table 3**).

We did not find a significant relationship between the increase in dermo-galvanic skin response and the number of risky decisions for subject in the Increasing Tax Justification group ($r = -0.04$, $p = 0.90$), nor for the participants in the No Justification group ($r = -0.01$, $p = 0.97$) (see **Figure 5**).

In order to assess the consistency between reported emotions and physiological response, we analyzed the correlations between self-reported emotions (more precisely, the emotional valence measured via the visual self-report query, labeled as valence from now on) and the general measure of emotional arousal (more precisely, the activation difference in SCR between test and baseline) per condition. This analysis shows that there is a significant strong correlation for the increasing tax condition, but not for the increasing demand condition (the same pattern occurs when considering only the test condition, see **Table 4**). This suggests that as the tax increases, people experience more negative affect,

[3]Notice that this effect size would imply a statistical power of 99% to detect the effect, which is unlikely and suggests the effect size is inflated (Hedges correction for bias gives the same effect size value). Given the exploratory nature of this study, we do not consider it an observation of the true effect, but an estimation of the existence of this effect in a setting with a possibly large sampling error.

TABLE 3 | Mixed effects model for SCR.

Predictor	Estimates	CI	*p*
(Intercept)	242.63	9.07–476.19	0.042*
Increasing Demand	−88.56	−418–241.74	0.599
Increasing Tax	30.19	−300.12–360.49	0.858
Decision	417.85	194–641.14	<0.001***
Condition Increasing Demand: Decision Phase	511.67	195.89–827.46	0.001**
Condition Increasing Tax: Decision Phase	37.36	−278.43–353.14	0.817
Random Effects			
σ^2	84375.45		
$\tau_{00\ Condition:participant_ID}$	17852.10		
$\tau_{00\ participant_ID}$	82388.69		
$ICC_{Condition:participant_ID}$	0.10		
$ICC_{participant_ID}$	0.45		
Observations	78		
Marginal R^2/Conditional R^2	0.374/0.714		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

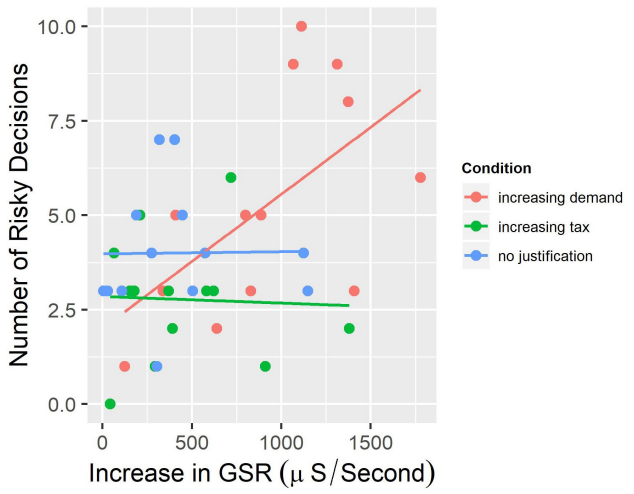

FIGURE 5 | Relationship between the increase in dermo-galvanic skin response and the number of risky decisions by condition.

preventing them from making investing decisions. Thus, emotion arousal fulfills a signaling role in our experimental setting. A linear model with self-reported emotion as a dependent variable, and condition and increase in arousal difference as predictors reveals only a significant interaction between condition and phase. That is, while mean valence does not differ between conditions or as a function of general measure of activation only, for the tax condition the expected mean is lower than for the other conditions only in the presence of the arousal difference $F(2,330) = 4.70$, $p < 0.05$, $\eta^2 = 0.19$.

TABLE 4 | Correlations between self-reported emotions and valence.

Frame	1	2	3
(1) Increasing Demand	0.0771		
(2) Increasing Tax		−0.595*	
(3) No Justification			0.395

*Valence = activation difference in SCR between test and baseline. *$p < 0.01$.*

GENERAL DISCUSSION

The results of these two studies indicate that individuals who read that prices are increasing because people are buying more of a given asset will take more risks than those who read that prices are increasing due to an alternative reason or are not given a reason for the price increase (Hypothesis 1) and individuals who read that prices are increasing because people are buying more of a given asset will have a greater emotional response than those who read that prices are increasing due to an alternative reason or are not given a reason for the price increase (Hypothesis 2).

In study 1, we found that the average number of participants and the average number of risky decisions that these participants made were both considerably higher in the Increasing Demand Justification condition compared with the other two conditions. The fact that the Increasing Demand Justification constitutes, in essence, a narration of herd behavior provides support for the idea that this justification might work as a sort of social influence signaling mechanism that generates a feedback loop in which people become progressively less risk averse. This effect might be even stronger in real economic bubbles where investors can corroborate the veracity of an increasing demand. Herd behavior engenders behavior imitation. Such imitation allows learning of particular behaviors in a quicker and more effective manner than comparable individual learning mechanisms in social environments (Baddeley, 2010). Herd behavior might also work as an instrument for fast information broadcasting concerning the localization and availability of resources (Danchin et al., 2004; Surowiecki, 2004) and even allows for social cohesion, which Simon (1990) considered an evolutionary advantage. In this sense, receptivity to social influence would allow a group of individuals to behave in an altruistic manner, promoting empathy while avoiding selfish behavior. Hence, behavior would therefore contribute to the resolution of social conflicts and help to overcome environmental obstacles.

