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CURRENT ADVANCES IN AFFECTIVE NEUROSCIENCE

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Editorial: Current Advances in Affective Neuroscience

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Keywords: affective neurocircuitry, affective neuroscience, depression, oxytocin, serotonin

Editorial on the Research Topic

Current Advances in Affective Neuroscience

Affective neuroscience is a rapidly progressing field and researchers are developing and employing innovative strategies to understand the neural circuitry and transmitter systems involved in affective processes and how they become dysfunctional in mental disorders. Recent epidemiological surveys have established disorders characterized by affective dysfunctions as one of the leading causes of disabilities worldwide and it is therefore of great importance to gain better insights into the key neural and molecular mechanisms underlying processing of affective stimuli and responses, how these can become dysfunctional and how they can be targeted by drug or other therapies. Our Frontiers Research Topic *Current Advances in Affective Neuroscience* aimed at providing an overview on the current developments and methodological advances in the field, encompassing animal models, and preclinical as well as clinical research in humans. The articles that were published in the Research Topic emphasize the breath-taking methodological advances in the field and the increasing aim of the community to contribute to the development of brain-based markers and novel treatments for disorders characterized by emotional dysregulations, as well as to bridge the gap between laboratory experiments and real life.

A number of authors emphasized the integration of novel methodological approaches rooted in computational sciences to facilitate progress in affective neuroscience. As such Jiang et al. proposed and evaluated a novel computational framework that employs a cortical folding pattern-guided approach to further delineate the emotional brain networks in the human brain, Markett et al. provided an overview and framework for the integration and application of recent developments in network neuroscience to describe emotional brain processes on the network level, and, Shani et al., proposed a machine learning based approach to optimize the efficacy of cognitive trainings, thus bridging the gap between methodological developments and clinical application. The increasing interest in the field to apply affective neuroscience inspired approaches to clinical questions, particularly novel treatment development, is further underscored by a number of preclinical studies. Employing sophisticated methodological strategies in rodent models Yuan et al. emphasized the importance of social company as a resource to attenuate fear renewal, Chen et al. demonstrated that the anxiolytic effects of Gan-Mai-Da-Zao, a traditional Chinese medicine, may be mediated by GABAergic and serotonergic receptors, while Rafa-Zablocka et al. reported evidence for the pivotal role of CREB in serotonergic neurons in the regulation of BDNF which may maintain the anti-depressive effects of Fluoxetine, a finding that resonates with the report from Han et al. that a BDNF-related imbalance of Copine 6 expression and synaptic plasticity may play a crucial role in the development of stress-associated depressive behavior. A review by Davis and Montag bridged the gap between animal models and human research by providing a cross-species perspective on Jaak Panksepp's affective neuroscience approach.

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The submissions involving human subjects spanned a broad methodological range further emphasizing the rapid growth and extension of the field, both in terms of basic and clinical research. Submissions included behavioral genetic and psychophysiological approaches, such that Lischke, Weippert et al. demonstrated that inter-individual differences in heart-rate variability associate with emotion regulation capacities and that specifically individual variations in genotype-dependent catecholamine metabolism may account for facial emotion recognition performance (Lischke, Pahnke et al.), while other studies employed fMRI alone to determine the neural basis of positive and negative social interactions (Gao et al.) or in combination with pharmacological challenge strategies to demonstrate that the role of the neuropeptide oxytocin in emotional empathy is mediated by the amygdala and generalizes across cultures (Geng et al.) and that it modulates cognitive appraisal of close intimate relationships (Aguilar-Raab et al.), whereas a tryptophan depletion genetic imaging study by Klasen et al. emphasized the role of serotonergic amygdala-frontal connections in aggression. The increasing translation of affective neuroscience approaches to emotional brain disorders was reflected in the submission of studies employing fMRI to determine the importance of altered insular intrinsic networks in autism spectrum disorder (Xu et al.), deficient functional interactions between the ventral striatum and paralimbic regions (Bai et al.) as well as global functional communication deficits (Zhang et al.) in depression, while intrinsic connectivity measures may also represent a treatment sensitive marker for Electroconvulsive Therapy in this disorder (Wei et al.). Finally, two studies aimed at bridging the gap between experiments in the laboratory and the real-world, with a review by Leibold and Schruers stressing the importance of real-life assessments and epigenetic approaches in panic disorders and Pahnke et al. suggesting that oral contraceptives may impair complex emotion recognition in women.

In conclusion the Research Topic reflects that Affective Neuroscience increasingly integrates different methodological developments to delineate the neural foundations of emotional processing across species and aims at translating these findings in the context of determining brain-based markers for mental disorders and developing novel treatments for these disorders.

AUTHOR CONTRIBUTIONS

BB and KK drafted the manuscript. JW and MB critically revised the manuscript.

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frontiers in Neuroscience

ORIGINAL RESEARCH
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Both Hypo-Connectivity and Hyper-Connectivity of the Insular Subregions Associated With Severity in Children With Autism Spectrum Disorders

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Some studies identified hypo-connectivity, while others showed hyper-connectivity of the insula in the autism spectrum disorders (ASD). These contradictory findings leave open the question of whether and to what extent functional connectivity of the insula is altered and how functional connectivity of the insula is associated with the severity of ASD. A newly emerging insular atlas that comprises multiple functionally differentiated subregions provides a new framework to interpret the functional significance of insular findings and uncover the mechanisms underlying the severity of ASD. Using the new insular atlas, the present study aimed to investigate the distinct functional connectivity of the insular subregions and their associations with ASD severity in a cohort of 49 children with ASD and 33 typically developing (TD) subjects. We found that compared with TD group, the ASD group showed different connectivity patterns in the left ventral agranular insula, right ventral dysgranular and granular insula, and dorsal dysgranular insula, characterized by significant hyper-connectivity and/or hypo-connectivity with special brain regions. Furthermore, both the hypo-connectivity and hyper-connectivity patterns of the insular subregions were significantly associated with the severity of ASD symptoms. Our research demonstrated distinct functional connectivity patterns of the insular subregions and emphasized the importance of the subdivisions within the insula to the potential impact of functional difference in children with ASD. Moreover, these results might help us to better understand the mechanisms underlying the symptoms in children with ASD and might elucidate potential biomarkers for clinical applications.

Keywords: autism spectrum disorders, hypo-connectivity, hyper-connectivity, insula, brainnetome atlas

INTRODUCTION

Autism spectrum disorders (ASD) are prevalent neurodevelopmental disorders characterized by deficits in social interaction; verbal and nonverbal communication; and restricted and stereotyped patterns of behavior, interests, and activities (Minshew and Williams, 2007). Although previous studies have directly linked aberrant intrinsic brain connectivity in ASD to specific symptoms, such

as poor social functioning, severe restricted, and repetitive behaviors (Monk et al., 2009; Weng et al., 2010), the exact mechanisms underlying these symptoms are poorly understood. To date, broad brain regions were thought to specially contribute to ASD symptoms. The insula is one such brain regions with the suggestion that the atypical functional connectivity of the insula may be important in the neuropathology of ASD (Uddin and Menon, 2009; Uddin et al., 2013b). Specifically, it has been demonstrated that adolescents with ASD show decreased regional homogeneity in the right insula (Paakki et al., 2010). Functionally, a comprehensive meta-analysis of functional imaging studies has revealed hypo-activation in the right anterior insula during various social tasks in ASD (school-age children, adolescents, and adults) (Di Martino et al., 2009). Regarding functional connectivity, reduced functional connectivity of the anterior, middle, and posterior insula with specific brain regions involved in different brain networks was identified in adolescent and adult ASD (Ebisch et al., 2011; Von Dem Hagen et al., 2013; Di Martino et al., 2014). These results all confirmed the hypo-connectivity of the insula and strongly supported the hypo-connectivity theory of the ASD. However, accumulating evidence of brain hyper-connectivity also exists in the domains of visual processing, emotion processing, memory, and language in ASD (Noonan et al., 2009; Shih et al., 2010, 2011). Specifically, Uddin et al. (2013b) observed stronger functional connectivity of the insula in 20 children with ASD and replicated this finding in an independent cohort, suggesting that the hyper-connectivity of the salience network including the bilateral insula may be a distinguishing feature in children with ASD. Although Uddin et al. proposed that discrepancies between findings of ASD related hypo-connectivity and hyper-connectivity might be reconciled by taking developmental changes into account (Uddin et al., 2013a), it remains unclear whether the insular subregions may account for observed inconsistencies in ASD. It is noteworthy that the insula is comprised of separate subregions, only assessing it as a whole region may obscure individual differences of functional connectivity with insula in ASD.

The insula is reported to be involved in diverse functions, including gustatory and olfactory processing, components of somatosensation, interoception, motivation, and the maintenance of homeostasis (Critchley, 2005; Seminowicz and Davis, 2007; Craig, 2009). Moreover, the insula also showed left/right differences, which could be related to the hypothesis that the two side of the insula subserve different functions and are linked to different circuits (Craig, 2009; Cauda et al., 2012). For example, the right insula has been proposed as a key node between the default mode network (DMN) and the central executive/attentional network (Sridharan et al., 2008). In addition, other studies (Cauda et al., 2011, 2012) identified that the anterior part related to salience network was found to be frankly lateralized on the right and the visuomotor integration network (posterior cluster) found to have a mild right lateralization. Given these variable functions and laterality of the insula, multiple functionally differentiated subregions with distinct patterns of connectivity were identified in the insula using k-means clustering of insula voxels (Jakab et al., 2012; Kelly et al., 2012), structural connections (Cloutman et al., 2012), clustering of a priori instantiated regions of interest (Cauda et al., 2011), meta-analytic approaches (Kurth et al., 2010b; Cauda et al., 2012), clustering of resting state functional connectivity patterns (Deen et al., 2011; Chang et al., 2013; Gordon et al., 2016), dynamic functional network connectivity (Nomi et al., 2016), and anatomy connectivity patterns (Fan et al., 2016). Among all these atlas of the insula, the insular subregions in Brainnetome atlas has been not only well established to reflect functional segregation of the insula, but also related well to other functional and histological maps of the insular cortex (Kurth et al., 2010a; Kelly et al., 2012; Chang et al., 2013; Morey et al., 2013). Moreover, different connectional, functional connectivity patterns, and behavioral domains of insular subregions were identified and shown along with the atlas (http://atlas.brainnetome.org/bnatlas.html), suggesting the possibility that the insular subregions may differ in their vulnerability to the ASD and may play different roles in the core symptoms of ASD. Thus, investigating functional connectivity of insula using the brainnetome atlas in the ASD will provide further insights to better understand the mechanisms underlying the core symptoms of ASD and may lead to identifying potential biomarkers that could be used in clinical situations. In addition, this atlas was successfully used in a recent study, which showed disrupted functional connectivity patterns of the insular subregions involved in different neural circuits associated with the contrary impacts on the depressive symptoms in drug-free major depressive disorder (Wang et al., 2017).

Using the new insula atlas (Fan et al., 2016), the present study aimed to investigate the distinct functional abnormalities in each of the insular subregions in a cohort of 49 children with ASD and 33 typically developing (TD) subjects. To further examine the relationship between the functional connectivity of each subregion of the insula and the severity of ASD, correlations were calculated between scores on the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) and altered functional connectivity of insular subregions.

MATERIALS AND METHODS

Participants

We used the dataset of the University of California, Los Angeles, one of the subsamples in the Autism Brain Imaging Data Exchange database (http://preprocessed-connectomes-project.org/abide/download.html). In regards to inclusion criteria, ASD had a prior clinical diagnosis of autism based on criteria from the Diagnostic and Statistical Manual of Mental Disorders IV, which was confirmed with the ADOS (Lord et al., 2000) and/or ADI-R (Lord et al., 1994). The ADOS has subscores for social interaction (ADOS social) and communication (ADOS communication), which are combined into a total score (ADOS total). The ADI-R has subscores for social interaction (ADI-R social), verbal (ADI-R verbal), and repetive behaviors (ADI-R). TD participants had no history of any genetic, neurological, psychiatric, or developmental disorders. In addition, they could not have a first

degree relative with an ASD diagnosis. Verbal, performance, and full-scale intelligence quotients (IQs) were assessed for each participant using the four subsets of the Wechsler Abbreviated Scale of Intelligence or the full Wechsler Intelligence Scale for Children (Wechsler, 1999). Handedness was assessed via parental reports on a questionnaire. This study was carried out in accordance with the recommendation of Institutional Review Board of University of California, Los Angeles with informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol wad approved by the Institutional Review Board of University of California, Los Angeles.

The initial dataset includes 62 ASD and 47 TD individuals. Based on the criteria from the Quality Assessment Protocol (http://preprocessed-connectomes-project.org/quality-assessment-protocol/ and http://preprocessed-connectomes-project.org/abide/quality_assessment.html), we only included subjects whose functional quality are all ok after manual checking. Moreover, subjects whose mean frame-wise displacement (FD) is >1 mm was also excluded. Finally, the sample included 49 ASD (age ranged from 8 to 17) and 33 TD subjects (age ranged from 9 to 17) (**Table 1**). The two groups did not significantly differ based on age, gender, mean FD, full-scale, verbal, and performance IQs. In the final sample, ADOS and ADI-R scores were available for only 48 ASD patients. Of the subjects in the ASD group, 23 individuals reported the use of one or more psychotropic medications.

TABLE 1 | Demographics and clinical characteristics.

Characteristic	TD	ASD	*p*-value
Sample size	33	49	–
Gender (female/male)[a]	6/27	6/43	0.456
Handness (left/right)[a]	3/30	5/44	0.868
Mean FD[b]:mean ± SD	0.13 ± 0.18	0.19 ± 0.19	0.114
Age[b]:mean ± SD	13.30 ± 2.04	13.05 ± 2.46	0.639
Verbal IQ[b]:mean ± SD	105.21 ± 10.74	102.93 ± 13.66	0.424
Performance IQ[b]:mean ± SD	101.63 ± 10.58	100.30 ± 13.91	0.643
Full scale IQ[b]:mean ± SD	103.81 ± 9.56	101.42 ± 13.33	0.378
ADOS total:mean ± SD	–	10.66 ± 3.54 ($n = 48$)	–
ADOS communication:mean ± SD	–	3.16 ± 1.43 ($n = 48$)	–
ADOS social:mean ± SD	–	7.50 ± 2.45 ($n = 48$)	–
ADI-R social:mean ± SD	–	20.02 ± 5.33 ($n = 48$)	–
ADI-R verbal:mean ± SD	–	16.29 ± 4.65 ($n = 48$)	–
ADI-R repetitive behaviors: mean ± SD	–	7.14 ± 2.55 ($n = 48$)	–

[a] *The p-value was obtained by a chi-square test.*

[b] *The p-value was obtained by a two-tailed two-sample t-test; –, indicates no data available. TD, typically developing; ASD, autism spectrum disorders; FD, framewise displacement; IQ, intelligence quotients; ADOS, Autism Diagnostic Observation Schedule; and ADI-R, Autism Diagnostic Interview-Revised.*

MRI Data Acquisition

All resting-state fMRI scans were acquired on a Siemens 3 T Trio at the University of California, Los Angeles. During data acquisition, subjects were asked to relax, keep their eyes open, and keep their head still. A white screen with a black fixation cross in the middle of the screen was presented. The T2-weighted functional images were collected with the following settings: repeat time = 3,000 ms, echo time = 28 ms, matrix size = 64 × 64, field of view = 192 mm, and thickness = 4 mm, no gap, interleaved acquisition, with an in-plane voxel dimension of 3 × 3 mm. The T1-weighted magnetization-prepared rapid gradient-echo images were collected with the following settings: repeat time = 2,300 ms, echo time = 2.84 ms, field of view = 256 mm, flip angle = 9°, and thickness = 1.2 mm, interleaved acquisition, with an in-plane voxel dimension of 1 × 1 mm.

Resting-State fMRI Data Preprocessing

The fMRI data were preprocessed under the Preprocessed Connectomes Project (http://preprocessed-connectomes-project.org/) with the Data Processing Assistant for Resting-State fMRI (DPARSF, http://preprocessed-connectomes-project.org/abide/dparsf.html). For each participant, the preprocessing steps were as follows: (1) all volume slices were corrected for different signal acquisition times; (2) the time series of images for each subject were realigned using a six-parameter (rigid body) linear transformation; (3) individual structural images were co-registered to the mean functional image after realignment using a six degrees-of-freedom linear transformation without resampling; (4) the transformed structural images were then segmented into gray matter, white matter, and cerebrospinal fluid; (5) the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) tool (Ashburner, 2007) was used to compute transformations from individual native space to MNI space; (6) the Friston 24-parameter model (Friston et al., 1996) was utilized to regress out head motion effects from the realigned data (Satterthwaite et al., 2013; Yan et al., 2013); (7) the white matter, cerebrospinal fluid, and global signals were regressed out; (8) linear and quadratic trends and temporal band pass filtering (0.01–0.1 Hz) were performed; (9) corresponding maps were then registered into MNI space with 3 mm three cubic voxels by using transformation information acquired from DARTEL; and (10) the maps were further smoothed by a kernel of 6 mm.

Definition of the Insular Subregions

The bilateral insula subregions were defined by the 50% probability maps in the Brainnetome Atlas. Six subregions of insula in each brain hemisphere were defined as seed areas, including the hypergranular insula, ventral agranular insula, dorsal agranular insula, ventral dysgranular and granular insula,

dorsal granular insula, and dorsal dysgranular insula. For resting-state functional connectivity (RSFC) analyses, the insula subregions were resampled into $3 \times 3 \times 3$ mm^3 in MNI space.

The Whole Brain RSFC Patterns in the ASD and TD Groups

For all the subjects, the RSFC was defined by Pearson correlation coefficients between the mean time series of each seed region and that of each voxel in the rest of the brain. We used the binary gray matter mask in SPM before computing the whole brain RSFC. Correlation coefficients were converted to z-values using Fisher's z transformation to improve normality. Next, one-sample t-test was performed to identify voxels which showed significantly positive or negative correlations with the seed region in these normalized correlation maps. For all the above voxel-wise comparisons, significance was determined with a voxel-level corrected threshold of $p < 0.001$ and a cluster-level corrected threshold of $p < 0.05$ using the Gaussian random field (GRF) correction in the DPABI (http://rfmri.org/dpabi).

Altered RSFC of the Insular Subregions in ASD

First, two-sample t-tests were implemented using DPABI to map group difference of RSFC between ASD and TD groups with the age, gender, handedness, verbal IQs, performance IQs, and full-scale IQs as covariates. Significance was determined with a voxel-level corrected threshold of $p < 0.001$ and a cluster-level corrected threshold of $p < 0.05$ using the GRF correction in the DPABI.

Then, we calculated the mean RSFC of the regions which showed significantly altered RSFC with subregions of the insula in the ASD and TD group. To exclude the effects of global signal, we re-analyzed the mean RSFC using rs-fMRI data with global signal.

Correlation Analyses Between the RSFC and the Severity of ASD

Finally, the partial correlation analyses between the average z-score of the region (RSFC) and the severity scores of ASD (ADOS and ADI-R scores) were performed in the ASD group with the age, gender, handedness, verbal IQs, performance IQs, and full-scale IQs as covariates using SPSS. The statistical level with $p < 0.05$ was considered as significant.

RESULTS

Distinct RSFC Patterns of Insular Subregions Between the ASD and TD Groups

RSFC analyses based on the insular subregions resulted in distinct connectivity maps in the TD and ASD groups (**Figure 1**). Statistical comparisons between these maps showed significant differences of RSFC between the TD and ASD groups in the left ventral agranular insula, right ventral dysgranular and granular insula, and left dorsal dysgranular insula (**Figure 2** and **Table 2**). Specifically, children with ASD showed hypo-connectivity between the left ventral agranular insula and the bilateral precuneus (PCUN), between ventral dysgranular and granular insula and the right supramarginal gyrus (SMG.R), and between the left dorsal dysgranular insula and the right cuneus (CUN.R). Moreover, children with ASD also showed hyper-connectivity between the left dorsal dysgranular insula and the left superior temporal gyrus (STG.L).

The Relationship Between RSFC and the Severity of ASD in Children

Importantly, significantly partial correlations between the RSFC of the insular subregions and the clinical characteristics of the children with ASD were identified with age, gender, handedness, full-scale IQs, verbal IQs, and performance IQs as covariates (**Figure 3**). The hypo-connectivity between the left ventral agranular insula and PCUN.R was negatively correlated with the ADOS total/social scores in the ASD group. Moreover, the hyper-connectivity between the left dorsal dysgranular insula and STG.L was positively correlated with the ADI-R social scores in the ASD group.

DISCUSSION

In the present study, we investigated the distinct functional alterations of the insular subregions between the children with ASD and TD groups. Compared with the TD group, the ASD group showed different connectivity patterns in the left ventral agranular insula, right ventral dysgranular and granular insula, and left dorsal dysgranular insula, characterized by hypo-connectivity and/or hyper-connectivity with specific brain regions. Furthermore, both the hypo-connectivity and hyper-connectivity of the insular subregions were significantly associated with the core symptoms of ASD.

ASD is a complex neurodevelopmental disorder that affects multiple cognitive domains. Recent theoretical models have highlighted the need to consider ASD as a disorder associated with several large-scale networks (Belmonte et al., 2004; Welchew et al., 2005; Geschwind and Levitt, 2007). Using independent component analysis, Uddin et al. demonstrated that compared with TD children, children with ASD exhibited altered functional connectivity of the salience network, DMN, frontotemporal network, motor network, and visual network (Uddin et al., 2013b). Our results support this notion by demonstrating hyper-connectivity and/or hypo-connectivity of the insular subregions involved in different brain networks, supporting several behavioral domains known to be impaired in the complex symptoms of the ASD.

Specifically, hypo-connectivity was observed between the left ventral agranular insula and bilateral PCUN in the ASD group. The left ventral agranular insula is mainly located in the left anterior insula, which is a hub in the salience network. Previous studies demonstrated that the anterior insula plays a critical role in processing information relevant to social functioning as a sort of "hub" that mediates interactions between the DMN and the central-executive networks (Sridharan et al., 2008; Uddin and

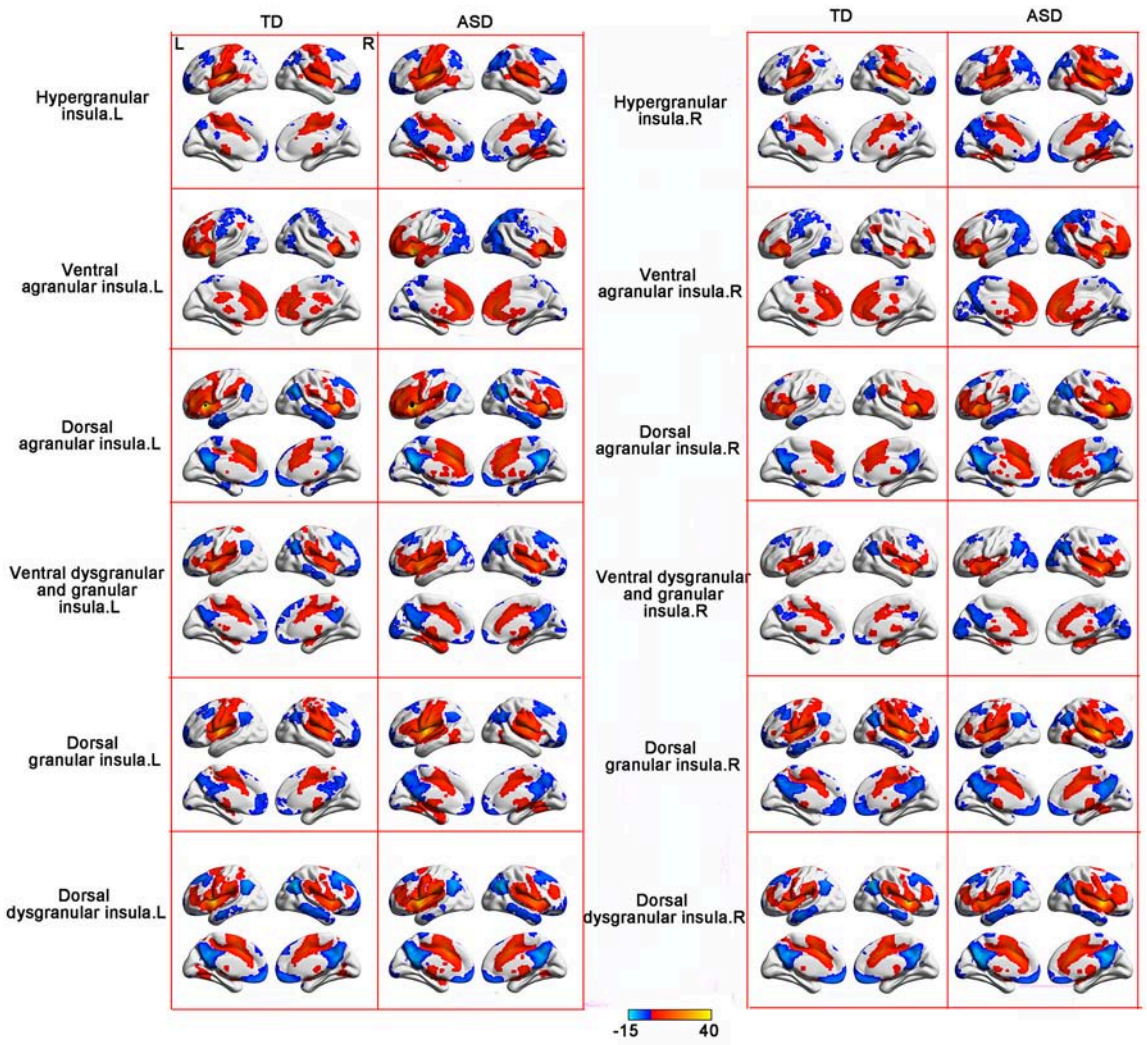

FIGURE 1 | RSFC patterns of the insular subregions in the ASD and TD groups. One sample *t*-tests were used to identify the group statistical maps. The significance level was determined with a voxel-level corrected threshold of $p < 0.001$ and a cluster-level corrected threshold of $p < 0.05$ using the Gaussian random field (GRF) corrections. The red and blue colors represent positive and negative functional connectivity with the seed regions.

Menon, 2009; Menon and Uddin, 2010). Apart from the key part of the DMN, the precuneus has a role in emotion, self-referential thinking, and projection processes critical for social development (Cavanna and Trimble, 2006) and has also been linked to atypical mentalizing or theory of mind in ASD (Castelli et al., 2002; Wang et al., 2007). Given the crucial role of both the anterior insula and the precuneus in some aspects of social cognition, the significant hypo-connectivity between the left ventral agranular insula and bilateral precuneus might contribute to social interaction deficits in ASD. Moreover, this suggestion was further supported by our result, which showed negative correlation between the hypo-connectivity of the left ventral agranular insula—PCUN.R with the ADOS total/social scores in the ASD group.

In addition, compared with the TD group, the ASD group showed hypo-connectivity between the right ventral dysgranular and granular insula and SMG.R. According to the behavioral results of the Brainnetome Atlas, the right ventral dysgranular and granular insula is mostly associated with emotion. The right insula is associated with sympathetic ("aroused") functions based on anatomical evidence of left-to-right asymmetry in peripheral autonomic efferent neurons and homeostatic afferent neurons, as well as a review of neuroimaging literature (Yamada et al., 2016). Moreover, SMG.R has been consistently shown to play a crucial role in emotion processing (Singer et al., 2009; Lamm et al., 2011). Since there is indeed good evidence that empathy may be impaired in ASD (Baron-Cohen and Wheelwright, 2004; Jones et al., 2010; Lockwood et al., 2013), it is reasonable to conclude that the hypo-connectivity between the right ventral dysgranular and granular insula and SMG.R might contribute to the deficits of emotion in ASD.

Furthermore, hypo-connectivity between the left dorsal dysgranular insula and the right cuneus was also identified in

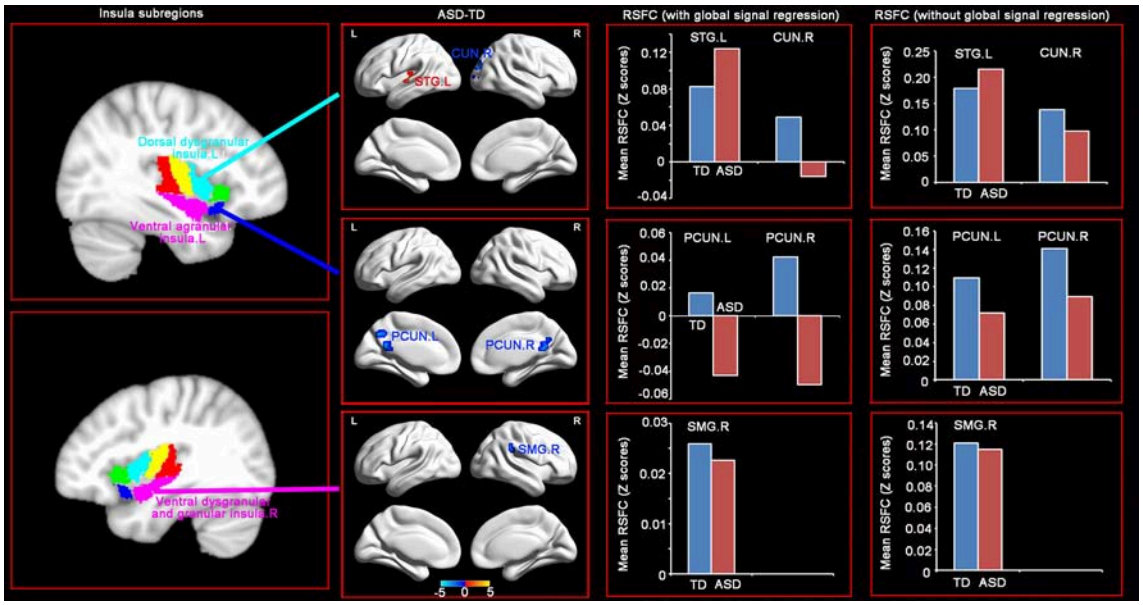

FIGURE 2 | Distinct RSFC patterns of the insular subregions in ASD. Two sample *t*-tests were used to identify the significant differences in functional connectivity between the ASD and TD groups with the age, gender, handedness, verbal IQs, performance IQs, and full-scale IQs as covariates. The significance was determined with a voxel-level corrected threshold of $p < 0.001$ and a cluster-level corrected threshold of $p < 0.05$ using the Gaussian random field (GRF) corrections. The red and blue colors represent increased and decreased functional connectivity respectively in the ASD group compared with the TD group. The results of mean RSFC with and without global signal showed similar patterns between the two groups.

TABLE 2 | Brain regions showing significant difference of functional connectivity with the insular subregions.

Seed regions	Abnormal regions	Types of connectivity	Number of voxels	Peak intensity	Peak coordinates		
Left ventral agranular insula	The right precuneus	Hypo-connectivity	46	−4.316	12	−56	31
	The left precuneus	Hypo-connectivity	36	−4.46	−12	−59	43
Right ventral dysgranular and granular insula	The right supramarginal gyrus	Hypo-connectivity	28	−4.1961	45	−32	31
Right dorsal dysgranular insula	The left superior temporal gyrus	Hyper-connectivity	46	4.8826	−48	−23	4
	The right cuneus	Hypo-connectivity	58	−4.1796	21	−86	4

the ASD. According to the behavioral results of the Brainnetome Atlas, both the left dorsal dysgranular insula and the right cuneus are mostly associated with perception, particularly the visual processing. When engaged in visual processing, ASD often exhibit enhanced perceptual abilities with more activity in the occipital regions, such as visual search (Keehn et al., 2008; Joseph et al., 2009; Samson et al., 2012) and visual discrimination (Bertone et al., 2005). However, a fMRI study of visual search (Keehn et al., 2013) is inconsistent with previous studies by showing neither group differences of any behavioral search measures nor differential patterns of activation in ASD. Due to these contradictory results and lack of visual measurements in our research, further investigation is needed to explain the result of the hypo-connectivity between the left dorsal dysgranular insula and the right cuneus in the ASD.

In addition, significant hyper-connectivity was identified between the left dorsal dysgranular insula and the STG.L in ASD. In line with the hyper-connectivity of our result, previous studies showed increased activity during social reward learning (Choi et al., 2015), sentence comprehension task (Just et al., 2004), and facial emotion processing (Dalton et al., 2008), as well as increased gray matter volume in STG.L in ASD (Waiter et al., 2004). Moreover, our finding of functional hyper-connectivity is also supported by the positive correlation with the ADI-R social scores, which implied that children with greater connectivity exhibited more severe impairment in the social domain. Considering that the left dorsal dysgranular insula is mostly associated with perception according to the behavioral results of the Brainnetome Atlas, this brain–behavior relationship suggests that aberrant functional connectivity may underlie the deficits of social perception in the ASD. Notably, the relationship

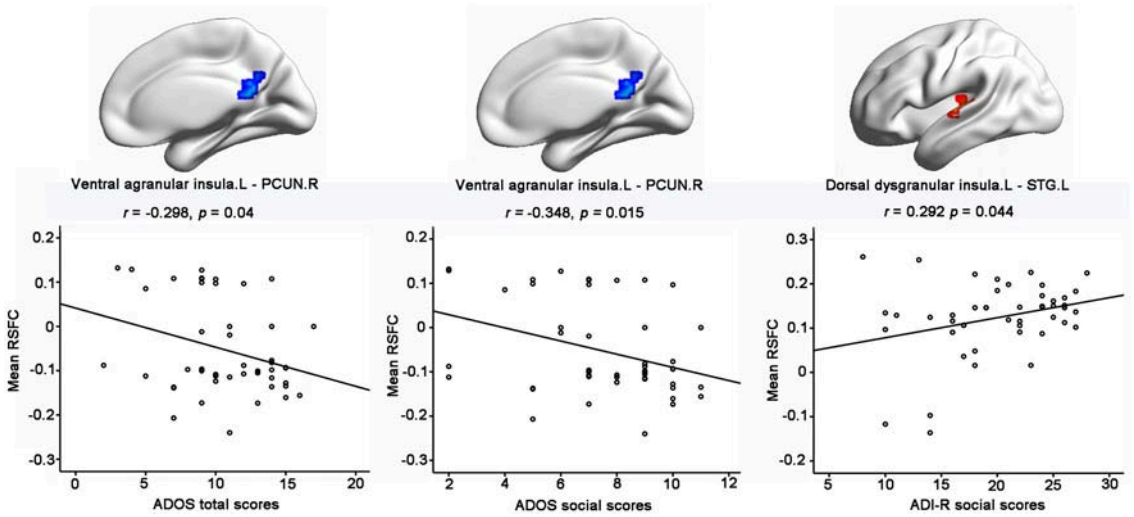

FIGURE 3 | Significant correlations between RSFC of insular subregions and clinical characteristics of ASD with the age, gender, handedness, verbal IQs, performance IQs, and full-scale IQs as covariates. Significance was determined with a threshold of $p < 0.05$.

between functional connectivity abnormalities of the left dorsal dysgranular insula and social severity was limited to the ADI-R social score, which is based on early social development, but not the ADOS score, which is a complimentary measure that rates current social functioning (Kleinhans et al., 2008). This pattern of correlation may imply that early development history plays an important part in the hyper-connectivity between the left dorsal dysgranular insula and the STG.L in ASD. Moreover, our finding is relatively novel. Further studies with large samples are needed to confirm this association and investigate its causes and clinical implications.

Several limitations should be acknowledged in our current study. First, some of the patients with ASD were given one or more psychotropic medications. Studies of drug-naïve patients to exclude the effects of medication on our findings are warranted. Second, given the high possibility that the RSFC can be effected by age (Dosenbach et al., 2010), we involved only children with a narrow range of age. However, this restriction left us no chance to address the developmental effects in the ASD. Thus, it remains a crucial topic for further investigation on the interaction between developmental changes and alterations of the RSFC in ASD.

In conclusion, compared with the TD group, the ASD group showed different connectivity patterns in the left ventral agranular insula, right ventral dysgranular and granular insula, and left dorsal dysgranular insula, characterized by significant hyper-connectivity and/or hypo-connectivity with specific brain regions. Furthermore, both the hypo-connectivity and hyper-connectivity of the insular subregions were significantly associated with the severity of ASD in children. Our research demonstrated distinct abnormalities in the RSFC patterns of the insular subregions and emphasized the importance of the subdivisions within the insula to potentially impact the functional difference in children with ASD. Moreover, these results might help us to better understand the mechanisms underlying the symptoms in children with ASD and might elucidate potential biomarkers for clinical applications.

AUTHOR CONTRIBUTIONS

HW and ZhZ acquired the data. JX, ZX, TL, and ZfZ analyzed data. JX, QH, LZ, and YG conceived this study and designed experiments. JX wrote the article with help of TL and ZfZ. All authors were involved in data interpretation and critically revising the manuscript.

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REFERENCES

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage* 38, 95–113. doi: 10.1016/j.neuroimage.2007.07.007

Baron-Cohen, S., and Wheelwright, S. (2004). The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. *J. Autism Dev. Disord.* 34, 163–175. doi: 10.1023/B:JADD.0000022607.19833.00

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., and Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *J. Neurosci.* 24, 9228–9231. doi: 10.1523/JNEUROSCI.3340-04.2004

Bertone, A., Mottron, L., Jelenic, P., and Faubert, J. (2005). Enhanced and diminished visuo-spatial information processing in autism depends on stimulus complexity. *Brain* 128, 2430–2441. doi: 10.1093/brain/awh561

Castelli, F., Frith, C., Happe, F., and Frith, U. (2002). Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. *Brain* 125, 1839–1849. doi: 10.1093/brain/awf189

Cauda, F., Costa, T., Torta, D. M., Sacco, K., D'agata, F., Duca, S., et al. (2012). Meta-analytic clustering of the insular cortex: characterizing the meta-analytic connectivity of the insula when involved in active tasks. *Neuroimage* 62, 343–355. doi: 10.1016/j.neuroimage.2012.04.012

Cauda, F., D'agata, F., Sacco, K., Duca, S., Geminiani, G., and Vercelli, A. (2011). Functional connectivity of the insula in the resting brain. *Neuroimage* 55, 8–23. doi: 10.1016/j.neuroimage.2010.11.049

Cavanna, A. E., and Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564–583. doi: 10.1093/brain/awl004

Chang, L. J., Yarkoni, T., Khaw, M. W., and Sanfey, A. G. (2013). Decoding the role of the insula in human cognition: functional parcellation and large-scale reverse inference. *Cereb. Cortex* 23, 739–749. doi: 10.1093/cercor/bhs065

Choi, U. S., Kim, S. Y., Sim, H. J., Lee, S. Y., Park, S. Y., Jeong, J. S., et al. (2015). Abnormal brain activity in social reward learning in children with autism spectrum disorder: an fMRI study. *Yonsei Med. J.* 56, 705–711. doi: 10.3349/ymj.2015.56.3.705

Cloutman, L. L., Binney, R. J., Drakesmith, M., Parker, G. J., and Lambon Ralph, M. A. (2012). The variation of function across the human insula mirrors its patterns of structural connectivity: evidence from *in vivo* probabilistic tractography. *Neuroimage* 59, 3514–3521. doi: 10.1016/j.neuroimage.2011.11.016

Craig, A. D. (2009). How do you feel–now? The anterior insula and human awareness. *Nat. Rev. Neurosci.* 10, 59–70. doi: 10.1038/nrn2555

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *J. Comp. Neurol.* 493, 154–166. doi: 10.1002/cne.20749

Dalton, K. M., Holsen, L., Abbeduto, L., and Davidson, R. J. (2008). Brain function and gaze fixation during facial-emotion processing in fragile X and autism. *Autism Res.* 1, 231–239. doi: 10.1002/aur.32

Deen, B., Pitskel, N. B., and Pelphrey, K. A. (2011). Three systems of insular functional connectivity identified with cluster analysis. *Cereb. Cortex* 21, 1498–1506. doi: 10.1093/cercor/bhq186

Di Martino, A., Ross, K., Uddin, L. Q., Sklar, A. B., Castellanos, F. X., and Milham, M. P. (2009). Functional brain correlates of social and nonsocial processes in autism spectrum disorders: an activation likelihood estimation meta-analysis. *Biol. Psychiatry* 65, 63–74. doi: 10.1016/j.biopsych.2008.09.022

Di Martino, A., Yan, C. G., Li, Q., Denio, E., Castellanos, F. X., Alaerts, K., et al. (2014). The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. *Mol. Psychiatry* 19, 659–667. doi: 10.1038/mp.2013.78

Dosenbach, N. U. F., Nardos, B., Cohen, A. L., Fair, D. A., Power, J. D., Church, J. A., et al. (2010). Prediction of individual brain maturity using fMRI. *Science* 329, 1358–1361. doi: 10.1126/science.1194144

Wechsler, D (1999). *Wechsler abbreviated scale of intelligence*. San Antonio, TX: Psychological Corporation.