In study 2, we confirmed that the Increased Demand Justification elicited greater emotional arousal from individuals, inferred from the corresponding galvanic skin response. We also found a correlation between the number of risky decisions and the emotional response. This might indicate that the decision process has a crucial emotional component. Nevertheless, it is impossible to confirm whether the emotional response was triggered by the decision or whether the emotion preceded and (in part or in whole) determined the decision. Regardless, the magnitude of the emotional response is directly related to the justification for the price increase. The crucial role that emotions play in decision making in cases of herd behavior might be better explained as a fast

and frugal heuristic (Gigerenzer and Goldstein, 1996), which provides adaptive benefits in various forms. In this case, emotions would be playing a fundamental role in the preservation of herd behavior and would therefore be valuable from an evolutionary point of view. As Frank (1988) suggests, emotions would be acting as strategic commitment devices forcing individuals to act in an apparently irrational way, but ultimately fulfilling a very important adaptive function. This would be in line with theories of risky decision making driven by emotional responses (Loewenstein et al., 2001), emotional contagion precipitating herd behavior (Kameda et al., 2015), and emotions potentiating risky decision making via herd behavior (Baddeley, 2010).

A vital component of these two studies was that participants never knew whether they had won or lost their money in a given round. In this sense, these two studies manage to discern between the justification framing effect and the potential effect caused by winning and/or losing. Irrespective of their own decisions, the prices of both products kept rising over the course of the ten rounds and participants gained no information whatsoever concerning the results of their decisions. Hence, we can infer that the justification for the price increase alone managed to influence the decisions of the participants.

The next steps to validate our finding include the use of more fine-grained emotion measures. Valence and arousal are only two of the dimensions of an emotion, and recent literature in decision making has highlighted the need to include discrete emotion models in the explanation of decision making (Lerner et al., 2015). Similarly, emotion inductions can shed light on the causal direction of the association detected between price justification and arousal. Likewise, as a complement to the study of positive valence emotions, future research would greatly benefit by separately analyzing the correlation with or influence of distinct negative valence emotions on risky decision making.

There are four main limitations of this study that should be considered in interpreting these results. First, small sample sizes limit the possibility of interpretation of effect sizes. This study should not be read as a confirmatory but as an exploratory study and as such, it is contributing to generating a hypothesis, not as its litmus test. Second, the nature of emotion we have assumed must be considered. With our measures we assume a dimensional nature of emotion (Russell and Barrett, 1999), thus focusing on arousal in experiment 1 and on arousal and valence in experiment 2. These should be more carefully studied and dissociated in future research. Third, our main manipulation. Even though the tax and demand manipulations are equivalent in both their payoff matrices and the linear increases associated, it is possible that participants considered the demand condition to be true within the terms of the experiment but not the tax condition. If this was the case, risky decisions and emotional activation variation would have been a function of the assumed realism of one of the conditions, not of the bubble effect ***per se***. However, in this situation, the differences between the tax and control conditions would remain unexplained. This alternative interpretation highlights, however, that the mechanism that might underlie herd behavior in bubble situations has to do with the perception of a relevant group of reference against which one's risky behavior appears normal. Finally, even though our findings are in line with value-based frameworks of decision making, whereby emotional responses precipitate herd behavior and drive risky decision making, our study is not able to discern a causal relationship between emotional response and risky decision making or herd behavior. Furthermore, recent research has highlighted the importance of task relevance on the relationship between emotional valence and behavior (Mirabella, 2018; Wispinski et al., 2018). Hence, future studies should uncover how emotional valence impacts decision making within sequential monetary choice paradigms, on the one hand, and understand the direction and nature of the relationship between emotion, herd behavior and risky decision making on the other.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethical committee of the Faculty of Social Sciences of the Universidad de los Andes, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. IRB Committee: Comité de Ética de la Facultad de Ciencias Sociales de la Universidad de los Andes. The experiments were approved by the Committee in their session of September 2015. All participants signed and informed consent provided either electronically (experiment 1) or in paper (experiment 2).

AUTHOR CONTRIBUTIONS

JS proposed the hypothesis, conducted the experiments, and drafted the manuscript. Both authors designed the experiments, contributed to data analysis, and approved the final version of the manuscript for submission. WJ-L provided the critical revisions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2019.01883/full#supplementary-material

TABLE S1 | Raw data experiment 1.

TABLE S2 | Raw data experiment 2.