Ebisch, S. J., Gallese, V., Willems, R. M., Mantini, D., Groen, W. B., Romani, G. L., et al. (2011). Altered intrinsic functional connectivity of anterior and posterior insula regions in high-functioning participants with autism spectrum disorder. *Hum. Brain Mapp.* 32, 1013–1028. doi: 10.1002/hbm.21085

Fan, L. Z., Li, H., Zhuo, J. J., Zhang, Y., Wang, J. J., Chen, L. F., et al. (2016). The Human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb. Cortex* 26, 3508–3526. doi: 10.1093/cercor/bhw157

Friston, K. J., Williams, S., Howard, R., Frackowiak, R. S., and Turner, R. (1996). Movement-related effects in fMRI time-series. *Magn. Reson. Med.* 35, 346–355. doi: 10.1002/mrm.1910350312

Geschwind, D. H., and Levitt, P. (2007). Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.* 17, 103–111. doi: 10.1016/j.conb.2007.01.009

Gordon, E. M., Laumann, T. O., Adeyemo, B., Huckins, J. F., Kelley, W. M., and Petersen, S. E. (2016). Generation and evaluation of a cortical area parcellation from resting-state correlations. *Cereb. Cortex* 26, 288–303. doi: 10.1093/cercor/bhu239

Jakab, A., Molnar, P. P., Bogner, P., Beres, M., and Berenyi, E. L. (2012). Connectivity-based parcellation reveals interhemispheric differences in the insula. *Brain Topogr.* 25, 264–271. doi: 10.1007/s10548-011-0205-y

Jones, A. P., Happe, F. G. E., Gilbert, F., Burnett, S., and Viding, E. (2010). Feeling, caring, knowing: different types of empathy deficit in boys with psychopathic tendencies and autism spectrum disorder. *J. Child Psychol. Psychiatry* 51, 1188–1197. doi: 10.1111/j.1469-7610.2010.02280.x

Joseph, R. M., Keehn, B., Connolly, C., Wolfe, J. M., and Horowitz, T. S. (2009). Why is visual search superior in autism spectrum disorder? *Dev. Sci.* 12, 1083–1096. doi: 10.1111/j.1467-7687.2009.00855.x

Just, M. A., Cherkassky, V. L., Keller, T. A., and Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain* 127, 1811–1821. doi: 10.1093/brain/awh199

Keehn, B., Brenner, L., Palmer, E., Lincoln, A. J., and Muller, R. A. (2008). Functional brain organization for visual search in ASD. *J. Int. Neuropsychol. Soc.* 14, 990–1003. doi: 10.1017/S1355617708081356

Keehn, B., Shih, P., Brenner, L. A., Townsend, J., and Muller, R. A. (2013). Functional connectivity for an island of sparing in autism spectrum disorder: an fMRI study of visual search. *Hum. Brain Mapp.* 34, 2524–2537. doi: 10.1002/hbm.22084

Kelly, C., Toro, R., Di Martino, A., Cox, C. L., Bellec, P., Castellanos, F. X., et al. (2012). A convergent functional architecture of the insula emerges across imaging modalities. *Neuroimage* 61, 1129–1142. doi: 10.1016/j.neuroimage.2012.03.021

Kleinhans, N. M., Richards, T., Sterling, L., Stegbauer, K. C., Mahurin, R., Johnson, L. C., et al. (2008). Abnormal functional connectivity in autism spectrum disorders during face processing. *Brain* 131, 1000–1012. doi: 10.1093/brain/awm334

Kurth, F., Eickhoff, S. B., Schleicher, A., Hoemke, L., Zilles, K., and Amunts, K. (2010a). Cytoarchitecture and probabilistic maps of the human posterior insular cortex. *Cereb. Cortex* 20, 1448–1461. doi: 10.1093/cercor/bhp208

Kurth, F., Zilles, K., Fox, P. T., Laird, A. R., and Eickhoff, S. B. (2010b). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct. Funct.* 214, 519–534. doi: 10.1007/s00429-010-0255-z

Lamm, C., Decety, J., and Singer, T. (2011). Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. *Neuroimage* 54, 2492–2502. doi: 10.1016/j.neuroimage.2010.10.014

Lockwood, P. L., Bird, G., Bridge, M., and Viding, E. (2013). Dissecting empathy: high levels of psychopathic and autistic traits are characterized by difficulties in different social information processing domains. *Front. Hum. Neurosci.* 7:760. doi: 10.3389/fnhum.2013.00760

Lord, C., Risi, S., Lambrecht, L., Cook, E. H. Jr., Leventhal, B. L., Dilavore, P. C., et al. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J. Autism Dev. Disord.* 30, 205–223. doi: 10.1023/A:1005592401947

Lord, C., Rutter, M., and Le Couteur, A. (1994). Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J. Autism Dev. Disord.* 24, 659–685. doi: 10.1007/BF02172145

Menon, V., and Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct. Funct.* 214, 655–667. doi: 10.1007/s00429-010-0262-0

Minshew, N. J., and Williams, D. L. (2007). The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch. Neurol.* 64, 945–950. doi: 10.1001/archneur.64.7.945

Monk, C. S., Peltier, S. J., Wiggins, J. L., Weng, S. J., Carrasco, M., Risi, S., et al. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage* 47, 764–772. doi: 10.1016/j.neuroimage.2009.04.069

Morey, L. C., Bender, D. S., and Skodol, A. E. (2013). Validating the proposed diagnostic and statistical manual of mental disorders, 5th edition, severity indicator for personality disorder. *J. Nerv. Ment. Dis.* 201, 729–735. doi: 10.1097/NMD.0b013e3182a20ea8

Nomi, J. S., Farrant, K., Damaraju, E., Rachakonda, S., Calhoun, V. D., and Uddin, L. Q. (2016). Dynamic functional network connectivity reveals unique and overlapping profiles of insula subdivisions. *Hum. Brain Mapp.* 37, 1770–1787. doi: 10.1002/hbm.23135

Noonan, S. K., Haist, F., and Muller, R. A. (2009). Aberrant functional connectivity in autism: evidence from low-frequency BOLD signal fluctuations. *Brain Res.* 1262, 48–63. doi: 10.1016/j.brainres.2008.12.076

Paakki, J. J., Rahko, J., Long, X., Moilanen, I., Tervonen, O., Nikkinen, J., et al. (2010). Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. *Brain Res.* 1321, 169–179. doi: 10.1016/j.brainres.2009.12.081

Samson, F., Mottron, L., Soulieres, I., and Zeffiro, T. A. (2012). Enhanced visual functioning in autism: an ALE meta-analysis. *Hum. Brain Mapp.* 33, 1553–1581. doi: 10.1002/hbm.21307

Satterthwaite, T. D., Elliott, M. A., Gerraty, R. T., Ruparel, K., Loughead, J., Calkins, M. E., et al. (2013). An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *Neuroimage* 64, 240–256. doi: 10.1016/j.neuroimage.2012.08.052

Seminowicz, D. A., and Davis, K. D. (2007). Interactions of pain intensity and cognitive load: the brain stays on task. *Cereb. Cortex* 17, 1412–1422. doi: 10.1093/cercor/bhl052

Shih, P., Keehn, B., Oram, J. K., Leyden, K. M., Keown, C. L., and Muller, R. A. (2011). Functional differentiation of posterior superior temporal sulcus in autism: a functional connectivity magnetic resonance imaging study. *Biol. Psychiatry* 70, 270–277. doi: 10.1016/j.biopsych.2011.03.040

Shih, P., Shen, M., Ottl, B., Keehn, B., Gaffrey, M. S., and Muller, R. A. (2010). Atypical network connectivity for imitation in autism spectrum disorder. *Neuropsychologia* 48, 2931–2939. doi: 10.1016/j.neuropsychologia.2010.05.035

Singer, T., Critchley, H. D., and Preuschoff, K. (2009). A common role of insula in feelings, empathy and uncertainty. *Trends Cogn. Sci.* 13, 334–340. doi: 10.1016/j.tics.2009.05.001

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12569–12574. doi: 10.1073/pnas.0800005105

Uddin, L. Q., and Menon, V. (2009). The anterior insula in autism: under-connected and under-examined. *Neurosci. Biobehav. Rev.* 33, 1198–1203. doi: 10.1016/j.neubiorev.2009.06.002

Uddin, L. Q., Supekar, K., and Menon, V. (2013a). Reconceptualizing functional brain connectivity in autism from a developmental perspective. *Front. Hum. Neurosci.* 7:458. doi: 10.3389/fnhum.2013.00458

Uddin, L. Q., Supekar, K., Lynch, C. J., Khouzam, A., Phillips, J., Feinstein, C., et al. (2013b). Salience network-based classification and prediction of symptom severity in children with autism. *JAMA Psychiatry* 70, 869–879. doi: 10.1001/jamapsychiatry.2013.104

Von Dem Hagen, E. A., Stoyanova, R. S., Baron-Cohen, S., and Calder, A. J. (2013). Reduced functional connectivity within and between social resting state networks in autism spectrum conditions. *Soc. Cogn. Affect. Neurosci.* 8, 694–701. doi: 10.1093/scan/nss053

Waiter, G. D., Williams, J. H., Murray, A. D., Gilchrist, A., Perrett, D. I., and Whiten, A. (2004). A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. *Neuroimage* 22, 619–625. doi: 10.1016/j.neuroimage.2004.02.029

Wang, C. N., Liu, Y., Wei, X. H., and Li, L. X. (2007). [Effects of electroacupuncture combined with behavior therapy on intelligence and behavior of children of autism]. *Zhongguo Zhen Jiu* 27, 660–662. Available online at: http://opentcm.com/Article1181.html

Wang, C., Wu, H., Chen, F., Xu, J., Li, H., Li, H., et al. (2017). Disrupted functional connectivity patterns of the insula subregions in drug-free major depressive disorder. *J. Affect. Disord.* 234, 297–304. doi: 10.1016/j.jad.2017.12.033

Welchew, D. E., Ashwin, C., Berkouk, K., Salvador, R., Suckling, J., Baron-Cohen, S., et al. (2005). Functional disconnectivity of the medial temporal lobe in Asperger's syndrome. *Biol. Psychiatry* 57, 991–998. doi: 10.1016/j.biopsych.2005.01.028

Weng, S. J., Wiggins, J. L., Peltier, S. J., Carrasco, M., Risi, S., Lord, C., et al. (2010). Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain Res.* 1313, 202–214. doi: 10.1016/j.brainres.2009.11.057

Yamada, T., Itahashi, T., Nakamura, M., Watanabe, H., Kuroda, M., Ohta, H., et al. (2016). Altered functional organization within the insular cortex in adult males with high-functioning autism spectrum disorder: evidence from connectivity-based parcellation. *Mol. Autism* 7, 41. doi: 10.1186/s13229-016-0106-8

Yan, C. G., Cheung, B., Kelly, C., Colcombe, S., Craddock, R. C., Di Martino, A., et al. (2013). A comprehensive assessment of regional variation in the impact of head micromovements on functional connectomics. *Neuroimage* 76, 183–201. doi: 10.1016/j.neuroimage.2013.03.004

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frontiers in Neuroscience

ORIGINAL RESEARCH
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Decreased Connection Between Reward Systems and Paralimbic Cortex in Depressive Patients

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Despite decades of research on depression, the underlying pathophysiology of depression remains incompletely understood. Emerging evidence from task-based studies suggests that the abnormal reward-related processing contribute to the development of depression. It is unclear about the function pattern of reward-related circuit during resting state in depressive patients. In present study, seed-based functional connectivity was used to evaluate the functional pattern of reward-related circuit during resting state. Selected seeds were two key nodes in reward processing, medial orbitofrontal cortex (mOFC) and nucleus accumbens (NAcc). Fifty depressive patients and 57 healthy participants were included in present study. Clinical severity of participants was assessed with Hamilton depression scale and Hamilton anxiety scale. We found that compared with healthy participants, depressive patients showed decreased connectivity of right mOFC with left temporal pole (TP_L), right insula extending to superior temporal gyrus (INS_R/STG) and increased connectivity of right mOFC with left precuneus. Similarly, decreased connectivity of left mOFC with TP_L and increased connectivity with cuneus were found in depressive patients. There is also decreased connectivity of right NAcc with bilateral temporal pole, as well as decreased connectivity of left NAcc with INS_R/STG. In addition, the functional connectivity of right nucleus accumbens with right temporal pole (TP_R) was negatively correlated with clinical severity. Our results emphasize the role of communication deficits between reward systems and paralimbic cortex in the pathophysiology of depression.

Keywords: depression, reward system, orbitofrontal cortex, nucleus accumbens, temporal pole

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INTRODUCTION

Depression is among common psychiatric disorders and the leading causes of disability worldwide (Ferrari et al., 2013). Despite decades of research on depression, the pathological neural mechanisms of depression remains incompletely understood. As a heterogenous psychiatric disorder, diverse symptoms of depression may attribute to distinct pathophysiology (Drysdale et al., 2016; Guo et al., 2016). Increasingly, anhedonia is regarded as a cardinal feature of depression (Pizzagalli, 2014) and associated with increased risk for suicide (Ducasse et al., 2018) and poor

treatment outcome (Vrieze et al., 2014). Anhedonia is defined as reduced interest or pleasure previously rewarding activities, part of a spectrum of reward circuit abnormalities (Der-Avakian and Markou, 2012). Convergent studies implicated the dysfunction of reward brain system in the neurobiology of anhedonia (Auerbach et al., 2017; Karcher et al., 2017). Indeed, behavioral studies have revealed the abnormality during reward-related processing in depression (Rizvi et al., 2018), displayed several types such as reward response bias, impaired reward learning ability and increased risk avoidance (Smoski et al., 2009).

The orbitofrontal cortex (OFC) is a key node in processing salience and magnitude of rewards (O'Doherty et al., 2001; Gottfried et al., 2003; Schoenbaum et al., 2011). Besides, OFC also plays a critical role in integrating reward information based on its strong anatomical connection with reward-related regions sensory, limbic and ventral striatal cortex (Kahnt et al., 2012). Ventral striatum is another core region in reward-related processing (Bartra et al., 2013; Sescousse et al., 2013). As a part of the ventral striatum, the nucleus accumbens (NAcc) is an important component of the reward circuit in the brain (Misaki et al., 2016), mainly responsible for mediating hedonic perception of rewards. In addition to perception of rewards, NAcc also takes on as a modulator in motivation-related behavior, which can influence several symptoms in depression, such as lack of motivation, anergia, or psychomotor slowing (Salamone et al., 2005).

Indeed, evidence from task-based neuroimaging studies has validated the maladaptive neural response of OFC and NAcc in reward-related processing in patients with depression. Blunted activity in OFC and NAcc for reward outcomes was consistently identified in depression (Tremblay et al., 2005; Pizzagalli et al., 2009). Depressive patients also exhibit hypoactivation of medial OFC in the neural coding for reward prediction (Rothkirch et al., 2017), as well as the decreased activity in NAcc (Ubl et al., 2015). In addition, the aberrancy of OFC and NAcc in depression is also supported by evidence from structural findings (Kempton et al., 2011; Lu et al., 2017).

Besides task-based and structural methods, another functional neuroimaging tool, resting-state functional magnetic resonance imaging (RS-fMRI), enables the detection of spontaneous brain activity to identify brain dysfunction in diseases (Biswal et al., 1995; Zhang and Raichle, 2010; Ji et al., 2017). With the non-invasive and task-independent feature, RS-fMRI has been increasingly applied in several mental diseases, especially in depressive disorder (Jalbrzikowski et al., 2017; Anhoj et al., 2018; Chen et al., 2018). Dysfunction within reward circuit among depressive individuals has been recognized using RS-fMRI (Cheng et al., 2016; Gong et al., 2017). Seed-based functional connectivity is one of technique to measure multi-regional cooperation within a special network. With the seed of medial and lateral OFC, Cheng et al. (2016) found that depressive patients exhibit reduced functional connectivity of medial OFC (mOFC) with temporal gyrus but increased functional connectivity of lateral OFC with precuneus and angular gyrus. Accordingly, they concluded medial reward and lateral non-reward orbitofrontal cortex circuits in depression. Consistent with this view, Gong et al. (2017) found decreased connectivity of NAcc with OFC, dorsomedial prefrontal cortex, superior temporal gyrus and insular lobe in depression. However, there are also studies reported against this view (Avery et al., 2014; Rzepa and McCabe, 2016). Diversity of seeds selection in different studies may explain these discrepancies. There is few work used diverse seeds within reward network in a single study to validate the reward-network abnormality in depression.

In present study, we investigated the functional coupling of depressive patients within reward network with diverse seeds (mOFC and NAcc). We hypothesized that there are similar alteration of coupling pattern between the NAcc-based and mOFC-based reward circuit in depressive individuals. In addition, we also investigated the neural alteration of reward-circuits coupling and clinical severity.

MATERIALS AND METHODS

Participants

Fifty patients with depressive episode from Anhui Mental Health Center were included in present study. All patients diagnosed with depressive episode according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Patients were excluded if they met the following exclusion criteria: (1) a history of ECT in the last 3 months; (2) age $>$ 65 years; (3) diagnosed with substance misuse, schizoaffective disorder, or schizophrenia; (4) past or current neurological illness; (5) head motion exceeding 2 mm in translation or 2° in rotation during fMRI scanning; (6) other contraindications of MRI scan. Clinical severity of patients was assessed with Hamilton depression scale (HAMD) and Hamilton anxiety scale (HAMA). We also recruited 57 healthy participants who met the same exclusion criteria as depressive patients except the diagnosis of depressive disorder. This study was carried out in accordance with the recommendations of Human Brain Imaging Collection, Anhui Medical University Ethics Committee. The protocol was approved by the Anhui Medical University Ethics Committee. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

MRI Data Acquisition

Resting-state and structural images of participants were acquired at the First Affiliated Hospital of Anhui Medical University. Participants were instructed to keep their eyes closed and move and think as little as possible during the MRI scanning. Resting-state MRI scans were conducted under a 3.0 T MRI scanner (Signa HDxt 3.0 T, GE Healthcare, Buckinghamshire, United Kingdom) composed of 240 echo-planar imaging volumes with the following parameters: TR = 2000 ms; TE = 22.5 ms; flip angle = 30°; matrix size = 64 × 64, field of view = 220 mm × 220 mm; slice thickness = 4 mm; 33 continuous slices (one voxel = 3.4 mm × 3.4 mm × 4.6 mm). High resolution three-dimensional brain volume imaging (3D BRAVO) for each participant was also acquired as an anatomical reference with following parameters: TR = 8.676 ms; TE = 3.184 ms; inversion time = 800 ms; flip angle = 8°; field of view = 256 mm × 256 mm; slice thickness = 1 mm; voxel size = 1 mm × 1 mm × 1 mm.

Functional Data Preprocessing

Functional data pre-processing was conducted with the Data Processing Assistant for Resting-State Functional MR Imaging toolkit (DPARSF), a software package based on Statistical Parametric Mapping software (SPM8[1]) and Resting State Functional MR Imaging Toolkit (REST[2]). The first 10 volumes were discarded to exclude the influence of unstable longitudinal magnetization. The remaining volumes were processed using the following steps: slice timing correction; realignment; coregistering to respective structural images; smoothed with a Gaussian kernel of 6 mm × 6 mm × 6 mm. The resulting images were regressed out nuisance signals, including global mean, white matter, cerebrospinal fluid signals, and 24 head-motion parameters. Finally, the images were filtered with a temporal band-pass of 0.01–0.1 Hz.

Functional Connectivity of Reward Circuits

The bilateral masks of NAcc and mOFC were defined according to the Human Brainnetome Atlas (Fan et al., 2016), a new brain atlas build upon connectional architecture. The mOFC masks consist of three subregions, labeled as ID 41, 47, 49 (left mOFC) and ID 42, 48, 50 (right mOFC) in Human Brainnetome Atlas. All seeds were showed in **Figure 1**. For each seeds, the functional connectivity was acquired by Pearson correlation coefficients between the mean time series of seed region and all brain voxels (defined by the binary gray matter mask in SPM). A Fisher's Z transformation was applied to improve the normality of correlation coefficient values. Finally, two-sample *t*-tests were applied to map group difference of connectivity map for each seed between depressive and healthy participants.

Statistical Analysis

For group-level structural and functional connectivity analyses, we performed two-sample *t*-tests for comparisons between the depressive patients and healthy controls for connectivity map of each seed. All statistical maps were thresholded using the Gaussian random field (GRF) correction with a voxel-level threshold of $P < 0.001$ and a cluster-level threshold of $P < 0.05$. We also compared changes in functional connectivity with changes in clinical symptoms among depressed individual using SPSS. The statistical level with $P < 0.05$ was considered as significant of correlation analysis with no correction.

RESULTS

Demographic and Clinical Characteristic

Present study included 50 patients with current depressive episode and 57 healthy controls. Demographic characteristic of the two groups are shown in **Table 1**. There was no significant difference between two groups in terms of age or gender. Compared with the healthy controls, depressive individuals presented greater HAMD and HAMA scores.

[1]www.fil.ion.ucl.ac.uk/spm

[2]http://www.restfmri.net

Group-Level Comparison of Functional Connectivity of the Bilateral mOFC

We explored the difference of functional connectivity between the two groups based on the seed of bilateral mOFC (shown in **Figure 2** and **Table 2**). Compared with healthy controls, depressive individuals presented decreased connectivity of right mOFC with left temporal pole (TP_L) and right insula extending to superior temporal gyrus (INS_R/STG), as well as increased connectivity with left precuneus. For the left mOFC, depressive individuals presented decreased connectivity in TP_L and increased connectivity in right cuneus.

Group-Level Comparison of Functional Connectivity of the Bilateral NAcc

Figure 3 and **Table 2** showed the difference of functional connectivity based on bilateral NAcc between depressive individuals and healthy controls. Compared with healthy controls, depressive individuals presented decreased connectivity of right NAcc with right temporal pole (TP_R) and TP_L. There was also decreased connectivity of left NAcc with INS_R/STG.

The Relationship Between Functional Connectivity and the Clinical Severity of Depressive Individuals

A negative relationship ($r = -0.393$, $P = 0.005$) existed between right NAcc-TP_R connectivity and depressive symptomatology among depressive individuals as shown in **Figure 4**. There was also a negative relationship ($r = -0.305$, $P = 0.031$) between right NAcc-TP_R connectivity and anxiety severity among depressive individuals.

DISCUSSION

In the present study, we investigated the function-pattern alterations of reward-related circuit during resting state in depressive patients with two key nodes in reward network, mOFC and NAcc. Compared with healthy participants, with both seeds of mOFC and NAcc, depressive individuals displayed decreased connectivity of reward network with paralimbic cortex, including temporal pole (TP), insula, and superior temporal gyrus. Furthermore, decreased connectivity between reward network and paralimbic cortex was significantly associated with clinical severity in depressive individuals.

Consistent with previous task-related neuroimaging studies, our findings with RS-fMRI also revealed the disrupted reward circuits in depressive individuals, specially, a reduction on the connectivity of mOFC and NAcc with paralimbic cortex. It is well-established that mOFC and NAcc play a crucial role in the representation of value-based information (Bartra et al., 2013). Blunt neural responses of mOFC and NAcc toward reward-related stimulus were frequently reported in depressive individuals (Tremblay et al., 2005; Redlich et al., 2015). Reduced activities of mOFC and NAcc during reward processing are associated with clinical characteristic in depression, such as depressive severity, anhedonia severity, and suicide in depression

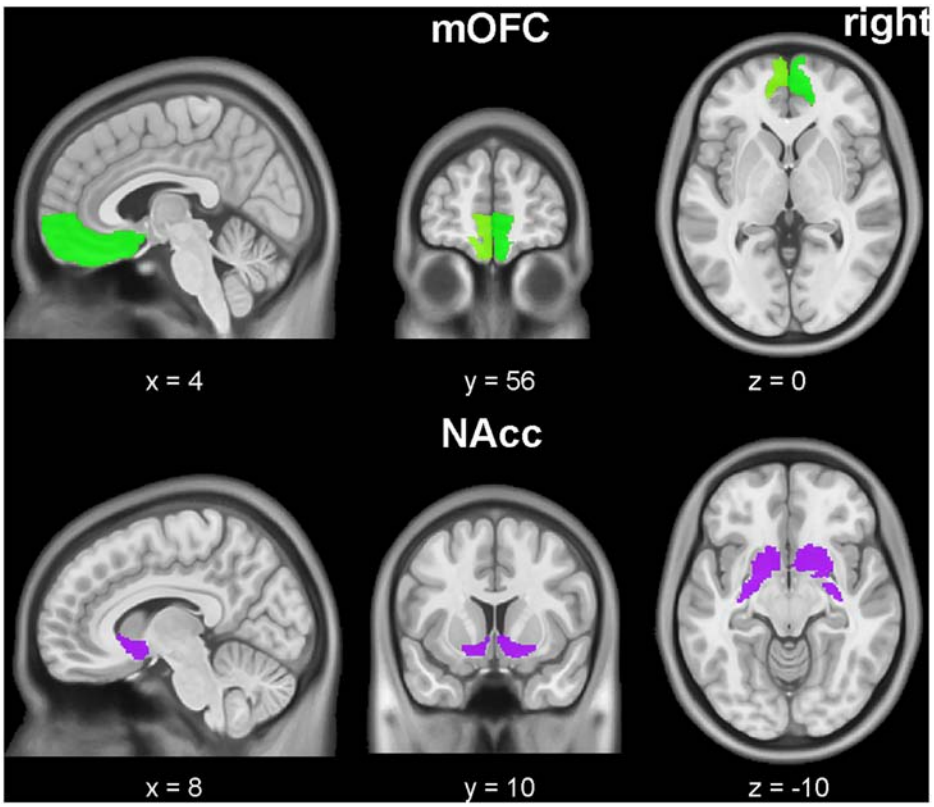

FIGURE 1 | Medial orbitofrontal cortex (mOFC) (right and left, green) and nucleus accumbens (NAcc) (right and left, purple) seed ROIs. The underlying brain template is from MNI152 standard-space structural image. The x, y, z values are in MNI coordinates.

(Misaki et al., 2016; Kim et al., 2017; Rothkirch et al., 2017). Recently, findings from resting-state studies also validated the abnormal coupling of mOFC or NAcc with other brain regions in depressive individuals (Baeken et al., 2017; Gong et al., 2017). Gong et al. (2017) found decreased connectivity of NAcc with OFC, dorsomedial prefrontal cortex, superior temporal gyrus, and insular lobe in depression. Similarly, another study with brain-wide voxel-level resting state neuroimaging analysis revealed reduced functional connectivity of mOFC with temporal gyrus in depression (Cheng et al., 2016). In line with previous studies, based on both the seeds of mOFC and NAcc, we found reduced connectivity of reward system with paralimbic cortex (temporal pole and insular lobe) in depression. Significantly, the connectivity between mOFC and TP is negatively associated with clinical severity.

The TP is a node of paralimbic system with strong connectivity with orbitofrontal cortex, striatum, insula, amygdala, and other emotion-related regions (Fan et al., 2014). As an association cortex, the TP enable multisensory integration and plays key roles in cognitive and socioemotional processing, such as memory, face processing and theory of mind (Olson et al., 2007). The disturbance of these processing is constantly correlated with depression (Zobel et al., 2010; Bistricky et al., 2011). Structural and functional abnormities of the TP have been detected in depression (Beauregard et al., 2006; Peng et al., 2011). Coupled with our findings, decreased mOFC-TP connectivity in depressive individuals has been demonstrated by prior works (Cheng et al., 2016; Rolls et al., 2018). Cheng et al. (2016) concluded that reduced functional connectivity between brain areas involved in pleasant feelings and rewards with memory

TABLE 1 | Demographic and clinical characteristic.

	Depressive individuals	Healthy controls	*t* or χ^2	*P*-value
Age (mean ± *SD*)	38.68 ± 11.33	36.68 ± 8.76	1.03[a]	0.31
Gender (M/F)	17/33	22/35	0.24[b]	0.62
HAMD (mean ± *SD*)	22.78 ± 3.96	2.93 ± 1.51	35.10[a]	<0.001
HAMA (mean ± *SD*)	15.10 ± 6.81	2.26 ± 1.34	33.42[a]	<0.001

[a]*t-Value;* [b]*chi-square; HAMD, Hamilton depression scale; HAMA, Hamilton anxiety scale; SD, standard deviation; M, male; F, female.*

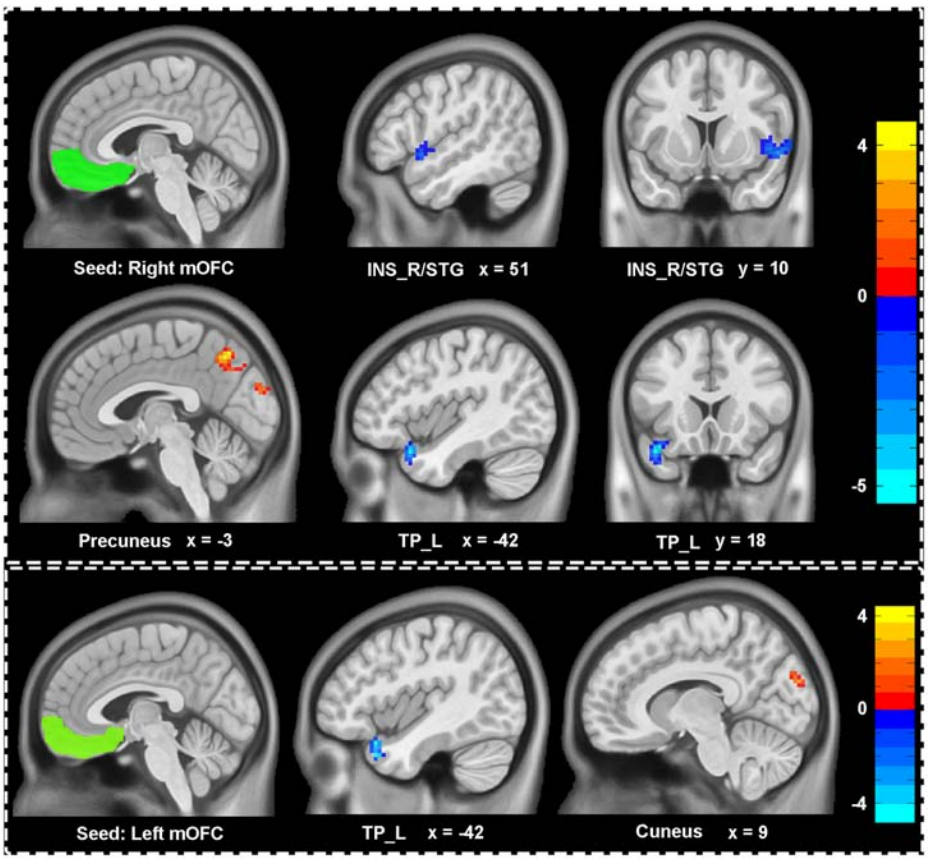

FIGURE 2 | Aberrant functional connectivity of mOFC in depressive patients compared with healthy controls. Depressive patients showed decreased connectivity of right mOFC with left temporal pole (TP_L), right insula extending to superior temporal gyrus (INS_R/STG) and increased connectivity of right mOFC with left precuneus. There were also decreased connectivity of left mOFC with LTP and increased with cuneus. All statistical maps were corrected with Gaussian random field (GRF) method at threshold of voxel $P < 0.001$, cluster $P < 0.05$. The *t* score bars are shown at right. The x, y values are in MNI coordinates.

systems, and that this may be part of the mechanism of depression. This hypothesis is strengthened by the significant relation between the depressive severity and the reduced connection between the mOFC and temporal lobe, revealed by both present and previous studies.

Along with the TP, the insula is another part of paralimbic system and involved in the evaluation of emotional or motivational salience of external and internal stimuli (Damasio et al., 2013). Specially, the insula participate in the representation of subjective value and act as a modulator for neural responses to losses and gains, which contribute to computing the costs and benefits in mixed valence scenarios (Bartra et al., 2013). Abnormal neural responses in insula during reward-related processes were closely related with depressive symptoms

TABLE 2 | Brain regions showing significant difference of functional connectivity with the reward network.

Seeds	Abnormal regions	Number of voxels	Peak intensity	Peak coordinates (x, y, z)[a]
Right mOFC	TP_L	45	−5.47	−42, 18, −24
	INS_R/STG	80	−4.46	54, 9, −6
	Precuneus	161	4.65	−3, −63, 48
Left mOFC	TP_L	50	−4.83	−42, 18, −33
	Cuneus	63	4.11	6, −87, 24
Right NAcc	TP_L	36	−4.59	−42, 18, −33
	TP_R	32	−4.38	33, 6, −36
Left NAcc	INS_R/STG	35	−4.36	33, 6, −6

[a] *The x, y, and z values are in Montreal Neurological Institute (MNI) coordinates. mOFC, medial orbitofrontal cortex; NAcc, nucleus accumbens; TP_L, left temporal pole; INS_R/STG, right insula extending to superior temporal gyrus; TP_R, right temporal pole.*

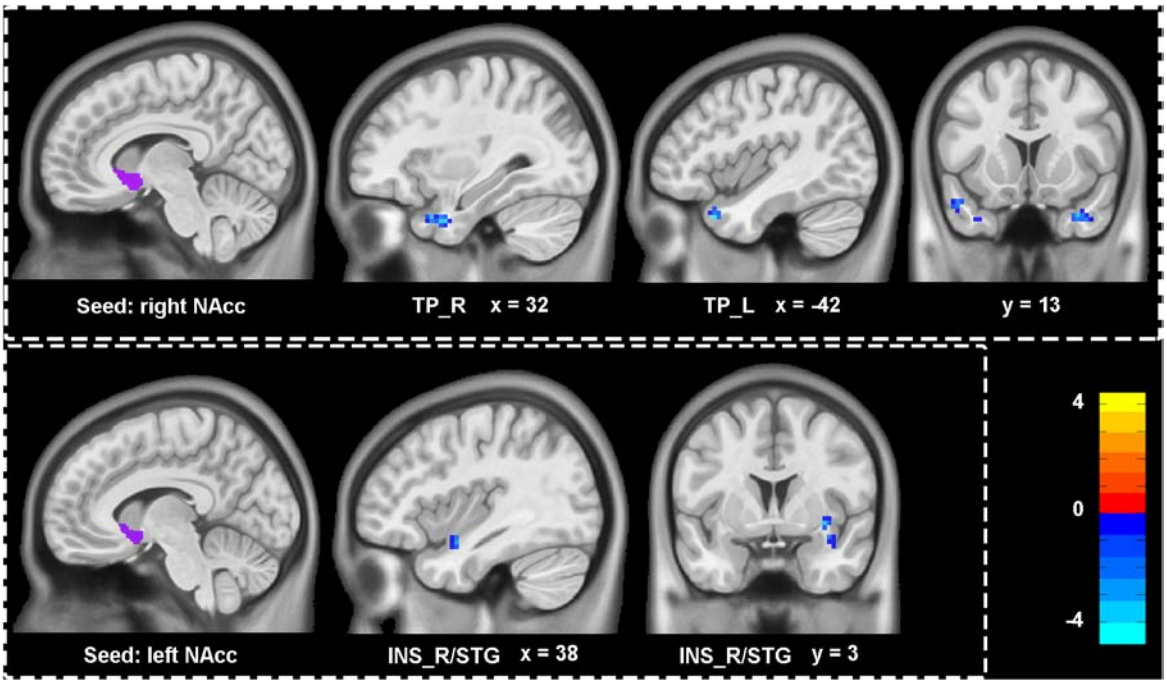

FIGURE 3 | Aberrant functional connectivity of NAcc in depressive patients compared with healthy controls. Depressive patients showed decreased connectivity of right NAcc with left and right temporal pole (TP_L, TP_R), as well as decreased connectivity of left NAcc with right insula extending to superior temporal gyrus (INS_R/STG). All statistical maps were corrected with GRF method at threshold of voxel $P < 0.001$, cluster $P < 0.05$. The t score bars are shown at right. The x, y values are in MNI coordinates.

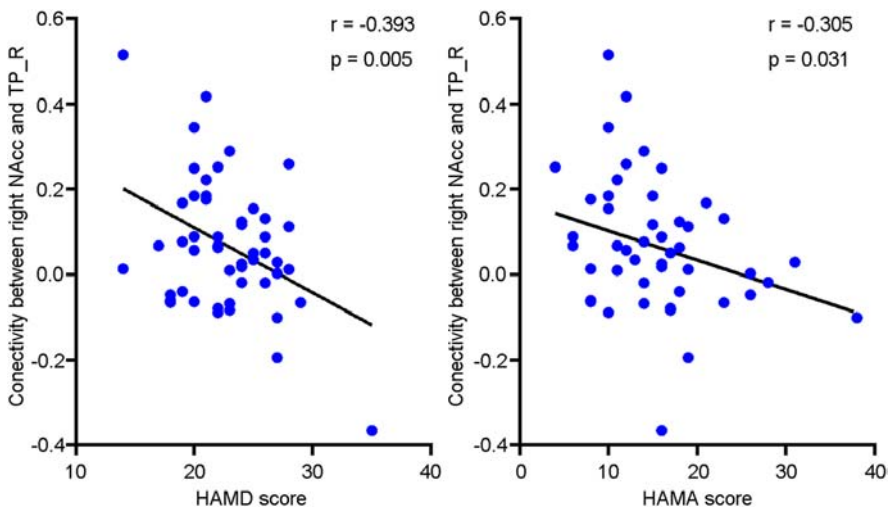

FIGURE 4 | Scatter plots of the negative relationship between clinical severity and functional connectivity of right NAcc with right temporal pole (TP_R). The clinical severity were assessed with Hamilton depression scale (HAMD) and Hamilton anxiety scale (HAMA).

(Engelmann et al., 2017). Further, our study found impaired connection between insula and acknowledged reward-related regions, OFC and ventral striatum, in depressive individuals. Indeed, there are strong structural connectivity between insula and acknowledged reward-related regions (Leong et al., 2016; Ghaziri et al., 2017). In line with our finding, previous neuroimaging study suggested that high risk for depression is related with the abnormal connection between OFC and insula during reward-related task (DelDonno et al., 2017). Based on the crucial role of insula in representation of emotional awareness and interoceptive signals, this connectional abnormity was interpreted that the impaired integration of the value of loss with the emotional and interoceptive awareness is correlated with the occurrence of depression (Rolls, 2016).

It is worthy of note that, besides decreased connectivity, increased connection of reward network among depressive individuals was also found within precuneus and cuneus. The precuneus is a key node of default mode network and involved in the sense of self and agency (Cavanna and Trimble, 2006). Increased functional connectivity during reward task between reward network and precuneus has been reported among patients with reward-related disease (Weiland et al., 2013). Studies with resting-state tool found the enhanced connectivity between precuneus and lateral OFC (defined as a non-reward/punishment system in depression) (Cheng et al., 2016). Along with precuneus, the cuneus is another prominent functional hub in the neural model of depression (Tomasi and Volkow, 2011). The cuneus is involved in the perception of facial emotion (Fusar-Poli et al., 2009), which is important for social interaction. Resting neuroimaging study in depression has suggested increased connectivity of reward network with the cuneus and that the enhanced connection was correlated with increased anhedonia severity (Yang et al., 2017). It is suggested be related to the explicit affectively negative sense of the self and increased anhedonia in depression (Rolls, 2016).

It is generally considered that there is high rate of suicide in patients with depression. Patients with suicidal behavior or ideation also presented aberrant reward processing and responses (Dombrovski et al., 2013; Silvers et al., 2016). The decreased connection between reward network and paralimbic cortex in depression may also attribute to the effect of suicide. Besides suicide, as a heterogeneous disease, depression manifest as diverse symptoms, such as anhedonia, low mood, anxiety, and somatic complaints. These symptoms have been associated with impaired reward-related processing (Avinun et al., 2017; Porreca and Navratilova, 2017; Nelson et al., 2018). For example, the increased anxiety symptoms were associated with decreased cortical activity while reward processing in both healthy participants and depressive patients (Nelson et al., 2018). Consistent with this finding, our results also implied the significant correlation between NAcc-TP connection and anxiety severity. In addition, different symptoms may have distinct effects on reward-based processing (Harle et al., 2017). Unfortunately, our study did not include the information about the specific symptoms of depressed patients. The absence of these informations restrains our attendance to understand what particular aspects of depression are associated with abnormal reward-network function. Further examinations are needed to explore the effect of specific symptoms on the reward network in depression.

It is must be acknowledged that there are several additional limitations in our study. On one hand, patients included in current study were given antidepressive medications. Future studies with drug-naïve patients to exclude the effects of medication are necessary. On the other hand, depressive patients enrolled into our study consisted of patients with both unipolar and bipolar depression. Given the different neural pattern of reward networks between unipolar and bipolar depression (Redlich et al., 2015), our findings mixed in factor of diagnostic types. Hence, our results should be interpreted with caution, and future investigations are needed divide participants into subgroups to clarify the distinct mechanisms underlying the specific subtypes of depressive individuals.

CONCLUSION

It compared with healthy participants, the depressive individuals showed decreased connectivity of reward network with paralimbic cortex, including TP, insula. The findings were validated with two key seeds of reward network, mOFC and NAcc. Significantly, the decreased connectivity between mOFC and TP was associated with depressive severity. Our study demonstrated reward-network abnormalities among depressive patients in resting-state functional pattern that underlies the pathogenesis of depression. These findings might also imply a potential biomarker for clinical applications.

AUTHOR CONTRIBUTIONS

TB and MZ performed the analysis and wrote the paper. YC, WX, CC, and QW helped to collect behavioral and imaging data. G-JJ help for the analysis of resting-state imaging data. YT and KW designed and supervised the present study.

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REFERENCES

Anhoj, S., Odegaard Nielsen, M., Jensen, M. H., Ford, K., Fagerlund, B., Williamson, P., et al. (2018). Alterations of intrinsic connectivity networks in antipsychotic-naive first-episode schizophrenia. *Schizophr. Bull.* doi: 10.1093/schbul/sbx171 [Epub ahead of print].

Auerbach, R. P., Pisoni, A., Bondy, E., Kumar, P., Stewart, J. G., Yendiki, A., et al. (2017). Neuroanatomical prediction of anhedonia in adolescents. *Neuropsychopharmacology* 42, 2087–2095. doi: 10.1038/npp.2017.28

Avery, J. A., Drevets, W. C., Moseman, S. E., Bodurka, J., Barcalow, J. C., and Simmons, W. K. (2014). Major depressive disorder is associated with abnormal

interoceptive activity and functional connectivity in the insula. *Biol. Psychiatry* 76, 258–266. doi: 10.1016/j.biopsych.2013.11.027

Avinun, R., Nevo, A., Knodt, A. R., and Elliott, M. L. (2017). Reward-related ventral striatum activity buffers against the experience of depressive symptoms associated with sleep disturbances. 37, 9724–9729. doi: 10.1523/jneurosci.1734-17.2017

Baeken, C., Duprat, R., Wu, G. R., De Raedt, R., and van Heeringen, K. (2017). Subgenual anterior cingulate-medial orbitofrontal functional connectivity in medication-resistant major depression: a neurobiological marker for accelerated intermittent theta burst stimulation treatment?. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 2, 556–565. doi: 10.1016/j.bpsc.2017.01.001

Bartra, O., McGuire, J. T., and Kable, J. W. (2013). The valuation system: a coordinate-based meta-analysis of BOLD fMRI experiments examining neural correlates of subjective value. *Neuroimage* 76, 412–427. doi: 10.1016/j.neuroimage.2013.02.063

Beauregard, M., Paquette, V., and Levesque, J. (2006). Dysfunction in the neural circuitry of emotional self-regulation in major depressive disorder. *Neuroreport* 17, 843–846. doi: 10.1097/01.wnr.0000220132.32091.9f

Bistricky, S. L., Ingram, R. E., and Atchley, R. A. (2011). Facial affect processing and depression susceptibility: cognitive biases and cognitive neuroscience. *Psychol. Bull.* 137, 998–1028. doi: 10.1037/a0025348

Biswal, B., Yetkin, F. Z., Haughton, V. M. and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn. Reson. Med.* 34, 537–541. doi: 10.1002/mrm.1910340409

Cavanna, A. E., and Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564–583. doi: 10.1093/brain/awl004

Chen, X., Ji, G. J., Zhu, C., Bai, X., Wang, L., He, K., et al. (2018). Neural correlates of auditory verbal hallucinations in schizophrenia and the therapeutic response to Theta-Burst transcranial magnetic stimulation. *Schizophrenia. Bull.* doi: 10.1093/schbul/sby054 [Epub ahead of print].

Cheng, W., Rolls, E. T., Qiu, J., Liu, W., Tang, Y., Huang, C. C., et al. (2016). Medial reward and lateral non-reward orbitofrontal cortex circuits change in opposite directions in depression. *Brain* 139, 3296–3309. doi: 10.1093/brain/aww255

Damasio, A., Damasio, H., and Tranel, D. (2013). Persistence of feelings and sentience after bilateral damage of the insula. *Cereb. Cortex* 23, 833–846. doi: 10.1093/cercor/bhs077

DelDonno, S. R., Jenkins, L. M., Crane, N. A., Nusslock, R., Ryan, K. A., Shankman, S. A., et al. (2017). Affective traits and history of depression are related to ventral striatum connectivity. *J. Affect. Disord.* 221, 72–80. doi: 10.1016/j.jad.2017.06.014

Der-Avakian, A., and Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci.* 35, 68–77. doi: 10.1016/j.tins.2011.11.005

Dombrovski, A. Y., Szanto, K., Clark, L., Reynolds, C. F., and Siegle, G. J. (2013). Reward signals, attempted suicide, and impulsivity in late-life depression. *JAMA Psychiatry* 70:1. doi: 10.1001/jamapsychiatry.2013.75

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., et al. (2016). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat. Med.* 23, 28–38. doi: 10.1038/nm.4246

Ducasse, D., Loas, G., Dassa, D., Gramaglia, C., Zeppegno, P., Guillaume, S., et al. (2018). Anhedonia is associated with suicidal ideation independently of depression: a meta-analysis. *Depress. Anxiety* 35, 382–392. doi: 10.1002/da.22709

Engelmann, J. B., Berns, G. S., and Dunlop, B. W. (2017). Hyper-responsivity to losses in the anterior insula during economic choice scales with depression severity. *Psychol. Med.* 47, 2879–2891. doi: 10.1017/s0033291717001428

Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., et al. (2016). The human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb. Cortex.*26, 3508–3526. doi: 10.1093/cercor/bhw157

Fan, L., Wang, J., Zhang, Y., Han, W., Yu, C., and Jiang, T. (2014). Connectivity-based parcellation of the human temporal pole using diffusion tensor imaging. *Cereb. Cortex* 24, 3365–3378. doi: 10.1093/cercor/bht196

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., et al. (2013). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med.* 10:e1001547. doi: 10.1371/journal.pmed.1001547

Fusar-Poli, P., Placentino, A., Carletti, F., Landi, P., Allen, P., Surguladze, S., et al. (2009). Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J. Psychiatry Neurosci.* 34, 418–432.

Ghaziri, J., Tucholka, A., Girard, G., Houde, J. C., Boucher, O., Gilbert, G., et al. (2017). The corticocortical structural connectivity of the human insula. *Cereb. Cortex* 27, 1216–1228. doi: 10.1093/cercor/bhv308

Gong, L., Yin, Y., He, C., Ye, Q., Bai, F., Yuan, Y., et al. (2017). Disrupted reward circuits is associated with cognitive deficits and depression severity in major depressive disorder. *J. Psychiatr. Res.* 84, 9–17. doi: 10.1016/j.jpsychires.2016.09.016

Gottfried, J. A., O'Doherty, J., and Dolan, R. J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science* 301, 1104–1107. doi: 10.1126/science.1087919

Guo, C. C., Hyett, M. P., Nguyen, V. T., Parker, G. B., and Breakspear, M. J. (2016). Distinct neurobiological signatures of brain connectivity in depression subtypes during natural viewing of emotionally salient films. *Psychol. Med.* 46, 1535–1545. doi: 10.1017/S0033291716000179

Harle, K. M., Guo, D., Zhang, S., Paulus, M. P., and Yu, A. J. (2017). Anhedonia and anxiety underlying depressive symptomatology have distinct effects on reward-based decision-making. *PLoS One* 12:e0186473. doi: 10.1371/journal.pone.0186473

Jalbrzikowski, M., Larsen, B., Hallquist, M. N., Foran, W., Calabro, F., and Luna, B. (2017). Development of white matter microstructure and intrinsic functional connectivity between the amygdala and ventromedial prefrontal cortex: associations with anxiety and depression. *Biol. Psychiatry* 82, 511–521. doi: 10.1016/j.biopsych.2017.01.008

Ji, G. J., Liao, W., Chen, F. F., Zhang, L., and Wang, K. (2017). Low-frequency blood oxygen level-dependent fluctuations in the brain white matter: more than just noise. *Sci. Bull.* 62, 656–657. doi: 10.1016/j.scib.2017.03.021

Kahnt, T., Chang, L. J., Park, S. Q., Heinzle, J., and Haynes, J. D. (2012). Connectivity-based parcellation of the human orbitofrontal cortex. *J. Neurosci.* 32, 6240–6250. doi: 10.1523/jneurosci.0257-12.2012

Karcher, N. R., Bartholow, B. D., Martin, E. A., and Kerns, J. G. (2017). Associations between electrophysiological evidence of reward and punishment-based learning and psychotic experiences and social anhedonia in at-risk groups. *Neuropsychopharmacology* 42, 925–932. doi: 10.1038/npp.2016.192

Kempton, M. J., Salvador, Z., Munafo, M. R., Geddes, J. R., Simmons, A., Frangou, S., et al. (2011). Structural neuroimaging studies in major depressive disorder. meta-analysis and comparison with bipolar disorder. *Arch. Gen. Psychiatry* 68, 675–690. doi: 10.1001/archgenpsychiatry.2011.60

Kim, K., Kim, S. W., Myung, W., Han, C. E., Fava, M., Mischoulon, D., et al. (2017). Reduced orbitofrontal-thalamic functional connectivity related to suicidal ideation in patients with major depressive disorder. *Sci. Rep.* 7;15772. doi: 10.1038/s41598-017-15926-0

Leong, J. K., Pestilli, F., Wu, C. C., Samanez-Larkin, G. R., and Knutson, B. (2016). White-matter tract connecting anterior insula to nucleus accumbens correlates with reduced preference for positively skewed gambles. *Neuron* 89, 63–69. doi: 10.1016/j.neuron.2015.12.015

Lu, Y., Shen, Z., Cheng, Y., Yang, H., He, B., Xie, Y., et al. (2017). Alternations of white matter structural networks in first episode untreated major depressive disorder with short duration. *Front. Psychiatry* 8:205. doi: 10.3389/fpsyt.2017.00205

Misaki, M., Suzuki, H., Savitz, J., Drevets, W. C., and Bodurka, J. (2016). Individual variations in nucleus accumbens responses associated with major depressive disorder symptoms. *Sci. Rep.* 6:21227. doi: 10.1038/srep21227

Nelson, B. D., Kessel, E. M., Klein, D. N., and Shankman, S. A. (2018). Depression symptom dimensions and asymmetrical frontal cortical activity while anticipating reward. *Psychophysiology* 55:e12892. doi: 10.1111/psyp.12892

O'Doherty, J., Kringelbach, M. L., Rolls, E. T., Hornak, J., and Andrews, C. (2001). Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat. Neurosci.* 4, 95–102. doi: 10.1038/82959

Olson, I. R., Plotzker, A., and Ezzyat, Y. (2007). The enigmatic temporal pole: a review of findings on social and emotional processing. *Brain* 130, 1718–1731. doi: 10.1093/brain/awm052

Peng, J., Liu, J., Nie, B., Li, Y., Shan, B., Wang, G., et al. (2011). Cerebral and cerebellar gray matter reduction in first-episode patients with major depressive

disorder: a voxel-based morphometry study. *Eur. J. Radiol.* 80, 395–399. doi: 10.1016/j.ejrad.2010.04.006

Pizzagalli, D. A. (2014). Depression, stress, and anhedonia: toward a synthesis and integrated model. *Annu. Rev. Clin. Psychol.* 10, 393–423. doi: 10.1146/annurev-clinpsy-050212-185606

Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., et al. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am. J. Psychiatry* 166, 702–710. doi: 10.1176/appi.ajp.2008.08081201

Porreca, F., and Navratilova, E. (2017). Reward, motivation, and emotion of pain and its relief. *Pain* 158(Suppl. 1), S43–S49. doi: 10.1097/j.pain.0000000000000798

Redlich, R., Dohm, K., Grotegerd, D., Opel, N., Zwitserlood, P., Heindel, W., et al. (2015). Reward processing in unipolar and bipolar depression: a functional MRI study. *Neuropsychopharmacology* 40, 2623–2631. doi: 10.1038/npp.2015.110

Rizvi, S. J., Lambert, C., and Kennedy, S. (2018). Presentation and neurobiology of anhedonia in mood disorders: commonalities and distinctions. *Curr. Psychiatry Rep.* 20:13. doi: 10.1007/s11920-018-0877-z

Rolls, E. T. (2016). A non-reward attractor theory of depression. *Neurosci. Biobehav. Rev.* 68, 47–58. doi: 10.1016/j.neubiorev.2016.05.007

Rolls, E. T., Cheng, W., Gilson, M., Qiu, J., Hu, Z., Ruan, H., et al. (2018). Effective connectivity in depression. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 3, 187–197. doi: 10.1016/j.bpsc.2017.10.004

Rothkirch, M., Tonn, J., Kohler, S., and Sterzer, P. (2017). Neural mechanisms of reinforcement learning in unmedicated patients with major depressive disorder. *Brain* 140, 1147–1157. doi: 10.1093/brain/awx025

Rzepa, E., and McCabe, C. (2016). Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology. *J. Psychiatr. Res.* 82, 40–47. doi: 10.1016/j.jpsychires.2016.07.013

Salamone, J. D., Correa, M., Mingote, S. M., and Weber, S. M. (2005). Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Curr. Opin. Pharmacol.* 5, 34–41. doi: 10.1016/j.coph.2004.09.004

Schoenbaum, G., Takahashi, Y., Liu, T. L., and McDannald, M. A. (2011). Does the orbitofrontal cortex signal value? *Ann. N. Y. Acad. Sci.* 1239, 87–99. doi: 10.1111/j.1749-6632.2011.06210.x

Sescousse, G., Caldu, X., Segura, B., and Dreher, J. C. (2013). Processing of primary and secondary rewards: a quantitative meta-analysis and review of human functional neuroimaging studies. *Neurosci. Biobehav. Rev.* 37, 681–696. doi: 10.1016/j.neubiorev.2013.02.002

Silvers, J. A., Hubbard, A. D., Chaudhury, S., Biggs, E., Shu, J., Grunebaum, M. F., et al. (2016). Suicide attempters with borderline personality disorder show differential orbitofrontal and parietal recruitment when reflecting on aversive memories. *J. Psychiatr. Res.* 81, 71–78. doi: 10.1016/j.jpsychires.2016.06.020

Smoski, M. J., Felder, J., Bizzell, J., Green, S. R., Ernst, M., Lynch, T. R., et al. (2009). fMRI of alterations in reward selection, anticipation, and feedback in major depressive disorder. *J. Affect. Disord.* 118, 69–78. doi: 10.1016/j.jad.2009.01.034

Tomasi, D., and Volkow, N. D. (2011). Functional connectivity hubs in the human brain. *Neuroimage* 57, 908–917. doi: 10.1016/j.neuroimage.2011.05.024

Tremblay, L. K., Naranjo, C. A., Graham, S. J., Herrmann, N., Mayberg, H. S., Hevenor, S., et al. (2005). Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. *Arch. Gen. Psychiatry* 62, 1228–1236. doi: 10.1001/archpsyc.62.11.1228

Ubl, B., Kuehner, C., Kirsch, P., Ruttorf, M., Diener, C., and Flor, H. (2015). Altered neural reward and loss processing and prediction error signalling in depression. *Soc. Cogn. Affect. Neurosci.* 10, 1102–1112. doi: 10.1093/scan/nsu158

Vrieze, E., Demyttenaere, K., Bruffaerts, R., Hermans, D., Pizzagalli, D. A., Sienaert, P., et al. (2014). Dimensions in major depressive disorder and their relevance for treatment outcome. *J. Affect. Disord.* 155, 35–41. doi: 10.1016/j.jad.2013.10.020

Weiland, B. J., Welsh, R. C., Yau, W. Y., Zucker, R. A., Zubieta, J. K., and Heitzeg, M. M. (2013). Accumbens functional connectivity during reward mediates sensation-seeking and alcohol use in high-risk youth. *Drug Alcohol Depend.* 128, 130–139. doi: 10.1016/j.drugalcdep.2012.08.019

Yang, X. H., Tian, K., Wang, D. F., Wang, Y., Cheung, E. F. C., Xie, G. R., et al. (2017). Anhedonia correlates with abnormal functional connectivity of the superior temporal gyrus and the caudate nucleus in patients with first-episode drug-naive major depressive disorder. *J. Affect. Disord.* 218, 284–290. doi: 10.1016/j.jad.2017.04.053

Zhang, D., and Raichle, M. E. (2010). Disease and the brain's dark energy. *Nat. Rev. Neurol.* 6, 15–28. doi: 10.1038/nrneurol.2009.198

Zobel, I., Werden, D., Linster, H., Dykierek, P., Drieling, T., Berger, M., et al. (2010). Theory of mind deficits in chronically depressed patients. *Depress. Anxiety* 27, 821–828. doi: 10.1002/da.20713

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Oxytocin Enhancement of Emotional Empathy: Generalization Across Cultures and Effects on Amygdala Activity

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Accumulating evidence suggests that the neuropeptide oxytocin (OXT) can enhance empathy although it is unclear which specific behavioral and neural aspects are influenced, and whether the effects are modulated by culture, sex, and trait autism. Based on previous findings in Caucasian men, we hypothesized that a single intranasal dose of OXT would specifically enhance emotional empathy (EE) via modulatory effects on the amygdala in an Asian (Chinese) population and explored the modulatory role of sex and trait autism on the effects. We first conducted a double-blind, randomized between-subject design experiment using a modified version of the multifaceted empathy task to determine whether OXT's facilitation of EE can be replicated in Chinese men (n = 60). To further explore neural mechanisms behind and potential sex differences, functional MRI and skin conductance measures were acquired in an independent experiment incorporating men and women (n = 72). OXT enhanced EE across experiments and sex, an effect that was accompanied by reduced amygdala activity and increased skin conductance responses. On the network level OXT enhanced functional coupling of the right amygdala with the insula and posterior cingulate cortex for positive valence stimuli but attenuated coupling for negative valence stimuli. The effect of OXT on amygdala functional connectivity with the insula was modulated by trait autism. Overall, our findings provide further support for the role of OXT in facilitating EE and demonstrate that effects are independent of culture and sex and involve modulatory effects on the amygdala and its interactions with other key empathy regions.

Keywords: amygdala, autism, cognitive empathy, culture, emotional empathy, oxytocin

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INTRODUCTION

Empathy is a key social-cognitive capacity that facilitates interpersonal functioning by allowing us to recognize, understand, and respond appropriately to mental and affective states experienced by others (Decety and Jackson, 2004; Dziobek et al., 2008; Reniers et al., 2010). Impaired empathy is a core deficit in psychiatric disorders characterized by interpersonal dysfunctions, including autism (Dziobek et al., 2008), schizophrenia (Shamay-Tsoory et al., 2007; Lee et al., 2011; Rosenfeld et al., 2011), and personality disorders (Herpertz and Bertsch, 2014).

Empathy is a multidimensional construct, entailing cognitive processes of perspective-taking, to make inferences about others' mental states (cognitive empathy, CE), as well as emotional processes reflecting a direct affective reaction involving understanding, sharing, and responding appropriately to others' feelings (emotional empathy, EE) (Shamay-Tsoory et al., 2009; Shamay-Tsoory, 2011; Bernhardt and Singer, 2012). EE has been further divided into a direct component (direct emotional empathy, EED), referring to explicit emotional evaluation and empathic concern, and an indirect component (indirect emotional empathy, EEI), referring to a more general physiological arousal response to both person and context (Dziobek et al., 2008). Although the cognitive and emotional components of empathy represent partly dissociable systems (Shamay-Tsoory et al., 2009), integrative approaches propose that the experience of empathy evolves as a dynamic interplay between them requiring an explicit representation of the specific affective state of the other person, thereby making CE a prerequisite for EE (Decety and Jackson, 2004; Hillis, 2014). On the neural level the functional organization of empathy is partially mirrored in shared and separable anatomical representations (Lamm et al., 2007, 2011; Schulte-Ruther et al., 2007; Singer and Lamm, 2009; Bernhardt and Singer, 2012; Leigh et al., 2013), with the bilateral insula, posterior cingulate cortex (PCC), and anterior cingulate cortex (ACC) contributing to both (Fan et al., 2011), and the amygdala contributing to the emotional component of empathy (Cox et al., 2012; Leigh et al., 2013).

Converging evidence suggests that the hypothalamic neuropeptide oxytocin (OXT) facilitates empathy (Rosenfeld et al., 2011; Striepens et al., 2011; Riem, 2012). Genetic approaches have consistently revealed associations between individual variations in the OXT receptor gene and levels of trait empathy in Caucasian (Rodrigues et al., 2009; Smith et al., 2014) and Chinese populations (Wu et al., 2012), with more recent studies suggesting that the specific associations evolve in interaction with other factors, particularly culture (Luo et al., 2015b; Montag et al., 2017) and sex (Weisman et al., 2015). Studies investigating the behavioral effects of intranasal OXT administration on CE have reported enhanced accuracy in the reading the mind in the eyes test (RMET) (Domes et al., 2007b) and a paradigm requiring participants to infer the intensity of positive or negative emotions expressed by subjects portrayed in videos (Bartz et al., 2010). However, findings in the domain of CE have been variable, with OXT effects in the RMET being either restricted to difficult items (Feeser et al., 2015) or unable to be reproduced at all even when taking into account stimulus difficulty and valence (Radke and de Bruijn, 2015). Other studies have also reported that effects were more pronounced in individuals with poor baseline performance (Riem et al., 2014) or high trait autism (Bartz et al., 2010). Studies that aimed specifically at determining effects of OXT on EE focused on empathy for pain, an evolutionary conserved primary emotional component (Decety, 2011; Panksepp and Panksepp, 2013), and found no effect on pain empathy toward a partner (Singer et al., 2008), although an enhanced pain empathic response toward members of an out-group (Shamay-Tsoory et al., 2013). In contrast, another study in men using the multifaceted empathy test (MET) (Dziobek et al., 2008), which assesses both CE and EE, observed that OXT specifically enhanced both EED and EEI, but not CE (Hurlemann et al., 2010). This latter study additionally demonstrated selective EE deficits in amygdala lesion patients and therefore suggested that the amygdala may mediate the EE enhancing effects of OXT. Although several neuroimaging studies have demonstrated modulatory effects of intranasally administered OXT on the core neural components of the empathy network, including the insula, ACC, and amygdala and their functional interactions, across different task paradigms (Bakermans-Kranenburg and van IJzendoorn, 2013; Wigton et al., 2015; Herpertz and Bertsch, 2016), to date only two studies have directly explored the neural mechanisms underlying OXT's empathy enhancing effects. The first reported that OXT increased activation in the superior temporal gyrus and insula during the RMET task (Riem et al., 2014), whereas the second reported reductions in left insula activity during pain empathic processing (Bos et al., 2015).

In summary, although the empathy enhancing effects of OXT are central to its proposed social-cognitive and therapeutic properties, it remains unclear whether it selectively enhances CE or EE, and which specific neural substrates are involved. To systematically address these questions, we employed two independent pharmacological between-subject placebo (PLC) controlled experiments in healthy Chinese individuals investigating the effects of intranasal OXT on CE and EE and the underlying neural basis of this effects during the MET (Dziobek et al., 2008).

Previous studies on the empathy enhancing potential of intranasal OXT are entirely based on observations in Caucasian populations. However, there is accumulating evidence from OXT-administration studies either employing comparable experimental protocols in Caucasian and Chinese subjects (Hurlemann et al., 2010; Hu et al., 2015) or examining moderating effects of key cultural orientation differences such as a collectivistic orientation (Pfundmair et al., 2014; Xu et al., 2017), suggesting culture-dependent social-cognitive effects of OXT. To this end, the first experiment aimed to replicate findings in a male Caucasian sample showing that OXT enhances EE but not CE (Hurlemann et al., 2010) in a male Chinese sample. In a second independent sample, male and female Chinese participants performed the same MET paradigm during functional magnetic resonance imaging (fMRI) to determine the neural substrates involved. Analyses on the neural level focused on the insula, amygdala, and ACC as core empathy regions (Lamm et al., 2007, 2011; Schulte-Ruther et al., 2007; Singer and Lamm, 2009; Bernhardt and Singer, 2012; Leigh et al., 2013). Given that the amygdala has been specifically (Cox et al., 2012; Leigh et al., 2013) and critically (Hurlemann et al., 2010) associated with emotional facets of empathy, we expected that OXT's enhancement of EE would be accompanied by altered regional activity and network level connectivity of the amygdala. Previous studies reported increased as well as decreased amygdala activity and connectivity following OXT (Domes et al., 2007a; Striepens et al., 2012; Hu et al., 2015; Wigton et al., 2015; Tully et al., 2018) therefore

no directed hypothesis with respect to OXT's neural effect was formulated.

Based on a growing number of findings suggesting sex-dependent effects of OXT on social cognition (Chen et al., 2016; Gao et al., 2016; Luo et al., 2017), the second experiment additionally explored whether OXT differentially affects empathic processing in men and women. In line with a previous study reporting that sex does not affect OXT's modulation of empathy (Shamay-Tsoory et al., 2013), we hypothesized that OXT facilitation of EE would generalize across sexes. Finally, in the context of increasing interest in the therapeutic application of OXT as a potential treatment to improve social cognitive deficits, including empathy, in autism spectrum disorders (Young and Barrett, 2015), and in line with previous studies in healthy subjects (Bartz et al., 2010; Scheele et al., 2014; Xu et al., 2015), the modulatory role of trait autism (assessed by the Autism Spectrum Quotient questionnaire, ASQ, Baron-Cohen et al., 2001) was explored.

MATERIALS AND METHODS

Participants

To fully replicate the previous study on Caucasian participants (Hurlemann et al., 2010), only males were recruited in the first experiment but both males and females were enrolled in the second experiment to explore potential sex-dependent effects of OXT on empathy. Experiment 1 (Exp 1) included 60 participants ($M \pm$ SD, mean age = 22.42 $\pm$ 2.23 years, all male) and Experiment 2 (Exp 2) included an independent sample of 72 participants (34 females, mean age = 21.18 $\pm$ 1.95 years, 38 males, mean age = 22.61 $\pm$ 2.01 years). Both experiments incorporated a double-blind, between-participant design, with participants being randomly assigned to receive either OXT or PLC nasal-spray, resulting in n = 30 (Exp 1) and n = 36 (Exp 2, female = 17) participants treated with OXT. The experimental groups in both experiments were of comparable age (Exp 1, p = 0.53, T_{58} = 0.63; Exp 2, p = 0.66, T_{70} = −0.44), education (Exp 1, p = 0.66, T_{58} = 0.44; Exp 2, p = 0.63, T_{70} = −0.49) and, in Exp 2, of equivalent sex distribution (chi-square < 0.001, df = 1, p = 1). Exclusion criteria for all participants were past or current physical, neurological, or psychiatric disorders, regular or current use of medication or tobacco.

Participants were required to abstain from alcohol, caffeine, or nicotine for at least 12 h before the experiment. None of the females in Exp 2 were taking oral contraceptives or were tested during their menstrual period. Menstrual cycle phase was determined using validated procedures as described in Penton-Voak et al. (1999). The proportion of females estimated to be in their follicular or luteal phases did not differ significantly between the treatment groups (chi-square = 0.12, df = 1, p = 0.73). In Exp 1, one participant (in the OXT group) and in Exp 2, three participants (in the PLC group) failed to understand task instructions and were consequently excluded from all further analysis, leading to a total of n = 59 participants in Exp 1 and n = 69 participants in Exp 2.

Before the experiment, written informed consent was obtained from all participants. The study was approved by the local ethics committee of the University of Electronic Science and Technology of China and all procedures and the informed consent for study participation were in accordance with the latest revision of the declaration of Helsinki.

Experimental Protocol

To control for potential confounding variables, all participants initially completed the following questionnaires: Becks Depression Inventory (BDI; Beck et al., 1961), WLEIS-C Emotional Intelligence Scale (Wleis-C; Wong and Law, 2002), State Trait Anxiety Inventory (STAI; Spielberger et al., 1970), Empathy Quotient (EQ; Baron-Cohen and Wheelwright, 2004), and Positive and Negative Affect Scale (PANAS; Watson et al., 1988). To examine associations with trait autism, the ASQ questionnaire (Baron-Cohen et al., 2001) was administered. Intranasal treatment (OXT nasal spray, Sichuan Meike Pharmacy Co., Ltd., China, or PLC nasal spray with identical ingredients except OXT) was administered in line with recommendations for the intranasal administration of OXT in humans (Guastella et al., 2013) and 45 min before the start of the experimental paradigm. In Exp 1, three puffs per nostril (at 30 s intervals) were administered (24 IU) and in Exp 2, five puffs per nostril (40 IU). Both doses are in the typical range employed by other studies (Striepens et al., 2011; Guastella et al., 2013) with the rationale for increasing the dose in Exp 2 being to explore dose-dependent behavioral effects of OXT. In a previous study we found equivalent behavioral and neural effects of 24 and 48 IU OXT doses (Zhao et al., 2017). However, it should be noted that findings from some other studies investigating dose-dependent effects of intranasal OXT have suggested an inverted-U-shaped dose–response curve (Cardoso et al., 2013; Quintana et al., 2016, 2017; Spengler et al., 2017) and thus a stronger enhancement of EE with 24 IU relative to 40 IU is conceivable. In post experiment interviews, participants were unable to guess better than chance whether they had received the OXT nasal spray, confirming successful blinding.

Experimental Paradigm

In line with a previous study on male Caucasian participants (Hurlemann et al., 2010), empathy was assessed using the MET (Dziobek et al., 2008; Hurlemann et al., 2010; Domes et al., 2013; Edele et al., 2013; Wingenfeld et al., 2014), which assesses both EE and CE components using ecologically valid photo-based stimuli of either negative or positive valence. To account for potential confounding effects of OXT on in-group versus out-group empathy (De Dreu and Kret, 2016) and a cultural empathy bias (Cao et al., 2015; Luo et al., 2015a), the original Caucasian MET stimuli were exchanged with corresponding pictures displaying Chinese protagonists. The Chinese stimuli were initially evaluated in an independent sample (Supplementary Materials) and the final set of Chinese stimuli (30 positive, 30 negative valence) closely resembled the Caucasian stimuli depicting daily life scenarios and conveying emotional mental states via facial expression, body posture, and contextual cues. To assess CE, participants were instructed to infer the

emotional state of the protagonist in each scene and choose the corresponding answer from four options listed. The four options presented similar but distinct emotional states to ensure at least 70% accuracy for each stimulus picture. For EED, participants were required to rate how they felt for the protagonist in the depicted scene (1–9 scale, 1 = not at all, 9 = very strong), for EEI participants were required to rate how much they were aroused by the scene (1–9 scale, 1 = very calm, 9 = very aroused). Details of the paradigm are visually presented in **Figure 1**.

The different components of empathy were presented in a mixed event/block-design. Following a 3 s instruction cue and a jittered inter-trial interval of 3.9 s (2.3–5.9 s), 10 stimuli per block were each presented for 3 s followed by either a choice of the emotion depicted for the CE condition (displayed for 4 s) or a rating scale (1–9) for the EED and EEI conditions (displayed for 5 s). Six blocks were presented for each condition, resulting in a total of 18 blocks. The order of blocks was counterbalanced across the experimental conditions, and the fMRI experiment was divided into six runs, each containing one block per empathy component. During fMRI (Exp 2) electrodermal activity was simultaneously acquired as an index of autonomic sympathetic activity (Stern et al., 2001) (technical details on the electrodermal data acquisition are provided in the Supplementary Materials). To allow baseline recovery of the electrodermal signal a mean inter-trial interval of 5 s (4–6 s) and a mean interval separating stimulus presentation and behavioral response of 4 s (3–5 s) was adopted for the fMRI experiment.

fMRI Data Acquisition

The fMRI data in Exp 2 were collected using a GE (General Electric Medical System, Milwaukee, WI, United States) 3.0T Discovery 750 MRI scanner. fMRI time series were acquired using a T2*-weighted echo planar imaging pulse sequence (repetition time, 2000 ms; echo time, 30 ms; slices, 39; thickness, 3.4 mm; gap, 0.6 mm; field of view, 240 × 240 mm^2; resolution, 64 × 64; flip angle, 90°). Additionally, a high resolution T1-weighted structural image was acquired using a 3D spoiled gradient recalled (SPGR) sequence (repetition time, 6 ms; echo time, 2 ms; flip angle 9°; field of view, 256 × 256 mm^2; acquisition matrix, 256 × 256; thickness, 1 mm without gap) to exclude participants with apparent brain pathologies and to improve normalization of the fMRI data.

fMRI Data Processing

Functional magnetic resonance imaging data were analyzed using SPM12 (Wellcome Trust Center of Neuroimaging, University College London, London, United Kingdom). The first five volumes were discarded to allow T1 equilibration and images

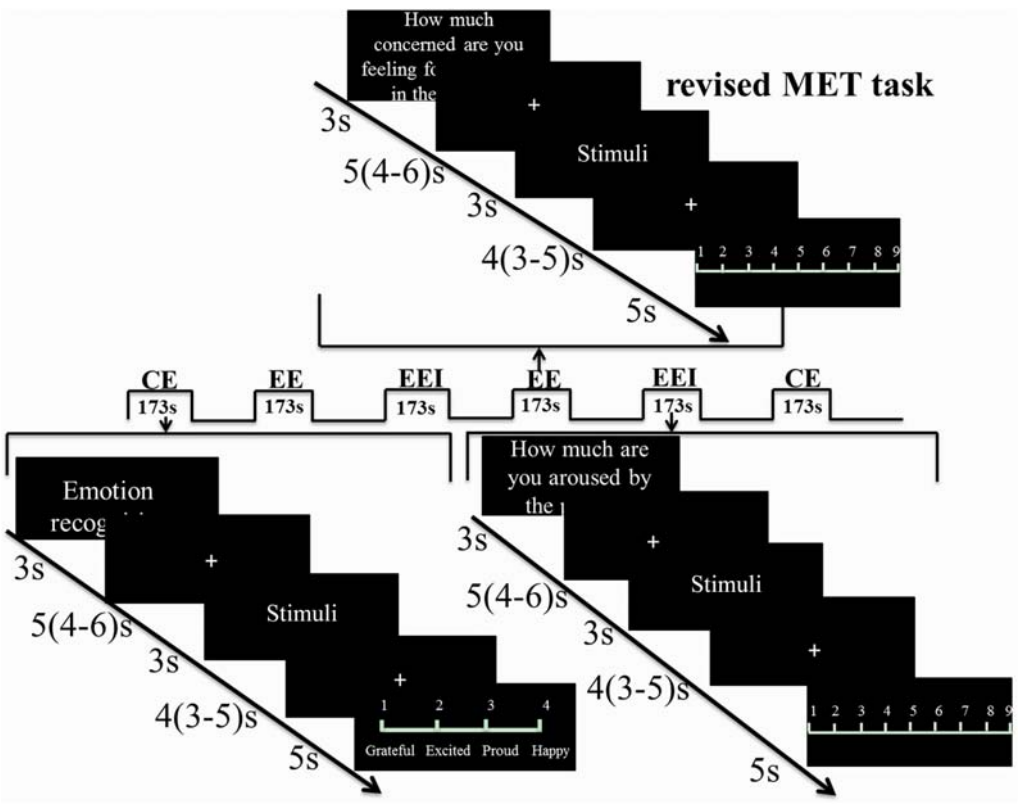

FIGURE 1 | Revised MET paradigm used in the fMRI experiment. Three blocks for CE, EED, and EEI were presented and in a balanced order. In each block, after 3 s of cue presentation, a jittered fixation (4–6 s) followed. Stimuli were shown for 3 s followed by another jittered fixation (3–5 s) and then a 5 s rating phase in order to separate the viewing and rating phases for fMRI analysis. Each block lasted for 173 s. There was a total of six runs with each run including three blocks, one block for CE, one for EED, and one for EEI.

were realigned to the first image to correct for head motion. Tissue segmentation, bias-correction, and skull-stripping were done for the high-resolution structural images. The functional time series were co-registered with the skull-stripped anatomical scan and normalized to MNI space with a voxel size of $3 \times 3 \times 3$ mm. Normalized images were then spatially smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8 mm. On the first level, event-related responses were modeled and subsequently convolved with the standard hemodynamic response function (HRF). The first level design matrix included valence- (positive, negative) and empathy type- (CE, EED, EEI) specific regressors for the viewing phases as main experimental conditions. In addition, regressors for the cue presentation, valence-, and empathy type-specific regressors for the rating phases, and for viewing and rating phases of incorrect trials as well as the six movement regressors were included. The experimental contrasts were next submitted to a second level random effects analysis.

To evaluate empathy-type specific main and interaction effects of treatment and valence, repeated-measured ANOVAs were employed in a flexible-factorial design. Based on our regional hypothesis and the core empathy network (Hurlemann et al., 2010; Bernhardt and Singer, 2012; Bakermans-Kranenburg and van IJzendoorn, 2013; Leigh et al., 2013; Hillis, 2014; Wigton et al., 2015; Shamay-Tsoory and Abu-Akel, 2016), the analyses focused on the bilateral amygdala, insula, and ACC which were structurally defined using 60% probability maps from the Harvard-Oxford (sub)cortical atlas. For the regionally focused analysis approach condition-specific parameter estimates were extracted from these regions of interest (ROI) using the Marsbar toolbox (Brett et al., 2002) and subjected to empathy type-specific ANOVAs with the between-participant factor treatment (OXT, PLC) and the within-participant factor valence (positive, negative) in SPSS (Statistical Package for the Social Sciences, Version 22). *P*-values for the *post hoc* tests of the ROI analysis were Bonferroni-corrected ($P < 0.05$). An exploratory voxel-wise whole-brain analysis in SPM that served to determine contributions of brain regions outside of the predefined network of interest was thresholded at $P < 0.05$, corrected using the family-wise error (FWE) approach.

To investigate the effects of OXT on the network level, a generalized form of psychophysiological interaction analysis (gPPI[1]; McLaren et al., 2012) was conducted using regions showing significant OXT effects in the BOLD level analysis as seeds and implementing an empathy-type specific voxel-wise whole-brain ANOVA approach including the between-participant factor treatment (OXT, PLC) and the within-participant factor valence (positive, negative) thresholded at $P < 0.05$, FWE-corrected at the cluster level. In line with recent recommendations for the control of false-positives in cluster-based correction approaches an initial cluster forming threshold of $P < 0.001$ was applied to data with a resolution of $3 \times 3 \times 3$ mm (Eklund et al., 2016; Slotnick, 2017). Parameter estimates were extracted from the significant regions to disentangle the specific effects in *post hoc* comparisons. Finally, associations between neural indices and trait autism (ASQ scores) were conducted in SPSS using Pearson correlation analysis.

[1]http://brainmap.wisc.edu/PPI

RESULTS

In both experiments, there were no significant differences in trait and mood questionnaire scores between OXT and PLC treatment groups (Supplementary Table S1 for Exp 1, Supplementary Table S2 for Exp 2). In line with previous studies in Chinese populations (Melchers et al., 2015; Montag et al., 2017), no significant sex differences in ASQ and EQ scores were observed in Exp 2 (Supplementary Table S3).

Behavioral Results

Based on previous conceptualizations of empathy, proposing that CE is a prerequisite for EE (Decety and Jackson, 2004; Hillis, 2014), for EED and EEI measures only trials for which subjects successfully recognized the emotions displayed by the protagonist were analyzed [for a similar approach see Luo et al. (2015a)]. To this end, correctly recognized trials were initially determined based on the CE performance, with only correct trials subsequently entering the analyses for the EED and EEI facets.

There were no significant differences in CE accuracy between the two treatment groups in both experiments (Exp 1, OXT, 77.53 ± 6.02%, PLC, 78.94 ± 5.78%, $T_{57} = 0.92$, $P = 0.36$; Exp 2, OXT, 80.10 ± 6.18%, PLC, 81.57 ± 5.83%, $T_{67} = 1.02$, $P = 0.31$). In Exp 1, there was a main effect of treatment [$F(1,57) = 6.46$, $P = 0.01$, $\eta_p^2 = 0.10$] for EED indicating that OXT generally enhanced EED (**Figure 2A**). There was no significant treatment × valence interaction [$F(1,57) = 0.96$, $P = 0.33$, $\eta_p^2 = 0.02$]. Analysis of EEI did not reveal a treatment main effect [$F(1,57) = 2.19$, $P = 0.14$, $\eta_p^2 = 0.04$] or valence × treatment interaction effect [$F(1,57) = 3.16$, $P = 0.08$, $\eta_p^2 = 0.05$].

Consistent with the findings for EED in Exp 1, Exp 2 also yielded a significant main effect of treatment on EED [$F(1,67) = 5.81$, $P = 0.02$, $\eta_p^2 = 0.08$] with higher ratings following OXT compared to PLC (**Figure 2B**). There was also a significant valence × treatment interaction [$F(1,67) = 4.18$, $P = 0.05$, $\eta_p^2 = 0.06$] with more pronounced effects of OXT on negative compared to positive valence stimuli [positive: $F(1,67) = 1.68$, $P = 0.2$, $\eta_p^2 = 0.02$; negative: $F(1, 67) = 9.96$, $P = 0.002$, $\eta_p^2 = 0.13$]. For EEI there was also a significant main effect of treatment [$F(1,67) = 4.84$, $P = 0.03$, $\eta_p^2 = 0.07$] but no treatment × valence interaction [$F(1,67) = 2.02$, $P = 0.16$, $\eta_p^2 = 0.03$). For CE, there were neither significant main effects nor interactions [$F(1,65) = 0.80$, $P = 0.37$, $\eta_p^2 = 0.01$). In Exp 2, no significant main or interaction effects involving sex were observed (all *P*s > 0.18) arguing against sex-dependent effects of OXT on empathy.

Associations Between Behavior and Trait Autism

In Exp 1, there was a trend toward a negative correlation between the ASQ score and the total EED and EEI scores in the OXT

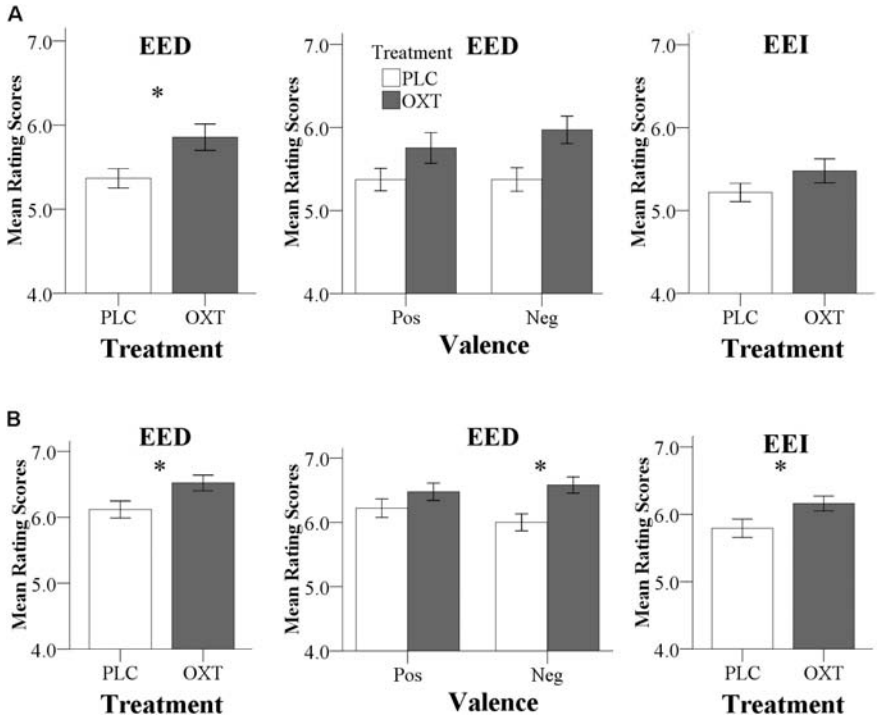

FIGURE 2 | Behavioral results for Exp 1 **(A)** and Exp 2 **(B)**. **(A)** In Exp 1 (24 IU administration), OXT significantly increased EED ratings. To allow for a better comparison with Exp 2, effects of OXT on positive and negative EED trials, as well as on EEI are also shown. **(B)** In Exp 2 (40 IU administration), OXT increased both EED and EEI ratings; an effect of OXT on negative EED drove the significant treatment by valence interaction. (*$P < 0.05$).

group (ASQ Total: EED $r = -0.47$, $P = 0.09$; EEI $r = -0.37$, $P = 0.19$) but not the PLC group (EED $r = 0.319$, $P = 0.18$; EEI $r = 0.17$, $P = 0.48$). The correlation significantly differed between the PLC and OXT groups for EED (Fisher's $z = -2.13$, $P = 0.03$) although not for EEI (Fisher's $z = -1.43$, $P = 0.15$). In Exp 2, there was a similar pattern of correlation differences between EED and EEI scores and total ASQ scores, although these associations did not reach statistical significance other than for EED under OXT (EED – PLC $r = 0.03$, $P = 0.85$, OXT $r = -0.34$, $P = 0.04$, Fisher's $z = 1.53$, $P = 0.13$; EEI – PLC $r = 0.03$, $P = 0.85$; OXT $r = -0.28$, $P = 0.09$, Fisher's $z = 1.29$, $P = 0.20$). Regression plots are shown in **Figure 3** and suggest that OXT is producing its main behavioral effects in participants with lower autism traits.

Dose-Dependent Effects Between Experiments 1 and 2 (24 vs. 40 IU)

Dose effects were explored by combining the data from male participants in Exp 1 (24 IU) and Exp 2 (40 IU). To initially explore potential effects of the different experimental environments (Exp 1, 24 IU, behavioral testing room; Exp 2, 40 IU, inside the MRI-scanner) on empathy *per se*, a first analysis focused on the PLC-treated subjects. A repeated ANOVA with environment (behavioral vs. MRI room) as a between-subject factor and valence as a within-subject factor revealed a significant environment main effect for both EE facets, indicating elevated EE ratings in the MRI room [main effects: EED: $P = 0.003$, $F(1,47) = 10.19$, $\eta_p^2 = 0.18$; EEI: $P = 0.02$, $F(1,47) = 5.74$, $\eta_p^2 = 0.11$, both interactions with valence > 0.38, non-significant, **Figure 4**], but no effects on CE [main effect: $P = 0.15$, $F(1,47) = 2.19$, $\eta_p^2 = 0.05$; interaction: $P = 0.9$, $F(1,47) = 0.02$, $\eta_p^2 < 0.001$]. These findings suggest that the MRI-environment *per se* increased EE, an effect possibly related to elevated levels of stress during the MRI assessments, which would be in line with a previous study reporting that stress-induction specifically increased EE, but not CE in the MET (Wolf et al., 2015). The environmental differences and potential interactions with OXT preclude the interpretation of dose-related differences between the experiments.

Oxytocin Effects on SCR

One participant was excluded from SCR analysis due to low skin impedance and thus a total of 68 participants from Exp 2 were included. Analyses of the SCR data paralleled the analyses of the empathy ratings, using ANOVAs with the between-subject factors treatment (OXT, PLC) and sex (male, female), and the within-subject factor valence. There was a marginal main effect of treatment on SCR during EED trials and significant during EEI trials [EED: $F(1,66) = 3.77$, $P = 0.06$, $\eta_p^2 = 0.05$; EEI: $F(1,66) = 4.50$, $P = 0.04$, $\eta_p^2 = 0.06$], but not during CE trials [$F(1,66) = 2.14$, $P = 0.15$, $\eta_p^2 = 0.03$]. This was due to SCR responses being increased in the OXT group during EE and EEI trials (**Figure 5**). There were no significant main effects of valence or treatment × valence or treatment × sex interactions for CE, EE, or EEI trials (all Ps > 0.2).

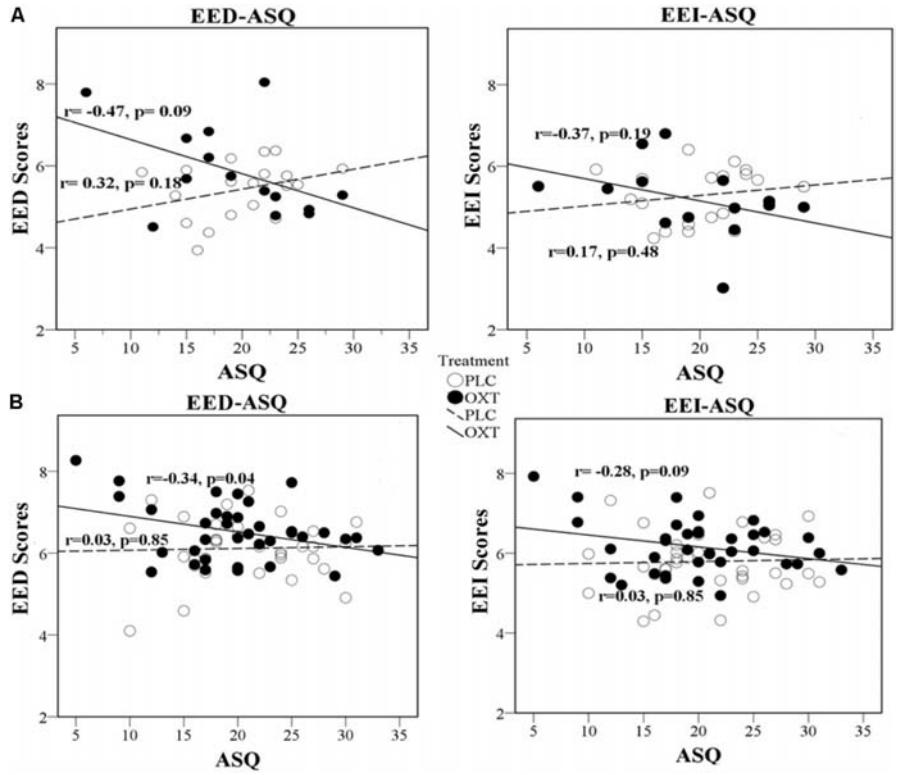

FIGURE 3 | Regression plots for ASQ score and EED and EEI ratings. Exp 1 **(A)** and Exp 2 **(B)** in OXT and PLC groups.

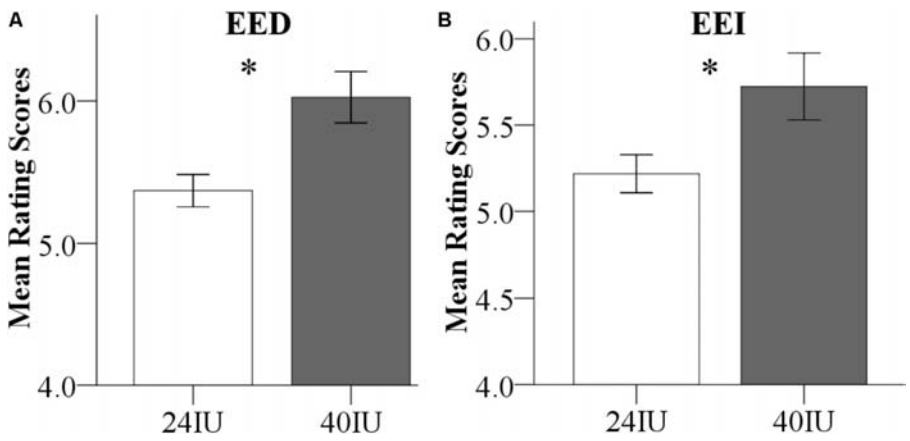

FIGURE 4 | (A) Direct emotional empathy (EED) and **(B)** indirect emotional empathy (EEI) ratings in the placebo (PLC) groups. Emotional empathy differences between Exp 1 and Exp 2 in the PLC-treated subjects. Examining the PLC-treated male subjects from the two experiments revealed that EE was significantly increased in the MRI environment (Exp 2) compared to the behavioral testing room (Exp 1).

Oxytocin Effects on Neural Activity

In view of the absence of sex-dependent effects in the behavioral analysis, and to increase the statistical power to determine OXT effects on the neural level, the data from male and female participants were pooled for the fMRI analyses. Four further participants were excluded from the fMRI analysis due to excessive head motion (head motion > 3 mm). The neural mechanisms underlying the behavioral effects of OXT were initially explored in the different priori ROIs (amygdala, insula, and ACC) using separate repeated measures ANOVAs for the three empathy (CE, EE, and EEI) conditions. Main treatment effects were only observed in the amygdala (**Figure 6A**) for EED [left amygdala: $F(1,63) = 6.55$, $P = 0.01$, $\eta_p^2 = 0.09$; right amygdala: $F(1,63) = 5.18$, $P = 0.03$, $\eta_p^2 = 0.08$]. There were no

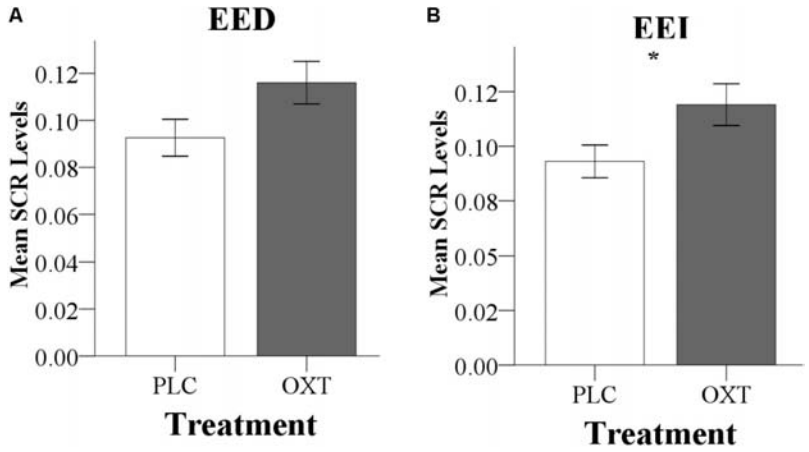

FIGURE 5 | Effects of oxytocin (OXT) on the SCR during **(A)** direct emotional empathy, EED, and **(B)** indirect emotional empathy, EEI. In Exp 2, OXT increased the SCR during both EED ($P = 0.06$) and EEI ($P < 0.05$). *Indicates $P < 0.05$.

significant main effects for CE or EEI or any treatment × valence interactions for CE, EED, or EEI. An exploratory whole brain analysis revealed no regions that showed significant treatment-dependent changes under CE, EED, or EEI (all $P_{FDR\_corrected} > 0.05$) outside of the prior defined ROIs.

Oxytocin Effects on Functional Connectivity

Repeated measures ANOVA models in SPM that included the between-subject factors treatment (OXT, PLC) and sex (male, female) and the within-subject factor valence (positive, negative) revealed a significant Treatment × Valence interaction effect for EED-associated functional coupling of the right amygdala with the bilateral insula and the bilateral PCC (left insula peak located at $x/y/z$, −33/6/−15, $P_{FWE} = 0.02$, cluster size = 143 voxels; right insula peak located at 45/18/−12, $P_{FWE} = 0.03$, cluster size = 121 voxels; left PCC peak located at −30/−33/30, $P_{FWE} = 0.003$, cluster size = 213 voxels; right PCC peak located at 21/−36/33, $P_{FWE} = 0.01$, cluster size = 149 voxels; coordinates given in MNI-space). Extraction of parameter estimates further revealed that OXT increased functional connectivity for positive valence stimuli whereas it decreased connectivity for negative valence ones [left insula: positive, $F(1,61) = 10.52$, $P = 0.002$, $\eta_p^2 = 0.15$, negative, $F(1,61) = 3.86$, $P = 0.05$, $\eta_p^2 = 0.06$; right insula: positive, $F(1,61) = 3.34$, $P = 0.07$, $\eta_p^2 = 0.05$, negative, $F(1,61) = 5.53$, $P = 0.02$, $\eta_p^2 = 0.08$; left PCC: positive, $F(1,61) = 4.34$, $P = 0.04$, $\eta_p^2 = 0.07$, negative, $F(1,61) = 5.67$, $P = 0.02$, $\eta_p^2 = 0.09$; right PCC: positive, $F(1,61) = 8.00$, $P = 0.006$, $\eta_p^2 = 0.12$, negative, $F(1,61) = 5.48$, $P = 0.02$, $\eta_p^2 = 0.08$] (**Figure 7**).

Associations Between Neural and SCR Effects of OXT and Behavioral and Autism Trait Scores

There was a significant positive correlation between EED scores and bilateral amygdala responses in the PLC group (left $r = 0.49$, $P = 0.004$; right $r = 0.35$, $P = 0.04$) which was absent in the OXT group (left $r = 0.005$, $P = 0.98$; right $r = -0.007$, $P = 0.97$). The correlation difference between the PLC and OXT groups was significant for the left (Fisher's $Z = 2.05$, $P = 0.04$) but not the right (Fisher's $Z = 1.43$, $P = 0.15$) amygdala (**Figure 6B**). There was no correlation between left or right amygdala responses with total ASQ scores.