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Role of Emotional Appraisal in Episodic Memory in a Sample of Argentinean Preschoolers

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Emotional processing and episodic memory are closely related throughout childhood development. With respect to emotional episodic memory, available evidence shows that the consolidation of information is accompanied by an arousal that generates longer duration and persistence of the memory representations. In the case of early stages of development (i.e., first 5 years), it is less clear how these associations emerge and are modulated by individual and environmental factors. In this study, 116 4- to 5-years old Argentinean children from different socio-environmental contexts (i.e., favorable or unfavorable living conditions at home), performed a task of visual emotional memory in which they observed a set of 15 images with variable emotional valences (negative, neutral, and positive). The child's task was to appraise each image using one of the following three possible valences: (a) drawings of faces with smiles (positive valence), (b) drawings of faces with tears and round mouth with edges down (negative valence), or (c) drawings of faces with horizontal mouth (neutral valence). Five years-old children exhibited greater accuracy appraisal. Individual differences in emotional accuracy appraisal allowed us to observe different performances in free recall of negative visual images. Accuracy appraisal did not vary between children with respect to gender, living conditions at home, or language ability. Seven to ten days after the emotional appraisal children were asked to tell the experimenter all the images they remembered (variables of interest: free recall of negative, positive, or neutral images). Results showed individual (age) differences. Specifically, 5-years-old children evoked more images than 4-years-old children. These findings contribute to the understanding of emotional memory in early developmental stages and raise the need to include emotional appraisal in the assessment of episodic memory.

Keywords: emotions, episodic memory, appraisal, individual differences, preschoolers

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INTRODUCTION

People's lives are influenced by their experiences. Memory encodes, stores, and allows information to be evoked for future use (Baddeley, 1999). The memory of past events begins to develop early in life and changes throughout the preschool and kindergarten years (Bauer et al., 2012). An example of this is the number of events or items that children can retrieve, the length of that span, and the

association of memory representations and processes on external or internal keys for their retrieval (Bauer et al., 2010; Ghetti and Bunge, 2012).

There are several studies in adults, which emphasize the interaction between memory and emotions (Justel et al., 2013). In this sense, emotional events are better remembered than the most trivial ones (Christianson, 1992; Quas and Lench, 2007; Brainerd et al., 2010). For example, a childhood accident is remembered accurately, but dinner a week ago is unlikely to be remembered. However, the direction in which this differential effect occurs is not always clear. Emotions would function as a filter system of selection of events that are going to be stored in memory in a more lasting way (Rodríguez et al., 2004). Specifically, emotional memory is the result of storing information that was accompanied by stressful and/or activating factors through which that memory could have been fixed more easily. It is a type of long-term memory about information with emotional valence, positive or negative (Bermúdez-Rattoni and Prado-Alcalá, 2001; Justel et al., 2013; Guzmán-Ramos et al., 2018; Siller-Pérez et al., 2018).

Some studies analyzed the factors involved in the consolidation and retrieval of emotional experiences in children (Cordon et al., 2013). Specifically, age seems to be an influential factor in these memories because there are marked differences in the amount of information that children remember and the accuracy with which they do it (Goodman et al., 1994; Merritt et al., 1994; Salmon et al., 2002; Brainerd et al., 2010; Leventon et al., 2014; Langnes et al., 2018). Because of this, age is a reliable predictor of memory performance, both for emotional (Goodman et al., 1997) and non-emotional information (Courage and Cowan, 2008). For example, children who are 4- and 5-years-old can encode and evoke more specific and integrated emotional information than children who are 3-years-old (Goodman et al., 1997; Brainerd et al., 2008; Wang, 2008).

As children develop their linguistic abilities, an increase in their performance in memory tasks occurs, which indicates the importance of verbal language when analyzing changes in memory development. Studies in children of 3- to 5-years old, in which they provided information on events of daily life, found that older children remembered more information than younger ones and used conditional verb tenses in their stories due to the greater development of their language ability (Hudson and Nelson, 1986; Fivush et al., 2003, 2008). In addition to age, the emotions that accompanied the performance during memory tasks can be another modulator. Emotionally significant experiences that occurred during childhood can be retained and remembered for a significant length of time (Cordon et al., 2013). In the same sense, Channell and Barth (2013) found that the accuracy of memories about emotional information was significantly related to the emotional knowledge of the children, suggesting that this association could not be explained simply by maturation, but individual differences also played an important role in memory processing.

When considering possible individual differences in the emotional appraisal, gender could be considered an intervening factor. There are few studies in children considering this variable. However, in adults there is some consensus in the literature about a greater reactivity of women to negative valence stimuli (Lang et al., 1993; Bradley and Lang, 2000). Specifically, a greater reactivity of the amygdala was verified before threatening images in women than in men (Canli et al., 2002). This reaction was also founded in emotional expression recognition in women with anxiety disorders (Ohrmann et al., 2010). In addition, on the assessment of affective images, there is a higher tendency in adult women to value negative images as such than men (Bradley and Lang, 2000). In accordance to these results, various physiological factors (such as skin conductance and shock boosted by fear) suggest that girls aged 7 to 10-years are more sensitive to initiate a physiological defensive mobilization than boys (McManis et al., 2011), because they also tend to value more negative images as such. Regarding development of the emotional recognition of facial expressions in children from 4 to 9-years old, an effect of gender was identified, with more recognition in the girls' performance (Widen and Russell, 2010). In another study on recognition of expressions of varied intensity in children from 4 to 18-years old the same study also founded that girls recognize facial expressions better than men, especially those of anger and disgust (Montirosso et al., 2010).