For the functional connections showing OXT effects for EED in terms of a treatment × valence interaction, coupling strength between the right amygdala and left insula during positive valence EED trials was positively correlated with the total ASQ in the PLC group (total ASQ – $r = 0.40$, $P = 0.02$) but not in the OXT group (total ASQ – $r = -0.22$, $P = 0.22$, Fisher's $Z = 2.50$, $P = 0.01$; **Figure 8A**). OXT particularly appears to increase the strength of right amygdala functional connections with the insula in individuals with lower ASQ scores, although only for positive valence EED. The strength of link between the right amygdala and left PCC during negative valence EED trials was positively correlated with the total ASQ score in the PLC group but not the OXT, although the difference between the groups was not significant (total ASQ – PLC $r = 0.36$, $P = 0.04$; OXT $r = 0.15$, $P = 0.41$; Fisher's $Z = 0.85$, $P = 0.39$; **Figure 8B**). There were no significant correlations between SCR values and ASQ scores during either EED or EEI trials (all Ps > 0.41).

DISCUSSION

The present study confirmed in two independent samples that intranasal OXT specifically facilitates EE but not CE as assessed by the MET paradigm in Chinese participants, thereby replicating previous findings in Caucasian participants (Hurlemann et al., 2010). Our findings also demonstrated for the first time that the OXT-induced enhancement of EED is associated with decreased bilateral amygdala reactivity and enhanced functional coupling of the right amygdala with the insula and PCC for positive valence stimuli but attenuated coupling for negative valence stimuli.

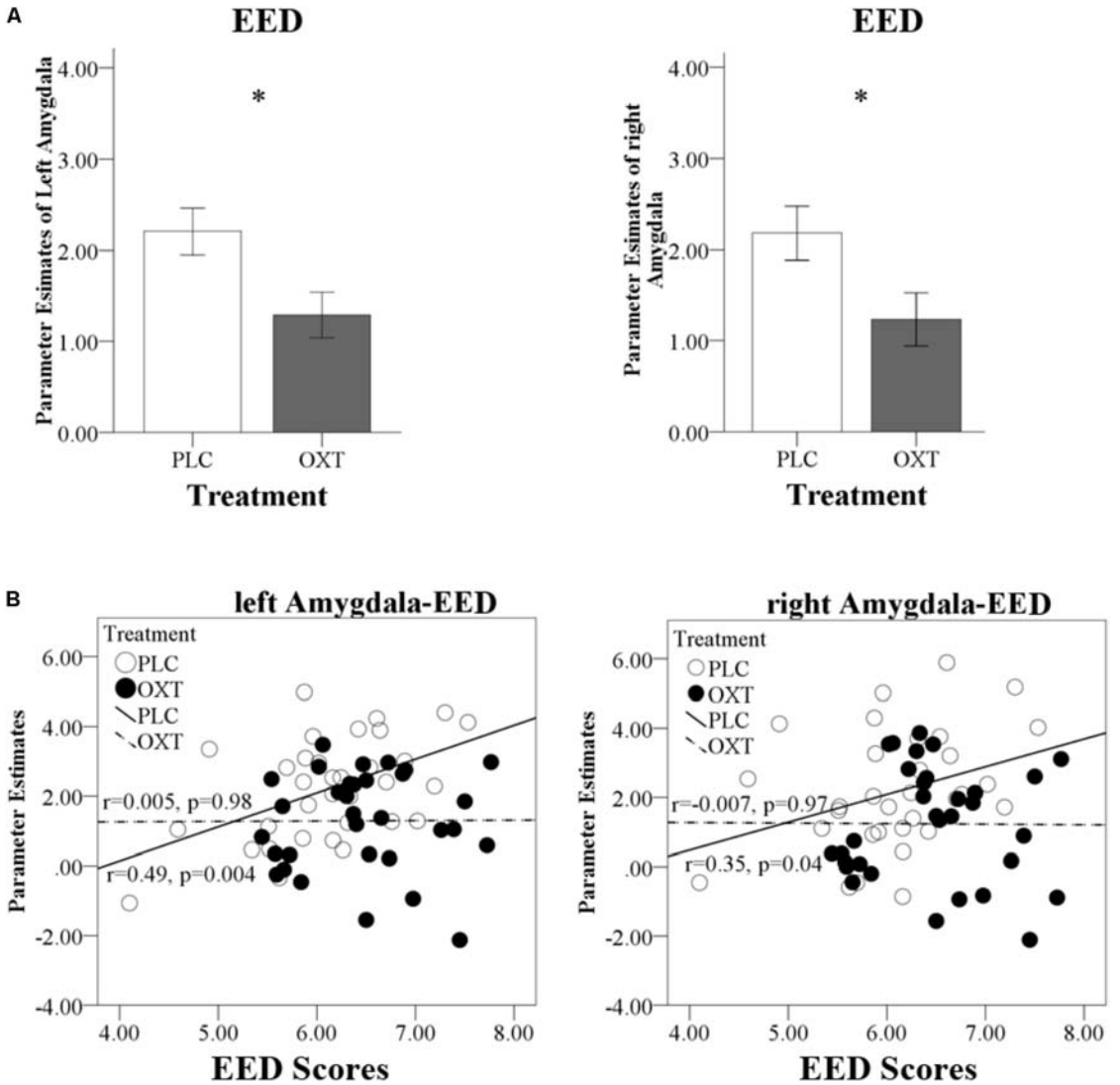

FIGURE 6 | Effects of OXT on bilateral amygdala responses. **(A)** Region of interest analysis results for left and right amygdala responses during EED trials. **(B)** Regression plots show correlations between left and right amygdala responses and EED scores in OXT and PLC groups. *$P < 0.05$.

These behavioral and neural effects were not modulated by subject sex, suggesting a generalization across men and women. Finally, an exploratory analysis of associations with trait autism revealed that both behavioral and neural effects of OXT were modulated to some extent by trait autism scores.

Although many studies have reported cultural differences between Asian and Caucasian participants in the context of OXT receptor polymorphisms and empathy (Kim et al., 2010; Luo et al., 2015b; Jessica et al., 2016), we did not find any substantive difference with respect to the effects of intranasal OXT on empathy processing as assessed by MET. Thus, in both cultures, OXT enhanced EE but not CE (Hurlemann et al., 2010) for both valences, although in our second experiment we found stronger effects for negative valence stimuli. Effects of the scanning environment on EE ratings *per se* precluded the direct evaluation of dose–response effects between the two experiments; however, OXT specifically increased EE in both suggesting that its effects generalize across 24 and 40 IU doses, in line with our previous finding (Zhao et al., 2017). In general, the magnitude of the reported behavioral OXT effect on both EED and EEI reported in Caucasian participants was however somewhat stronger compared to both 24 and 40 IU doses administered in our study, although different MET stimuli were used.

In agreement with other studies, there were no sex-differences in EE, trait empathy (Wu et al., 2012) or trait autism (Kawamura et al., 2011; Montag et al., 2017) scores in our Chinese study cohort, whereas in Caucasian participants we found that females scored significantly higher than males for both positive and negative valence stimuli (Hurlemann et al., 2010). Thus, it is conceivable that in Caucasian females the effects of OXT in the MET might not be as pronounced as in males. The absence of

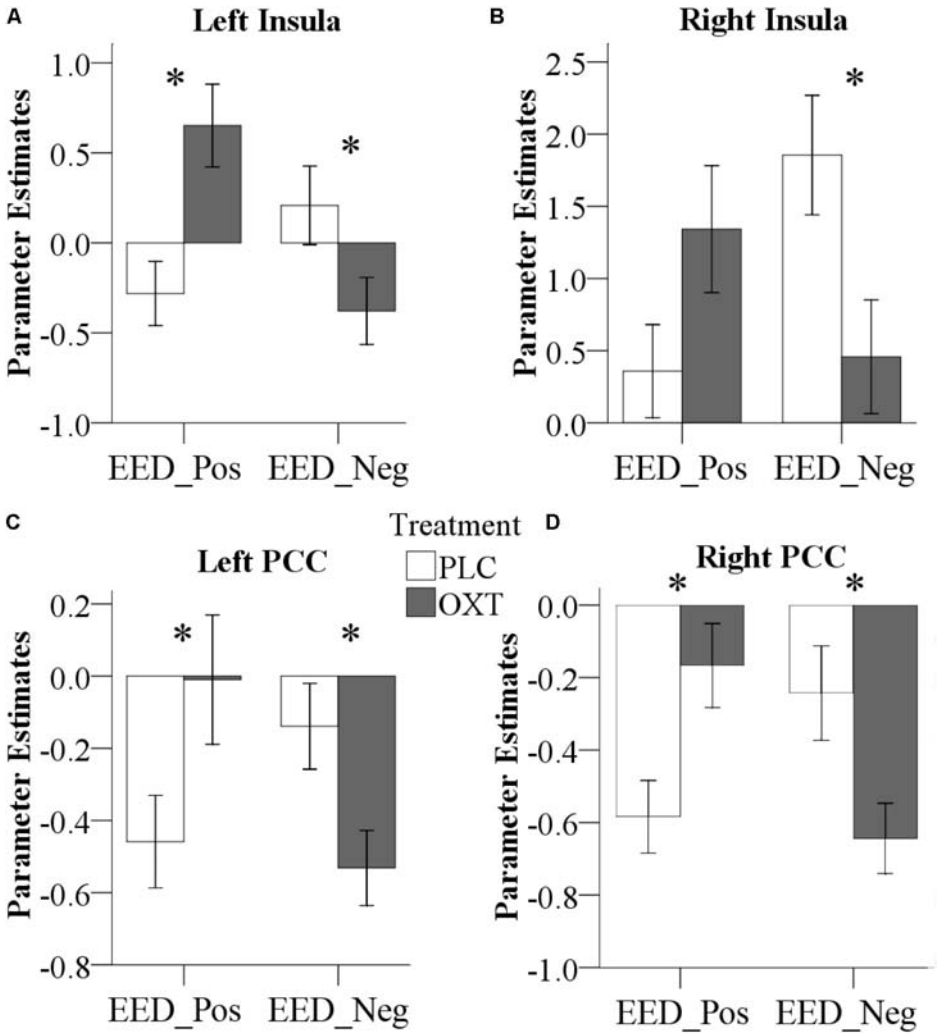

FIGURE 7 | Effects of OXT effect on right amygdala functional connectivity. Effects of treatment on functional connectivity of the right amygdala, indicating valence-dependent effects of OXT on the coupling of the right amygdala with the left **(A)** and right **(B)** insula, as well as the left **(C)** and right **(D)** PCC (*$P < 0.05$) (Pos, Positive; Neg, Negative).

an effect of OXT on CE in the MET contrasts with reports using other paradigms, notably the RMET (Domes et al., 2007b; Feeser et al., 2015). However, the robustness of these findings has been questioned by another study which failed to replicate them even when taking into account both item difficulty and valence (Radke and de Bruijn, 2015). Moreover, there are also notable differences between the MET and RMET with the images in the MET including more complex natural scenes and emotions conveyed by multiple cues (face, body posture, and context) whereas in the RMET emotions are only interpreted from pictures of eye regions and are also often more subtle. Thus, OXT can facilitate CE in some contexts, particularly with cues restricted to eyes, but not in others where multiple cues are present. Additionally, and in contrast to previous studies, we measured SCR responses during trials involving the three empathy components and OXT only increased the SCR in EE and not CE trials. Thus, OXT enhancement of EE is paralleled by increased physiological arousal not only in EEI trials (where participants are asked to score how aroused they are by the stimulus picture) but also in EED trials (where they are scoring the strength of their feelings toward to protagonist in the picture).

In line with the specific, and critical contribution of the amygdala to emotional, rather than cognitive aspects of empathy (Hurlemann et al., 2010), OXT's enhancement of EE was accompanied by a reduction of associated amygdala activity. Exploratory analyses revealed that EED scores were positively associated with the magnitude of amygdala responses during positive valence trials in the PLC group, whereas this association was absent under OXT, possibly reflecting an enhancement of amygdala processing efficiency. While some previous studies found that OXT specifically reduced amygdala responses to negative emotional stimuli (Kirsch et al., 2005; Gamer et al., 2010), the suppression of EED-associated amygdala activity was observed irrespective of valence. A similar pattern of

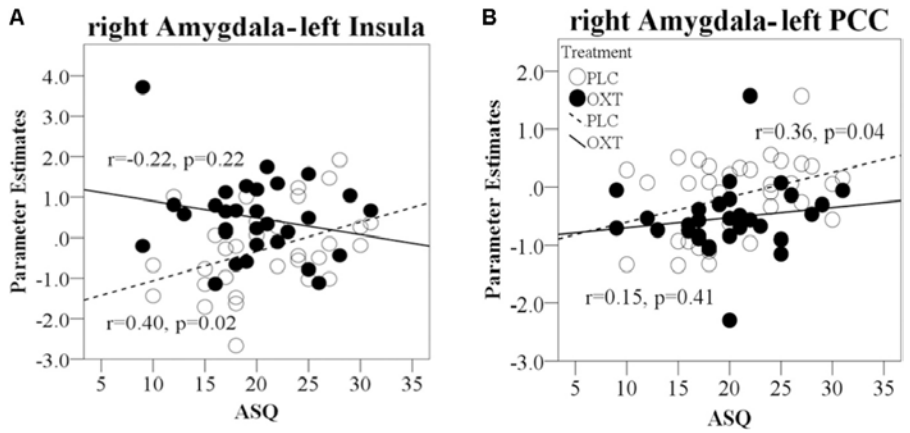

FIGURE 8 | Regression plots for correlations of ASQ scores with amygdala responses and functional connectivity during EE trials in OXT and PLC groups. **(A)** Correlation between right amygdala–left PCC functional connectivity during negative EED trials and total ASQ score. **(B)** Correlation of right amygdala functional connectivity with left insula cortex during positive EE trials and total ASQ score. In all cases, significant positive correlations during PLC administration are absent in the OXT group.

OXT-induced valence-independent suppression of amygdala activity has previously been suggested to reflect reduced uncertainty of a social stimulus which in turn motivates approach behavior (Domes et al., 2007a). In line with this interpretation, the valence-independent EED-associated amygdala suppression may reflect that OXT's approach-facilitating properties (Arakawa et al., 2010) promote EE regardless of whether the emotions expressed by the protagonist are positive or negative, which is also in line with a rodent study reporting an overall reduction of amygdala EEG power following OXT (Sobota et al., 2015). Other studies have found that OXT's modulation of amygdala responses dependent upon sex (Gao et al., 2016; Luo et al., 2017) and it is generally considered that the salience of cues as well as their context may play an important role in determining OXT's effects (Shamay-Tsoory and Abu-Akel, 2016). In the present study, neither sex nor valence influenced amygdala reactivity. This possibly reflects the fact that both salience and context are broadly similar for EE responses in the two sexes.

Oxytocin also differentially altered the functional connectivity between the right amygdala and bilateral insula in a valence-dependent manner. In EED trials, the strength of the functional connectivity between the right amygdala and insula following OXT was significantly increased during positive valence stimuli but decreased during negative ones. A few previous studies have also reported OXT effects on functional connectivity between the insula and amygdala (Rilling et al., 2012; Striepens et al., 2012; Hu et al., 2015; Gao et al., 2016) and these two regions are key hubs of the brain salience network (Uddin, 2015). Thus, in the current context OXT may have acted to increase the salience of both positive and negative valence stimuli during EED trials by differentially altering the functional connectivity between the amygdala and insula. Rilling et al. (2012) have also previously suggested that the stronger the functional coupling between amygdala and insula, the more the amygdala is able to elicit subjective feeling states in response to salient social stimuli.

The effect of OXT on increasing functional connectivity between the right amygdala and bilateral PCC for positive valence stimuli and decreasing it for negative ones in EED trials may similarly reflect a modulatory influence on salience processing. A previous study has reported that OXT enhanced functional connectivity between amygdala and PCC during exposure to infant laughter (Riem et al., 2012), suggesting that it increased the incentive salience of infant laughter. In our current study, the consistent patterns of functional connectivity changes elicited by OXT for positive and negative valence stimuli for amygdala functional connectivity with the insula and PCC may indicate that these three regions comprise an integrated network mediating valence-dependent OXT effects.

Both the behavioral and neural effects of OXT were modified to some extent by trait autism scores, as measured by the ASQ. In both experiments OXT tended to produce a negative correlation between EE and ASQ scores, whereas this correlation was absent in the PLC group. However, this effect of OXT only achieved significance in Exp 1, which included only male participants, and indicates that increased EE scores were more evident in individuals with lower ASQ scores. For the neural associations, functional connectivity between the amygdala and insula was positively associated with total ASQ scores for positive valence EE trials in the PLC group, but this was absent in the OXT group. This indicates that OXT effects on functional connections between the right amygdala and left insula (for positive valence stimuli) were also strongest in individuals with lower ASQ scores. Thus overall, while both behavioral and neural OXT effects on EE were modified by ASQ scores, the extent to which these findings represent support for possible therapeutic use in ASD remains unclear. Indeed, a recent study on OXT enhancement of behavioral and neural responses to affective touch also reported stronger effects in individuals with lower ASQ scores (Scheele et al., 2014).

There are several limitations which should be acknowledged in the current study. Firstly, we were unable to directly compare

behavioral and neural responses during the MET task in Caucasian as well Chinese participants, so we cannot totally exclude the possibility that some cultural differences in response to OXT during empathic processing may exist. Secondly, we only investigated effects using the MET paradigm and it is possible that OXT effects on CE as well as EE would have been found using other paradigms. Thirdly, no endogenous levels of OXT were assessed in the present study. Comparing the PLC-treated subjects between EXP 1 and EXP 2 suggests that the scanner environment *per se* may have had an influence on EE ratings, possibly due to elevated stress levels in the MRI environment. Given that previous studies reported endogenous OXT release in response to stress (Lang et al., 1983; Jong et al., 2015) we cannot completely rule out interactions between differential endogenous OXT levels in EXP 1 and EXP 2 and treatment. Lastly, the absence of sex-differences in OXT effects in the current study might have been contributed to by our Chinese male and female participants exhibiting similar EE scores, in contrast to Caucasian participants (Hurlemann et al., 2010), and also similar ASQ scores.

In summary, in the current study we have shown that in the MET paradigm, OXT enhances EE but not CE in Chinese participants, similar to Caucasian ones, and additionally that this occurs in female as well as male participants. Furthermore, we have shown for the first time that this EE effect of OXT is associated with decreased amygdala responses and differentially altered functional connectivity between the amygdala and insula and PCC for positive and negative valence stimuli. Finally, we have shown that both behavioral and neural effects of OXT are modified to some extent by trait autism scores, although behavioral and functional connectivity effects were strongest in individuals with lower scores.

AUTHOR CONTRIBUTIONS

YG, RH, and KK designed the experiments. YG collected the data. YG, WZ, FZ, KK, and BB analyzed the data. YG, WZ, XM, SY, RH, BB, and KK interpreted the results. YG, BB, and KK wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2018.00512/full#supplementary-material

REFERENCES

Arakawa, H., Arakawa, K., and Deak, T. (2010). Oxytocin and vasopressin in the medial amygdala differentially modulate approach and avoidance behavior toward illness-related social odor. *Neuroscience* 171, 1141–1151. doi: 10.1016/j.neuroscience.2010.10.013

Bakermans-Kranenburg, M. J., and van IJzendoorn, M. H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Transl. Psychiatry* 3:e258. doi: 10.1038/tp.2013.34

Baron-Cohen, S., and Wheelwright, S. (2004). The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. *J. Autism Dev. Disord.* 34, 163–175. doi: 10.1023/b:jadd.0000022607.19833.00

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., and Clubley, E. (2001). The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J. Autism Dev. Disord.* 31, 5–17. doi: 10.1023/A:1005653411471

Bartz, J. A., Zaki, J., Bolger, N., Hollander, E., Ludwig, N. N., Kolevzon, A., et al. (2010). Oxytocin selectively improves empathic accuracy. *Psychol. Sci.* 21, 1426–1428. doi: 10.1177/0956797610383439

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., and Erbaugh, J. (1961). An inventory for measuring depression. *Arch. Gen. Psychiatry* 4, 561–571. doi: 10.1001/archpsyc.1961.01710120031004

Bernhardt, B. C., and Singer, T. (2012). The neural basis of empathy. *Annu. Rev. Neurosci.* 35, 1–23. doi: 10.1146/annurev-neuro-062111-150536

Bos, P. A., Montoya, E. R., Hermans, E. J., Keysers, C., and van Honk, J. (2015). Oxytocin reduces neural activity in the pain circuitry when seeing pain in others. *Neuroimage* 113, 217–224. doi: 10.1016/j.neuroimage.2015.03.049

Brett, M., Anton, J.-L., Valabregue, R., and Poline, J.-B. (2002). Region of interest analysis using the MarsBar toolbox for SPM 99. *Neuroimage* 16:S497.

Cao, Y., Contreras-Huerta, L. S., McFadyen, J., and Cunnington, R. (2015). Racial bias in neural response to others' pain is reduced with other-race contact. *Cortex* 70, 68–78. doi: 10.1016/j.cortex.2015.02.010

Cardoso, C., Ellenbogen, M. A., Orlando, M. A., Bacon, S. L., and Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. *Psychoneuroendocrinology* 38, 399–407. doi: 10.1016/j.psyneuen.2012.07.013

Chen, X., Hackett, P. D., DeMarco, A. C., Feng, C., Stair, S., Haroon, E., et al. (2016). Effects of oxytocin and vasopressin on the neural response to unreciprocated cooperation within brain regions involved in stress and anxiety in men and women. *Brain Imaging Behav.* 10, 581–593. doi: 10.1007/s11682-015-9411-7

Cox, C. L., Uddin, L. Q., Di Martino, A., Castellanos, F. X., Milham, M. P., and Kelly, C. (2012). The balance between feeling and knowing: affective and cognitive empathy are reflected in the brain's intrinsic functional dynamics. *Soc. Cogn. Affect. Neurosci.* 7, 727–737. doi: 10.1093/scan/nsr051

De Dreu, C. K., and Kret, M. E. (2016). Oxytocin conditions intergroup relations through upregulated in-group empathy, cooperation, conformity, and defense. *Biol. Psychiatry* 79, 165–173. doi: 10.1016/j.biopsych.2015.03.020

Decety, J. (2011). The neuroevolution of empathy. *Ann. N. Y. Acad. Sci.* 1231, 35–45. doi: 10.1111/j.1749-6632.2011.06027.x

Decety, J., and Jackson, P. L. (2004). The functional architecture of human empathy. *Behav. Cogn. Neurosci. Rev.* 3, 71–100. doi: 10.1177/1534582304267187

Domes, G., Heinrichs, M., Glascher, J., Buchel, C., Braus, D. F., and Herpertz, S. C. (2007a). Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biol. Psychiatry* 62, 1187–1190. doi: 10.1016/j.biopsych.2007.03.025

Domes, G., Heinrichs, M., Michel, A., Berger, C., and Herpertz, S. C. (2007b). Oxytocin improves "mind-reading" in humans. *Biol. Psychiatry* 61, 731–733.

Domes, G., Hollerbach, P., Vohs, K., Mokros, A., and Habermeyer, E. (2013). Emotional empathy and psychopathy in offenders: an experimental study. *J. Personal. Disord.* 27, 67–84. doi: 10.1521/pedi.2013.27.1.67

Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H. R., Wolf, O. T., et al. (2008). Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the multifaceted empathy test (MET). *J. Autism Dev. Disord.* 38, 464–473. doi: 10.1007/s10803-007-0486-x

Edele, A., Dziobek, I., and Keller, M. (2013). Explaining altruistic sharing in the dictator game: the role of affective empathy, cognitive empathy, and justice sensitivity. *Learn. Individ. Differ.* 24, 96–102. doi: 10.1016/j.lindif.2012.12.020

Eklund, A., Nichols, T. E., and Knutsson, H. (2016). Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7900–7905. doi: 10.1073/pnas.1602413113

Fan, Y., Duncan, N. W., de Greck, M., and Northoff, G. (2011). Is there a core neural network in empathy? An fMRI based quantitative meta-analysis. *Neurosci. Biobehav. Rev.* 35, 903–911. doi: 10.1016/j.neubiorev.2010.10.009

Feeser, M., Fan, Y., Weigand, A., Hahn, A., Gärtner, M., Böker, H., et al. (2015). Oxytocin improves mentalizing–pronounced effects for individuals with attenuated ability to empathize. *Psychoneuroendocrinology* 53, 223–232. doi: 10.1016/j.psyneuen.2014.12.015

Gamer, M., Zurowski, B., and Buchel, C. (2010). Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9400–9405. doi: 10.1073/pnas.1000985107

Gao, S., Becker, B., Luo, L., Geng, Y., Zhao, W., Yin, Y., et al. (2016). Oxytocin, the peptide that bonds the sexes also divides them. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7650–7654. doi: 10.1073/pnas.1602620113

Guastella, A. J., Hickie, I. B., McGuinness, M. M., Otis, M., Woods, E. A., Disinger, H. M., et al. (2013). Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology* 38, 612–625. doi: 10.1016/j.psyneuen.2012.11.019

Herpertz, S. C., and Bertsch, K. (2014). The social-cognitive basis of personality disorders. *Curr. Opin. Psychiatry* 27, 73–77. doi: 10.1097/YCO.0000000000000026

Herpertz, S. C., and Bertsch, K. (2016). Oxytocin effects on brain functioning in humans. *Biol. Psychiatry* 79, 631–632. doi: 10.1016/j.biopsych.2016.02.004

Hillis, A. E. (2014). Inability to empathize: brain lesions that disrupt sharing and understanding another's emotions. *Brain* 137, 981–997. doi: 10.1093/brain/awt317

Hu, J. H., Qi, S., Becker, B., Luo, L. Z., Gao, S., Gong, Q. Y., et al. (2015). Oxytocin selectively facilitates learning with social feedback and increases activity and functional connectivity in emotional memory and reward processing regions. *Hum. Brain Mapp.* 36, 2132–2146. doi: 10.1002/hbm.22760

Hurlemann, R., Patin, A., Onur, O. A., Cohen, M. X., Baumgartner, T., Metzler, S., et al. (2010). Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *J. Neurosci.* 30, 4999–5007. doi: 10.1523/JNEUROSCI.5538-09.2010

Jessica, L. Y., Sasaki, J. Y., Keiko, I., Shinada, M., and Kim, H. S. (2016). Gene-culture interaction influence of culture and oxytocin receptor gene polymorphism on loneliness. *Cult. Brain* 4, 21–37. doi: 10.1007/s40167

Jong, T. R., Menon, R., Bludau, A., Grund, T., Biermeier, V., Klampfl, S. M., et al. (2015). Salivary oxytocin concentrations in response to running, sexual self-stimulation, breastfeeding and the TSST: the Regensburg oxytocin challenge (ROC) study. *Psychoneuroendocrinology* 62, 381–388. doi: 10.1016/j.psyneuen.2015.08.027

Kawamura, Y., Liu, X., Shimada, T., Otowa, T., Kakiuchi, C., Akiyama, T., et al. (2011). Association between oxytocin receptor gene polymorphisms and autistic traits as measured by the Autism-Spectrum Quotient in a non-clinical Japanese population. *Asia Pac. Psychiatry* 3, 128–136. doi: 10.1111/j.1758-5872.2011.00132.x

Kim, H. S., Sherman, D. K., Sasaki, J. Y., Xu, J., Chu, T. Q., Ryu, C., et al. (2010). Culture, distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15717–15721. doi: 10.1073/pnas.1010830107

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., et al. (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *J. Neurosci.* 25, 11489–11493. doi: 10.1523/JNEUROSCI.3984-05.2005

Lamm, C., Decety, J., and Singer, T. (2011). Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. *Neuroimage* 54, 2492–2502. doi: 10.1016/j.neuroimage.2010.10.014

Lamm, C., Nusbaum, H. C., Meltzoff, A. N., and Decety, J. (2007). What are you feeling? Using functional magnetic resonance imaging to assess the modulation of sensory and affective responses during empathy for pain. *PLoS One* 2:e1292 doi: 10.1371/journal.pone.0001292

Lang, R. E., Heil, J. W., Ganten, D., Hermann, K., Unger, T., and Rascher, W. (1983). Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinology* 37, 314–316. doi: 10.1159/000123566

Lee, J., Zaki, J., Harvey, P. O., Ochsner, K., and Green, M. F. (2011). Schizophrenia patients are impaired in empathic accuracy. *Psychol. Med.* 41, 2297–2304. doi: 10.1017/S0033291711000614

Leigh, R., Oishi, K., Hsu, J., Lindquist, M., Gottesman, R. F., Jarso, S., et al. (2013). Acute lesions that impair affective empathy. *Brain* 136, 2539–2549. doi: 10.1093/brain/awt177

Luo, L., Becker, B., Geng, Y., Zhao, Z., Gao, S., Zhao, W., et al. (2017). Sex-dependent neural effect of oxytocin during subliminal processing of negative emotion faces. *Neuroimage* 162, 127–137. doi: 10.1016/j.neuroimage.2017.08.079

Luo, S. Y., Li, B. F., Ma, Y., Zhang, W. X., Rao, Y., and Han, S. H. (2015a). Oxytocin receptor gene and racial ingroup bias in empathy-related brain activity. *Neuroimage* 110, 22–31. doi: 10.1016/j.neuroimage.2015.01.042

Luo, S. Y., Ma, Y. N., Liu, Y., Li, B. F., Wang, C. B., Shi, Z. H., et al. (2015b). Interaction between oxytocin receptor polymorphism and interdependent culture values on human empathy. *Soc. Cogn. Affect. Neurosci.* 10, 1273–1281. doi: 10.1093/scan/nsv019

McLaren, D. G., Ries, M. L., Xu, G., and Johnson, S. C. (2012). A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. *Neuroimage* 61, 1277–1286. doi: 10.1016/j.neuroimage.2012.03.068

Melchers, M., Li, M., Chen, Y., Zhang, W., and Montag, C. (2015). Low empathy is associated with problematic use of the Internet: empirical evidence from China and Germany. *Asian J. Psychiatry* 17, 56–60. doi: 10.1016/j.ajp.2015.06.019

Montag, C., Sindermann, C., Melchers, M., Jung, S., Luo, R., Becker, B., et al. (2017). A functional polymorphism of the OXTR gene is associated with autistic traits in Caucasian and Asian populations. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 174, 808–816. doi: 10.1002/ajmg.b.32596

Panksepp, J., and Panksepp, J. B. (2013). Toward a cross-species understanding of empathy. *Trends Neurosci.* 36, 489–496. doi: 10.1016/j.tins.2013.04.009

Penton-Voak, I. S., Perrett, D. I., Castles, D. L., Kobayashi, T., Burt, D. M., Murray, L. K., et al. (1999). Menstrual cycle alters face preference. *Nature* 399, 741–742. doi: 10.1038/21557

Pfundmair, M., Aydin, N., Frey, D., and Echterhoff, G. (2014). The interplay of oxytocin and collectivistic orientation shields against negative effects of ostracism. *J. Exp. Soc. Psychol.* 55, 246–251. doi: 10.1016/j.jesp.2014.07.016

Quintana, D. S., Westlye, L. T., Alnaes, D., Rustan, O. G., Kaufmann, T., Smerud, K. T., et al. (2016). Low dose intranasal oxytocin delivered with Breath Powered device dampens amygdala response to emotional stimuli: a peripheral effect-controlled within-subjects randomized dose-response fMRI trial. *Psychoneuroendocrinology* 69, 180–188. doi: 10.1016/j.psyneuen.2016.04.010

Quintana, D. S., Westlye, L. T., Hope, S., Naerland, T., Elvsashagen, T., Dorum, E., et al. (2017). Dose-dependent social-cognitive effects of intranasal oxytocin delivered with novel Breath Powered device in adults with autism spectrum disorder: a randomized placebo-controlled double-blind crossover trial. *Transl. Psychiatry* 7:e1136. doi: 10.1038/tp.2017.103

Radke, S., and de Bruijn, E. R. (2015). Does oxytocin affect mind-reading? A replication study. *Psychoneuroendocrinology* 60, 75–81. doi: 10.1016/j.psyneuen.2015.06.006

Reniers, R., Corcoran, R., Drake, R., Shryane, N., and Vollm, B. (2010). The QCAEP: a questionnaire of cognitive and affective empathy. *J. Pers. Assess.* 93, 84–95. doi: 10.1080/00223891.2010.528484

Riem, M. M., van IJzendoorn, M. H., Tops, M., Boksem, M. A., Rombouts, S. A., and Bakermans-Kranenburg, M. J. (2012). No laughing matter: intranasal oxytocin administration changes functional brain connectivity during exposure to infant laughter. *Neuropsychopharmacology* 37, 1257–1266. doi: 10.1038/npp.2011.313

Riem, M. M. E. (2012). Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: a randomized controlled trial. *Biol. Psychiatry* 70, 660–660.

Riem, M. M. E., Bakermans-Kranenburg, M. J., Voorthuis, A., and van IJzendoorn, M. H. (2014). Oxytocin effects on mind-reading are moderated by experiences of maternal love withdrawal: an fMRI study. *Prog. Neuro Psychopharmacol. Biol. Psychiatry* 51, 105–112. doi: 10.1016/j.pnpbp.2014.01.014

Rilling, J. K., DeMarco, A. C., Hackett, P. D., Thompson, R., Ditzen, B., Patel, R., et al. (2012). Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. *Psychoneuroendocrinology* 37, 447–461. doi: 10.1016/j.psyneuen.2011.07.013

Rodrigues, S. M., Saslow, L. R., Garcia, N., John, O. P., and Keltner, D. (2009). Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21437–21441. doi: 10.1073/pnas.0909579106

Rosenfeld, A. J., Lieberman, J. A., and Jarskog, L. F. (2011). Oxytocin, dopamine, and the amygdala: a neurofunctional model of social cognitive deficits in schizophrenia. *Schizophr. Bull.* 37, 1077–1087. doi: 10.1093/schbul/sbq015

Scheele, D., Kendrick, K. M., Khouri, C., Kretzer, E., Schlapfer, T. E., Stoffel-Wagner, B., et al. (2014). An oxytocin-induced facilitation of neural and emotional responses to social touch correlates inversely with autism traits. *Neuropsychopharmacology* 39, 2078–2085. doi: 10.1038/npp.2014.78

Schulte-Ruther, M., Markowitsch, H. J., Fink, G. R., and Piefke, M. (2007). Mirror neuron and theory of mind mechanisms involved in face-to-face interactions: a functional magnetic resonance imaging approach to empathy. *J. Cogn. Neurosci.* 19, 1354–1372. doi: 10.1162/jocn.2007.19.8.1354

Shamay-Tsoory, S. G. (2011). The neural bases for empathy. *Neuroscientist* 17, 18–24. doi: 10.1177/1073858410379268

Shamay-Tsoory, S. G., and Abu-Akel, A. (2016). The social salience hypothesis of oxytocin. *Biol. Psychiatry* 79, 194–202. doi: 10.1016/j.biopsych.2015.07.020

Shamay-Tsoory, S. G., Abu-Akel, A., Palgi, S., Sulieman, R., Fischer-Shofty, M., Levkovitz, Y., et al. (2013). Giving peace a chance: oxytocin increases empathy to pain in the context of the Israeli-Palestinian conflict. *Psychoneuroendocrinology* 38, 3139–3144. doi: 10.1016/j.psyneuen.2013.09.015

Shamay-Tsoory, S. G., Aharon-Peretz, J., and Levkovitz, Y. (2007). The neuroanatomical basis of affective mentalizing in schizophrenia: comparison of patients with schizophrenia and patients with localized prefrontal lesions. *Schizophr. Res.* 90, 274–283. doi: 10.1016/j.schres.2006.09.020

Shamay-Tsoory, S. G., Aharon-Peretz, J., and Perry, D. (2009). Two systems for empathy: a double dissociation between emotional and cognitive empathy in inferior frontal gyrus versus ventromedial prefrontal lesions. *Brain* 132(Pt 3), 617–627. doi: 10.1093/brain/awn279

Singer, T., and Lamm, C. (2009). The social neuroscience of empathy. *Ann. N. Y. Acad. Sci.* 1156, 81–96. doi: 10.1111/j.1749-6632.2009.04418.x

Singer, T., Snozzi, R., Bird, G., Petrovic, P., Silani, G., Heinrichs, M., et al. (2008). Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. *Emotion* 8, 781–791. doi: 10.1037/a0014195

Slotnick, S. D. (2017). Cluster success: fMRI inferences for spatial extent have acceptable false-positive rates. *Cogn. Neurosci.* 8, 150–155. doi: 10.1080/17588928.2017.1319350

Smith, K. E., Porges, E. C., Norman, G. J., Connelly, J. J., and Decety, J. (2014). Oxytocin receptor gene variation predicts empathic concern and autonomic arousal while perceiving harm to others. *Soc. Neurosci.* 9, 1–9. doi: 10.1080/17470919.2013.863223

Sobota, R., Mihara, T., Forrest, A., Featherstone, R. E., and Siegel, S. J. (2015). Oxytocin reduces amygdala activity, increases social interactions, and reduces anxiety-like behavior irrespective of NMDAR antagonism. *Behav. Neurosci.* 129, 389–398. doi: 10.1037/bne0000074

Spengler, F. B., Schultz, J., Scheele, D., Essel, M., Maier, W., Heinrichs, M., et al. (2017). Kinetics and dose dependency of intranasal oxytocin effects on amygdala reactivity. *Biol. Psychiatry* 82, 885–894. doi: 10.1016/j.biopsych.2017.04.015

Spielberger, C. D., Gorsuch, R. L., and Lushene, R. E. (1970). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA Consulting Psychologists Press.

Stern, R. M., Ray, W. J., and Quigley, K. S. (2001). *Psychophysiological Recording*. Oxford: Oxford University Press.

Striepens, N., Kendrick, K. M., Maier, W., and Hurlemann, R. (2011). Prosocial effects of oxytocin and clinical evidence for its therapeutic potential. *Front. Neuroendocrinol.* 32, 426–450. doi: 10.1016/j.yfrne.2011.07.001

Striepens, N., Scheele, D., Kendrick, K. M., Becker, B., Schafer, L., Schwalba, K., et al. (2012). Oxytocin facilitates protective responses to aversive social stimuli in males. *Proc. Natl. Acad. Sci. U.S.A.* 109, 18144–18149. doi: 10.1073/pnas.1208852109

Tully, J., Gabay, A. S., Brown, D., Murphy, D. G. M., and Blackwood, N. (2018). The effect of intranasal oxytocin on neural response to facial emotions in healthy adults as measured by functional MRI: a systematic review. *Psychiatry Res.* 272, 17–29. doi: 10.1016/j.pscychresns.2017.11.017

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nat. Rev. Neurosci.* 16, 55–61. doi: 10.1038/nrn3857

Watson, D., Clark, L. A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070. doi: 10.1037/0022-3514.54.6.1063

Weisman, O., Pelphrey, K. A., Leckman, J. F., Feldman, R., Lu, Y., Chong, A., et al. (2015). The association between 2D:4D ratio and cognitive empathy is contingent on a common polymorphism in the oxytocin receptor gene (OXTR rs53576). *Psychoneuroendocrinology* 58, 23–32. doi: 10.1016/j.psyneuen.2015.04.007

Wigton, R., Radua, J., Allen, P., Averbeck, B., Meyer-Lindenberg, A., McGuire, P., et al. (2015). Neurophysiological effects of acute oxytocin administration: systematic review and meta-analysis of placebo-controlled imaging studies. *J. Psychiatry Neurosci.* 40, E1–E22. doi: 10.1503/jpn.130289

Wingenfeld, K., Kuehl, L. K., Janke, K., Hinkelmann, K., Dziobek, I., Fleischer, J., et al. (2014). Enhanced emotional empathy after mineralocorticoid receptor stimulation in women with borderline personality disorder and healthy women. *Neuropsychopharmacology* 39, 1799–1804. doi: 10.1038/npp.2014.36

Wolf, O. T., Schulte, J. M., Drimalla, H., Hamacher-Dang, T. C., Knoch, D., and Dziobek, I. (2015). Enhanced emotional empathy after psychosocial stress in young healthy men. *Stress* 18, 631–637. doi: 10.3109/10253890.2015.1078787

Wong, C.-S., and Law, K. S. (2002). The effects of leader and follower emotional intelligence on performance and attitude: an exploratory study. *Leadersh. Q.* 13, 243–274. doi: 10.1016/S1048-9843(02)00099-1

Wu, N., Li, Z., and Su, Y. J. (2012). The association between oxytocin receptor gene polymorphism (OXTR) and trait empathy. *J. Affect. Disord.* 138, 468–472. doi: 10.1016/j.jad.2012.01.009

Xu, L., Ma, X., Zhao, W., Luo, L., Yao, S., and Kendrick, K. M. (2015). Oxytocin enhances attentional bias for neutral and positive expression faces in individuals with higher autistic traits. *Psychoneuroendocrinology* 62, 352–358. doi: 10.1016/j.psyneuen.2015.09.002

Xu, X., Yao, S., Xu, L., Geng, Y., Zhao, W., Ma, X., et al. (2017). Oxytocin biases men but not women to restore social connections with individuals who socially exclude them. *Sci. Rep.* 7:40589. doi: 10.1038/srep40589

Young, L. J., and Barrett, C. E. (2015). Neuroscience. Can oxytocin treat autism? *Science* 347, 825–826. doi: 10.1126/science.aaa8120

Zhao, W., Geng, Y., Luo, L., Zhao, Z., Ma, X., Xu, L., et al. (2017). Oxytocin increases the perceived value of both self- and other-owned items and alters medial prefrontal cortex activity in an endowment task. *Front. Hum. Neurosci.* 11:272. doi: 10.3389/fnhum.2017.00272

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Social Company Disrupts Fear Memory Renewal: Evidence From Two Rodent Studies

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Renewal of fear outside treatment context is a challenge for behavioral therapies. Prior studies suggest a social buffering effect that fear response is attenuated in the presence of social company. However, few studies have examined the role of social company in reducing fear renewal. Here, we used a Pavlovian fear conditioning procedure including acquisition, extinction and test stages to examine social buffering effect on fear memory renewal in male rats. The test context was manipulated to be either different from the extinction one in ABC model, or same as that in ACC model. All conditioned subjects underwent extinction individually in Experiment 1 but with a partner in Experiment 2. In test, both experiments manipulated social company (alone vs. accompanied) and context (ABC vs. ACC). Experiment 1 showed more freezing in ABC than in ACC model during the test-alone condition, indicating a fear renewal effect which, however, was absent during the test-accompanied condition. Also, accompanied subjects showed less freezing compared to alone subjects in the ABC model. In Experiment 2, animals showed a similar freezing in ABC and ACC models despite being tested alone, implying that social company offered at extinction disrupted fear renewal. Again, we observed reduced freezing in accompanied relative to alone subjects in the test. These results suggest that social company is effective in disrupting fear renewal after leaving treatment context.

Keywords: social company, social buffering effect, fear conditioning, renewal, context updating, extinction

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is characterized by the intrusive flashback of fear memories or difficulty in fear memory extinction (Jovanovic and Ressler, 2010). Exposure therapy, which is based on extinction theory, aims to treat PTSD patients through repeated presence of trauma-related stimuli in the absence of real threats (Mark and Lovell, 1998). However, approximately 30–50% of patients experience renewal of anxiety symptom when the therapy is finished and patients leave the therapeutic context (Choy et al., 2007). Thus, treatments do not completely erase the original fear memory, but instead it forms context-dependent learning. In other words, the extinguished fear memory tends to renew when fear-related stimulus is presented outside of the extinction context (Bouton and Bolles, 1979). Accordingly, fear memory renewal is defined as the recovery of an extinguished fear response when test occurs in a novel context different from that of extinction (Boschen et al., 2009; Polack et al., 2013).

To simulate PTSD and explore its therapies in the laboratory setting, the Pavlovian fear conditioning paradigm is often used with animal models (Herry et al., 2010; Goode and Maren, 2014). In the Pavlovian fear conditioning paradigm, exposing an animal to the pair of a neutral conditioned stimulus (CS), such as a light or white noise, with an unconditioned stimulus (US), such as a footshock, will lead to a conditioned fear response (CR) when the animal receives the CS alone. The typical renewal procedure includes three stages, that is, the acquisition (presentation of the CS-US), extinction (presentation of the CS alone), and the test stage (presentation of the CS alone) (Bouton, 2002; Boschen, 2009). For example, if acquisition and extinction of fear occurs in context A and B, respectively, then fear response in test phase is usually less intense when the animal is tested in context B than in context A and C. That is, the animal would easily retrieve extinction memory if test occurs in the extinction context (i.e., Context B), while extinction memory is hard to be retrieved when the animal is tested in a novel (C) or in the fear acquisition (A) context. In animal studies, fear memory renewal is defined as the freezing increase during the "different" (e.g., ABC) compared to the "same" (e.g., ABB) context in the test (Wang et al., 2016).

Accumulating evidence shows that mere presence of conspecific partner, whether familiar or unfamiliar, is able to reduce one's stress-related response in both human and animal subjects (Fontana et al., 1999; Hennessy et al., 2000). This phenomenon is called social buffering, which has been extensively observed across species, including rodents, sheep, pigs, non-human primates and also humans (Hennessy et al., 2000, 2009; Kikusui et al., 2006). For example, rodent studies indicate that the locomotor activity decreases when fear conditioned rats are subject to the CS alone, but the activity increases when they are accompanied by another non-fearful rat (Davitz and Mason, 1955). It was found that the rats' freezing to contextual stimulus conditioned with shock was blocked by the presence of a partner (Kiyokawa et al., 2004). Furthermore, the freezing, and corticosterone level both decreased in the presence of a conspecific to fear-conditioned rats (Kiyokawa et al., 2014a), and it was reported that the presence of a conspecific animal suppresses CS-induced activation of amygdala (Fuzzo et al., 2015). These evidence indicates that social company is effective in reducing conditioned fear in rats.