In relation to gender, ratings of emotional valence and arousal did not differ significantly as a function of participant gender (Cordon et al., 2013). Because of that gender differences did not examined in the children's emotional memory (Leventon and Bauer, 2016). Furthermore, due to gender differences in the socialization of emotion (e.g., Brody and Hall, 2010) and mixed evidence for gender differences in appraisal measured in children (e.g., Dennis and Hajcak, 2009), are necessary to examine gender differences in emotional appraisal and emotional memory expression.

On the other hand, socio-environmental conditions also can modulate the performance of children in learning and memory tasks (Principe et al., 2017). For example, Tessler and Nelson (1994) found that the conversation between mothers and children during a visit to a museum influenced the content of the memories in children of 3-years-old; none of them reported aspects of the visit that had not been discussed. In other study, Haden et al. (2001) organized three events in the homes of 30-, 36-, and 42-months-old children and recorded mothers and children's conversations during shared activities. The children recalled more of the activities that had been discussed with their mothers compared with those that were only spoken of by the mother or that were not discussed at all. In another study, children's memories of external situations with emotional valence (e.g., natural disasters, abuse episodes), which were usually negative, enhanced their performance during a memory task (Haden et al., 2001).

As previously mentioned, negative or positive stimuli are generally processed differently from non-emotional (neutral) stimuli, and they are remembered more clearly over time in adults and children (Bradley et al., 1992; Cahill and Alkire, 2003; Cordon et al., 2013). However, it is not yet clear in what sense the emotional valence of events is associated with memory processing in preschoolers. Emotional appraisal can be defined as the process through which different responses are generated

from the same event, based on subjective assessment of events or stimuli (Nieto and Delgado, 2006; Kuppens et al., 2012; Moors, 2013, 2017; Moors and Scherer, 2013; Fernando et al., 2015; Parsafar and Davis, 2018; Scherer and Fontaine, 2018). People constantly perform emotional assessments of events and stimuli according to what it implies for their own well-being, objectives, and concerns (Kuppens et al., 2012). These appraisal responses are considered the closest psychological determinants of emotions (Ellsworth and Scherer, 2003; Kuppens et al., 2012), so these would be one of the results of the assessment (Ellsworth and Scherer, 2003; Parsafar and Davis, 2018).

There is agreement among several researchers that emotions include different components, such as appraisal, the tendency to act or to cope, behavioral actions, physiological changes, and subjective expressions (Scherer and Fontaine, 2018). In this sense, Russell (2009) proposed a two-dimensional theory of emotion that is composed of appraisal and intensity (valence and arousal) of a stimulus, where various phenomena occurred in each emotional episode (i.e., changes in facial expressions, vocal tone, functioning of the peripheral nervous system, emotional appraisal, behavior, subjective experience, and emotional regulation; Scherer and Fontaine, 2018). The appraisal process would be integrated into emotional knowledge, which also would include aspects such as understanding of emotions, expression of emotions, and the subsequent recovery of that information to be integrated into memories (Channell and Barth, 2013; Fidalgo et al., 2018).

However, children and adults may react differently to the same stimulus (Davies and Logie, 1993; Roseman and Smith, 2001; Nieto and Delgado, 2006; Fernando et al., 2015). This implies, on the one hand, that appraisal is crucial to defining emotional processing and its consequent response rather than the properties of the stimulus itself (Fernando et al., 2015). On the other hand, this also means that this appraisal would be modulated by individual factors (e.g., age, gender, temperament, development of language skills) and socio-environmental factors (e.g., demographic and economic characteristics of the family context) (Davies and Logie, 1993; Fivush and Baker-Ward, 2005; Nieto and Delgado, 2006; Wang, 2008). Despite the increase in the number of studies that address these issues, there are few that focus on the emotional appraisal of visual stimuli in preschoolers and its association with memory processes (Kuppens et al., 2013).

The aim of this paper is to analyze emotions and memory associations through an emotional memory paradigm in preschoolers. In this study, 4 and 5 years old children from different sociodemographic contexts (favorable or unfavorable) were tested in one task to analyze their processing to visual images with emotional information (negative and positive valences) or non-emotional images (neutral valence). Specifically, we analyzed the role of the emotional appraisal accuracy on the later expression of emotional memory. Additionally, we analyzed whether gender, socio-environmental conditions, and the children's language skills modulated their performances in accuracy appraisal. *Accuracy of appraisal* is the variable that can be considered, which arises as a result of the comparison between the children appraisal and the task appraisal as defined by researchers (valence of each stimulus). According to the literature, it is expected: (a) that age and the memory will be associated, that is, that 5-years-old children will evoke more images than those of 4-years-old (Goodman et al., 1997; Cordon et al., 2013); (b) differences in the episodic memory will meet: greater number of images will be recalled for emotional (negative and positive) compared to neutral content (Bradley et al., 1992; Cordon et al., 2013); and (c) modulation of the emotional free recall by the emotional appraisal and the socio-environmental conditions at home (Ellsworth and Scherer, 2003; Kuppens et al., 2012; Principe et al., 2017). With regard to gender (differences between girls and boys) and language skills (low, moderate and high performance) associations with emotional memory performances (based on children's own appraisal, and on task appraisal) will be explored.