For human studies, the psychotherapists require PTSD patients and their family members to receive therapeutic training together, in order to optimize the intervention effects for PTSD symptoms (Glynn, 1999; Sautter et al., 2009; Monson and Fredman, 2012). This implies that social company may disrupt the fear renewal in patients with PTSD. Therefore, we hypothesize that social company may play a critical role in suppressing fear renewal when an animal model is used. In a recent study close to this theme, Mikami et al. (2016) investigated how social company alters the effect of extinction training on fear retention in rats. The rats were firstly subjected to a fear conditioning procedure, and then experienced extinction procedure with or without social company. The results showed that social company enhanced the extinction effect in suppressing fear retention in ACC but not in ABC model (Mikami et al., 2016). However, the research purpose of this work determines that social company should be given to the extinction rather than to the test stage. To our knowledge, currently no study has examined the social buffering effect on fear renewal, by varying the time points to deliver company. Specifically, it is important to know when to offer social company may generate an optimal suppression of fear renewal. On the other hand, as the rehabilitated patients may experience various new situations after leaving a specific therapeutic situation, it is impossible to give social support in every new situation. In this regard, it is important to examine whether social company given to the extinction stage could reduce fear renewal in a novel context. Specifically, it requires examination whether social company given to the extinction stage may replace the fear suppression effect of social company offered to the test stage.

To address this issue, the present study used both ABC and ACC model to isolate a fear renewal effect. ACC, instead of ABB, is used in order to equate the test context during both conditions. Consequently, differential freezing among the ABC and ACC model is not attributable to physical differences in the test context, as all tests are conducted in an identical context. ABC model is used due to its close resemblance to the situation of fear memory renewal of PTSD patients in real life, in that novel contexts outside of the extinction one are common and the original context for fear acquisition is difficult to replicate (Balooch et al., 2012). We used two experiments to investigate social buffering effect on fear memory renewal. In the first experiment, all conditioned subjects underwent extinction individually and we manipulated social company at the test stage. We hypothesize that social company at the test stage could suppress the subjects' fear renewal in the ABC model. In the second experiment, all the conditioned subjects underwent extinction with a conspecific animal, to examine how social company at extinction may alter the strength of fear renewal and its modulation by social company in the test. Based on the abundant evidence for context-dependent learning and social buffering phenomenon, we predict that animals in the test would exhibit higher fear response (i.e., more freezing) in ABC than in ACC model; while social company may disrupt the fear renewal effect, irrespective of the stage to offer social company.

MATERIALS AND METHODS

Subjects and Housing

Both experiments were conducted in strict accordance with the recommendations of "Regulations for the Administration of Affairs Concerning Experimental Animals," the State Science and Technology Commission, China. All animal procedures were approved by the animal care and use committee at Southwest University, China. 64 male Sprague-Dawley rats (180–220 g, postnatal age: about 60 days) including 52 subjects and 12 partners were purchased from the Institute of Traditional Medicine, Chongqing, China. Animals were housed in pairs in transparent cages (47 cm × 32 cm × 21cm) with corn-cob granule for bedding in a room maintained on a 12-h light/dark cycle (lights on at 8:00 a.m.) and were allowed to freely access food and water in their home cages. In each cage,

two rats were assigned to be either subject or partner (i.e., rats placed with the subject during extinction or test), which ensured unfamiliarity between the subject and the accompanying rats. All the accompanying rats were manipulated to be unfamiliar to the subjects in this study, in order to control for the possible amplification of social buffering effect by familiarity (Hennessy et al., 2000; Kiyokawa et al., 2014b). All rats were handled (5 min per rat per day) for 5 days to habituate to the experimenters. All behavioral procedures were performed at 8:30 a.m.

Apparatus

Four identical and standard rodent conditioning observation chambers (30.1 cm × 24.7 cm × 23.3 cm; Clever System Inc., Vienna, VA, United States) were used in the experiments. The chambers consisted of aluminum (side walls) and Plexiglas (rear wall, ceiling, and detachable front door). Digital video cameras were mounted on top of each chamber to videotape rats' behavior. A speaker was mounted outside the wall of each chamber and was used for delivery of white noise.

Context A was a semi-circular chamber made by placing a curved plastic board into the standard rodent conditioning chamber. The floor of each chamber consisted of 18 stainless steel rods (5 mm diameter) spaced 1.6 cm apart. The rods were wired to a shock source for delivery of footshocks. The white light within the chambers was provided for illumination and the experimental room was dark. Stainless steel pans were placed underneath the grid floors and the chambers were sprayed with 2% acetic acid before animals were placed into it.

Context B was an opaque cask (25 cm diameter and 23 cm height) with a transparent cover inside the standard rodent conditioning chamber. A hole (10 cm diameter) was designed in the cover, to facilitate the rats' breathing. Context B did not have stainless steel rods on the floor. To protect subjects' activity or freezing from being disturbed by partners when subjects received the white noise (CS), all casks were approximately bisected into 250-cm^2 with a transparent plastic PVC partition. There are 9 holes (2 cm diameter) in transparent plastic board for subject and partner to communicate by visual, olfactory or restricted tactile modalities (Kiyokawa et al., 2009). Olfactory communications were allowed because the main olfactory system, which underlies the processing of conspecific olfactory signals, has been verified to mediate the social buffering effect in rats (Kiyokawa et al., 2012).The partner was placed on one side of the cask before subject entered in. The white light within the chambers was provided for illumination and the experimental room was bright. The chambers were sprayed and cleaned with 75% ethyl alcohol before animals were placed into it.

Context C was formed by the standard rodent conditioning chamber, with the floor of each consisting of 16 stainless steel rods alternate in thickness. To protect subjects' activity or freezing from being disturbed by the partners when subjects received the white noise (CS), all chambers were bisected into approximately 360-cm^2 with a transparent plastic PVC partition. Similarly, there are 9 holes (2 cm diameter) in transparent plastic board for subject and partner to communicate by visual, olfactory or restricted tactile modalities. The partner was placed on one side of the box before subject entered in it. The white light within the chamber was provided for illumination and the experimental room was bright. The chambers were sprayed and cleaned with 2% isoamyl alcohol before animals were placed into it.

Experiment Design

In Experiment 1, there were three experimental phases: fear conditioning, extinction, and test. For fear conditioning (Day 1), each time a maximum of 4 subjects were transported to context A in pairs in their home cages, which were covered with a white trash bag. All the subjects received auditory fear conditioning which consisted of 4 trials of 30 s, 80 dB, white-noise (conditioned stimulus, CS) co-terminating with a 1 s, 0.5 mA foot shock (1 min intertrial interval, unconditioned stimulus, US). The white noise started 3 min after subjects were placed in context A. After the final trial was finished, the animals were immediately returned to the homecage and the shock grids and floor trays were cleaned. Partners stayed in the feeding room with no foot shock.

On Day 2 and Day 3, subjects were transported to context B in pairs in their homecages that were covered with a yellow trash bag in the ABC condition (**Figure 1A**). In the ACC condition, subjects were transported to context C in pairs in their homecages covered with a black trash bag. All the subjects, irrespective of condition, received 20 white-noise (30 s, 80 dB) in the absence of footshock, beginning 3 min after placement in their respective context without social company. After the final trial was finished, the animals were immediately returned to the homecage.

On Day 4, subjects were transported to context C in pairs in their homecages that were covered with a black trash bag. All the subjects received renewal test with or without partner. The accompanying rats were unfamiliar to the subjects. The subject and the accompanying rats were neither housed together nor had any physical contacts before experiment. The test consisted of 6 trials of white-noise (30 s, 80 dB) in the absence of footshock, beginning 3 min after placement in the context C. The animals were returned to the homecage immediately after the final trial was finished. The test occurred either in the extinction context (ACC) or in a novel context (ABC). This yields a total of 4 groups in a 2 * 2 design (context * company): ACC-Test alone (n = 8), ACC-Test accompanied (n = 7), ABC-Test alone (n = 7), and ABC-Test accompanied (n = 6).

Experiment 2 had the same procedure as described above with the exception that all the subjects underwent extinction training in the presence of a naïve unconditioned partner (with social company). It also yields a total of 4 groups in a 2 * 2 design (context * company): ACC-Test alone (n = 6), ACC-Test accompanied (n = 7), ABC-Test alone (n = 6), and ABC-Test accompanied (n = 5) (**Figure 2A**).

Data Collection and Analysis

Freezing was defined as an immobile posture and was measured during the 30-s period after the onset of each CS (trial). We calculated the percentage of time spent in a freezing posture with respect to the 30-s period in every CS trial during the acquisition phase. All of the freezing was recorded and analyzed with digital video cameras by using commercially available software (Freezescan, Clever System Inc., Vienna, VA, United States). The averaged freezing of every 4 CS was calculated as a block

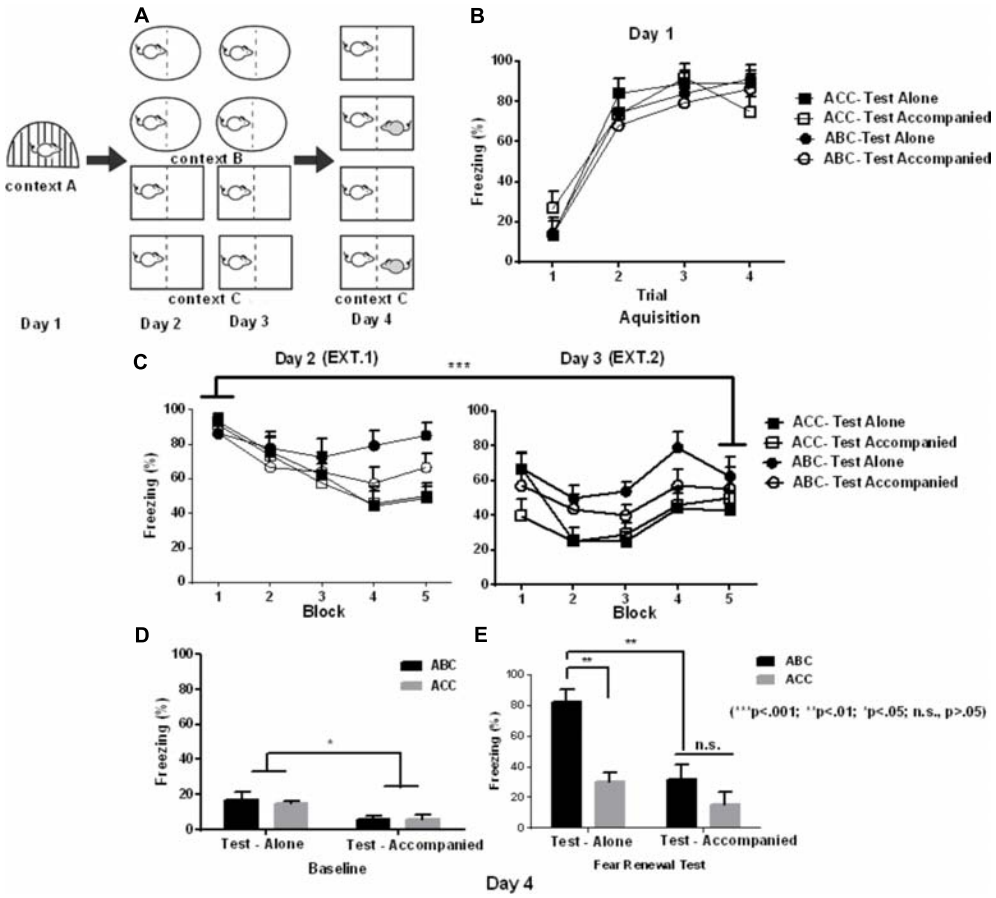

FIGURE 1 | Effects of social buffering on fear memory renewal in Experiment 1. **(A)** Schematic illustration of Experimental design (shape denotes context), yielding a total of 4 groups in a 2 * 2 design (context * company): ACC-Test alone (n = 8), ACC-Test accompanied (n = 7), ABC-Test alone (n = 7), and ABC-Test accompanied (n = 6). **(B)** Freezing in fear acquisition (Day 1) varying with the trial of shock in each group (CS-US). **(C)** Freezing in extinction averaged in blocks of 4 trials (4 CS) in Day 2 and 3. **(D)** Baseline freezing in the 180-s baseline period prior to CS onset. **(E)** Freezing in the Same (ACC) or updated (ABC) context in the first test block. It can be observed that the robust fear renewal effect was blocked by partner presentation. Error bar denotes ± SEM.

for the extinction and the averaged freezing of every 2 CS as a block for the test. Freezing during fear conditioning was analyzed via two-way repeated ANOVA with Group as the between-subjects factor and Trial as the within subjects factor. After this, animals were equally split into groups (ABC/ACC and test-alone/accompanied) based on their freezing level in the acquisition phase, ensuring a similar level of freezing before extinction. Freezing during extinction was analyzed via a three-way mixed-design ANOVA with Time (2 levels, the first block vs. the last block) as a repeated factor while Company and Context as two between-subjects factors. Baseline freezing in the test was analyzed via a two-way ANOVA with Company and Context as between-subjects factors. Baseline freezing was measured in the first 3 min before the onset of the first CS during test (Kiyokawa et al., 2007). Freezing in the test was analyzed via a three-way mixed-design ANOVA with Block (3 levels) as a repeated factor while Company (2 levels) and Context (2 levels; ABC vs. ACC) as two between-subjects factors. *Post hoc* comparisons with Bonferroni method were performed after a significant omnibus F-ratio was detected. Student's t-test was used to investigate the difference between EXT.1 ACC-test alone and EXT.2 ACC-test alone groups. The first block of two trials during test was chosen as the key indicator of fear renewal. The sample size of both experiments was determined by the principle (E = Total number of animals − Total number of groups ≥ 20) recommended by Charan and Kantharia (2013). Accordingly, 28 rats were used for Experiment 1 (E = 24) and 24 rats (E = 20) were used for Experiment 2, in order to obtain an E value no less than 20. *Post hoc* power analysis via G*power indicated that the current sample size of two experiment (5–8 rats for each condition) is sufficient to obtain a reliable statistical power for the key results reported in this study (all observed powers > 0.9). Statistical significance was accepted at $p < 0.05$, two-tailed.

RESULTS

In Experiment 1, **Figure 1B** shows that the subjects' freezing during acquisition was affected by the trial [context A; $F(3,72) = 67.82$; $p < 0.001$; $\eta_p^2 = 0.739$], with the percent of

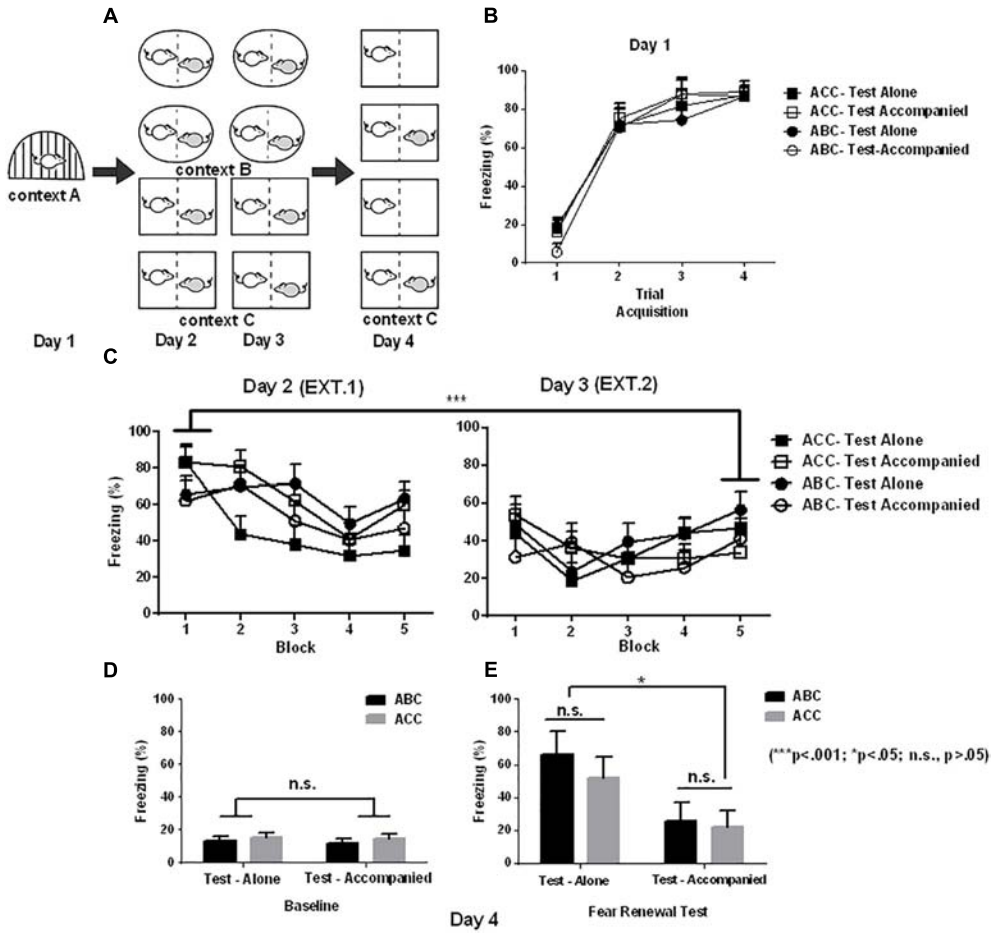

FIGURE 2 | Effects of social buffering on fear memory renewal in Experiment 2. **(A)** Schematic illustration of Experimental design (shape denotes context), yielding a total of 4 groups in a 2 * 2 design (context * company): ACC-Test alone (n = 6), ACC-Test accompanied (n = 7), ABC-Test alone (n = 6), and ABC-Test accompanied (n = 5). **(B)** Freezing in fear acquisition (Day 1) varying with the trial of shock in each group (CS-US). **(C)** Freezing in extinction averaged in blocks of 4 trials (4 CS) in Day 2 and 3. **(D)** Baseline freezing in the 180-s baseline period prior to CS onset. **(E)** Freezing in the Same (ACC) or updated (ABC) context in the first test block. It can be observed that the fear renewal effect, indexed by ABC-ACC difference in freezing, was no longer significant in the test after accompanied extinction. Error bar denotes ± SEM.

freezing significantly increased in the Trial 2–4 than in Trial 1. There is no significant main effect of group [$F(3,24) = 0.70$; $p = 0.56$] or significant interaction effect between group and trial [$F(9,72) = 1.56$; $p = 0.18$].

Freezing averaged in each of the 10 blocks during extinction is shown in **Figure 1C**. The data from the first and the last block were used for statistical analysis. A 2 (Context) * 2 (Company) * 2 (Block) mixed-design ANOVA of freezing during extinction revealed a significant main effect of Block [$F(1,24) = 34.46$; $p < 0.001$; $\eta_p^2 = 0.58$), with the percent of freezing significantly decreased in the last than in the first block. There were no significant main effects of Context [$F(1,24) = 0.42$; $p = 0.52$] and Company [$F(1,24) = 0.03$; $p = 0.85$]. There was no significant Context and Block interaction [$F(1,24) = 2.5$; $p = 0.12$], Context by Company interaction [$F(1,24) = 0.19$; $p = 0.67$], Context by Block interaction [$F(1,24) = 2.5$; $p = 0.12$], or Context by Block by Company [$F(1,24) = 0.24$; $p = 0.63$]. These data suggest that the extinction was successful, and the extinction effect was similar across the four samples before the test.

After extinction, rats were tested for fear renewal in the same context as extinction ("ACC" design) or shifted out of the extinction context ("ABC" renewal). **Figure 1D** shows the freezing in the baseline. A 2 (Company) * 2 (Context) ANOVA of the baseline freezing showed a main effect of Company [$F(1,24) = 9.55$; $p = 0.005$; $\eta_p^2 = 0.285$], while there was no significant main effect of Context [$F(1,24) = 0.00$; $p = 0.97$] or significant interaction between Company and Context [$F(1,24) = 0.42$; $p = 0.52$]. This suggests that the accompanied groups had less freezing than the alone groups during the baseline.

Figure 1E shows the freezing in the first block of the test, as the first test block has proven to exhibit the strongest fear renewal effect (Corcoran et al., 2005). For the freezing analysis in the test stage, there were significant main effects of Block [$F(2,48) = 9.20$; $p < 0.001$; $\eta_p^2 = 0.277$] and Context

[$F(1,24) = 9.16$; $p = 0.006$; $\eta_p^2 = 0.276$], and a significant Block by Context interaction [$F(2,48) = 9.33$; $p < 0.001$; $\eta_p^2 = 0.280$]. *Post hoc* comparisons indicates that subjects in ABC model showed a significantly higher freezing than subjects in ACC model from block 1 to block 3 (all $p < 0.05$), while this effect was most pronounced in block 1 ($p < 0.001$). This indicates that ABC model induced a robust fear renewal effect. There was a significant main effect of Company, with the accompanied subjects showing reduced freezing compared to the alone subjects [$F(1,24) = 7.81$; $p = 0.01$].

More importantly, there was a significant interaction between Company and Context [$F(1,24) = 5.42$; $p = 0.03$; $\eta_p^2 = 0.184$]. The *post hoc* comparisons revealed that the difference between accompanied and alone conditions was not significant in ACC model ($p = 0.74$); while the accompanied group showed significantly less freezing compared to the alone group in ABC model ($p = 0.002$). On the other hand, the alone subjects showed a higher percent of freezing in the ABC vs. ACC conditions ($p = 0.002$), while the accompanied subjects showed a similar level of freezing across ABC and ACC conditions ($p = 0.64$). The above company by context interaction was unaffected by block, shown by the non-significant company * context * block interaction [$F(2,48) = 1.47$; $p = 0.24$].

In Experiment 2, **Figure 2B** shows that the subjects' freezing during acquisition was more pronounced in the Trial 2–4 than in Trial 1 [context A; $F(3,60) = 114.60$; $p < 0.001$; $\eta_p^2 = 0.851$]. There was no significant main effect of Group [$F(3,20) = 0.19$; $p = 0.90$] or significant interaction between Group and Trial [$F(9,60) = 0.61$; $p = 0.73$].

The averaged freezing from block 1 to 10 in the extinction is depicted in **Figure 2C**. A 2 (Context) * 2 (Company) * 2 (Block) mixed-design ANOVA of freezing during extinction revealed a significant main effect of Block [$F(1,20) = 13.92$; $p < 0.001$; $\eta_p^2 = 0.41$], with the freezing rate significantly decreased in the last than in the first block. The main effects of Context [$F(1,20) = 0.78$; $p = 0.39$] and Company [$F(1,20) = 0.17$; $p = 0.19$] were non-significant. There was no significant Context and Block interaction [$F(1,20) = 3.1$; $p = 0.09$], Context by Company interaction [$F(1,20) = 0.06$; $p = 0.81$], Company by Block interaction [$F(1,20) = 0.63$; $p = 0.44$], and no significant Context by Block by Company interaction [$F(1,20) = 0.001$; $p = 0.98$]. These results suggest that the extinction was successful, and the extinction effect was similar across the four samples before the test.

After extinction, rats were tested for fear renewal in the same context as extinction ("ACC") or shifted out of the extinction context ("ABC"). **Figure 2D** shows the freezing in the baseline. The 2 (Company) * 2 (Context) ANOVA of baseline freezing showed no main effect of Company [$F(1,20) = 0.15$; $P = 0.70$] or Context [$F(1,20) = 0.87$; $P = 0.36$]. Also, there was no significant interaction between Company and Group [$F(1,20) = 0.001$; $P = 0.97$]. This suggests that distinct from that in Experiment 1, the baseline freezing in Experiment 2 was similar across the four groups when the extinction was performed with company (**Figure 2D**).

Figure 2E shows the freezing data for the first test block. For the freezing analysis in the test stage, we observed significant main effects of Company [$F(1,20) = 4.428$; $p < 0.05$; $\eta_p^2 = 0.181$] and Block [$F(2,40) = 7.14$; $p < 0.05$; $\eta_p^2 = 0.263$], and a significant Company by Block interaction [$F(2,40) = 4.7$; $p = 0.025$; $\eta_p^2 = 0.191$]. The accompanied groups exhibited less freezing than the alone groups, and this effect was most pronounced in the first block ($p = 0.01$). On the other hand, the accompanied groups showed similar freezing from the first to the third block ($p = 0.76$), while the alone groups showed less freezing from the first to the third block ($p < 0.05$).

There was no significant main effect of Context [$F(1,20) = 0.001$; $p = 0.97$], and no significant Company by Context [$F(1,20) = 0.02$; $p = 0.89$], or Company by Context by Block interaction [$F(2,40) = 0.50$; $p = 0.56$]. These data suggest that social company offered to the extinction stage disrupted the robust fear memory renewal effect as observed in Experiment 1.

In order to examine whether test alone in ACC of Experiment 2 induces freezing due to removal of partner in test compared to extinction context, we compared the test-alone freezing of ACC model between Experiment 1 and Experiment 2 by the Student's *t*-test. The results showed no significant differences between Experiment 1 and Experiment 2 [$t(7) = -1.484$, $p = 0.183$]. Thus, removal of partner in the test-alone condition of ACC model in Experiment 2 did not significantly induce fear renewal.

DISCUSSION

Giving social company at different stage in two experiments, the current study focuses on how social company modulates fear memory renewal elicited by contextual updating. Fear memory renewal was operationally defined by the contrast of freezing rate between ABC and ACC model. Experiment 1 manipulated social company at the test rather than the extinction stage, to examine how social company in the test phase alone may alter fear memory renewal. The results firstly showed that rats exhibited more freezing in ABC than in ACC model, which is consistent with previous finding of robust fear memory renewal in the ABC model (Wang et al., 2016). Then, we observed no significant freezing differences in the test between accompanied and alone conditions in ACC model. This is consistent with the reports of Nowak et al. (2013), which observed a similar freezing for the mice with the presence of naïve associate and those without associate in the ABB paradigm (equivalent to the current ACC paradigm) (Nowak et al., 2013). It is worth noting that the authors of this work used an experimental design allowing the mice to see, hear and smell the neighbor, but not to contact conspecific physically. The authors thought that absence of direct physical contact impeded the interaction between the animals, thereby they did not observe a significant difference in the levels of fear response between the mice with and without naïve associates. In our study the visual, auditory and limited olfactory contacts were allowed, to optimize the social buffering effect. In this regard, we infer that the lack of social buffering effect in ACC model should not be due to the insufficient provision of social company. Instead, this is most likely due to the floor effect of freezing, as the rats were tested in the same context as in the extinction, which

facilitates the retrieval of the fear unlearning memories (Myers and Davis, 2007).

Importantly, the accompanied group showed significantly less freezing than alone group during test in ABC model, which implies that social company during test could suppress fear memory renewal from context updating. This is consistent with prior reports that the presence of a conspecific, whether familiar or unfamiliar to the subject, ameliorated conditioned fear responses in behavioral (e.g., freezing) and physiological (e.g., corticosterone) measures when the animal was tested after a fear conditioning procedure (Kiyokawa et al., 2007, 2014a; Ishii et al., 2016). However, these studies did not design an extinction phase, thus unable to allow for the observation of a fear renewal effect and how it varies with social company. Thus, the current study extends previous studies by showing that social company does not only ameliorate fear responses evoked by the CS, but is also able to mitigate the fear renewal in response to the CS occurring in a novel context outside of the extinction one.

As described above, Experiment 1 showed a clear social buffering effect on fear memory renewal when social company was offered to the test stage. However, the practical implication of this finding is limited. This is due to the fact that the rehabilitated patients in the real-life settings may experience various new situations after leaving a specific therapeutic situation, as stated above. This prompted Experiment 2 that focused on whether social company given to the extinction stage may suppress the fear renewal effect in a novel context. The results showed that the subjects' freezing in the test stage was no longer significantly different between ABC and ACC model, implying that social company given to the extinction stage disrupted the robust fear memory renewal effect.

However, one may question that in Experiment 2, the lack of higher freezing from ACC to ABC model in the test-alone condition does not necessarily reflect social buffering effect due to providing company to the extinction stage. Instead, it may reflect enhanced freezing in the ACC model due to removal of company from the extinction to the test stage. This possibility should be considered as prior studies indicated that context includes not only external physical environment but also internal cognitive context (Bouton et al., 2006; Maren et al., 2013). However, this may not contaminate our conclusion as our comparison of test-alone freezing in ACC model showed no significant differences from Experiment 1 to Experiment 2. This suggests that partner removal did not significantly induce fear renewal, as freezing was similar no matter whether the test-alone context was constituted by partner removal or by the lack of partner all the time. This inference was further confirmed by our finding that, adding a partner to the test context did not alter the freezing rate when comparing accompanied to alone condition in the ACC model of Experiment 1 (see **Figure 1E**). It is still necessary to conduct confirmatory research manipulating Extinction Company in parallel with Context in the test-alone condition with a larger sample size. This allows a more rigid statistical examination of whether removal of partner from extinction to test indeed elicits no effect of fear renewal.

Another issue worth noting is that the current findings appear discrepant with those by Mikami et al. (2016). In this work, the authors manipulated the presence of social company, that is, providing a conspecific male rat or not to the subject, to investigate how the effect of extinction training on conditioned fear varies with social company. The results showed that the extinction training suppressed freezing elicited by the CS in the ABB paradigm (i.e., test and extinction context was the same) when social company was available. However, the above extinction effect vanished when social company is absent, or when social company is provided but the subject was tested in a novel context (Mikami et al., 2016). Thus, comparing these data with the current findings may leave an inconsistent impression, in that we observed social company given to the extinction stage disrupted fear renewal when the subject was tested in a novel context. However, the current study differs from this work in two important ways. Firstly, the extinction training of the two studies differs in both duration and intensities. The extinction training of Mikami et al. (2016) consisted of 24 trials of CS delivered in 1 day, while the current extinction procedure consisted of 40 trials of CS divided by 2 days.

More importantly, driven by the research purpose of how the effects of extinction training on fear response may be moderated by social company, Mikami et al. (2016) focused on whether the freezing differences between extinction and non-extinction in the recall test vary with social company and contextual updating. However, the current study focuses on how social company may modulate the effect of fear memory renewal elicited by context updating. Driven by this purpose, the current study did not manipulate extinction. Instead, we let all the subjects receive a 2-day extinction, in order to manipulate the context in the test (same/different). Accordingly, we mainly tested how ABC-ACC differences in freezing varied as a function of social company (accompanied vs. alone). In this regard, our observation that social company eliminated the fear renewal effect during ABC vs. ACC contrast, is not incompatible with the finding of Mikami and colleagues that 24 trials of accompanied extinction in 1 day mitigated the animals' fear-conditioned response in ABB but not in ABC model.

Prior studies in protection from extinction showed that pairing an inhibitory CS (a predictor for the absence of the US) with a fear-eliciting cue during extinction impedes extinction, leading to a return of fear response when the inhibitory CS is removed in the test (Rescorla, 2003; Lovibond et al., 2009). In Experiment 2, we observed that the presence of social conspecific during extinction inhibited the animal's fear renewal effect in the test stage, though the animal was tested alone and in a novel context. This result is not incompatible with the "protection from extinction" studies. Firstly, the role of an inhibitory CS in these studies is predicting the absence of the US. Thus, it is unsurprising that the individual's expectancy of the US would appear again if the inhibitory CS is removed. By contrast, the role of social company is to provide social supports and relieve the stress, instead of predicting environmental safety (Kikusui et al., 2006). Secondly, the inhibitory CS works based on a long-term training of subjects in the laboratory, to obtain a safety prediction. By contrast, social company by presenting conspecific

may inherently links to safety information (Hornstein et al., 2016).

To the best of our knowledge, previous studies have provided social company at different time points of fear conditioning, to examine how social company influences fear response, such as pre-fear conditioning (Guzman et al., 2009) or in fear acquisition (Lee and Noh, 2016), extinction (Mikami et al., 2016), or test (Kiyokawa et al., 2014a). However, little research has examined the role of social company in fear renewal by employing the ACC and ABC paradigms simultaneously. The benefit of using two models simultaneously is that the differential freezing between the ABC and ACC model is not attributable to physical differences in the test context because all tests are conducted in an identical context with the same CS. The current study extends prior studies by exploring the suppression of fear renewal by social company and how to maximize the suppression effect by manipulating the time points to offer company. Taken together, the two studies not only provide evidence that social company is able to mitigate fear renewal, but also suggests that social company given at the extinction stage generates an optimal suppression of fear renewal. Though massive extinction treatment has been suggested to attenuate the fear renewal effect (Denniston et al., 2003), it is more practical and economical to provide social company during treatment in the limited time of the therapist. Future studies need to examine the neural pathway subserving social buffering effect on fear renewal, in addition to the current understanding of neural pathway of social buffering (Kiyokawa et al., 2012). Another point worth noting is that the accompanying rats used in our study were all unfamiliar to the subjects, for isolating a unique effect of social buffering. A handful of studies have shown that familiar conspecifics elicit better social buffering effects compared to unfamiliar ones (Hennessy et al., 2000; Kiyokawa et al., 2014b). In this regard, future studies should consider using familiar partner to explore social buffering effects on clinical PTSD, in order to seek an optimal intervention effect.

AUTHOR CONTRIBUTIONS

JY, MY, and XW performed the experiments. WC helped in experimental design. JY and MY analyzed the data. JY, MY, and YX wrote the paper.

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REFERENCES

Balooch, S. B., Neumann, D. L., and Boschen, M. J. (2012). Extinction treatment in multiple contexts attenuates ABC renewal in humans. *Behav. Res. Ther.* 50, 604–609. doi: 10.1016/j.brat.2012.06.003

Boschen, M. J. (2009). Relapse of successfully treated anxiety and fear Theoretical issues and recommendations for clinical practice. *Aust. N. Z. J. Psychiatry* 43, 89–100. doi: 10.1080/00048670802607154

Boschen, M. J., Neumann, D. L., and Waters, A. M. (2009). Relapse of successfully treated anxiety and fear: theoretical issues and recommendations for clinical practice. *Aust. N. Z. J. Psychiatry* 43, 89–100. doi: 10.1080/00048670802607154

Bouton, M. E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol. Psychiatry* 52, 976–986.

Bouton, M. E., and Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learn. Motiv.* 10, 445–466.

Bouton, M. E., Westbrook, R. F., Corcoran, K. A., and Maren, S. (2006). Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biol. Psychiatry* 60, 352–360. doi: 10.1016/j.biopsych.2005.12.015

Charan, J., and Kantharia, N. D. (2013). How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* 4, 303–306.

Choy, Y., Fyer, A. J., and Lipsitz, J. D. (2007). Treatment of specific phobia in adults. *Clin. Psychol. Rev.* 27, 266–286. doi: 10.1016/j.cpr.2006.10.002

Corcoran, K. A., Desmond, T. J., Frey, K. A., and Maren, S. (2005). Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *J. Neurosci.* 25, 8978–8987.

Davitz, J. R., and Mason, D. J. (1955). Socially facilitated reduction of a fear response in rats. *J. Comp. Physiol. Psychol.* 48, 149–151. doi: 10.1037/H0046411

Denniston, J. C., Chang, R. C., and Miller, R. R. (2003). Massive extinction treatment attenuates the renewal effect. *Learn. Motiv.* 34, 68–86. doi: 10.1016/S0023-9690(02)00508-8

Fontana, A. M., Diegnan, T., Villeneuve, A., and Lepore, S. J. (1999). Nonevaluative social support reduces cardiovascular reactivity in young women during acutely stressful performance situations. *J. Behav. Med.* 22, 75–91.

Fuzzo, F., Matsumoto, J., Kiyokawa, Y., Takeuchi, Y., Ono, T., and Nishijo, H. (2015). Social buffering suppresses fear-associated activation of the lateral amygdala in male rats: behavioral and neurophysiological evidence. *Front. Neurosci.* 9:99. doi: 10.3389/fnins.2015.00099

Glynn, S. M. (1999). A test of behavioral family therapy to augment exposure for combat-related posttraumatic stress disorder. *J. Consult. Clin. Psychol.* 67, 243–251. doi: 10.1037//0022-006x.67.2.243

Goode, T. D., and Maren, S. (2014). Animal models of fear relapse. *ILAR J.* 55, 246–258. doi: 10.1093/ilar/ilu008

Guzman, Y. F., Tronson, N. C., Guedea, A., Huh, K. H., Gao, C., and Radulovic, J. (2009). Social modeling of conditioned fear in mice by non-fearful conspecifics. *Behav. Brain Res.* 201, 173–178. doi: 10.1016/j.bbr.2009.02.024

Hennessy, M., Maken, D., and Graves, F. (2000). Consequences of the presence of the mother or unfamiliar adult female on cortisol, ACTH, testosterone and behavioral responses of periadolescent guinea pigs during exposure to novelty. *Psychoneuroendocrinology* 25, 619–632.

Hennessy, M. B., Kaiser, S., and Sachser, N. (2009). Social buffering of the stress response: diversity, mechanisms, and functions. *Front. Neuroendocrinol.* 30, 470–482. doi: 10.1016/j.yfrne.2009.06.001

Herry, C., Ferraguti, F., Singewald, N., Letzkus, J. J., Ehrlich, I., and Luthi, A. (2010). Neuronal circuits of fear extinction. *Eur. J. Neurosci.* 31, 599–612. doi: 10.1111/j.1460-9568.2010.07101.x

Hornstein, E. A., Fanselow, M. S., and Eisenberger, N. I. (2016). A safe haven: investigating social-support figures as prepared safety stimuli. *Psychol. Sci.* 27, 1051–1060. doi: 10.1177/0956797616646580

Ishii, A., Kiyokawa, Y., Takeuchi, Y., and Mori, Y. (2016). Social buffering ameliorates conditioned fear responses in female rats. *Horm. Behav.* 81, 53–58. doi: 10.1016/j.yhbeh.2016.03.003

Jovanovic, T., and Ressler, K. J. (2010). How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *Am. J. Psychiatry* 167, 648–662. doi: 10.1176/appi.ajp.2009.09071074

Kikusui, T., Winslow, J. T., and Mori, Y. (2006). Social buffering: relief from stress and anxiety. *Philos. Trans. R. Soc. B Biol. Sci.* 361, 2215–2228. doi: 10.1098/rstb.2006.1941

Kiyokawa, Y., Hiroshima, S., Takeuchi, Y., and Mori, Y. (2014a). Social buffering reduces male rats' behavioral and corticosterone responses to a conditioned stimulus. *Horm. Behav.* 65, 114–118. doi: 10.1016/j.yhbeh.2013.12.005

Kiyokawa, Y., Honda, A., Takeuchi, Y., and Mori, Y. (2014b). A familiar conspecific is more effective than an unfamiliar conspecific for social buffering of conditioned fear responses in male rats. *Behav. Brain Res.* 267, 189–193. doi: 10.1016/j.bbr.2014.03.043

Kiyokawa, Y., Kikusui, T., Takeuchi, Y., and Mori, Y. (2004). Partner's stress status influences social buffering effects in rats. *Behav. Neurosci.* 118, 798–804. doi: 10.1037/0735-7044.118.4.798

Kiyokawa, Y., Takeuchi, Y., and Mori, Y. (2007). Two types of social buffering differentially mitigate conditioned fear responses. *Eur. J. Neurosci.* 26, 3606–3613. doi: 10.1111/j.1460-9568.2007.05969.x

Kiyokawa, Y., Takeuchi, Y., Nishihara, M., and Mori, Y. (2009). Main olfactory system mediates social buffering of conditioned fear responses in male rats. *Eur. J. Neurosci.* 29, 777–785. doi: 10.1111/j.1460-9568.2009.06618.x

Kiyokawa, Y., Wakabayashi, Y., Takeuchi, Y., and Mori, Y. (2012). The neural pathway underlying social buffering of conditioned fear responses in male rats. *Eur. J. Neurosci.* 36, 3429–3437. doi: 10.1111/j.1460-9568.2012.08257.x

Lee, H., and Noh, J. (2016). Pair exposure with conspecific during fear conditioning induces the link between freezing and passive avoidance behaviors in rats. *Neurosci. Res.* 108, 40–45. doi: 10.1016/j.neures.2016.01.005

Lovibond, P. F., Mitchell, C. J., Minard, E., Brady, A., and Menzies, R. G. (2009). Safety behaviours preserve threat beliefs: protection from extinction of human fear conditioning by an avoidance response. *Behav. Res. Ther.* 47, 716–720. doi: 10.1016/j.brat.2009.04.013

Maren, S., Phan, K. L., and Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat. Rev. Neurosci.* 14, 417–428. doi: 10.1038/nrn3492

Mark, I., and Lovell, K. (1998). Treatment of posttraumatic stress disorder by exposure and or cognitive restructuring A controlled study. *Arch. Gen. Psychiatry* 55, 317–325. doi: 10.1001/archpsyc.55.4.317

Mikami, K., Kiyokawa, Y., Takeuchi, Y., and Mori, Y. (2016). Social buffering enhances extinction of conditioned fear responses in male rats. *Physiol. Behav.* 163, 123–128. doi: 10.1016/j.physbeh.2016.05.001

Monson, C. M., and Fredman, S. J. (2012). Effect of cognitive-behavioral couple therapy for ptsd a randomized controlled trial. *J. Am. Med. Assoc.* 308, 700–709. doi: 10.1001/jama.2012.9307

Myers, K. M., and Davis, M. (2007). Mechanisms of fear extinction. *Mol. Psychiatry* 12, 120–150. doi: 10.1038/sj.mp.4001939

Nowak, A., Werka, T., and Knapska, E. (2013). Social modulation in extinction of aversive memories. *Behav. Brain Res.* 238, 200–205. doi: 10.1016/j.bbr.2012.10.031

Polack, C. W., Laborda, M. A., and Miller, R. R. (2013). On the differences in degree of renewal produced by the different renewal designs. *Behav. Process.* 99, 112–120. doi: 10.1016/j.beproc.2013.07.006

Rescorla, R. (2003). Protection from extinction. *Learn. Mem.* 31, 124–132. doi: 10.3758/BF03195975

Sautter, F. J., Glynn, S. M., Thompson, K. E., Franklin, L., and Han, X. (2009). A couple-based approach to the reduction of PTSD avoidance symptoms. *J. Marital Fam. Ther.* 35, 343–349. doi: 10.1111/j.1752-0606.2009.00125.x

Taylor, G. T. (1981). Fear and affiliation in domesticated male-rats. *J. Comp. Physiol. Psychol.* 95, 685–693.

Wang, Q., Jin, J., and Maren, S. (2016). Renewal of extinguished fear activates ventral hippocampal neurons projecting to the prelimbic and infralimbic cortices in rats. *Neurobiol. Learn. Mem.* 134(Pt A), 38–43. doi: 10.1016/j.nlm.2016.04.002

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A Cortical Folding Pattern-Guided Model of Intrinsic Functional Brain Networks in Emotion Processing

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There have been increasing studies demonstrating that emotion processing in humans is realized by the interaction within or among the large-scale intrinsic functional brain networks. Identifying those meaningful intrinsic functional networks based on task-based functional magnetic resonance imaging (task fMRI) with specific emotional stimuli and responses, and exploring the underlying functional working mechanisms of interregional neural communication within the intrinsic functional networks are thus of great importance to understand the neural basis of emotion processing. In this paper, we propose a novel cortical folding pattern-guided model of intrinsic networks in emotion processing: gyri serve as global functional connection centers that perform interregional neural communication among distinct regions via long distance dense axonal fibers, and sulci serve as local functional units that directly communicate with neighboring gyri via short distance fibers and indirectly communicate with other distinct regions via the neighboring gyri. We test the proposed model by adopting a computational framework of dictionary learning and sparse representation of emotion task fMRI data of 68 subjects in the publicly released Human Connectome Project. The proposed model provides novel insights of functional mechanisms in emotion processing.

Keywords: emotion, task fMRI, intrinsic functional network, cortical gyri and sulci, functional model

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INTRODUCTION

Understanding the neurobiological basis of emotions (e.g., fear, anger, sadness, etc.) in humans has received extensive interests in the affective neuroscience field (Lindquist and Barrett, 2012; Lindquist et al., 2012). With the advancement of *in-vivo* functional neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (Logothetis, 2008; Friston, 2009) as well as the development of advanced image analysis and computational modeling methodologies, researchers are able to examine the neural circuitry of emotion processing for a better understanding of the functional architecture of brain emotion. Specifically, based on task fMRI with specific emotional stimuli and responses, specific brain regions or brain networks involved in such emotion processing can be identified; in other words, it is assumed that different kinds of emotion processing can be localized to specific brain regions/networks (Vytal and Hamann, 2010; Panksepp, 2011; Lindquist et al., 2012; Murphy et al., 2012). Recently, mounting evidence has shown that human brain is intrinsically organized into multiple functional networks such as default mode, visual,

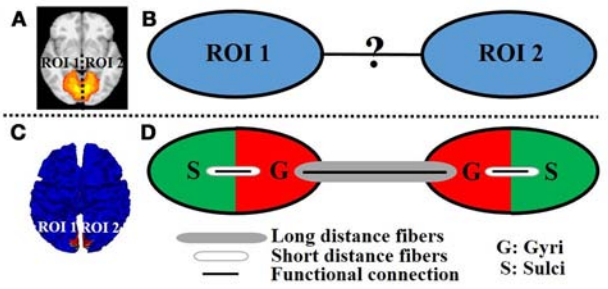

FIGURE 1 | An example intrinsic network composed of two distinct regions of interest (ROI 1 and 2 in left and right hemisphere, respectively) viewed in volume space **(A)** and on cortical surface **(C)**. **(B)** The unknown functional working mechanisms of interregional neural communication within one network. **(D)** Illustration of the proposed model. The red/green color represents the gyral/sulcal regions within the ROI of intrinsic network.

motor, auditory, cognitive control, etc., each of which is spatially distributed across specific neuroanatomical areas (Fox et al., 2005; Bullmore and Sporns, 2009; Duncan, 2010; Pessoa, 2012; Fedorenko et al., 2013); the emotion processing is realized by the interaction within or among those intrinsic functional brain networks (Bressler and Menon, 2010; Lindquist and Barrett, 2012; Barrett and Satpute, 2013). As a consequence, identifying meaningful intrinsic functional brain networks based on task fMRI data, as well as exploring its underlying functional working mechanisms of interregional neural communication, is of great importance to understand the neural basis of emotion processing.