MATERIALS AND METHODS

Participants

A sample of 116 children (56 girls and 60 boys) that were 4 ($n = 50$) or 5 ($n = 66$) years old was conformed. The children were students at one of three kindergartens from the Autonomous City of Buenos Aires: two private schools ($n = 54$) and one public school ($n = 62$). The children included in the sample had no developmental disorders in terms of their perinatal and postnatal health history.

Ethical Considerations

Informed consents were obtained from parents/caregivers, and ethical approval was obtained from the Ethical Review Committee of CEMIC (Directorate of Research. Av. Galván 4102, 1st Floor, C1431FWO, Autonomous City of Buenos Aires. Protocol N° 961). The study was conducted according to American Psychological Association ethical standards and international and national children rights laws. Once the authorization was obtained from the school institutions where the activities were carried out, information meetings were organized for parents in which they had the opportunity to interact with the members of the research group, who informed them about the objectives and activities of the study, and then gave them a written information consent with the same content. Consequently, they were invited to participate and to authorize the participation of their children, for which the signing of informed consent for each of the evaluations was a necessary condition.

Tasks and Assessment Procedures

Two tasks with were administered in this study. The first was emotional memory task with two component.

Component A. *Emotional appraisal*. This component assesses the attribution of emotional expressions to emotional images. The instrument consisted of two sets of stimuli. The first set was composed of 15 images with different valences (five negative, five positive, and five neutral) that was obtained from Development Affective Photographs System to children (DAPS; Cordon et al., 2013). All the images were in color, they had people (i.e., children, women), animals (i.e., dogs, cockroach), objects (i.e., book, cup),

or people performing actions (i.e., children playing with a ball, girl jumping the rope). These images were presented in counterbalanced order on a tablet or on a notebook. The second set consisted of three images (in white and black) with emotional expressions (happy, sad, or neutral faces). Children observed the images of the first set and then had to choose one expression to show how they felt when they saw the images. The three images of the faces appeared on a sheet, which the children had available while they watched each image. Each photograph lasted on the screen until the children chose a face, and decided to move forward with the images, that is, the time varied among the children, but lasted approximately 2 s. The faces with emotional expressions were available all the time for the children to decide which one to choose. The participants did not have a time limit to decide. The choice was made by pointing with one finger, one of the three faces with emotional expressions. Before beginning the task, the participants observed two example images. The variable of interest was children appraisal response to images that had different valences (negative, positive, or neutral). This variable can be defined as the attribution of valence by each child to each visual stimuli with different emotional contents.

Component B. *Emotional memory*. This component consisted of two phases. First, children were asked to evoke immediately (immediate emotional memory) the appraised images of set 1 described above (this variable was obtained to control that children codified the visual information, but it was not incorporated as a variable of interest in the study). Then, after a delay of 7–10 days, children were again request to evoke those images (emotional memory test). The memory test consisted in the evocation of visual information through free recall. Children said words or phrases about images that they observed during the task. The variables of interest were the free recall of negative, positive, or neutral images, which was defined as the number of images of each type evoked after a time interval since the occurrence of the task presentation.

The second task was Navarra's oral language test (PLON; Aguinaga et al., 1991) was used to control the relationship between memory capacity and linguistic expression. The sub-scales of understanding, expression, and social knowledge were administered. For 4 years old children, in the understanding sub-scale, a sheet of 10 images was presented and the child was asked to point to six objects, one at a time. For the expression sub-scale, a sheet of seven images was presented and the child was asked to name each one while pointing them out. For the social knowledge sub-scale, children were asked to answer four questions about basic needs (e.g., "What do you do when you are sleepy? What do you do when you are thirsty? What do you do when you got cold?"). For 5 years old children, in the understanding sub-scale, images were presented to the child, and he/she was asked to point to objects by their use (e.g., "Point out something that is good for playing"). In the expressive sub-scale, images were presented and the child was asked what the object was used for. I In the social knowledge sub-scale, three pictures of children performing actions were presented, and children were asked what they were doing. Variables of interest were the scores obtained in each sub-scale, which were categorized according to level into 0 (low: scores equal to zero in the subscales); 1 (moderate: scores 1–5 in the subscales); and 2 (high: scores $\geq$6 in the subscales).

Tasks were administered in two different sessions of 20–30 min each one. In the first session, the emotional appraisal component was administered. In the second session, the emotional memory component and the PLON task were administered.