A variety of fMRI time series analysis methodologies have been successfully applied in the brain mapping field for intrinsic functional network identification based on either task fMRI data or resting state fMRI data such as principal component analysis (PCA) (Andersen et al., 1999), independent component analysis (ICA) (McKeown et al., 1998), and dictionary learning/sparse representation (Abolghasemi et al., 2015; Lv et al., 2015a,b). The premise is that the activity patterns in fMRI blood-oxygen level-dependent (BOLD) signals among spatially distinct brain regions within an intrinsic network are temporally coupled. **Figure 1A** shows an example intrinsic network which is composed of two spatially distinct brain regions (regions of interest (ROI) 1 and 2 in left and right hemisphere, respectively). Although the distinct regions within one network are argued to be functionally linked and interacting with each other, the underlying functional working mechanisms of interregional neural communication among those regions within one network are still largely unknown (**Figure 1B**).

In the literature of brain network analysis, however, there has been little effort devoted to adding the factor of cortical folding patterns into consideration. Actually, the cortical folding pattern, which is composed of highly convoluted convex gyri and concave sulci, is one of the most prominent features of human brain (Barron, 1950; Welker, 1990). A variety of studies have demonstrated that there are both structural and functional differences between cortical gyral and sulcal regions (Nie et al., 2012; Chen et al., 2013; Deng et al., 2014; Zhang et al., 2014; Jiang et al., 2015, 2018). For example, it is reported that gyral regions are connected by much denser diffusion tensor imaging (DTI) or high angular resolution diffusion imaging (HARDI) derived axonal fiber bundles than sulcal regions in the whole cortex, indicating that gyri are structural connection center of the cortex (Nie et al., 2012; Chen et al., 2013; Zhang et al., 2014). Another studies report that gyral regions have stronger functional connectivity and more spatial overlap patterns of global functional networks than sulcal regions, indicating that gyri are global functional center of the cortex (Deng et al., 2014; Jiang et al., 2015, 2018).

As an attempt to modeling the interregional neural communication of intrinsic networks in emotion processing, and inspired by the abovementioned structural/functional differences between gyral and sulcal regions as well as the previous finding that distinct regions within one intrinsic network are interconnected by DTI-derived fiber bundles (Greicius et al., 2009; Van den Heuvel et al., 2009), in this paper, we propose a novel cortical folding pattern-guided model of the intrinsic network (**Figure 1C**) in emotion processing: gyri serve as global functional connection centers that perform interregional neural communication among distinct regions via long distance dense fibers, and sulci serve as local functional units that directly communicate with neighboring gyri via short distance fibers (inter-column cortico-cortical fibers) and indirectly communicate with other distinct regions via the neighboring gyri with the dense fibers (**Figure 1D**). We test the proposed model by assessing the task fMRI signal representation accuracy via a computational framework of dictionary learning and sparse representation of whole-brain emotion task fMRI signals. We hypothesize that the sparse representation accuracy value of task fMRI signals, which indicates the degree of interregional neural communication among distinct regions, is significantly larger on gyri than on sulci within the intrinsic network in emotion processing.

MATERIALS AND METHODS

Data Acquisition and Pre-processing

We adopt the emotion task fMRI data of 68 subjects in the publicly released Human Connectome Project (HCP, Q1 release) (Barch et al., 2013) as a testbed in this paper. This emotion task is similar with the one in Hariri et al. (2002). Participants were presented and asked to match either two different shapes or faces (with angry or fearful expressions) at the bottom of the screen with the one at the top of the screen. There were six blocks (of face or shape alternatively), each of which was preceded by a 3 s task cue (shape or face) and 6 trials of the same match task (face or shape, 3 s for each trial). There were 3 face blocks and 3 shape blocks for each of the two runs. More details of the task design are referred to Barch et al. (2013).

The acquisition parameters of the task fMRI data are as follows (Barch et al., 2013): 220 mm FOV, 90 × 104 matrix, 72 slices, TR = 0.72 s, 176 volumes (time points), TE = 33.1 ms, flip angle = 52, in-plane FOV = 208 × 180 mm, 2.0 mm isotropic voxels. The pre-processing steps using FSL FEAT are referred to Barch et al. (2013) which mainly include skull removal, motion

correction, slice time correction, spatial smoothing, and global drift removal (high-pass filtering).

Sparse Representation of Whole-Brain Task fMRI Signals

We adopt a computational framework of dictionary learning and sparse representation of whole-brain task fMRI signals to test the proposed model. The rationales are 2-fold. First, the dictionary learning and sparse representation framework has been demonstrated as an efficient and effective data-driven approach in identifying concurrent intrinsic networks based on task fMRI signals (Abolghasemi et al., 2015; Lv et al., 2015a,b) (detailed in Section Intrinsic network identification and representation on cortical surface). This is also the premise to model the working mechanisms of intrinsic networks. Second, the dictionary learning and sparse representation framework can learn meaningful functional activity basis patterns from hundreds of thousands of whole-brain task fMRI signals effectively and represent the task fMRI signals based on the learned basis efficiently and compactly (Abolghasemi et al., 2015; Lv et al., 2015a,b). Assessing the task fMRI signals representation accuracy based on the learned basis in gyral/sulcal regions within one intrinsic network is reasonable to validate the proposed cortical folding pattern-guided model of intrinsic network in emotion processing as detailed in Section Signal representation accuracy assessment on gyri/sulci within one intrinsic network.

As illustrated in **Figure 2**, for each subject, first, the fMRI signals of whole-brain voxels are extracted, and normalized to zero mean and standard deviation of 1 (Mairal et al., 2010). Second, all n normalized signals, each of which has t time points, are aggregated into a 2D matrix $\mathbf{X} = [\mathbf{x}_1, \mathbf{x}_2,\ldots,\mathbf{x}_n] \in \mathbb{R}^{t\times n}$ (**Figure 2A**). Third, by applying the widely adopted online dictionary learning method (Mairal et al., 2010), $\mathbf{X}$ is decomposed into an over-complete dictionary matrix $\mathbf{D} = [\mathbf{d}_1, \mathbf{d}_2,\ldots,\mathbf{d}_m] \in \mathbb{R}^{t\times m}$ (m is the dictionary size, $m>t$ and $m<<n$) and a sparse coefficient matrix $\boldsymbol{\alpha}=[\boldsymbol{\alpha}_1, \boldsymbol{\alpha}_2,\ldots, \boldsymbol{\alpha}_n] \in \mathbb{R}^{m\times n}$ (**Figure 2B**). In this way, each fMRI signal is represented as a linear combination of all learned dictionary atoms in $\mathbf{D}$, i.e., $\mathbf{x}_i=\mathbf{D}\times\boldsymbol{\alpha}_i+\boldsymbol{\varepsilon}$ ($\boldsymbol{\varepsilon}$ is error term). Specifically, an empirical cost function $f_n(\mathbf{D})$ of $\mathbf{X}$ is defined to assess the average loss of regression of all n signals based on $\mathbf{D}$:

$$f_n(\mathbf{D}) \triangleq \frac{1}{n}\sum_{i=1}^{n} l(\mathbf{x}_i, \mathbf{D}) \tag{1}$$

where $l(\mathbf{x}_i, \mathbf{D}) \triangleq \min_{\alpha_i\in\mathbb{R}^m} \frac{1}{2}||\mathbf{x}_i - \mathbf{D}\alpha_i||_2^2 + \lambda||\alpha_i||_1$. Note that the l_1 regularization is adopted for a sparse solution of α_i. λ is used to regularize regression loss and sparsity level. We also defined a constraint for $\mathbf{D}$ to make the coefficients in $\boldsymbol{\alpha}$ comparable:

$$C \triangleq \left\{\mathbf{D}\in\mathbb{R}^{t\times m} \;\; s.t. \;\; i = 1,\ldots m, \; (\mathbf{d}_i)^T\mathbf{d}_i \le 1\right\} \tag{2}$$

In this way, Equation (1) can be rewritten as a matrix factorization problem:

$$\min_{\mathbf{D}\in C,\alpha\in\mathbb{R}^{m\times n}} \frac{1}{2}||\mathbf{X} - \mathbf{D}\alpha||_F^2 + \lambda||\alpha||_{1,1} \tag{3}$$

We learn $\mathbf{D}$ in Equation (3) using the effective online dictionary learning method (Mairal et al., 2010). $\boldsymbol{\alpha}$ is then solved based on $\mathbf{D}$ as an l_1 regularized linear least-squares problem (Mairal et al., 2010). We use the parameter setting of the same HCP data in Lv et al. (2015b) as $m = 400$ and $\lambda = 1.5$. From brain science perspective, the learned dictionary atoms in $\mathbf{D}$ represent a set of signal basis (**Figure 2B**) derived from whole-brain task fMRI signals. Each original fMRI signal can be represented by these relevant signal basis patterns via linear combination.

Intrinsic Network Identification and Representation on Cortical Surface

As demonstrated in Section Sparse representation of whole-brain task fMRI signals, each column of $\boldsymbol{\alpha}$ stores the sparse coefficients of representing each original fMRI signal based on $\mathbf{D}$. Moreover, each row of $\boldsymbol{\alpha}$ can be mapped back to the original brain volume space to represent the spatial volumetric pattern that has reference to each dictionary atom (**Figure 2C**). To identify the meaningful intrinsic networks from all spatial patterns, we adopt the publicly available intrinsic network template (Smith et al., 2009) as references. This template provides nine stable and meaningful intrinsic networks on cortical area including three visual, default mode, motor, auditory, executive control, and bilateral frontal/parietal networks (Smith et al., 2009). Specifically, the spatial pattern similarity is defined as the overlap rate R:

$$R(S,T) = |S\cap T| / |T| \tag{4}$$

where S is a set of cortical vertices involving in a spatial pattern that has reference to a dictionary atom, T is a set of cortical vertices involving in the spatial pattern of a specific intrinsic network template (Smith et al., 2009). The spatial pattern with the highest R with the intrinsic network template is identified as the corresponding intrinsic network in this individual subject as previous studies (Lv et al., 2015b). Note that the task-induced network can also be effectively identified and separated with intrinsic networks by means of considering both temporal and spatial patterns of dictionary atoms in the dictionary learning and sparse representation framework as detailed in Jiang et al. (2015, 2018) and Lv et al. (2015a,b). We then map the identified intrinsic networks in task fMRI volume space (**Figure 2C**) to T1 cortical surface (**Figure 2D**) in order to utilize the cortical folding pattern information. Specifically, the network is firstly converted into T1 volume space and then mapped onto the cortical surface using an in-house tool by localizing each voxel involved in the network to its nearest cortical mesh vertex.

Signal Representation Accuracy Assessment on Gyri/Sulci Within One Intrinsic Network

As demonstrated in Section Sparse representation of whole-brain task fMRI signals, the learned over-complete $\mathbf{D}$ represents a set of all basis components of neural activities from whole-brain task fMRI signals. Each original fMRI signal $\mathbf{x}_i$ is approximately

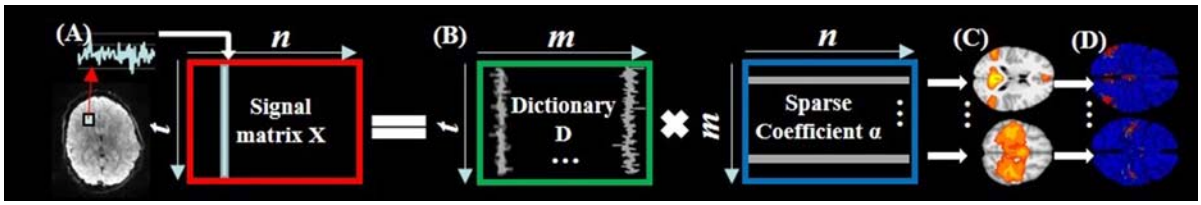

FIGURE 2 | The illustration of sparse representation of whole-brain rsfMRI signals. **(A)** The whole-brain rsfMRI signals of an example subject which are aggregated into a 2D matrix **X**. **(B)** The decomposed dictionary matrix **D** and sparse coefficient matrix **α** based on **X**. **(C)** The identified intrinsic networks in task fMRI volume space. **(D)** The corresponding intrinsic networks on cortical surface.

represented as $\bar{\mathbf{x}}_i = \mathbf{D} \times \alpha_i$. Here we assess the task fMRI signal representation accuracy P as:

$$P_{\mathbf{x}_i} = corr(\bar{\mathbf{x}}_i, \mathbf{x}_i) \tag{5}$$

where corr(.) is the Pearson's correlation coefficient between $\mathbf{x}_i$ and $\mathbf{x}_i$ and ranges from 0 to 1. The larger the P is, the better the signal representation is for $\mathbf{x}_i$, i.e., $\mathbf{x}_i$ can be well represented by the basis components of neural activities in $\mathbf{D}$. In other words, $\mathbf{x}_i$ well participates in or follow the neural activities in emotion processing. Since the distinct regions within one intrinsic network theoretically have similar neural activities and are functionally linked, the assessment of the task fMRI signal representation accuracy (Equation 5) in these distinct regions within the intrinsic network is thus indicative of the degree of interregional neural communication among distinct regions within the intrinsic network.

Specifically, for each distinct region $V = \forall v_i$ (v_i is the cortical vertex in the region) within one intrinsic network on cortical surface, we first calculate the signal representation accuracy value P_{v_i} (Equation 5) for the task fMRI signals of all v_i. Second, based on the principal curvature value of v_i to delineate gyral/sulcal regions as $pcurv_{v_i} \begin{cases} \geq 0, & v_i \epsilon gyri \\ < 0, & v_i \epsilon sulci \end{cases}$ provided in the HCP data (Barch et al., 2013), we separate V into gyral and sulcal regions as $V_{gyri} = \forall v_i \; s.t. \; pcurv_{v_i} \geq 0$ and $V_{sulci} = \forall v_i \; s.t. \; pcurv_{v_i} < 0$, respectively. Note that $V = V_{gyri} + V_{sulci}$. Finally, the set of all signal representation accuracy values in gyral and sulcal regions is represented as $P_{V_{gyri}} = \forall P_{v_i} \; s.t. \; v_i \in V_{gyri}$ and $P_{V_{sulci}} = \forall P_{v_i} \; s.t. \; v_i \in V_{sulci}$, respectively. By evaluating the possible mean accuracy value difference between gyral and sulcal regions in each of the distinct regions within one intrinsic network, the proposed cortical folding pattern-guided model of the intrinsic network in emotion processing is validated.

RESULTS

Signal Representation Accuracy Difference on Gyri/Sulci in Default Mode Network

We adopted the proposed framework to examine the signal representation accuracy difference on gyri/sulci in default mode network (DMN), which is one of the most recognized intrinsic network (Smith et al., 2009). As illustrated in **Figure 3**, there are four spatially distinct regions of interest (ROIs) in DMN including left inferior parietal lobule (ROI 1), right inferior parietal lobule (ROI 2), bilateral medial prefrontal gyrus/anterior cingulate cortex (ROI 3), and bilateral posterior cingulate cortex (ROI 4) (Smith et al., 2009). For each of the four ROIs, we can see that both gyral and sulcal regions have reasonably high accuracy value (the mean accuracy value is 0.82 for gyri and 0.76 for sulci) since the sparse representation approach (Section Sparse representation of whole-brain task fMRI signals) can relatively effectively represent whole-brain rsfMRI signals. However, there is still accuracy difference between gyral and sulcal regions. A two-sample one-tailed t-test between the set of accuracy values of gyri and sulci ($p < 0.05$, Bonferroni corrected) shows that the signal representation accuracy value on gyri is significantly larger than that on sulci for each of the four ROIs (**Figure 3**).

Signal Representation Accuracy Difference on Gyri/Sulci in Other Intrinsic Networks

We assessed the signal representation accuracy on gyri/sulci in the other eight intrinsic networks to examine the generality of the proposed working model of intrinsic networks in emotion processing. As shown in **Figure 4**, the eight intrinsic networks (Smith et al., 2009) include three visual networks (Network 1–3), motor (Network 4), auditory (Network 5), executive control (Network 6), and bilateral frontal/parietal networks (Network 7–8). The mean accuracy value across all ROIs in all eight intrinsic networks is 0.75 for gyri and 0.71 for sulci. A two-sample one-tailed t-test between the set of accuracy values of gyri and sulci ($p < 0.05$, Bonferroni corrected) shows that the signal representation accuracy value on gyri is significantly larger than that on sulci in each of the intrinsic network.

Reproducibility and Structural Substrates of the Intrinsic Functional Network Model

We assessed the signal representation accuracy on gyri/sulci in all nine intrinsic networks in emotion processing in all 68 subjects. **Table 1** indicates that the signal representation accuracy on gyri is consistently larger than sulci in each of the nine intrinsic networks across a majority of subjects. Moreover, we performed the permutation test for each intrinsic network to separate all signal representation accuracy values within the intrinsic network into gyri and sulci groups and to calculate the mean difference between the two groups for 1000 times. The p-value based on the 1,000-time permutation t-test is $p < 0.05$ for all intrinsic networks and subjects, indicating the signal representation accuracy of gyri is truly larger than sulci within all intrinsic networks in emotion processing.

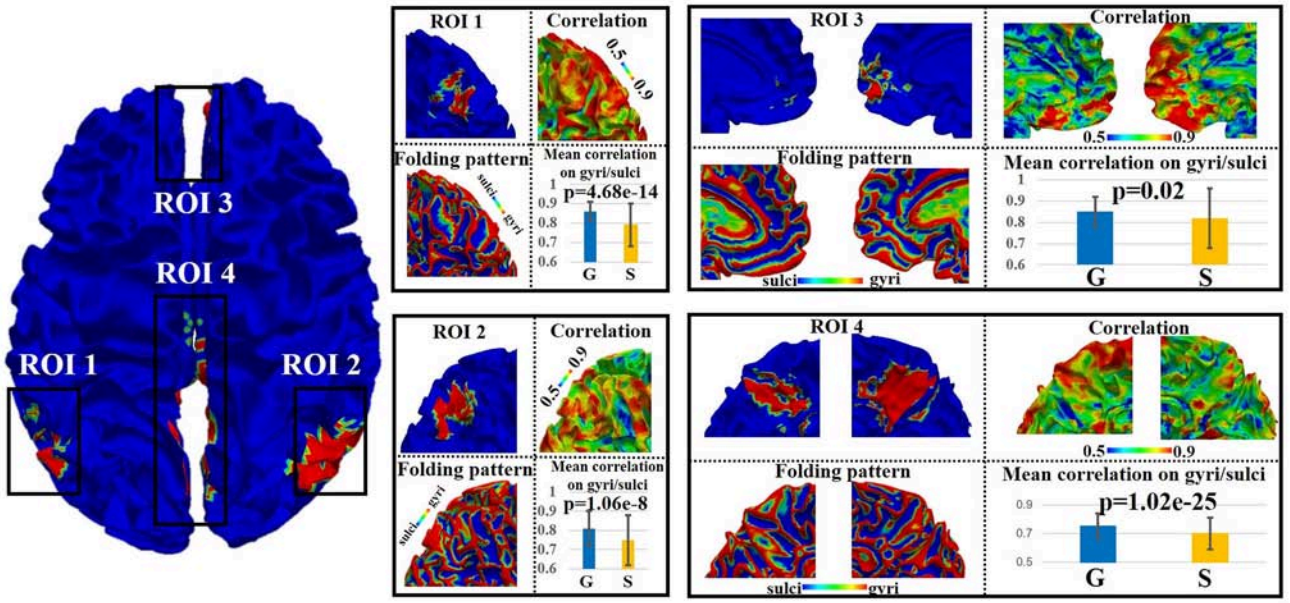

FIGURE 3 | Emotion task fMRI signal representation accuracy difference between gyral and sulcal regions in default mode network (DMN) of one subject. The detailed assessment of each of the four distinct regions (ROI 1–4) within DMN is in zoomed-in view. G, gyri; S, sulci. *P*-value: two-sample one-tailed *t*-test (gyri > sulci, $p = 0.05$, Bonferroni corrected).

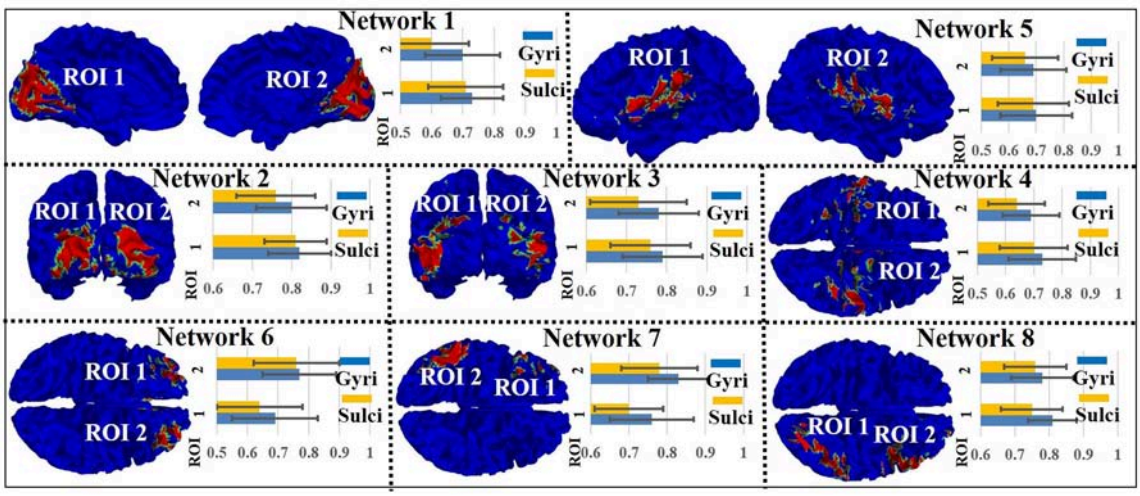

FIGURE 4 | Emotion task fMRI signal representation accuracy difference between gyral and sulcal regions in the other eight intrinsic networks.

We further adopted the DTI data of the same 68 subjects in the HCP data set to examine the correlation between DTI FA value and the signal representation accuracy in gyri/sulci. The experimental results show that across all intrinsic networks and subjects, the FA values are positively correlated with the emotion task signal representation accuracy in gyri/sulci (*r*-value ranges from 0.3 to 0.6 across different intrinsic networks and subjects, *p*-value < 0.01), indicating that gyri has both more structural fiber connections and higher task fMRI signal representation accuracy than sulci.

DISCUSSION

We proposed a novel cortical folding pattern-guided model of intrinsic functional brain networks in emotion processing. This model is evaluated and validated via the proposed computational framework of dictionary learning and sparse representation of emotion task fMRI signals, and the task fMRI signal representation accuracy assessment on gyral and sulcal regions within the intrinsic network. Experimental results based on the HCP emotion task fMRI data demonstrated that the fMRI signal

TABLE 1 | Proportion of number of subjects with significant gyral/sulcal signal representation accuracy difference (two-sample t-test, $p < 0.05$, Bonferroni corrected) in the intrinsic networks in emotion processing.

Network	1	2	3	4	5	6	7	8	9
Proportion	0.67	0.82	0.76	0.78	0.75	0.99	0.82	0.82	0.97

Network 1–3, three visual networks; Network 4, motor; Network 5, auditory; Network 6, executive control; Network 7–8, bilateral frontal/parietal networks; Network 9, default mode.

representation accuracy value in gyri is significantly larger than that on sulci across all nine major cortical intrinsic networks. Our results provide novel insights of functional mechanisms in emotion processing.

We identified nine meaningful intrinsic functional networks which mainly locate on cortical regions based on the emotion task fMRI data. Note that we focus on the intrinsic networks on cortical regions in this work in order to take advantage of the cortical folding pattern information. There are also meaningful and important intrinsic networks in subcortical area (e.g., amygdala, etc.) in emotion processing for future studies. Our finding is consistent with previous studies showing that there are "*domain-general, distributed*" intrinsic functional networks in human brain, and emotion processing arises from the interaction within or among these intrinsic functional brain networks (Lindquist and Barrett, 2012; Barrett and Satpute, 2013).

We found that the emotion task fMRI signal representation accuracy value is significantly larger on gyral regions than sulcal regions within the intrinsic network, indicating that gyri might directly participate more than sulci in functional activities/interactions among distinct regions within the intrinsic networks in emotion processing. As demonstrated in Section Signal representation accuracy assessment on gyri/sulci within one intrinsic network, the learned dictionary matrix represents a set of all basis neural activities of emotion task fMRI signals in the whole-brain. The gyral regions with higher signal representation accuracy based on all basis neural activities are thus of more neural communication among distinct regions within the intrinsic network, i.e., gyral regions serve as global neural communication centers among distinct regions within the intrinsic network in emotion processing. The sulcal regions with lower signal representation accuracy are thus of less neural communication among distinct regions within the intrinsic network and serve as local centers within the single regions of the intrinsic network in emotion processing. This finding, to some extent, is in agreement with the previous studies arguing that gyral regions have stronger interregional functional connectivity than sulcal regions (Deng et al., 2014). It is also in agreement with other studies demonstrating that gyral regions have more spatial overlap patterns of functional networks than sulcal regions in both temporally stationary and dynamic states (Jiang et al., 2015, 2018). Moreover, we found that the DTI FA values are positively correlated with the emotion task signal representation accuracy in gyri/sulci. Given the fact that brain structure predicts its function (Passingham et al., 2002; Zhang et al., 2011), this finding as well as other mounting evidences that gyral regions have more interregional axonal fiber connections than sulcal regions (Nie et al., 2012; Chen et al., 2013; Zhang et al., 2014) provide the structural substrates for the abovementioned functional observations. In conclusion, based on both structural and functional evidences, we argue that the emotion task fMRI signal representation accuracy difference between gyri and sulci within the intrinsic network reasonably supports our proposed cortical folding pattern-guided model; that is, within an intrinsic network in emotion processing, gyri are the global functional connection centers which perform interregional neural communication among distinct regions, and sulci are the local functional units which directly communicate with neighboring gyri and indirectly communicate with other distinct regions via the neighboring gyri.

In this work, we adopted the emotion task fMRI data in the HCP datasets as a testbed. The proposed model showed reproducibility and generality across different subjects under the same emotion task design. In the future, we plan to test our model on other task fMRI data sets with different emotion processing paradigms to examine if there is any common finding among different emotion processing paradigms. It would be also interesting to explore the potential general principle of our proposed cortical folding pattern-guided model using different task data sets.

AUTHOR CONTRIBUTIONS

XJ designed the experiment, analyzed part of the data, generated figures, and wrote the manuscript. LZ analyzed part of the data. HL pre-processed and analyzed part of the data. LG guided HL and LZ on this work. KK interpreted the results and revised the manuscript. TL designed the experiment and critically revised the manuscript.

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REFERENCES

Abolghasemi, V., Ferdowsi, S., and Sanei, S. (2015). Fast and incoherent dictionary learning algorithms with application to fMRI. *Signal Image Video Process.* 9, 147–158. doi: 10.1007/s11760-013-0429-2

Andersen, A. H., Gash, D. M., and Avison, M. J. (1999). Principal component analysis of the dynamic response measured by fMRI: a generalized linear systems framework. *Magn. Reson. Imaging* 17, 795–815. doi: 10.1016/S0730-725X(99)00028-4

Barch, D. M., Burgess, G. C., Harms, M. P., Petersen, S. E., Schlaggar, B. L., Corbetta, M., et al. (2013). Function in the human connectome: task-fMRI and individual differences in behavior. *Neuroimage* 80, 169–189. doi: 10.1016/j.neuroimage.2013.05.033

Barrett, L. F., and Satpute, A. (2013). Large-scale brain networks in affective and social neuroscience: Towards an integrative functional architecture of the brain. *Curr. Opin. Neurobiol.* 23, 361–372. doi: 10.1016/j.conb.2012.12.012

Barron, D. (1950). An experimental analysis of some factors involved in the development of the fissure pattern of the cerebral cortex. *J. Exp. Zool.* 113, 553–581. doi: 10.1002/jez.1401130304

Bressler, S. L., and Menon, V. (2010). Large-scale brain networks in cognition: emerging methods and principles. *Trends Cogn. Sci. (Regul. Ed).* 14, 277–290. doi: 10.1016/j.tics.2010.04.004

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci*. 10, 186–198. doi: 10.1038/nrn2575

Chen, H., Zhang, T., Guo, L., Li, K., Yu, X., Li, L., et al. (2013). Coevolution of gyral folding and structural connection patterns in primate brains. *Cereb. Cortex* 23, 1208–1217. doi: 10.1093/cercor/bhs113

Deng, F., Jiang, X., Zhu, D., Zhang, T., Li, K., Guo, L., et al. (2014). A functional model of cortical gyri and sulci. *Brain Struct. Funct.* 219, 1473–1491. doi: 10.1007/s00429-013-0581-z

Duncan, J. (2010). The multiple-demand (MD) system of the primate brain: mental programs for intelligent behavior. *Trends Cogn. Sci.* 14, 172–179. doi: 10.1016/j.tics.2010.01.004

Fedorenko, E., Duncan, J., and Kanwisher, N. (2013). Broad domain generality in focal regions of frontal and parietal cortex. *Proc. Nat. Acad. Sci. U.S.A.* 110, 16616–16621. doi: 10.1073/pnas.1315235110

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Nat. Acad. Sci. U.S.A.* 102, 9673–9678. doi: 10.1073/pnas.0504136102

Friston, K. J. (2009). Modalities, modes, and models in functional neuroimaging. *Science* 326, 399–403. doi: 10.1126/science.1174521

Greicius, M. D., Supekar, K., Menon, V., and Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb. Cortex* 19, 72–78. doi: 10.1093/cercor/bhn059

Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., and Weinberger, D. R. (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage* 17, 317–323. doi: 10.1006/nimg.2002.1179

Jiang, X. (2016). *Modeling Functional Interactions Between Cortical Gyri and Sulci.* Dissertation, The University of Georgia, Athens.

Jiang, X., Li, X., Lv, J., Zhang, T., Zhang, S., Guo, L., et al. (2015). Sparse representation of HCP grayordinate data reveals novel functional architecture of cerebral cortex. *Hum. Brain Mapp.* 36, 5301–5319. doi: 10.1002/hbm.23013

Jiang, X., Li, X., Lv, J., Zhao, S., Zhang, S., Zhang, W., et al. (2018). Temporal dynamics assessment of spatial overlap pattern of functional brain networks reveals novel functional architecture of cerebral cortex. *IEEE Trans. Biomed. Eng.* 65, 1183–1192. doi: 10.1109/TBME.2016.2598728

Lindquist, K. A., and Barrett, L. F. (2012). A functional architecture of the human brain: emerging insights from the science of emotion. *Trends Cogn. Sci. (Regul. Ed).* 16, 533–540. doi: 10.1016/j.tics.2012.09.005

Lindquist, K. A., Wager, T. D., Kober, H., Bliss-Moreau, E., and Barrett, L. F. (2012). The brain basis of emotion: a meta-analytic review. *Behav. Brain Sci.* 35, 121–143. doi: 10.1017/S0140525X11000446

Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature* 453, 869–878. doi: 10.1038/nature06976

Lv, J., Jiang, X., Li, X., Zhu, D., Chen, H., Zhang, T., et al. (2015a). Sparse representation of whole-brain fMRI signals for identification of functional networks. *Med. Image Anal.* 20, 112–134. doi: 10.1016/j.media.2014.10.011

Lv, J., Jiang, X., Li, X., Zhu, D., Zhang, S., Zhao, S., et al. (2015b). Holistic atlases of functional networks and interactions reveal reciprocal organizational architecture of cortical function. *IEEE Trans. Biomed. Eng.* 62, 1120–1131. doi: 10.1109/TBME.2014.2369495

Mairal, J., Bach, F., Ponce, J., and Sapiro, G. (2010). Online learning for matrix factorization and sparse coding. *J. Mach. Learn. Res.* 11, 19–60. doi: 10.1145/1756006.1756008

McKeown, M. J., Jung, T. P., Makeig, S., Brown, G., Kindermann, S. S., Lee, T. W., et al. (1998). Spatially independent activity patterns in functional MRI data during the Stroop color-naming task. *Proc. Nat. Acad. Sci. U.S.A.* 95, 803–810. doi: 10.1073/pnas.95.3.803

Murphy, F. C., Ewbank, M. P., and Calder, A. J. (2012). Emotion and personality factors influence the neural response to emotional stimuli. *Behav. Brain Sci.* 35, 156–157. doi: 10.1017/S0140525X11001725

Nie, J., Guo, L., Li, K., Wang, Y., Chen, G., Li, L., et al. (2012). Axonal fiber terminations concentrate on gyri. *Cereb. Cortex* 22, 2831–2839. doi: 10.1093/cercor/bhr361

Panksepp, J. (2011). The basic emotional circuits of mammalian brains: do animals have affective lives?. *Neurosci. Biobehav. Rev.* 35, 1791–1804. doi: 10.1016/j.neubiorev.2011.08.003

Passingham, R. E., Stephan, K. E., and Kötter, R. (2002). The anatomical basis of functional localization in the cortex. *Nat. Rev. Neurosci.* 3, 606–616. doi: 10.1038/nrn893

Pessoa, L. (2012). Beyond brain regions: network perspective of cognition–emotion interactions. *Behav. Brain Sci.* 35, 158–159. doi: 10.1017/S0140525X11001567

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., et al. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13040–13045. doi: 10.1073/pnas.0905267106

Van den Heuvel, M. P., Mandl, R. C., Kahn, R. S., and Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Hum. Brain Mapp.* 30, 3127–3141. doi: 10.1002/hbm.20737

Vytal, K., and Hamann, S. (2010). Neuroimaging support for discrete neural correlates of basic emotions: a voxel-based meta-analysis. *J. Cogn. Neurosci.* 22, 2864–2885. doi: 10.1162/jocn.2009.21366

Welker, W. (1990). Why does cerebral cortex fissure and fold? a review of determinants of gyri and sulci. *Cereb. Cortex* 8, 3–136. doi: 10.1007/978-1-4615-3824-0_1

Zhang, T., Chen, H., Guo, L., Li, K., Li, L., Zhang, S., et al. (2014). Characterization of U-shape streamline fibers: methods and applications. *Med. Image Anal.* 18, 795–807. doi: 10.1016/j.media.2014.04.005

Zhang, T., Guo, L., Li, K., Jing, C., Yin, Y., Zhu, D., et al. (2011). Predicting functional cortical ROIs via DTI-derived fiber shape models. *Cereb. Cortex* 22, 854–864. doi: 10.1093/cercor/bhr152

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Encoding Praise and Criticism During Social Evaluation Alters Interactive Responses in the Mentalizing and Affective Learning Networks

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Verbal communication with evaluative characters of different emotional valence has a considerable impact on the extent to which social relations are facilitated or undermined. Here using functional magnetic resonance imaging, we investigated how the brain acts in response to social praise and criticism, leading to differential affective judgments. We engaged thirty men and women in a task associating sex-balanced, neutral faces with praising or criticizing comments targeting others or objects. A whole-brain analysis revealed that criticism as compared to praise enhanced the activation in the medial prefrontal cortex (mPFC), particularly its dorsal portion, whereas the right amygdala displayed an opposite pattern of changes. Comments on others relative to objects increased the reactivity in the left posterior superior temporal sulcus and posterior cingulate cortex (PCC) such that both praise and criticism of others produced stronger activation in these two regions than their object-targeted counterparts. The interaction of valence and target was identified in the mPFC with greater reactivity in the contrasts of criticism vs. praise in the social context and others- vs. object-targeted criticism. Comments also modulated the functional connectivity of prior activated regions with the left temporoparietal junction, bilateral caudate and left PCC/precuneus showing reduced connectivity in response to social criticism but greatly strengthened connectivity for social praise as compared to non-social counterparts. These neural effects subsequently led to altered likeability ratings for the faces. Neither behavioral nor neural effects observed were influenced by the gender of participants. Taken together, our findings suggest a fundamental interactive role of the mentalizing and affective learning networks in differential encoding of individuals associated with praising or criticizing others, leading to learning of valenced traits and subsequent approach or avoidance responses in social interactions.

Keywords: praise, criticism, social inference, the mentalizing network, affective learning

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INTRODUCTION

Language has been proposed to function as a context that shapes human perception (Barrett et al., 2007). When an individual talks, verbal information conveying different affective value may promote inference of character traits, affect how this person is judged (Bliss-Moreau et al., 2008; Baron et al., 2011; Schwarz et al., 2013), and thus alter the balance between approach and avoidance behavior (Todorov et al., 2008). In everyday communication, those who tend to criticize often may display their negative (e.g., anti-social) attributes and lead to others disfavoring and avoiding them socially; in contrast, praising comments may play the opposite role (Blair et al., 2008; Gao et al., 2016; Miedl et al., 2016). To facilitate favorable social impressions and interactions, it is of great importance to examine how people respond neurally and behaviorally to a person's criticizing and praising. In particular, it is important to distinguish the impact of verbal comments made concerning others rather than objects, since previous research suggests a stronger impact of social relative to non-social contexts (Bliss-Moreau et al., 2008; Anderson et al., 2011; Gao et al., 2016).

Learning or making inferences about other people including their intentions, beliefs, and traits implicates a brain network supporting mentalizing (Mitchell, 2009; Van Overwalle, 2009; Ma et al., 2011). A wealth of evidence has shown that this network comprises the medial prefrontal cortex (mPFC), including its dorsal portion, posterior cingulate cortex (PCC) or precuneus, temporoparietal junction (TPJ) and posterior superior temporal sulcus (pSTS) (Frith and Frith, 2006; Mitchell, 2009; Muscatell et al., 2012). Here we associated neutral faces of different individuals with praising and criticizing comments to cue social inferences and thus hypothesized that the mentalizing network would be activated and modulated by encoding of verbal praise and criticism. We also hypothesized that the mentalizing network might respond differentially to person- and object-directed comments since this neural circuitry appears sensitive to how person-related the judgment context is (Mitchell et al., 2002). In the mentalizing network, the mPFC was our primary candidate region given that it has been suggested to be tuned to social valence (Harris et al., 2007). However, we did not have further predictions on whether the responses of this region would be more intense to social criticism or to social praise since previous findings in this regard remained contradictory such that some showed stronger responses to negative (Perry et al., 2012) whilst some to positive social information (Harris et al., 2007).

Verbal comments in our paradigm contain emotional properties, which may give rise to the recruitment of the amygdala. This region is sometimes involved in mentalizing tasks due to its essential role in emotion processing (Mitchell et al., 2005). It has also been implicated in processing positive and negative emotional value assigned to neutral agents via associative learning (Everitt and Robbins, 1992; Phelps and LeDoux, 2005; Schiller et al., 2009). Given the differentiated emotional and motivational values of praise and criticism in our social judgment context we were therefore interested in assessing how amygdala responses would be modulated and how these responses would interact with those in the mentalizing network.

Gender differences in social behavior have long been postulated (Eagly, 1987) but not yet well established in the use of verbal cues for social inferences. However, they have been reported in understanding others by young children (Dunn et al., 1991) and in the activity of neural correlates of mentalizing (Krach et al., 2009). We therefore exploratorily investigated whether men and women would encode praise and criticism differently, leading to divergent affective judgments.

MATERIALS AND METHODS

Participants

Thirty healthy Chinese participants (15 males; age range, 20–25; $M \pm$ SD, 22.73 $\pm$ 1.57 years) were recruited by local advertisement. All participants were right-handed, had no vision problems or language disabilities, and reported no history of neurological or psychiatric disorders. Participants with MRI-contraindications were excluded from participation. The experiment had full ethical approval from the local ethics committee at the University of Electronic Science and Technology of China and all participants gave written informed consent in accordance with the latest revision of the Declaration of Helsinki.

Stimuli

Our paradigm used facial pictures of 36 individuals (balanced for sex) and 144 verbal comments of the same length (in Chinese). In a pre-test incorporating an independent sample (n = 36, 18 males) all faces were rated as emotionally neutral ($M \pm$ SD, 4.985 $\pm$ 0.149) and average in attractiveness (4.378 $\pm$ 0.351) and trustworthiness (4.871 $\pm$ 0.324) using 9-point Likert scales. All comments in the four categories (criticizing-others/praising-others/criticizing-objects/praising-objects) were also assessed by an independent sample (n = 30, 15 males). A two-way repeated-measures analysis of variance (ANOVA) showed that criticizing and praising comments significantly differed in terms of valence ($F_{1,29}$ = 144.11, $P < 0.001$, η_p^2 = 0.832); no other valence differences were found. Moreover, the four categories of comments did not differ in arousal, likelihood and comprehension ratings (all Ps > 0.1; **Supplementary Table S1**).

Procedure

Faces and comments were presented in a pseudorandom order using E-prime 2.0 software. Four comments were sequentially assigned to one face in order to form an evaluative impression. One third of the faces were paired with criticizing comments, one third praising comments, and the rest were coupled with both criticism and praise (first two criticism, next two praise; or, vice versa). All comments paired with one face remained constant in terms of target (either other people or non-social objects). In each trial of the evaluation task (**Figure 1**), a face-comment combination was presented for 5 s. Successive two face-comment combinations were followed by a 10 s face-alone interval and then another two face-comment combinations. After they learned face-comment pairs participants were shown a scale for 10 s indicating they were required to rate the likeability of the

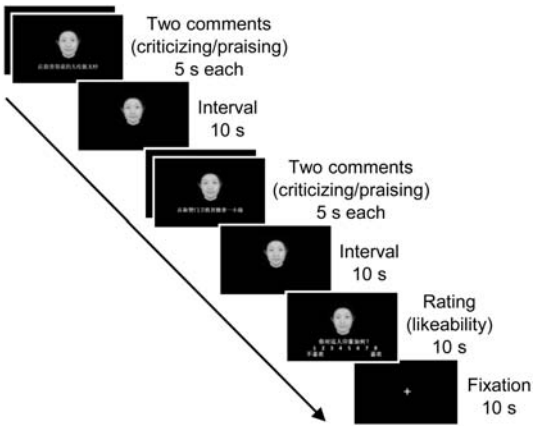

FIGURE 1 | An example of a trial in the affective evaluation task. The individual whose face image is presented here provided written informed consent for the publication of the image.

person involved on an 8-point scale (1 = I don't like the person at all; 8 = I like the person very much). Following a 10 s fixation cross the next trial was initiated.

Acquisition and Analysis of fMRI Data

During the evaluation task fMRI employing a blood oxygenation level-dependent (BOLD) contrast was conducted on a whole-body 3.0 T MRI scanner (Siemens Trio, Erlangen, Germany) with a 12-channel head coil as signal receiver. Echo planar images were acquired with a gradient-echo planar imaging sequence (TR, 2000 ms; TE, 30 ms; slices, 32; thickness, 4 mm; gap, 0 mm; field of view, 240 mm × 240mm; flip angle, 90°; matrix size, 64 × 64; voxel size, 3.8 mm × 3.8 mm × 4 mm). High-resolution whole-brain structural T1-weighted images were also obtained using a magnetization prepared gradient echo sequence (TR, 1,900 ms; TE, 2.26 ms; thickness,1 mm; sagittal field of view, 256 mm × 256 mm; flip angle, 9°; matrix, 256 × 256 × 176; voxel size, 1 mm × 1 mm × 1 mm) in order to control for any anatomic abnormalities and increase normalization accuracy during fMRI data pre-processing.

fMRI data was preprocessed using DPARSF v2.3 (Data Processing Assistant for Resting-State fMRI software[1]) and analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, United Kingdom[2]) implemented in Matlab 7 (MathWorks). The first five volumes of each functional time series were discarded to allow for T1 equilibration. Images were corrected for head movement between scans by an affine registration. A two-pass procedure was used, by which images were initially realigned to the first image of the time series and subsequently realigned to the mean of all images. For spatial normalization the mean T1 image of each subject was normalized to the current Montreal Neurological Institute (MNI) template using Dartel. All functional images were hereby transformed into standard MNI space and resampled at 3 mm × 3 mm × 3 mm voxel size. The normalized images were spatially smoothed using an 8-mm FWHM Gaussian kernel.

On the first level, six conditions "criticizing-others," "praising-others," "criticizing-objects," "praising-objects," "face-alone," and "rating" were modeled by a stick function convolved with the hemodynamic response function (HRF) (Maldjian et al., 2003). Head movement parameters were included in the design matrix to control for movement related artifacts. On the second level, a paired *t*-test was used to examine the effects of verbal comments vs. face-alone baseline. Due to the absence of gender effects in behavioral results, two-sample *t*-tests were primarily conducted in the second level analysis to confirm, at the neural level, the absence of a main effect of gender on all other experimental manipulations (all face-comment combinations vs. face-alone presentations), and its two-way interactions, respectively, with valence (criticizing vs. praising conditions) and with target (others vs. objects conditions), and three-way interaction with the other two factors [(criticizing- vs. praising-others) vs. (criticizing- vs. praising-objects)]. Subsequent analyses focused on main and interactive effects of valence and target using a flexible factorial ANOVA based on four contrasts (criticizing-others/praising-others/criticizing-objects/praising-objects vs. face-alone). To disentangle significant effects individual parameter estimates were extracted from 8 mm radius spheres centered at the peak coordinates of the effects using MarsBar (Brett et al., 2002).

To further examine the interplay between brain regions underpinning the processing of different comments, a functional connectivity (gPPI) analysis (McLaren et al., 2012) was performed using 8 mm sphere seed regions centered at the peak coordinates of the significant activations in the mPFC, amygdala, pSTS and PCC/precuneus in the prior BOLD response analysis. Here, the coordinates representing the mPFC were the peak of the interactive effect since parameter estimates based on the peaks of both valence and interactive effects showed the same pattern regardless of slightly different coordinates. The data were subjected to flexible factorial ANOVAs followed by parameter estimate extraction using 8 mm radius spheres centered at the peak coordinates of the connectivity effects.

A peak-level family-wise error (FWE) corrected significance threshold of $P < 0.05$ was used on the whole-brain level for all the BOLD response and functional connectivity effects, except the valence × target interaction and amygdala activation in the BOLD response (not connectivity) analysis. Based on our observation of a valence-target interaction in likeability ratings and priori hypothesis for involvement of the amygdala in emotion-laden mentalizing (Mitchell et al., 2005), a more liberal threshold (FWE-corrected $P < 0.05$ adapted to a small-volume correction) was used to identify the potential interaction and modulation of amygdala activation. The small-volume correction was conducted based on the structural mask obtained from the Wake Forest University Pickatlas 3.0 (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003, 2004). All coordinates are reported in MNI space.