Characterization of Living Conditions

A Socio-Economic Level questionnaire (NES) was used to obtain child health history, socio-economic, and socio-environmental information on their living conditions at home. This information was obtained from the report of the families in individual interviews with the researchers. This scale was used in previous studies in Argentina (Lipina et al., 2004, 2005; Segretin et al., 2009, 2014; Prats et al., 2017). NES was also used to evaluate parents' education and occupational levels, housing and overcrowding, conditions, and to identify indicators of unsatisfied basic needs (UBN; Boltvinik, 1995). Scores were assigned directly to mothers and fathers for educational and occupational backgrounds; however, only the higher score was considered for the total scores. For housing conditions, scores were assigned based on type of dwelling, floor, water, bathroom, ceiling, external walls, and home property. Living conditions at home were considered *unfavorable* when at least one of the indicators of UBN was present; if none of these indicators was present, living conditions at home were considered *favorable*. Thus two groups were formed, and the unfavorable condition showed lower scores of the indicators (**Table 1**).

Data Analysis

First, we performed univariate analyses of the variables of interest, which included the mean, median, standard deviation, standard error, and sample size for each age group. Then, normality and homoscedasticity of the variables were evaluated. When the parametric requirement were not met, trigonometric transformations were applied to the variables, and assumptions were re-tested with the transformed variables. Non-parametric statistics were used, because some assumptions were indicators of UBN not met. The Mann–Whitney *U*-test was used for independent samples that considered the age (4 or 5 years), gender (girls, boys), and living conditions (favorable, unfavorable), and emotional appraisal (children appraisal, task appraisal) as grouping variables. The Kruskal–Wallis test was used when the grouping variable was language skills (low, moderate, high). Descriptive and inferential analyses were conducted using SPSS software (version 15.0).

RESULTS

Analysis of Variations of the Emotional Appraisal Responses

Because it was found that the children appraisal did not coincide with the stimuli valences (task appraisal), an analysis was made considering the accuracy appraisal as a variable of interest. We

TABLE 1 | Mean and standard deviation of education, occupation, housing, and overcrowding scores of living conditions homes.

	Favorable (*n* = 32)		Unfavorable (*n* = 40)			
	M	***SD***	***M***	***SD***	***Z***	***P***
Education	11	5.36	7	3.12	−6.08	0.000
Occupation	9	2.73	5	2.51	−5.96	0.000
Housing	12	0.00	10	1.90	−4.80	0.000
Overcrowding conditions	9	2.67	8	2.45	−3.26	0.001

Scores obtained from NES. The sample size is lower than the total sample (N = 116), however, no significant differences were found in the cognitive performance (emotional appraisal and emotional memory) of the children who have socio-environmental information, and children who do not ($p \geq 0.05$).