Statistics

Using IBM SPSS Statistics version 22 behavioral data and parameter estimates extracted from imaging data were analyzed

[1]http://www.restfmri.net/forum/DPARSF

[2]http://www.fil.ion.ucl.ac.uk/spm

by means of three-way repeated-measures ANOVAs with "comment valence" and "comment target" as within-participants variables and "participant gender" as a between-participants variable. Partial eta squared (η_p^2) was calculated as a measure of effect size. The assumption of sphericity was assessed with Mauchly's test, the Greenhouse-Geisser correction for non-sphericity was applied as required and Bonferroni correction was used when pairwise comparisons were applicable. Pearson correlations between the praise-criticism differences in likeability ratings and extraction of functional connectivity were computed and two-tailed *P*-values were reported. $P < 0.05$ was considered significant in all the analyses.

RESULTS

Likeability Ratings

The repeated-measures ANOVA with three factors "valence" (praise, criticism or both), "target" (others vs. objects) and "gender" (**Figure 2**) showed significant main effects of valence ($F_{2,56} = 79.585$, $P < 0.001$, $\eta_p^2 = 0.74$) and target ($F_{1,28} = 22.416$, $P < 0.001$, $\eta_p^2 = 0.445$). Faces paired with criticism were rated the least likeable ($M \pm SE$, 3.817 ± 0.149), those paired with praise the most (5.939 ± 0.152), and those paired with both criticism and praise ranked in the middle (4.958 ± 0.124). The overall likeability (for both praise and criticism) of individuals commenting on non-social objects (5.074 ± 0.114) was higher than that of those targeting other people (4.735 ± 0.105). There was a significant interaction between valence and target ($F_{2,56} = 6.002$, $P = 0.004$, $\eta_p^2 = 0.177$), which was driven by the presence of an others-objects difference in the ratings for faces associated with criticism or praise alone but not for those associated with both criticism and praise. That is, individuals always criticizing or praising others relative to objects were liked less (criticizing: $M_{others} - M_{objects} = -0.711$, $F_{1,28} = 20.317$, $P < 0.001$, $\eta_p^2 = 0.42$; praising: $M_{others} - M_{objects} = -0.289$, $F_{1,28} = 6.482$, $P = 0.017$, $\eta_p^2 = 0.188$). In contrast, the likeability of those who made mixed comments didn't differ between the social and non-social contexts ($M_{others} - M_{objects} = -0.017$, $F_{1,28} = 0.015$, $P = 0.903$, $\eta_p^2 = 0.001$). No significant main or interactive effects of gender were observed.

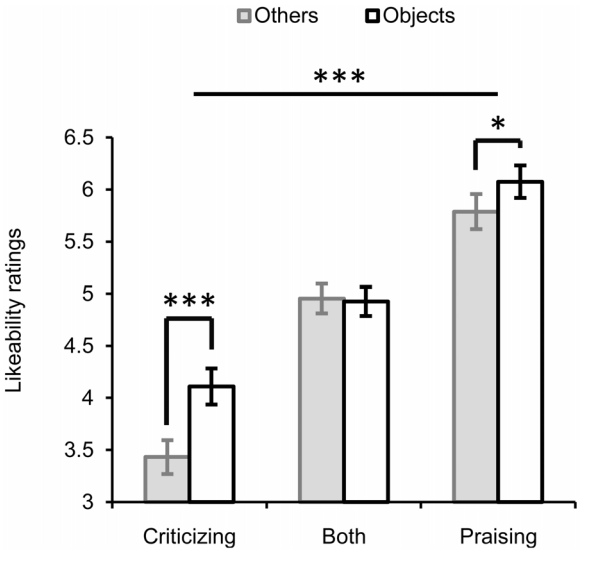

FIGURE 2 | Likeability ratings for faces of individuals making critical, praising, or mixed comments on either others or objects. *$P < 0.05$, *** $P < 0.001$. Bars depict $M \pm SE$.

BOLD Responses

The whole-brain analysis yielded robust comment-induced activation (**Supplementary Figure S1** and **Supplementary Table S2**) but no main or interactive effects of participant gender. We thus focused on the effects of "valence" and "target" in subsequent analyses. The flexible factorial ANOVA showed a main effect of valence (**Figure 3A**; also see **Supplementary Table S3** for more details) in the left mPFC ($F_{1,87} = 25.33$, $P_{FWE} = 0.045$) and right amygdala ($F_{1,87} = 10.04$, $P_{FWE} = 0.037$), a main effect of target (**Figure 3C**) in the left pSTS ($F_{1,87} = 37.69$, $P_{FWE} = 0.001$) and PCC/precuneus ($F_{1,87} = 30.9$, $P_{FWE} = 0.007$), and their interaction in the mPFC ($F_{1,87} = 14.67$, $P_{FWE} = 0.050$; **Figure 3E**). The extraction of parameter estimates (**Figures 3B,D,F**) revealed stronger reactivity in the mPFC to criticism relative to praise ($M_{criticism} - M_{praise} = 0.506$, $F_{1,28} = 12.698$, $P = 0.001$, $\eta_p^2 = 0.312$), but the opposite pattern for the valence main effect in the right amygdala ($M_{criticism} - M_{praise} = -0.125$, $F_{1,28} = 5.444$, $P = 0.027$, $\eta_p^2 = 0.163$). The main effect of target was confirmed by parameter estimates from the left pSTS ($M_{others} - M_{objects} = 0.765$, $F_{1,28} = 34.505$, $P < 0.001$, $\eta_p^2 = 0.543$) and PCC/precuneus ($M_{others} - M_{objects} = 0.714$, $F_{1,28} = 19.095$, $P < 0.001$, $\eta_p^2 = 0.405$) with both regions displaying enhanced activation in response to comments targeting others as compared to objects. A similar target main effect was also found in the mPFC with greater reactivity to others-targeted relative to object-targeted comments ($M_{others} - M_{objects} = 0.7$, $F_{1,28} = 20.599$, $P < 0.001$, $\eta_p^2 = 0.424$), which did not survive the correction threshold in the previous whole-brain analysis. The extraction further disentangled the interaction between valence and target in the mPFC ($F_{1,28} = 12.334$, $P = 0.002$, $\eta_p^2 = 0.306$), with pairwise comparisons showing that social criticism produced stronger activation than social praise ($M_{criticism} - M_{praise} = 0.952$, $F_{1,28} = 20.989$, $P < 0.001$, $\eta_p^2 = 0.428$). However, this effect was not observed in the non-social context ($M_{criticism} - M_{praise} = -0.057$, $F_{1,28} = 0.045$, $P = 0.833$, $\eta_p^2 = 0.002$). On the other hand, criticism targeting others, relative to those targeting objects, increased mPFC activation ($M_{others} - M_{objects} = 1.204$, $F_{1,28} = 28.653$, $P < 0.001$, $\eta_p^2 = 0.506$) while the responses to praise did not differ as a function of target ($M_{others} - M_{objects} = 0.195$, $F_{1,28} = 0.998$, $P = 0.326$, $\eta_p^2 = 0.034$).

Functional Connectivity

The gPPI analysis (**Table 1** and **Figure 4A**) identified a significant main effect of valence and its interaction with target in the functional connectivity of the mPFC with the left TPJ,

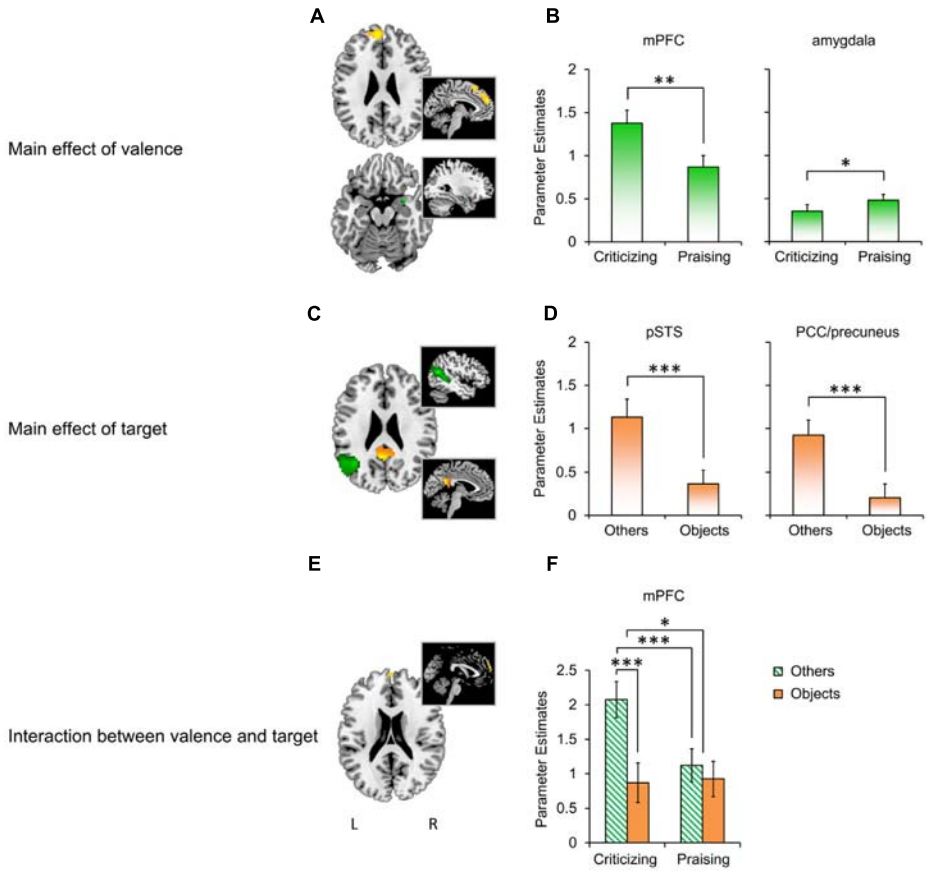

FIGURE 3 | BOLD responses to people- and object-targeted criticism and praise. **(A)** The main effect of valence in the mPFC (yellow; $k = 205$, thresholded at $P < 0.001$ uncorrected for viewing) and right amygdala (green; $k = 7$, $P < 0.05$ uncorrected for viewing). **(B)** Parameter estimates extracted based on the peak of the effect in the mPFC ($x = –6$, $y = 54$, $z = 27$) and amygdala ($x = 30$, $y = 3$, $z = –18$). **(C)** The main effect of target in the left pSTS (green; $k = 187$, $P_{FWE} < 0.05$) and PCC/precuneus (yellow; $k = 21$, $P_{FWE} < 0.05$). **(D)** Extraction based on the peak of the effect in the pSTS ($x = –45$, $y = –60$, $z = 18$) and PCC/precuneus ($x = –3$, $y = –54$, $z = 21$). **(E)** The interaction between valence and target in the mPFC ($k = 14$, $P < 0.001$ uncorrected for viewing). **(F)** Extraction based on the peak of the effect in the mPFC ($x = 0$, $y = 63$, $z = 24$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Bars depict $M \pm$ SE. L, left; R, right; mPFC, medial prefrontal cortex; pSTS, posterior superior temporal sulcus; PCC, posterior cingulate cortex.

TABLE 1 | Significant effects on the connectivity of the mPFC, amygdala, PCC/precuneus and pSTS.

Seeds	Connected regions	Coordinates			Valence		Valence × Target	
		x	*y*	*z*	*F*	P_{FWE}	*F*	P_{FWE}
mPFC	Left TPJ	−42	−45	27	33.12	0.004	34.8	0.002
	Left caudate	−21	15	15	30.12	0.012	31.54	0.007
	Right caudate	15	15	12	26.74	0.036	27.99	0.024
	Left PCC (precuneus)	−15	−39	45	32.65	0.005	34.18	0.003
amygdala	Left TPJ	−39	−45	30	37.25	0.001	38.96	0.001
	Left caudate	−21	15	15	30.9	0.009	32.07	0.006
	Right caudate	15	15	12	32.19	0.006	33.45	0.004
PCC/	Left TPJ	−39	−45	30	38	0.001	39.82	<0.001
Precuneus	Left caudate	−21	15	15	30.87	0.009	32.21	0.006
	Right caudate	15	15	12	28.98	0.017	30.37	0.011
pSTS	Left TPJ	−42	−48	30	35.66	0.002	36.97	0.001
	Left caudate	−21	15	15	31.01	0.009	32.21	0.006
	Right caudate	15	15	12	32.2	0.006	33.5	0.004
	Precuneus	0	−48	48	26.85	0.035	28.48	0.02

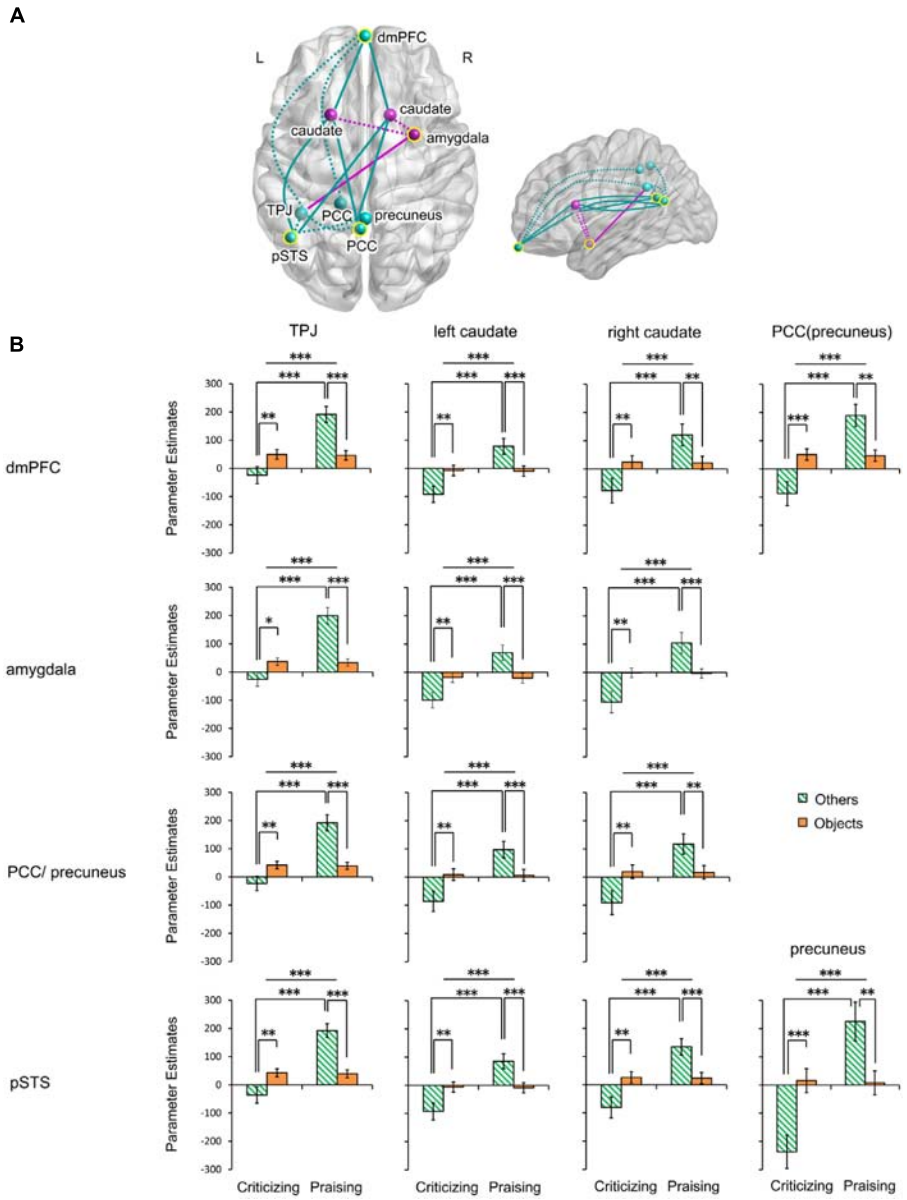

FIGURE 4 | Functional connectivity of the mPFC, right amygdala, PCC/precuneus and left pSTS modulated by people- and object-targeted criticism and praise. **(A)** Axial and sagittal views of altered connections. Yellow circles indicate seed regions. Turquoise, regions and altered connections of seed regions in the mentalizing network; fuchsia, regions and altered connections of seed regions in the affective learning network. Dotted lines, within-network interactions; solid lines, between-network interactions. **(B)** Parameter estimates extracted from the connections. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Bars depict $M \pm$ SE. L, left; R, right; dmPFC, dorsal medial prefrontal cortex; PCC, posterior cingulate cortex; pSTS, posterior superior temporal sulcus; TPJ, temporoparietal junction.

bilateral caudate and left PCC including the precuneus. Very similar effects were found in the connectivity of the right amygdala and the PCC/precuneus with the left TPJ and bilateral caudate. These valence main and valence-target interaction effects were also observed in the pSTS coupling with the left TPJ, bilateral caudate and precuneus. No functional connections of the four seed regions showed a significant main effect of target. Parameter estimates extracted from all the significant effects further disentangled the changes in the connections (**Figure 4B** and **Supplementary Table S4**). All the connections were greatly strengthened in response to praise as compared to criticism regardless of target ($Ps < 0.001$). All the couplings involving the TPJ were strengthened for others-targeted relative to object-targeted comments ($Ps < 0.05$), while no main effect of target was found on the extraction from other connections. Praising comments strengthened the connectivity as compared to critical ones when targeting other people ($Ps < 0.001$), although this was not observed in the non-social context. On the other hand, praising people relative to objects strengthened all the connections ($Ps < 0.01$). In contrast, when criticizing, targeting

others weakened the connections as compared to targeting objects ($Ps < 0.05$).

Correlations Between Neural and Behavioral Results

To confirm the modulation of likeability by positive and negative verbal cues, Pearson correlations were computed between praise-criticism differences in functional connectivity and differences in ratings. Only in the social condition did the cross-valence differences in ratings correlate negatively with the differences in the connectivity of the four seed regions with the left TPJ (mPFC, $r = -0.441$, $P = 0.015$; amygdala, $r = -0.372$, $P = 0.043$; PCC, $r = -0.405$, $P = 0.026$; pSTS: $r = -0.379$, $P = 0.039$; **Figure 5**), indicating that the larger the social praise-criticism differences in the mPFC-, amygdala-, PCC- and pSTS-TPJ connectivity, the smaller the differences in the likeability ratings.

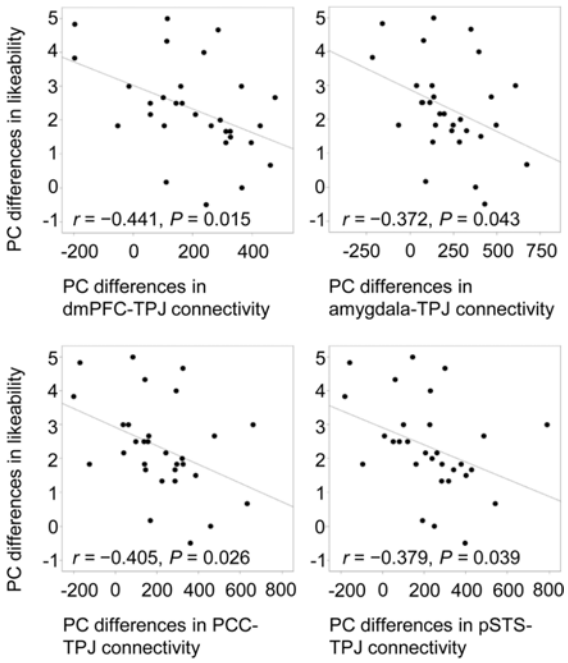

FIGURE 5 | Negative correlations between cross-valence differences in likeability and functional connectivity of the mPFC, right amygdala, PCC and left pSTS with the left TPJ. All cross-valence differences were calculated by subtracting criticism from praise. PC, praise vs. criticism.

DISCUSSION

The present study provides the first evidence for how the brain responds to associating specific individuals with socially and non-socially targeted praise and criticism leading to differential effects on their likeability. Overall, our manipulated verbal cues exert, as hypothesized, markedly distinct effects on neural responses and functional connections involving a network of brain regions supporting mentalizing (mPFC, PCC/precuneus, left pSTS and TPJ) (Frith and Frith, 2006; Mitchell, 2009; Muscatell et al., 2012) and those implicated in affective valuation and approach/avoidance behavior (right amygdala and bilateral caudate).

The activation of the mPFC, largely the dorsal portion, was greatly enhanced in response to individuals associated with criticism, particularly others-targeted criticism, as compared to complimentary counterparts. These valence main and interactive effects suggest a central role of the mPFC in encoding valenced social information during impression formation. Not only has this region been extensively engaged in the processing of social information (Mitchell et al., 2002; Amodio and Frith, 2006), but also this processing is valence-dependent when understanding affective states or making affective judgments of others socially (Harris et al., 2007; Altmann et al., 2012; Perry et al., 2012). On the other hand, using videos showing self-targeted comments, Miedl et al. (2016) failed to identify any praise-criticism differences in the mPFC responses despite strong activation of this region to the valenced cues as compared to neutral ones. Blair et al. (2008) also did not find any valence-specific mPFC responses in healthy individuals to either self- or other-referential praise and criticism, although its reactivity to self-referential criticism differed in individuals with and without generalized social phobia. However, both of these situations primarily involve self-attribution of examples of praising or criticizing characteristics to self or others but not social affective judgments of others as in our study. Thus, the mPFC may differentially encode praising and criticizing comments in the context of evaluating the likeability of others displaying these characteristics.

The activation of other regions of mentalizing, the left pSTS and PCC/precuneus displayed more target-oriented effects. These two regions seem more responsive to different levels of sociality rather than valence-sociality interactions (Schiller et al., 2009; Lahnakoski et al., 2012). While the activation of the mPFC, left pSTS and PCC/precuneus was influenced by different aspects of verbal information manipulation, their functional connectivity changes showed a very similar pattern of modulation by praise and criticism targeting others and objects in terms of the valence main effect and its interaction with target. Indeed, these effects were driven by a decrease of connectivity for social criticism and an increase for social praise. The latter even contributed to the target main effect observed in the connections engaging the left TPJ. The mPFC, TPJ, PCC/precuneus and pSTS have been reported extensively in mentalizing (Frith and Frith, 2006; Mitchell, 2009; Muscatell et al., 2012). Particularly the mPFC, together with the TPJ, are the core regions of the mentalizing network although they may have differentiated functions in trait inferences (Van Overwalle, 2009; Ma et al., 2011). Our findings in connectivity between these regions shed light on the interactions within the mentalizing network in affective evaluation of social others. Here the mPFC, pSTS and PCC/precuneus process valence-dependent social cues via their dynamic interplay with the TPJ. The results may suggest facilitation of making inferences about the individuals involved

when they are praising but rejection in understanding them when they are criticizing, particularly in a more socially oriented context.

The right amygdala, unlike the mPFC, was activated less strongly in response to critical relative to praising comments. Both animal and human studies point to reciprocal relations between these two regions, with evidence for them responding inversely, particularly in fearful conditions (Garcia et al., 1999; Quirk et al., 2003; Shin et al., 2005). The valence-induced changes in the mPFC and amygdala here may suggest different but complementary roles of the two regions in learning about the social characteristics of another person. While the amygdala is generally more associated with the processing of negative valence stimuli it can also respond to positive and neutral valence ones. It has consequently been argued that the amygdala is involved more in processing motivational needs and it is this that determines which different valenced stimuli are responded to (Cunningham et al., 2008; Cunningham and Brosch, 2012). Moreover, like the amygdala, the striatum including caudate also plays a critical role in learning emotional and motivational values of both aversive and rewarding stimuli (Delgado et al., 2004; Fareri and Tottenham, 2016). Amygdalostriatal coupling has been implicated in promotion of reward-based valuation and approach behavior (Popescu et al., 2009; Villablanca, 2010; Fareri and Tottenham, 2016). In the current study, functional connectivity between the right amygdala and the bilateral caudate was differentially influenced by valence, being strengthened during exposure to individuals who praise and weakened by ones who criticize. It is possible that strengthened functional connectivity primarily reflects learning of the positive association between praising individuals and social reward, resulting in increased approach behavior. Conversely, weakened connections may indicate reward devaluation and thus avoidance. Taken together, the differential effects of praise and criticism on functional connectivity between the amygdala and TPJ, and additionally between all of the mentalizing regions and the bilateral caudate, further suggest that the mentalizing and affective processing networks are interacting to facilitate learning of both positive and negative associations based on trait inferences, guiding subsequent social preferences and behavioral adaptation.

Interestingly, the differences in functional connectivity strengths between praise and criticism conditions within the mentalizing regions and between the TPJ and amygdala were negatively correlated with corresponding differences in likeability ratings. That is, the larger the cross-valence differences in the functional connections, the smaller such differences were in likeability ratings. This may suggest that the interplay within regions in the mentalizing network and between them and the amygdala may contribute to how much valenced social cues inform affective judgments. In other words, this provides further evidence that interactions between the metalizing and reward learning networks underlie affective evaluation of social others cued by praise and criticism. And in this interactive process, the TPJ may function as a hub connecting these networks, which consists with evidence from brain-damaged patients indicating a role of the TPJ as a necessary mediator of social inference (Samson et al., 2004) and extends this role into emotional and motivational social inference.

In line with the fMRI findings likeability ratings for individuals associated with praise and criticism showed significant main and interactive effects of both valence and target, suggesting the role of verbal cues in biasing affective judgments. The neutral faces of individuals associated with negative attributes (critical toward others/objects) were robustly evaluated as less likeable compared to those associated with positive traits (praising/prosocial). In particular, individuals who criticized others were liked even less than those critical of objects. It is notable that even though the critical comments used in the present study are not extreme, and highly likely to be made in everyday, real-life social settings, they nevertheless still have a strong negative impact on how individuals producing them are judged.

Although some of our fMRI results displayed enhanced brain responses to praise of others, this unexpectedly did not result in individuals associated with them being liked more than ones associated with object-targeted praise. It is possible that individuals who constantly praise other people may be viewed as trying deliberately to please them, and this could act to devalue its positive impact. Indeed, this is supported by previous findings in a binocular rivalry task that visual dominance did not differ between faces previously paired with positive behaviors involving others relative to objects although this person-object difference was observed in faces associated with negative behaviors (Anderson et al., 2011). Praising evaluations are also not always positive for everyone and can evoke fearful responses. Indeed, a recent study hypothesized that while social criticism hurts everybody, responses to social praise may be heterogeneous due to variant levels of fear for praise (Miedl et al., 2016). Our findings in this regard may indicate that person-directed praise is more complicated than criticism in terms of understanding its intention or making inferences based upon it.

We did not observe gender-specific effects of our manipulated verbal cues at either neural or behavioral levels. Possibly gender differences would occur in heterosexual interactions. Given the complexity of our experiment design, however, further analysis of potential heterosexual interaction effects were not really allowed in respect of statistical power. The sex of both comment makers (faces) and receivers (participants) should both be considered in future work.

CONCLUSION

Using naturalistic verbal comments the present study demonstrates that in both men and women encoding faces paired praising and criticizing cues involves person inferences and associative learning supported by interactions within and between mentalizing and affective processing networks, which facilitate social preferences and social approach or avoidance decisions in future interpersonal interactions. In social communication therefore, caution should be exercised when evaluative comments, particularly negative ones, are made about others.

AUTHOR CONTRIBUTIONS

SG, YG, JL, and YZ collected the data. SG, YG, and SY analyzed the data. SG and SY conceived the study, interpreted the results, and wrote the paper. All authors discussed the results and commented on the 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/fnins.2018.00611/full#supplementary-material

REFERENCES

Altmann, U., Bohrn, I., Lubrich, O., Menninghaus, W., and Jacobs, A. (2012). The power of emotional valence—from cognitive to affective processes in reading. *Front. Hum. Neurosci.* 6:192. doi: 10.3389/fnhum.2012.00192

Amodio, D. M., and Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nat. Rev. Neurosci.* 7, 268–277. doi: 10.1038/nrn1884

Anderson, E., Siegel, E. H., Bliss-Moreau, E., and Barrett, L. F. (2011). The visual impact of gossip. *Science* 332, 1446–1448. doi: 10.1126/science.1201574

Baron, S. G., Gobbini, M. I., Engell, A. D., and Todorov, A. (2011). Amygdala and dorsomedial prefrontal cortex responses to appearance-based and behavior-based person impressions. *Soc. Cogn. Affect. Neurosci.* 6, 572–581. doi: 10.1093/scan/nsq086

Barrett, L. F., Lindquist, K. A., and Gendron, M. (2007). Language as context for the perception of emotion. *Trends Cogn. Sci.* 11, 327–332. doi: 10.1016/j.tics.2007.06.003

Blair, K., Geraci, M., Devido, J., McCaffrey, D., Chen, G., Vythilingam, M., et al. (2008). Neural response to self- and other referential praise and criticism in generalized social phobia. *Arch. Gen. Psychiatry* 65, 1176–1184. doi: 10.1001/archpsyc.65.10.1176

Bliss-Moreau, E., Barrett, L. F., and Wright, C. I. (2008). Individual differences in learning the affective value of others under minimal conditions. *Emotion* 8, 479–493. doi: 10.1037/1528-3542.8.4.479

Brett, M., Anton, J. L., Valabregue, R., and Poline, J. B. (2002). Region of interest analysis using the MarsBar toolbox for SPM 99. *Neuroimage* 16:S497. doi: 10.1016/S1053-8119(02)90013-3

Cunningham, W. A., Bavel, J. J., and Johnsen, I. R. (2008). Affective flexibility: evaluative processing goals shape amygdala activity. *Psychol. Sci.* 19, 152–160. doi: 10.1111/j.1467-9280.2008.02061.x

Cunningham, W. A., and Brosch, T. (2012). Motivational salience: amygdala tuning from traits, needs, values, and goals. *Curr. Dir. Psychol. Sci.* 21, 54–59. doi: 10.1177/0963721411430832

Delgado, M. R., Stenger, V. A., and Fiez, J. A. (2004). Motivation-dependent responses in the human caudate nucleus. *Cereb. Cortex* 14, 1022–1030. doi: 10.1093/cercor/bhh062

Dunn, J., Brown, J., Slomkowski, C., Tesla, C., and Youngblade, L. (1991). Young children's understanding of other people's feelings and beliefs: individual differences and their antecedents. *Child Dev.* 62, 1352–1366. doi: 10.1111/j.1467-8624.1991.tb01610.x

Eagly, A. H. (1987). *Sex Differences in Social Behavior: A Social-Role Interpretation*. Hillsdale, NJ: Erlbaum.

Everitt, B. J., and Robbins, T. W. (1992). "Amygdala-ventral striatal interactions and reward-related processes," in *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*, ed. J. P. Aggleton (New York, NY: Wiley-Liss), 401–429.

Fareri, D. S., and Tottenham, N. (2016). Effects of early life stress on amygdala and striatal development. *Dev. Cogn. Neurosci.* 19, 233–247. doi: 10.1016/j.dcn.2016.04.005

Frith, C. D., and Frith, U. (2006). The neural basis of mentalizing. *Neuron* 50, 531–534. doi: 10.1016/j.neuron.2006.05.001

Gao, S., Becker, B., Luo, L., Geng, Y., Zhao, W., Yin, Y., et al. (2016). Oxytocin, the peptide that bonds the sexes also divides them. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7650–7654. doi: 10.1073/pnas.1602620113

Garcia, R., Vouimba, R.-M., Baudry, M., and Thompson, R. F. (1999). The amygdala modulates prefrontal cortex activity relative to conditioned fear. *Nature* 402, 294–296. doi: 10.1038/46286

Harris, L. T., McClure, S. M., van den Bos, W., Cohen, J. D., and Fiske, S. T. (2007). Regions of the MPFC differentially tuned to social and nonsocial affective evaluation. *Cogn. Affect. Behav. Neurosci.* 7, 309–316. doi: 10.3758/cabn.7.4.309

Krach, S., Blümel, I., Marjoram, D., Lataster, T., Krabbendam, L., Weber, J., et al. (2009). Are women better mindreaders? Sex differences in neural correlates of mentalizing detected with functional MRI. *BMC Neurosci.* 10:9. doi: 10.1186/1471-2202-10-9

Lahnakoski, J., Glerean, E., Salmi, J., Jääskeläinen, I., Sams, M., Hari, R., et al. (2012). Naturalistic fMRI mapping reveals superior temporal sulcus as the hub for the distributed brain network for social perception. *Front. Hum. Neurosci.* 6:233. doi: 10.3389/fnhum.2012.00233

Ma, N., Vandekerckhove, M., Van Overwalle, F., Seurinck, R., and Fias, W. (2011). Spontaneous and intentional trait inferences recruit a common mentalizing network to a different degree: spontaneous inferences activate only its core areas. *Soc. Neurosci.* 6, 123–138. doi: 10.1080/17470919.2010.485884

Maldjian, J. A., Laurienti, P. J., and Burdette, J. H. (2004). Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *Neuroimage* 21, 450–455. doi: 10.1016/j.neuroimage.2003.09.032

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., and Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 19, 1233–1239. doi: 10.1016/S1053-8119(03)00169-1

McLaren, D. G., Ries, M. L., Xu, G., and Johnson, S. C. (2012). A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. *Neuroimage* 61, 1277–1286. doi: 10.1016/j.neuroimage.2012.03.068

Miedl, S. F., Blechert, J., Klackl, J., Wiggert, N., Reichenberger, J., Derntl, B., et al. (2016). Criticism hurts everybody, praise only some: common and specific neural responses to approving and disapproving social-evaluative videos. *Neuroimage* 132, 138–147. doi: 10.1016/j.neuroimage.2016.02.027

Mitchell, J. P. (2009). Inferences about mental states. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 1309–1316. doi: 10.1098/rstb.2008.0318

Mitchell, J. P., Banaji, M. R., and Macrae, C. N. (2005). The link between social cognition and self-referential thought in the medial prefrontal cortex. *J. Cogn. Neurosci.* 17, 1306–1315. doi: 10.1162/0898929055002418

Mitchell, J. P., Heatherton, T. F., and Macrae, C. N. (2002). Distinct neural systems subserve person and object knowledge. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15238–15243. doi: 10.1073/pnas.232395699

Muscatell, K. A., Morelli, S. A., Falk, E. B., Way, B. M., Pfeifer, J. H., Galinsky, A. D., et al. (2012). Social status modulates neural activity in the mentalizing network. *Neuroimage* 60, 1771–1777. doi: 10.1016/j.neuroimage.2012.01.080

Perry, D., Hendler, T., and Shamay-Tsoory, S. G. (2012). Can we share the joy of others? Empathic neural responses to distress vs joy. *Soc. Cogn. Affect. Neurosci.* 7, 909–916. doi: 10.1093/scan/nsr073

Phelps, E. A., and LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 48, 175–187. doi: 10.1016/j.neuron.2005.09.025

Popescu, A. T., Popa, D., and Paré, D. (2009). Coherent gamma oscillations couple the amygdala and striatum during learning. *Nat. Neurosci.* 12, 801–807. doi: 10.1038/nn.2305

Quirk, G. J., Likhtik, E., Pelletier, J. G., and Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J. Neurosci.* 23, 8800–8807. doi: 10.1523/jneurosci.23-25-08800.2003

Samson, D., Apperly, I. A., Chiavarino, C., and Humphreys, G. W. (2004). Left temporoparietal junction is necessary for representing someone else's belief. *Nat. Neurosci.* 7, 499–500. doi: 10.1038/nn1223

Schiller, D., Freeman, J. B., Mitchell, J. P., Uleman, J. S., and Phelps, E. A. (2009). A neural mechanism of first impressions. *Nat. Neurosci.* 12, 508–514. doi: 10.1038/nn.2278

Schwarz, K. A., Wieser, M. J., Gerdes, A. B., Mühlberger, A., and Pauli, P. (2013). Why are you looking like that? How the context influences evaluation and processing of human faces. *Soc. Cogn. Affect. Neurosci.* 8, 438–445. doi: 10.1093/scan/nss013

Shin, L. M., Wright, C. I., Cannistraro, P. A., Wedig, M. M., McMullin, K., Martis, B., et al. (2005). A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch. Gen. Psychiatry* 62, 273–281. doi: 10.1001/archpsyc.62.3.273

Todorov, A., Baron, S. G., and Oosterhof, N. N. (2008). Evaluating face trustworthiness: a model based approach. *Soc. Cogn. Affect. Neurosci.* 3, 119–127. doi: 10.1093/scan/nsn009

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289. doi: 10.1006/nimg.2001.0978

Van Overwalle, F. (2009). Social cognition and the brain: a meta-analysis. *Hum. Brain Mapp.* 30, 829–858. doi: 10.1002/hbm.20547

Villablanca, J. R. (2010). Why do we have a caudate nucleus? *Acta Neurobiol. Exp.* 70, 95–105.

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Selective Depletion of CREB in Serotonergic Neurons Affects the Upregulation of Brain-Derived Neurotrophic Factor Evoked by Chronic Fluoxetine Treatment

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Neurotrophic factors are regarded as crucial regulatory components in neuronal plasticity and are postulated to play an important role in depression pathology. The abundant expression of brain-derived neurotrophic factor (BDNF) in various brain structures seems to be of particular interest in this context, as downregulation of BDNF is postulated to be correlated with depression and its upregulation is often observed after chronic treatment with common antidepressants. It is well-known that BDNF expression is regulated by cyclic AMP response element-binding protein (CREB). In our previous study using mice lacking CREB in serotonergic neurons (Creb1^{TPH2CreERT2} mice), we showed that selective CREB ablation in these particular neuronal populations is crucial for drug-resistant phenotypes in the tail suspension test observed after fluoxetine administration in Creb1^{TPH2CreERT2} mice. The aim of this study was to investigate the molecular changes in the expression of neurotrophins in Creb1^{TPH2CreERT2} mice after chronic fluoxetine treatment, restricted to the brain structures implicated in depression pathology with profound serotonergic innervation including the prefrontal cortex (PFC) and hippocampus. Here, we show for the first time that BDNF upregulation observed after fluoxetine in the hippocampus or PFC might be dependent on the transcription factor CREB residing, not within these particular structures targeted by serotonergic projections, but exclusively in serotonergic neurons. This observation may shed new light on the neurotrophic hypothesis of depression, where the effects of BDNF observed after antidepressants in the hippocampus and other brain structures were rather thought to be regulated by CREB residing within the same brain structures. Overall, these results provide further evidence for the pivotal role of CREB in serotonergic neurons in maintaining mechanisms of antidepressant drug action by regulation of BDNF levels.

Keywords: BDNF, NTF3, NGF, CREB, CREM, fluoxetine, serotonergic system

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INTRODUCTION

The majority of the current antidepressant therapies are based on the enhancement of monoaminergic transmission observed directly after drug administration, yet alleviation of depressive symptoms occur several weeks later. The mechanism of molecular changes underlying this phase of adaptation required for antidepressants to become effective remains elusive and is

intensively researched. Neurotrophic factors are regarded as crucial regulatory components in neuronal plasticity and are postulated to play an important role in depression pathology (Lee and Kim, 2010). The abundant expression of brain-derived neurotrophic factor (BDNF) in various brain structures seems to be of particular relevance in this context.

It is well-known that BDNF contributes to mechanisms of learning and memory by modulation of synaptic transmission and plasticity (Huang et al., 1999). Patients with depression are often associated with memory impairments, in particular regarding positive events, while memory for negative ones is potentiated (Dillon and Pizzagalli, 2018). According to the cognitive model of depression proposed by Beck, patients with major depressive disorder (MDD) are characterized by impaired cognitive processes, such as attention and memory, experiencing biased processing, rumination with dysfunctional attitudes and negative schemes (Disner et al., 2011). Alterations in BDNF levels may straightforwardly influence activity-dependent plasticity in the hippocampus, therefore having direct impact on memory and emotions in patients with MDD (Phillips, 2017).

In animal models, it has been shown that the expression of BDNF is downregulated by exposure to stress (an important factor contributing to depression) (Smith et al., 1995) and can be restored by antidepressant treatment (Warner-Schmidt and Duman, 2006). These studies were also supported by findings revealing that BDNF is upregulated after chronic treatment with common antidepressants (Russo-Neustadt et al., 1999). Overall, these and many similar observations have prompted the basis of the so-called neurotrophic hypothesis of depression, which presumes that the disease may be related to reduced BDNF levels (particularly in the hippocampus), and thus may be possible to treat with antidepressants that promote neurogenesis enhancement (Duman, 2002).

However, the relationship between antidepressants and neurotrophic factors (mainly BDNF) is complex and structure dependent. Indeed, in humans, it was shown that plasma levels of BDNF are decreased in depression (Cunha et al., 2006) but that downregulation of BDNF in the brain structures was restricted to the hippocampus and prefrontal cortex (PFC), while BDNF levels were increased in the nucleus accumbens and amygdala (Autry and Monteggia, 2012). On the other hand, the effects of antidepressants on BDNF exertion in the hippocampus are rather robust and concomitant (Russo-Neustadt and Chen, 2005), yet they may have opposite effects on other brain structures, i.e., the nucleus accumbens (Berton et al., 2006).

Brain-derived neurotrophic factor encoding gene belongs to the group of genes with a cyclic AMP (cAMP) response element in their promoter region, which is directly regulated by the cAMP response element-binding protein (CREB) cellular transcription factor. Therefore, it is not surprising that many studies have also pointed out an important role for CREB in the mechanisms of antidepressant drug action, although the data on this topic are inconclusive (Nibuya et al., 1996; Dowlatshahi et al., 1998; Rossby et al., 1999; Conti et al., 2002; Yamada et al., 2003; Blendy, 2006; Hisaoka et al., 2008).

Again, despite the variety of data showing that antidepressants upregulate CREB in the hippocampus (Gass and Riva, 2007), mice with hippocampal CREB deletion not only maintained their responsiveness to antidepressants in behavioral tests but also exhibited increased hippocampal neurogenesis observed after antidepressant treatment (Gundersen et al., 2013).

In our previous study, we investigated the role of CREB in the mechanism of antidepressant drug action using newly developed and characterized inducible transgenic mice lacking CREB selectively in serotonergic neurons ($Creb1^{TPH2CreERT2}$ mice). To avoid the well-known compensatory effects of another transcription factor, CREM (McPherson and Lawrence, 2007), which is often neglected by other knock-out studies of CREB function, the animals were maintained in a CREM-deficient background ($Creb1^{TPH2CreERT2}Crem-/-$ mice). Although the transgenic mice did not reveal any visible impairments at the basal state, we found that single $Creb1^{TPH2CreERT2}$ mutants resulted in a drug-resistant phenotype in the tail suspension test (TST) after fluoxetine administration and that this effect differed across sex in $Creb1^{TPH2CreERT2}Crem-/-$ mice, in that the anxiolytic effect of fluoxetine was restored in male but not female double mutants (Rafa-Zablocka et al., 2017).

The aim of the current study was to investigate the molecular changes in neurotrophin expression in $Creb1^{TPH2CreERT2}$ and $Creb1^{TPH2CreERT2}Crem-/-$ mice after chronic fluoxetine treatment, with a focus on the brain structures implicated in depression pathology and with profound innervation by serotonergic projections – the PFC and hippocampus.

MATERIALS AND METHODS

Animals

Selective ablation of CREB in serotonergic neurons ($Creb1^{TPH2Cre}$ mice) was achieved by the Cre/loxP recombination system as described previously (Rafa-Zablocka et al., 2017). Briefly, transgenic mice (C57Bl/6N background) hosting Cre recombinase under the tryptophan hydroxylase 2 (TPH2) promoter (TPH2CreERT2 mice) were crossed with animals harboring the floxed Creb1 gene in a CREM-deficient (Crem−/−) background. The mutation was triggered by application of tamoxifen (2 mg/mouse, 1x daily, five consecutive days; Sigma-Aldrich, United States). Therefore, the resulting transgenic line ($Creb1^{TPH2CreERT2}Crem-/-$ mice) possessed a functional deletion of CREB restricted to only serotonergic neurons. Genotyping was performed with a commercially available kit (AccuStart^{TM} II Mouse Genotyping Kit, QuantaBio/VWR) according to the manufacturer's protocol as described previously (Rafa-Zablocka et al., 2017).

The study was carried out on male and female $Creb1^{TPH2CreERT2}Crem-/-$ mice housed with their control (Cre-negative or/and CREM+/+) littermates of the same sex in self-ventilated cages (Allentown, PA, United States) under standard laboratory conditions (12 h light/dark cycle, with food and water *ad libitum*). The study was carried out following the guidance of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental procedures were approved by the Animal Ethical Committee

at the Institute of Pharmacology, Polish Academy of Sciences (Permit No. 1125, issued 11/24/2014).

Drugs and Tissue Collection

Three weeks after tamoxifen administration animals of all genotypes: wild-type, Creb1^{TPH2CreERT2}, Creb1^{TPH2CreERT2}Crem−/−, and Crem−/− were divided into two groups. Control group received saline and treatment group received fluoxetine (10 mg/kg, ip; CarboSynth, United Kingdom) 1x day for 21 consecutive days. Animals were sacrificed by cervical dislocation 24 h after last injection, and tissues were collected (hippocampus and PFC for mRNA/protein assessment, whole brains for immunofluorescence). Experimental scheme is summarized on **Figure 1**.

Immunofluorescence

The procedure was performed as described previously (Kiryk et al., 2013). Briefly, the brains were removed, fixed overnight in 4% paraformaldehyde (PFA), dehydrated, embedded in paraffin, and coronally sectioned (7 μm) on a rotary microtome (Leica, RM45). Select sections from the corresponding region of the PFC and hippocampus (HIP) were incubated overnight at 4°C with primary anti-BDNF (1:100, Abcam, United Kingdom) antibody and visualized with fluorescent anti-rabbit Alexa-488 secondary antibody (Invitrogen, United States). Stained sections were acquired and analyzed under a fluorescence microscope (Nikon Eclipse50i, Japan) equipped with a camera and NIS Elements software.