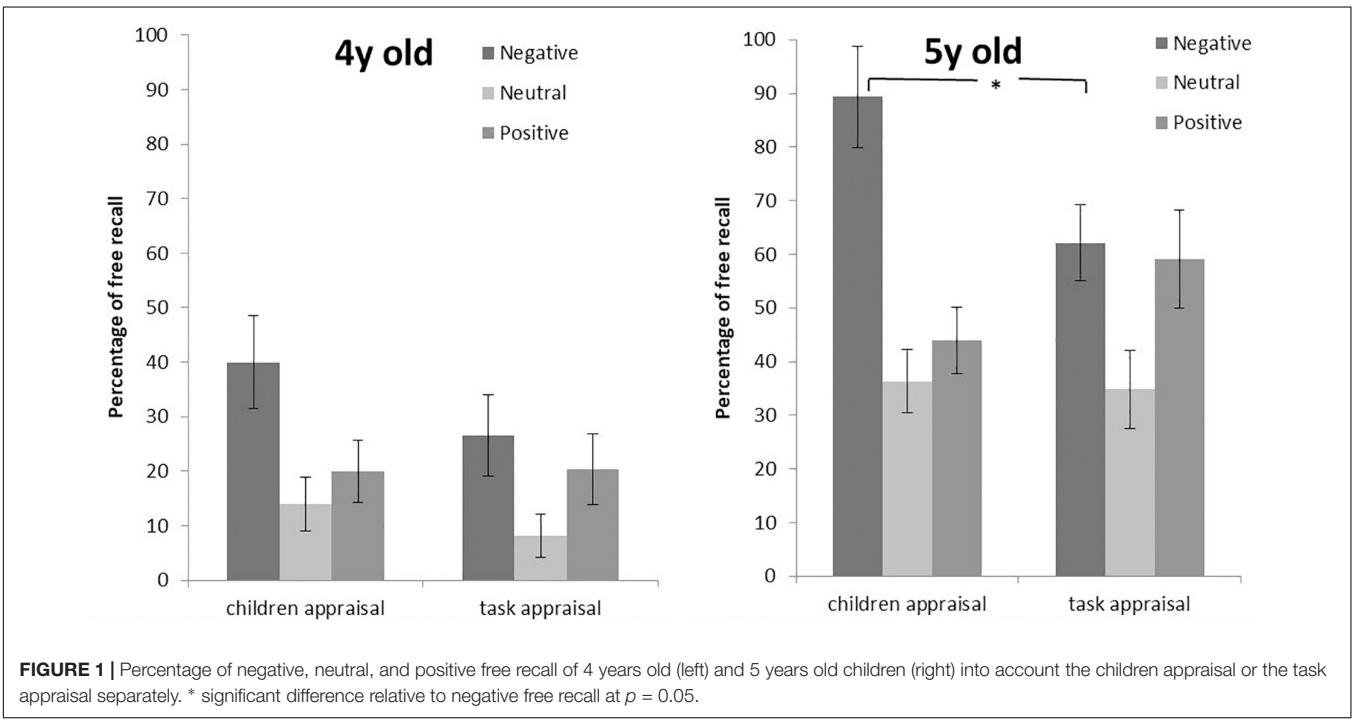

FIGURE 1 | Percentage of negative, neutral, and positive free recall of 4 years old (left) and 5 years old children (right) into account the children appraisal or the task appraisal separately. * significant difference relative to negative free recall at $p = 0.05$.

did not found that this variable was previously explored in the literature. In this way, the valences that children attributed to images were compared (children appraisal compared with task appraisal) and an accuracy appraisal was obtained. Accuracy appraisal varied from 0 to 5 in the negative ($Md = 2$), neutral ($Md = 2$), and positive valences ($Md = 3$).

Age Differences

An analysis using the Mann–Whitney *U*-test that compared the accuracy appraisal between ages, showed significant differences between 4- and 5 years olds in the positive ($Z = -3.14$, $p = 0.002$), but not in the negative ($Z = -1.35$, $p = 0.18$) and neutral valences ($Z = -0.610$, $p = 0.54$). The 5 years old children ($M = 1.74$, $SD = 0.85$) were more accurate in the positive valence, compared with the 4 years old children ($M = 2.27$, $SD = 0.89$).

Differences by Gender, Language Ability, and Living Conditions at Home

However, the accuracy appraisal did not vary between children with respect to gender ($p \geq 0.05$), living conditions at home ($p \geq 0.05$), or language ability ($p \geq 0.05$) as variables groups.

According to these results, appraisal accuracy should be incorporated into the analyses of expression memory.

Analysis of Emotional Memory Taking Into Account the Children Appraisal or the Task Appraisal Separately

According to the results described above, children appraisal and task appraisal were compared in the performance of the emotional memory. A global analysis with the Mann–Whitney *U*-Test indicated that the performance memory was significantly different for the negative images ($Z = -2.03$, $p = 0.04$), but it did not differ for neutral images ($Z = -1.04$, $p = 0.3$) and positive images ($Z = -0.42$, $p = 0.67$). In general, children remember more negative images compared to positive and neutral ones.

Age Differences

Separated analysis were run for each age group to test differences between children appraisal and task appraisal. Results indicated significant differences only in 5 years olds for negative images ($Z = -2.00$, $p = 0.04$), but not for positive or neutral images

($p \geq 0.05$). Children who were 5 years old recalled more negative images when they were evaluated taking into account their own assessment than when considering the valence of the task. **Figure 1** shows the emotional accuracy of the age groups (4 and 5 years old) to the emotional valence (positive, neutral and negative). Children evoke of negative images was different when comparing children appraisal with task appraisal. Those differences were not evidenced for positive or neutral images.

Differences by Gender, Language Skills, and Living Conditions at Home

When we examined the children's appraisal condition compared with the task appraisal condition as variable group, the Mann–Whitney *U*-Test showed no significant differences in the free recall of negative, neutral, and positive images ($p \geq 0.05$) for each category of the considered factors (gender, language skills, and living conditions). That is, for boys or girls, from favorable or unfavorable living conditions, and with low, moderate or high language skills, children had similar memories of the three valences regardless of whether we used children appraisal or task appraisal.

CONCLUSION AND GENERAL DISCUSSION

The objective of the present study was to analyze emotional and memory associations during the preschool period. We explored differences in those associations considering individual (i.e., age, gender, language skills) and socio-environmental (i.e., living conditions at homes) factors. We found that appraisal in 4-years-old did not coincide with task appraisal, and that children who were 5-years-old showed greater accuracy appraisal. This differential accuracy is presented in the appraisal of positive visual images. However, the accuracy appraisal did not vary with respect to gender, living conditions at home, or language ability.

With respect to the memory test performance (7–10 days after the session 1), results suggest differences in the number of free recall images between 4 and 5 years old children. In particular, older children evoked more images, regardless their valence. When individual and environmental factors were considered in the performance analysis, no differences were identified. Nevertheless, when the accuracy appraisal (children appraisal or task appraisal) was taken into account, differences in performance were identified for negative stimulus. Five-years-old children evoke more images which they had appraised as negative. This pattern of results provides preliminary evidence regarding the need of including appraisal as a specific aspect of processing in the emotional memory study at these ages. It also suggests that different aspects of emotional memory are possible to be evaluated in young children from different socio-environmental contexts.

Several studies have indicated that the emotional processing of images can generate variations in the consolidation of memory (Channell and Barth, 2013; Kuppens et al., 2013; Leventon and Bauer, 2016). In this sense, those images or events with emotional valences (positive or negative) were remembered more clearly than those with neutral valences (Christianson, 1992; Quas and Lench, 2007; Brainerd et al., 2010; Justel et al., 2013). Our study presents a different pattern of results in relation to other studies (Leventon and Bauer, 2016), in which children evoked images with negative and positive valences in a similar way. One of the differences of the present study is that it analyzes the emotional memory in children with different living conditions, even though the comparisons between the groups of children with favorable and unfavorable conditions were not statistically significant.

Regarding language development and its relation to memory expression, it would be relevant for future studies to analyze vocabulary with and without emotional content in children of these ages, in order to explore whether and how the valences of words are associated with their subsequent.

Higher memory performances of negative visual images when evaluated according to children appraisal may be due to the fact that people usually attribute emotions based on their own emotional states rather than those corresponding to task stimuli (Lerner and Keltner, 2000, 2001). It is also possible that due to this information would be better consolidated and evoked. These differences tend to be less pronounced when children are younger and when memory performance is evaluated according to task appraisal. On the other hand, these differences may be because at this stage the emotional categories are not yet consolidated (Nelson and Russell, 2016), so that children may exhibit variations in their initial appraisal of the stimuli. There are no studies of children that relate these variables, however, Kuppens et al. (2013) studied emotional appraisal of visual stimuli and its relationship with memory processes in adults. They examined how these prominent cognitive and affective components of emotional experience related dynamically to each other in daily life. Their findings revealed insightful patterns of the continuous interplay between core affect and appraisal. That is, appraisals and core affect were not independent properties of emotional experience, but were intricately related in a dynamic interplay that was characterized by congruency between appraisals and valence that suggested a central role in acting and responding to the environment for arousal (Kuppens et al., 2013). Therefore, emotions or moods should not be understood as static phases, but as a dynamic phenomenon that involves components that change and follow each other across time continuously (Kuppens et al., 2009; Boden and Berenbaum, 2010). Based on this reasoning, our study was limited by not including the arousal of visual stimuli. Future studies are proposed to incorporate the evaluation of arousal when analyzing the interaction between emotional appraisal and emotional memory.

On the other hand, appraisal accuracy was surprisingly low for the emotional appraisal task, especially for negative and neutral conditions (performance was around chance level). This brings into question the validity and reliability of this task in assessing emotional appraisal accuracy in 4–5 years olds. This task was used in different studies which reported similar results. However, these findings do not rule out the possibility that these responses are specific to the population that participated in these studies (in all cases, girls and boys residing in Buenos Aires City), and it is possible that cultural factors associated with the expression of

these responses could modulate their performance. All of these aspects should be explored in future studies.

The main contribution of our work is the inclusion of the appraisal as an important component to consider in a specific way in the expression of emotional memory of children at this stage of development. The modulation of appraisal on emotional memory opens questions about previous findings in which this component has not been taken in consideration and the implications on the consolidation and retrieval of the memories, especially the negative ones. In this sense, it is important to consider that several studies indicate the prevalence of negative memories during childhood. For example, negative events elicited more false memories than neutral event in a sample of 7-year-old children (Otgaar et al., 2008). In another study, children who developed negative pain memories were more likely to have experienced greater pain intensity and state anxiety during previous painful experiences (Noel et al., 2009, 2012). In a more recent study, emotion influenced the recognition memory of negative scenes in children (Leventon et al., 2014). Other studies provide evidence for negative emotions effects on memory in the school years (Davidson et al., 2006; Cordon et al., 2013). In this sense, the results of the current study also indicate a prevalence of negative memories, however, the performance is greater when evaluating the memories taking into account children's appraisal, compared to task appraisal. Thus, our results emphasize the need to include individual differences in emotional appraisal when analyzing the subsequent performance of children.

ETHICS STATEMENT

Informed consents were obtained from parents/caregivers, and ethical approval was obtained from the Ethical Review Committee (Protocol N° 961). This study was conducted in accordance with the American Psychological Association ethical standards, and international and national children rights laws. Once authorization was obtained from the schools where the activities were to be conducted, informative meetings were organized for parents in which they had an opportunity to interact with the research group. The researchers informed them about the objectives and activities of the study, and provided them with an information sheet. Consequently, they were invited to participate and to authorize the participation of their children, which required them to sign an informed consent for each of the evaluations. Likewise, prior to any evaluation, the permission of the children was requested.

AUTHOR CONTRIBUTIONS

ER participated as designer, operator, and supervisor, performed the tabulation and prepared the datasets, designed and executed the statistical analysis, and wrote the manuscript. MS and SL participated as designer and coordinator, designed and supervised the statistical analysis, and wrote the manuscript. VR collaborated in preparation of the datasets, statistical analysis design, and manuscript review.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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