Real-Time PCR

The procedure was performed as described previously (Diaz-Ruiz et al., 2008). Briefly, after dissection, the brain structures were preserved in RNAlater (Ambion, United States), RNA was extracted using an RNeasy Mini kit (Qiagen, United States), its quality was verified on an Agilent 2100 Bioanalyzer, and the quantity was spectrophotometrically determined. Reverse transcription was performed on 1000 ng of total RNA from each sample using MultiScribe Reverse Transcriptase (Applied Biosystems, United States). TaqMan qPCR was performed on 50 ng reverse-transcribed cDNA in a final volume of 20 μl using the Quant Studio Platform (Life Technologies, United States) following the manufacturer's protocol. The following predesigned TaqMan gene expression assays were used: Creb1 (Mm00501607_m1), Ntrk2 (Mm00435422_m1), Bdnf (Mm04230607_s1), Ntrk3 (Mm00456222_m1), Ntf3 (Mm00435413_s1), and Ngf (Mm00443039_m1). Hypoxanthine-phosphoribosyltransferase (Hprt1, Mm03024075_m1) was chosen as the housekeeping gene. The results were calculated as the fold change in expression compared to that in the control mice (wild-type littermates) using the $\Delta\Delta$Ct method.

Western Blot

For protein isolation, each sample was homogenized in RIPA buffer (Sigma, United States) containing a protease inhibitor cocktail (Sigma, United States) and phosphatase inhibitors (Thermo Fisher, United States), incubated for 2 h at 4°C and centrifuged at 18000 × *g*. The protein concentration was assessed by a BCA protein assay kit (Sigma, United States). Samples containing 15 μg protein each were run on a polyacrylamide gel (BioRad, United States) and transferred to nitrocellulose membranes. The membranes were blocked in 5% (w/v) non-fat dry milk in TBST, and the blots were incubated overnight with primary antibodies (dilution: 1:1000) against the following proteins: BDNF (ab108319, Abcam, United Kingdom), CREB (ab32515, Abcam, United Kingdom), phospho-CREB (06-519, Millipore, United States), NGF (sc-365944, Santa Cruz, United States) and NTF3 (PA5-14861, Thermo Fisher, United States). GAPDH (1:5000, MAB374, Millipore, United States) was used as the loading control. After incubation with the proper secondary antibody linked to horseradish peroxidase (dilution 1:5000, anti-mouse PI-2000 and anti-rabbit PI-1000, VECTOR Laboratories, United States), the signal was developed and visualized by WesternBright Quantum (Advansta, United States) HRP substrate with the help of the PXi 4 (Syngene, United Kingdom) imaging system. Densitometric analysis was performed using Multi-Gauge v.3.0 (Fujifilm, Japan) software.

Statistical Analysis

Data were analyzed using GraphPad Prism 7.0 software (GraphPad, United States). All comparisons were performed using two-way analysis of variance (ANOVA) followed by Fisher's least significant difference *post hoc* test. Changes with *p*-values lower than 0.05 were considered significant.

RESULTS

Selective Ablation of CREB in Serotonergic Neurons Does Not Influence the Expression Levels of mRNA Encoding for CREB and BDNF After Fluoxetine Treatment

To determine whether the mutation impacted the effects of fluoxetine administration on neurotrophic factors, we screened the mRNA expression of Creb1, Bdnf, Ntf3, and Ngf as well as of the receptors of Bdnf and Ntf3, Ntrk2 (TrkB), and Ntrk3 (TrkC), respectively. The experiments were performed on untreated and treated wild-type (w/t) C57BL/6N mice, Creb1^{TPH2CreERT2} mice (single mutants lacking CREB only) and Creb1^{TPH2CreERT2}Crem−/− mice (double mutants lacking CREB and CREM). We narrowed the analysis to the two structures widely implicated in depression pathophysiology and the effects of antidepressant drugs – the hippocampus and PFC (Dusi et al., 2015). Taking into account the growing awareness and importance of gender differences in neuropsychiatric disorders and reactiveness to antidepressant treatment (Kreiner et al., 2013), particularly those targeting the serotonergic system in both clinical and experimental studies (Martenyi et al., 2001; Jones and Lucki, 2005; Keers and Aitchison, 2010; Chmielarz et al., 2013), male and female cohorts were used in all experiments.

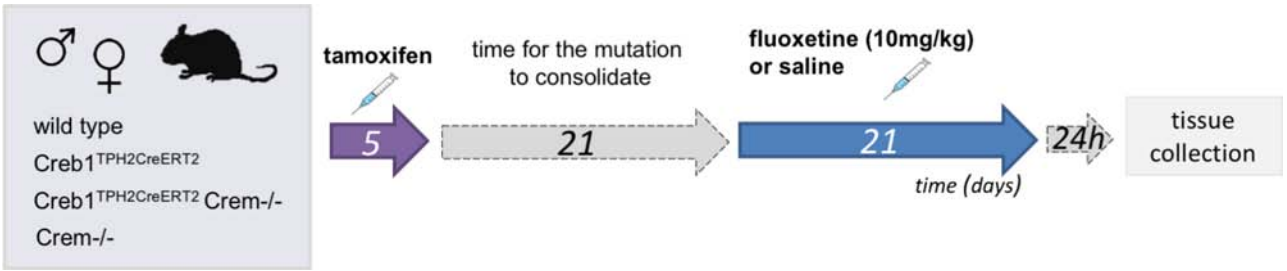

FIGURE 1 | Flowchart summarizing the experimental paradigm.

In the hippocampus, we did not observe any changes in the expression of the transcripts investigated, with regard to the genotype, treatment, or gender (**Figures 2A–E**). In the PFC, the outcome was similar, aside from enhanced expression of Ntf3 observed in all male mice (two-way ANOVA: genotype $F_{(3,38)} = 5{,}98$, $p < 0.01$) but not female mice (however, the results did not reach statistical significance in w/t and Creb1^{TPH2CreERT2} mice exposed to fluoxetine treatment) (**Figures 3A–E**). Additionally, in the PFC, we noticed enhanced expression of Ngf in only Creb1^{TPH2CreERT2} female single mutants (two-way ANOVA: genotype $F_{(3,40)} = 3{,}93$, $p < 0.05$) in both fluoxetine-treated (*post hoc*, $p < 0.05$) and non-treated (*post hoc*, $p < 0.05$) animals (**Figures 3B,C**).

Selective Ablation of CREB in Serotonergic Neurons Counteracts the Upregulation of BDNF Evoked by Chronic Fluoxetine Administration

Since the analysis of the set of basic genes associated with the neurotrophic theory of depression did not provide any conclusive feedback, the next step was to determine the expression of three neurotrophic factors, BDNF, NGF, and NTF3, on the protein level assessed by Western blot. Indeed, we were able to confirm profoundly enhanced expression of BDNF after 21 days of fluoxetine treatment in the hippocampus of both w/t male (two-way ANOVA, fluoxetine: $F_{(1,36)} = 4{,}39$, $p < 0.05$; *post hoc* $p < 0.05$) and female (two-way ANOVA, fluoxetine: $F_{(1,34)} = 4{,}50$, $p < 0.05$; *post hoc* $p < 0.05$) mice (**Figures 4A,B**), an effect that has been previously reported after chronic antidepressant treatment in this brain structure (Nibuya et al., 1995; Vaidya et al., 1999), including selective serotonin reuptake inhibitors (SSRIs) (Baj et al., 2012). Similar effects were observed in the PFC but in only w/t female mice (two-way ANOVA, fluoxetine: $F_{(1,34)} = 4{,}48$, $p < 0.05$; *post hoc* $p < 0.05$) (**Figures 4E,F**). This observation was counteracted by the mutation introduced into Creb1^{TPH2CreERT2} mice lacking CREB in the serotonergic system (**Figures 4A,B,E,F**). Surprisingly, we noticed a restoration of this effect in double mutants (Creb1^{TPH2CreERT2}Crem−/− mice). We were able to visualize these findings by immunofluorescent staining with anti-BDNF antibody performed on coronal slices of the PFC in female mice (**Supplementary Figure S1**). Additionally, in male single mutants (Creb1^{TPH2CreERT2} mice), we found enhanced expression of NGF (two-way ANOVA, genotype: $F_{(2,36)} = 3{,}47$, $p < 0.05$; *post hoc* $p < 0.05$ vs. w/t + FLX, $p < 0.01$ vs. Creb1^{TPH2CreERT2}Crem−/− + FLX) and NTF3 (two- way ANOVA, genotype: $F_{(2,36)} = 4{,}92$, $p < 0.05$; *post hoc* $p < 0.05$ vs. w/t + FLX, $p < 0.01$ vs. Creb1^{TPH2CreERT2}Crem−/− + FLX) evoked by chronic fluoxetine administration in PFC (**Figures 4E,G,H**), but not in the hippocampus (**Figures 4A,C,D**).

Selective Ablation of CREB in Serotonergic Neurons Does Not Influence the Level and Activity of CREB in the Hippocampus and Prefrontal Cortex

Since it is well-known that BDNF expression is regulated by CREB (Finkbeiner et al., 1997), we checked whether the abolition of fluoxetine-induced BDNF overexpression observed in Creb1^{TPH2CreERT2} mice might have also been reflected in the level of CREB functionality. Therefore, we examined the expression of CREB and CREB phosphorylation on Ser-133 by Western blot. However, thorough analysis of all experimental groups did not reveal any changes in CREB and phospho-CREB (pCREB) with regards to genotype, gender and treatment (**Figures 5A–F**).

The Effects of CREM Deletion on the Expression of Neurotrophic Factors in the Hippocampus and Prefrontal Cortex After Fluoxetine Treatment

Finally, to dissect whether the changes in the CREB-dependent regulation of BDNF observed in Creb1^{TPH2CreERT2} mice, but not – at least to the extent reaching statistical significance – in Creb1^{TPH2CreERT2}Crem−/− double mutants, were influenced by CREM deficiency, we performed a separate study of BDNF, NGF, and NTF3 protein expression by Western blot that was restricted to w/t and CREB−/− mice. These experiments revealed that indeed the CREM−/− background seems to be responsible for enhanced expression of these proteins observed in Creb1^{TPH2CreERT2}Crem−/− after fluoxetine treatment. Namely, in both the hippocampus and PFC of female and male mice, the levels of BDNF were upregulated when compared to those in w/t animals (**Figures 6B–D**); however, only the levels in the PFC of female CREM−/− mice were statistically significant

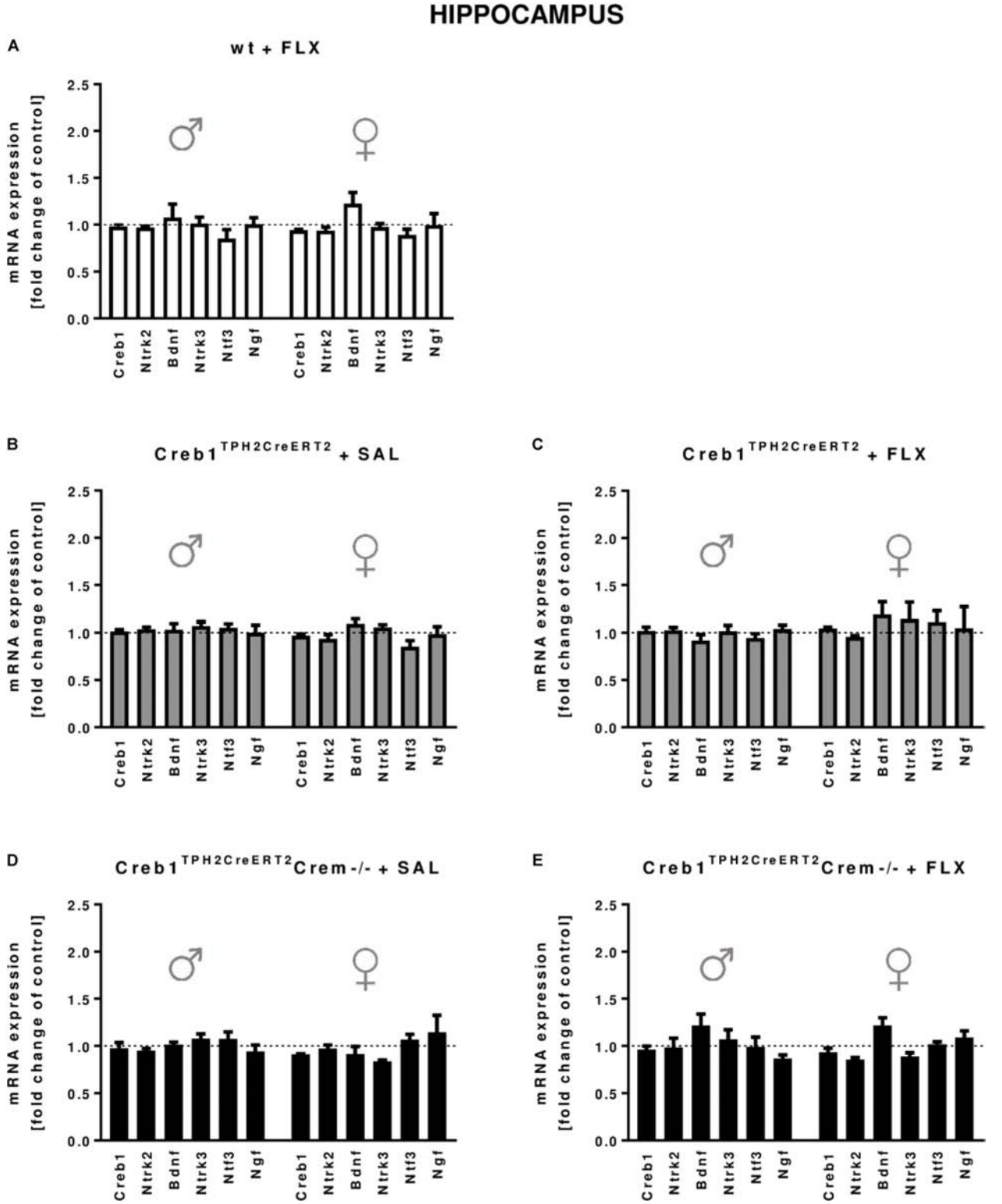

FIGURE 2 | mRNA expression of genes encoding for Creb1, neurotrophins (Bdnf, Ngf, and Ntf3) and their receptors (Ntrk2, Ntrk3) in the hippocampus of saline- and fluoxetine-treated wild-type, Creb1^{TPH2CreERT2}, and Creb1^{TPH2CreERT2}Crem–/– mice. Neither genotype nor fluoxetine influenced the mRNA expression of Creb1, Bdnf, Ngf, Ntf3, Ntrk2, and Ntrk3. Bars represent fold changes in the mRNA expression of Creb1, Bdnf, Ngf, Ntf3, Ntrk2, and Ntrk3 vs. that in wild-type non-treated animals (dot line) in the hippocampus of **(A)** wild-type fluoxetine-treated, **(B,C)** Creb1^{TPH2CreERT2} saline- and fluoxetine-treated, and **(D,E)** Creb1^{TPH2CreERT}Crem–/– saline- and fluoxetine-treated mice. All graphs show data from males (left) and females (right). W/t, wild-type; FLX, fluoxetine. Data are presented as the mean ± SEM.

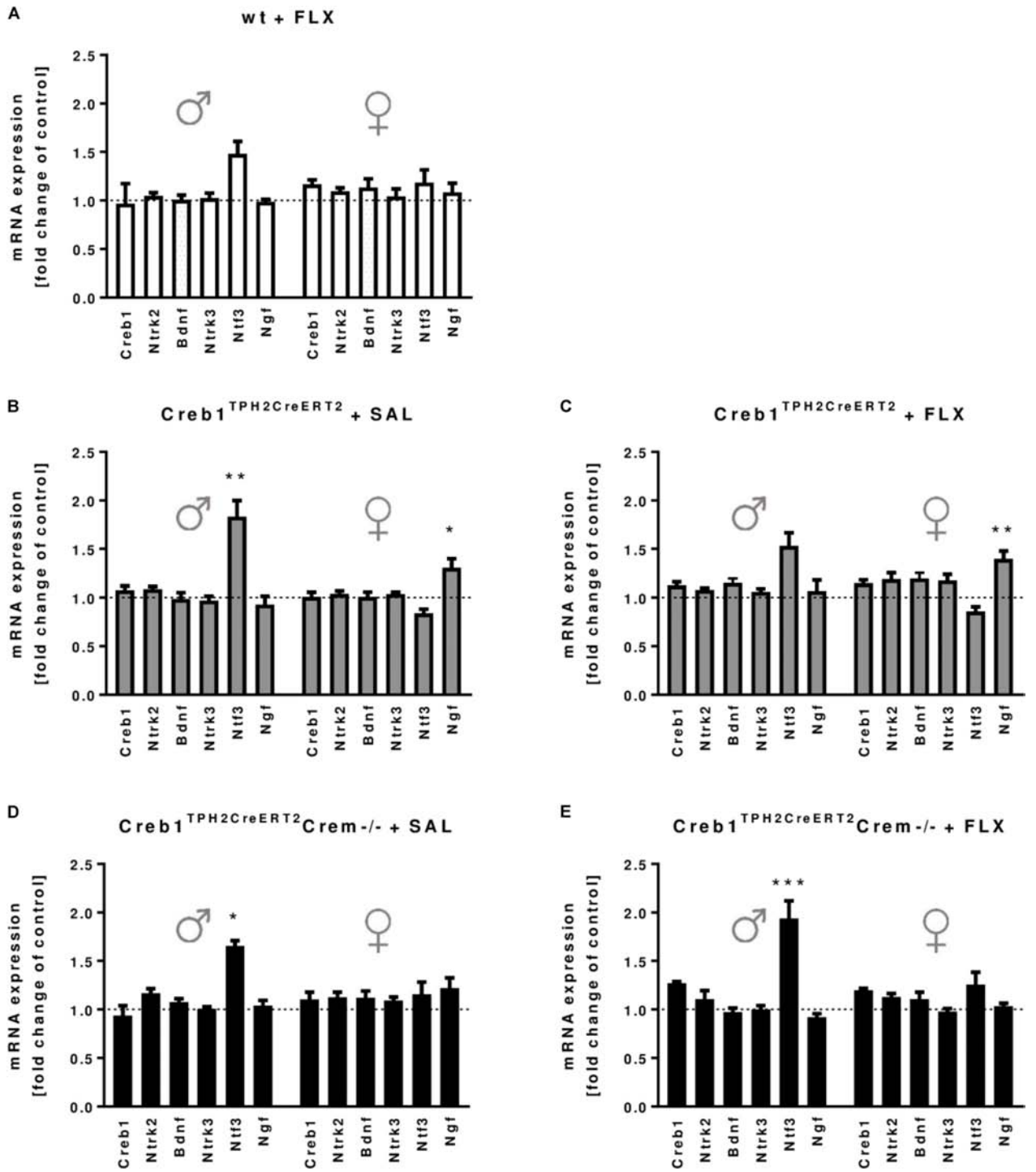

FIGURE 3 | mRNA expression of genes encoding for Creb1, neurotrophins (Bdnf, Ngf, and Ntf3) and their receptors (Ntrk2, Ntrk3) in the prefrontal cortexes (PFCs) of saline- and fluoxetine-treated wild-type, Creb1^{TPH2CreERT2}, and Creb1^{TPH2CreERT2}Crem–/– mice. In the PFC of male Creb1^{TPH2CreERT2} (saline-treated mice) and Creb1^{TPH2CreERT2}Crem–/– mutants (saline- and fluoxetine-treated mice), increased NTF3 mRNA levels were observed. However, Creb1^{TPH2CreERT2} females after both saline and fluoxetine treatment show increased Ngf mRNA expression levels. Bars represent fold changes in the mRNA expression of Creb1, Bdnf, Ngf, Ntf3, Ntrk2, and Ntrk3 vs. that in wild-type non-treated animals (dot line) in the PFC of **(A)** wild-type fluoxetine-treated, **(B,C)** Creb1^{TPH2CreERT2} saline- and fluoxetine-treated, and **(D,E)** Creb1^{TPH2CreERT}Crem–/– saline- and fluoxetine-treated mice. All graphs represent data from males (left) and females (right). Data are presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. saline-treated wild-type mice of the same sex. W/t, wild-type; FLX, fluoxetine.

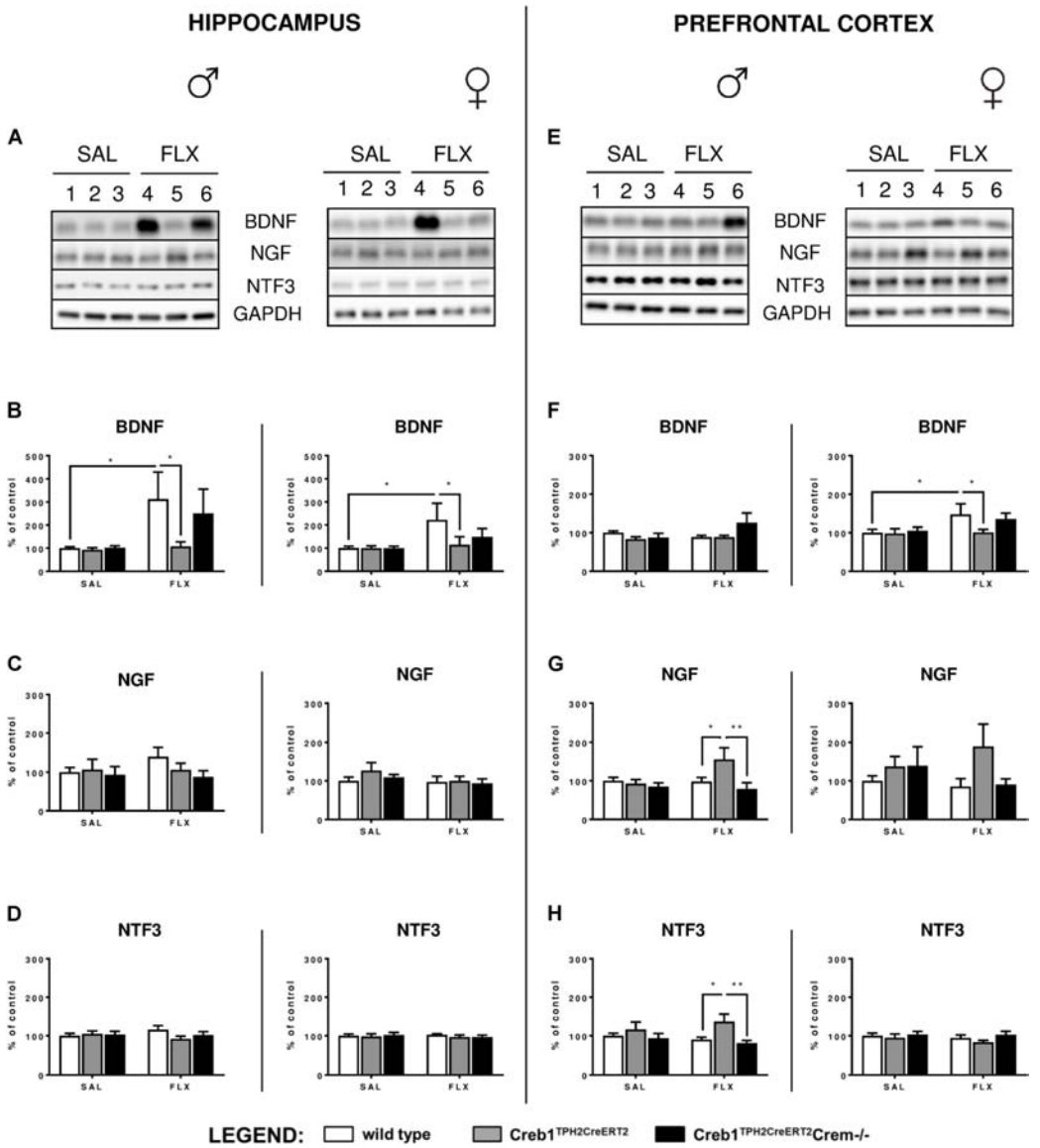

FIGURE 4 | Protein expression of BDNF, NGF, and NTF3 after chronic fluoxetine administration in the hippocampus and PFC of wild-type, Creb1^{TPH2CreERT2} and Creb1^{TPH2CreERT2}Crem–/– mice. Chronic fluoxetine treatment (10 mg/kg, ip, 1x daily, 21 days) induced BDNF upregulation in the hippocampus of male and female mice **(B)** and in the PFC of female **(F)** wild-type animals; the effect was abolished in Creb1^{TPH2CreERT2} mice **(B,F)**. Fluoxetine increased NGF **(G)** and NTF3 **(H)** levels in the PFC of male Creb1^{TPH2CreERT2} mutants. Western blot analyses of the effects of fluoxetine administration on the protein levels of **(B,F)** BDNF, **(C,G)** NGF, and **(D,H)** NTF3 in the hippocampus (left panel) and the PFC (right panel) of wild-type, Creb1^{TPH2CreERT2}, and Creb1^{TPH2CreERT2}Crem–/– mice. **(A,E)** Representative blots of BDNF, NGF, NTF3 and GAPDH in saline (wells 1–3)- and fluoxetine (wells 4–6)-treated wild-type (wells 1, 4), Creb1^{TPH2CreERT2} (wells 2, 5) and Creb1^{TPH2CreERT2}Crem–/– (wells 3, 6) mice. Data are presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$ vs. saline-injected wild-type mice of the same sex. W/t, wild-type; SAL, saline; FLX, fluoxetine.

(two-way ANOVA, fluoxetine $F_{(1,21)} = 8{,}44$, $p < 0.01$; *post hoc* $p < 0.01$ vs. w/t control) (**Figure 6D**). Apart from this effect, only the level of NTF3 in male CREM−/− mice was significantly enhanced related to that in w/t mice (two-way ANOVA, genotype × fluoxetine: $F_{(1,23)} = 18{,}90$, $p < 0.001$, *post hoc* $p < 0.001$ vs. w/t control) (**Figure 6D**).

It is worth noting that in all non-treated CREM−/− animals, the levels of all investigated neurotrophins were the same as in w/t mice (**Figures 6A–C**), which was concomitant to the data revealing the lack of any changes in BDNF, NGF, and NTF3 w/o fluoxetine treatment in Creb1^{TPH2CreERT2} and Creb1^{TPH2CreERT2}Crem−/− mice (**Figures 5A–F**).

DISCUSSION

The results of this study are a step forward in our research in dissecting the role of CREB in depression and antidepressant

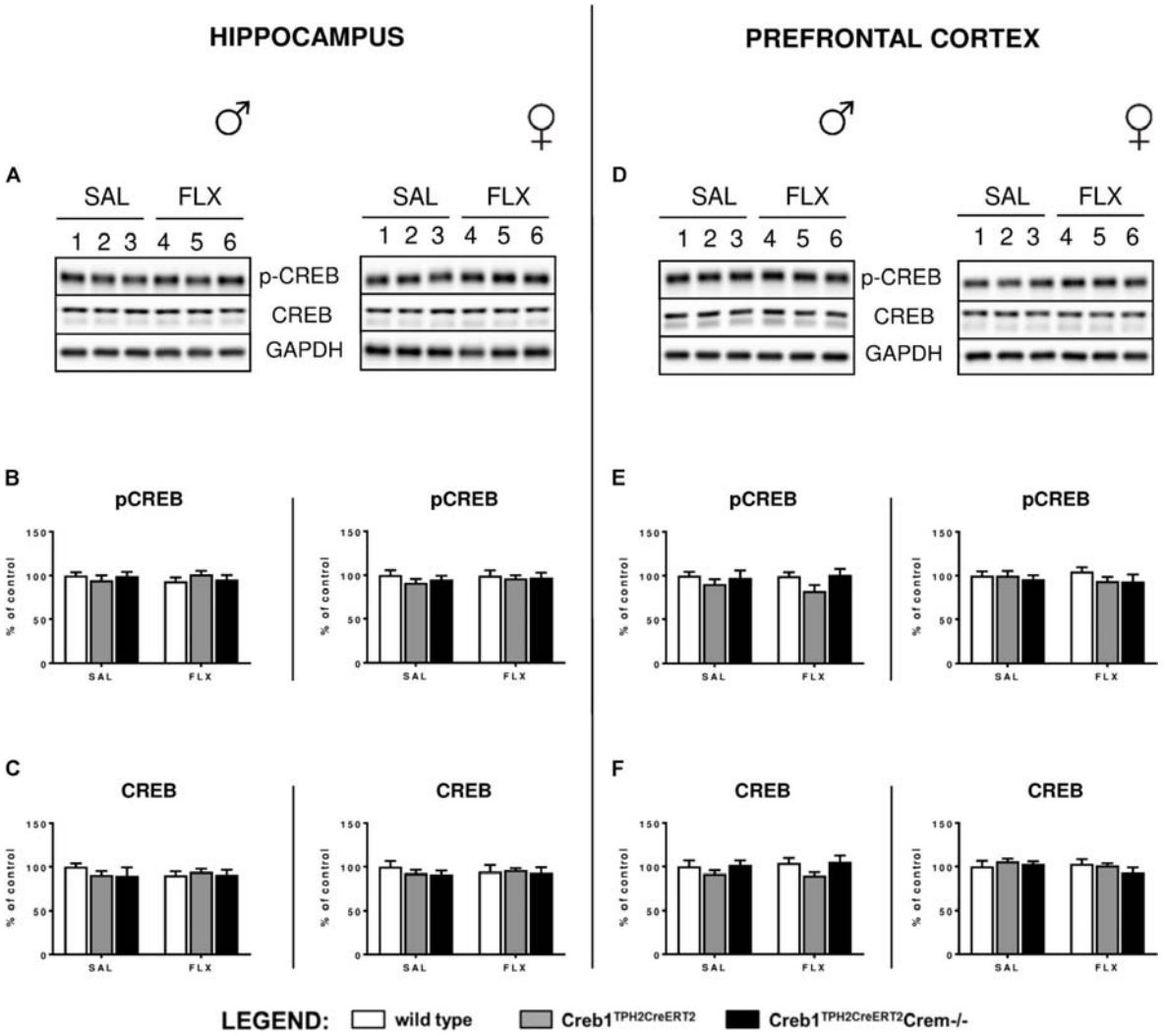

FIGURE 5 | Lack of effect of chronic fluoxetine administration on CREB protein expression and phosphorylation levels in wild-type, Creb1^{TPH2Cre} and Creb1^{TPH2CreERT2} mice. No changes in the protein levels of pCREB and CREB were observed with regard to treatment, genotype, and gender. Western blot analyses of the effects of fluoxetine administration on the protein levels of **(B,E)** phosphorylation at Ser133 CREB (p-CREB) and **(C,F)** total CREB in the hippocampus (left panel) and PFC (right panel) of wild-type, Creb1^{TPH2CreERT2}, and Creb1^{TPH2CreERT2}Crem–/– mice. **(A,D)** Representative blots of p-CREB, CREB, and GAPDH of saline (wells 1–3)- and fluoxetine (wells 4–6)-treated wild-type (wells 1, 4), Creb1^{TPH2CreERT2} (wells 2, 5) and Creb1^{TPH2CreERT2}Crem–/– (wells 3, 6) mice. Data are presented as the mean ± SEM. W/t, wild-type; SAL, saline; FLX, fluoxetine.

treatment. In our study we utilized a novel transgenic mouse model characterized by selective ablation of CREB restricted to chosen neuronal populations, which are important targets for common antidepressant therapies (Rafa-Zablocka et al., 2017).

Here, we show for the first time that BDNF upregulation in the hippocampus or PFC observed after antidepressants targeting the serotonergic system (i.e., fluoxetine) might be dependent on the transcription factor CREB residing not within these particular structures that are targeted by serotonergic projections, but exclusively in serotonergic neurons. This observation may bring a new perspective to the neurotrophic hypothesis of depression, in which the effects of BDNF observed after antidepressants in the hippocampus and other brain structures are thought to be regulated by CREB residing within the same brain structures (Nibuya et al., 1996). In particular, it has been shown that in the hippocampus CREB level is increased after chronic treatment with fluoxetine, which directly augments BDNF expression (Nibuya et al., 1996; Tiraboschi et al., 2004). Since CREB and BDNF both play an important role in neuronal plasticity, both molecules are often regarded as key factors in the pathophysiology of depression and as targets of antidepressant drugs (Nair and Vaidya, 2006). Furthermore, BDNF and serotonergic system are in tight interconnection in the brain regulating the neuronal plasticity, response to stress stimuli and antidepressants efficacy (Mattson et al., 2004; Martinowich and Lu, 2008; Homberg et al., 2014). However due to the heterogeneity of serotonergic transmission the direct interdependence is not so easy to define (Homberg et al., 2014).

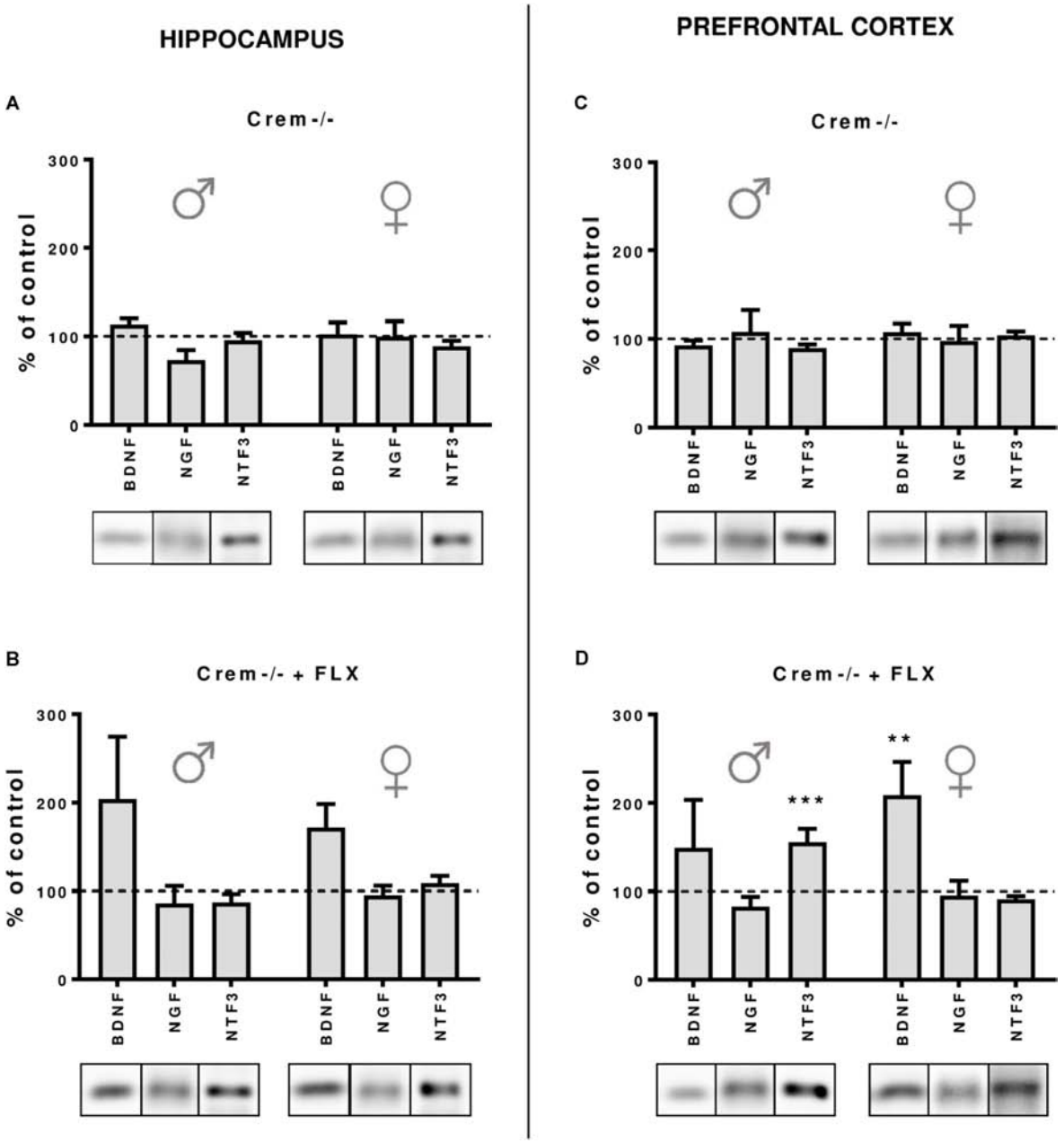

FIGURE 6 | Protein expression of BDNF, NGF and NTF3 after chronic fluoxetine administration in the hippocampus and PFC of wild-type and Crem–/– mice. CREM deletion did not cause any changes in neurotrophin levels in the hippocampus **(A)** or in the PFC **(C)** of male and female mutants. Fluoxetine administration increased BDNF levels in the PFC of females ($p < 0.01$) **(D)**, and a similar tendency was observed in the hippocampus of both Crem–/– males and females. Additionally, fluoxetine increased NTF3 levels in the PFC of Crem–/– males. Western blot analyses of the effects of fluoxetine administration on the protein levels of BDNF, NGF and NTF3 in the hippocampus of **(A)** CREM–/– saline-treated and **(B)** Crem–/– fluoxetine-treated mice and in the PFC of **(C)** Crem–/– saline-treated and **(D)** Crem–/– fluoxetine-treated mice vs. those in wild-type saline animals (dashed line). Representative blots of each group are shown below the corresponding bar. W/t, wild-type; SAL, saline; FLX, fluoxetine. Data are presented as the mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. saline-treated wild-type animals of the same sex.

It has been assured, that chronic administration of BDNF (both *in vitro* and *in vivo*) increases the serotonergic transmission (Celada et al., 1996; Siuciak et al., 1996; Deltheil et al., 2008). Concomitantly, reduced BDNF level observed in BDNF heterozygous KO mice results in the alteration of 5-HT receptor expression associated with decreased serotonin transmission (Lyons et al., 1999). In parallel, there are also existing evidences for regulating BDNF levels by evoking changes in serotonin transmission. Namely, serotonin application *in vitro* (Galter and Unsicker, 2000), and pharmacological stimulation of 5-HT2A receptor *in vivo* by 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) influenced BDNF expression (Vaidya et al., 1997). These

observations were supported by studies performed on knockout animals, showing reduced expression of BDNF in SERT KO rats (Molteni et al., 2010). However, it has to be mentioned, that this regulation is not completely understood and the data are inconsistent – in particular, SERT KO mice did not confirm this latter observation, and Tph2 KO mice were surprisingly characterized by elevated levels of hippocampal BDNF (Kronenberg et al., 2016). The gender differences in studying this issue, in particular in transgenic animals, have also been taken into consideration (Chan and Ye, 2017).

In our experimental model, we did not see any changes in CREB mRNA expression, protein expression or phosphorylation level in the hippocampus nor PFC (**Figures 2**, **3**, **5**). Nevertheless, we cannot exclude the role of CREB in these particular brain structures in raising the BDNF level, as it has been shown that Ser133 phosphorylation is not required for CREB-mediated transcription (Briand et al., 2015). However, it seems to be clear that the changes in BDNF observed after chronic fluoxetine treatment depend on CREB in serotonergic neurons, as the mice selectively lacking CREB in these particular neurons ($Creb1^{TPH2CreERT2}$) lost the ability to show enhanced expression of BDNF after drug administration.

The lack of change observed in CREB in w/t mice after fluoxetine administration does not necessarily constitute a denial of existing hypotheses in this topic. First, it is not a general rule that the correlation between mRNA and protein abundance is straightforward (Maier et al., 2009), and existing data on the effect of long-term antidepressant administration on CREB mRNA and protein expression are inconsistent. For example, both fluoxetine (SSRIs) and desipramine (a drug mainly acting on the noradrenergic system, similar in action to selective noradrenaline reuptake inhibitors, NSRI) have been shown to raise mRNA levels encoding CREB in the hippocampus, but an increase in CREB protein in this structure was observed only after fluoxetine administration (Nibuya et al., 1996). Moreover, other authors have not confirmed the effects of fluoxetine but showed that both antidepressants exacerbated the phosphorylation of CREB in the PFC (Tiraboschi et al., 2004; Laifenfeld et al., 2005). Furthermore, treatment with venlafaxine, a dual serotonin and noradrenaline reuptake inhibitor, significantly reduced pCREB in this brain structure without influence on total CREB expression (Rossby et al., 1999). A generally accepted statement is that chronically used antidepressants should contribute to the enhancement of both expression and activity of CREB (Blendy, 2006) mostly confirmed by data obtained from postmortem studies showing elevated CREB levels in patients that had undergone antidepressant therapy (Dowlatshahi et al., 1998) and decreased levels in those who had not undergone treatment (Yamada et al., 2003). Nevertheless, it remains an open question as to how the involvement of CREB is significant in antidepressant mechanisms and whether its activation is necessary for their effectiveness, as the experiments performed on transgenic animals lacking CREB showed rather opposing effects (genetic deletion of CREB unexpectedly contributed to the induction of an antidepressant phenotype) (Pliakas et al., 2001; Conti et al., 2002; Newton et al., 2002). It should be emphasized, however, that these models were subject to significant limitations that could affect animal behavior, developmental changes and, consequently, interpretation of the results as they were based on non-selective removal of the CREB encoding gene from multiple brain structures. Here, we took advantage of a far more advanced genetic tool allowing for selective and specific deletion of CREB in a chosen neuronal population, i.e., serotoninergic cells; therefore, the effects observed here can be confidently associated with CREB located in these neurons only.

In our experiments focused on the PFC, we found that only the females showed enhanced expression of BDNF after chronic treatment with fluoxetine (**Figures 4E,F**). The topic of gender differences in response to antidepressants emerged recently in clinical studies (Keers and Aitchison, 2010) and has also been observed in behavioral studies conducted on animal models with SSRIs, including our previous studies (Bhatnagar et al., 2004; Jones and Lucki, 2005; Chmielarz et al., 2013; Rafa-Zablocka et al., 2017). Moreover, it has also been shown that chronic fluoxetine, applied in the same dosage as in our experimental paradigm (10 mg/kg) with increased BDNF levels in the hippocampus of both sexes, and this effect was not correlated with cell proliferation, as female mice had higher levels of cell proliferation than their male counterparts. The differences in the pharmacokinetics of fluoxetine may contribute to this phenomenon, as females show higher concentrations of the norfluoxetine metabolite than males in both the plasma and brain (Hodes et al., 2010). Norfluoxetine acts as an SSRI similar to fluoxetine, but its increased half-life may lead to prolonged therapeutic coverage in females, which may translate to the higher brain plasticity observed by Hodes et al. (2010). This effect may also be reflected in our studies.

Gender differences were also observed in the regulation of another neurotrophic factor, neurotrophin 3 (NTF3) (**Figures 4E,G,H**). Here, we found upregulation of mRNA expression encoding for NTF3 in the PFC (but not in the hippocampus) in virtually all investigated male groups but not female groups in comparison to control untreated mice, though not always reaching statistical significance (**Figures 3A–E**). These effects were not always correlated in terms of the protein level, as Western blot performed on the protein samples extracted from the PFC showed enhanced NTF3 expression in only single mutants ($Creb1^{TPH2CreERT2}$ mice) after fluoxetine, while this upregulation ceased again in double mutants ($Creb1^{TPH2CreERT2}Crem-/-$ mice) receiving this drug (**Figures 4E,H**). Similar effects regarding the pattern of expression of nerve growth factor, NGF, were observed and do not seem to be gender dependent; however, statistical significance was not reached in females (**Figures 4E,G**). Overall, these observations were somewhat surprising, as the regulation of NTF3 and NGF seems to be opposite of that of BDNF in the mice lacking CREB in serotonergic neurons; however, one must take into consideration that the effects on NTF3 and NGF are restricted to the PFC but not to the hippocampus and to male mice but not to female mice. On the other hand, it is known that chronic treatment with fluoxetine itself (without combination with other drugs) does not increase the levels of NTF3 and NGF in the hippocampus or in the PFC (Agostinho et al., 2011); therefore, observed effects on single CREB-deficient mice ($Creb1^{TPH2CreERT2}$) cannot be regarded as an artifact. In fact, opposite regulation of these three neurotrophic factors was

reported previously in various experimental studies (Rocamora et al., 1994; Canals et al., 1998) as well as recently in clinical studies (Bilgic et al., 2017).

All the above described effects regarding the CREB-mediated response of BDNF after fluoxetine treatment were observed predominantly in single mutant mice ($Creb1^{TPH2CreERT2}$ mice), while in the double mutants ($Creb1^{TPH2CreERT2}$Crem−/− mice) also lacking cyclic AMP response element modulator (CREM), these effects were attenuated or even showed a tendency to be reversed (**Figures 4A,B,E,F** and **Table 1**). We included the latter cohort of mice in all the analyses due to the known compensatory properties of CREM in the absence of CREB (Hummler et al., 1994; Mantamadiotis et al., 2002). CREM belongs to the CREB/CREM/ATF family of transcription factors that bind to cAMP response elements (CREs) of cAMP-responsive genes (De Cesare and Sassone-Corsi, 2000).

Therefore, we wanted to make sure that CREM had no possibility of compensating for CREB in our model. In general, one should rather expect that the compensatory effect of CREM will prevent to observe any changes evoked by single CREB deletion. Apparently, it was not the case in this study, where the effects of chronic fluoxetine treatment on BDNF expression were counteracted already by single CREB removal from 5-HT neurons, and additional CREM KO background surprisingly seems to revert these changes. It is hard to explain why the lack of both CREB and CREM seems to be less harmful considering the effects of fluoxetine in our study. However, it must be mentioned that since the CREM negative background is not specific to the serotonergic neurons ($Creb1^{TPH2CreERT2}$ mutants were inbred into CREM KO mice) and this mutation is constitutive, this may have an unexpected impact on developmental stages evoking other compensatory mechanisms and so-called off-target effects that are often observed in non-spatiotemporal, classic KO animals (El-Brolosy and Stainier, 2017).

To exploit this topic in depth, we decided to compare all CREM lacking mice with/without fluoxetine treatment in order to determine whether the observed effects diminishing the role of CREB ablation in $Creb1^{TPH2CreERT2}$Crem−/− mice were truly related to the introduced CREM deletion. Indeed, this experiment confirmed the initial hypothesis, as all the CREM-deficient mice unveiled slightly enhanced expression of BDNF after fluoxetine irrespective of investigated structure or gender (however, it reached significance in only the PFC of females), while such effect was not observed in animals that did not receive the drug (**Figures 6A–D**). CREM KO mice are known to have impaired spermatogenesis processes, making the male CREM−/− mice infertile (Blendy et al., 1996), and they are also characterized by emotional and locomotor disturbances (Maldonado et al., 1999). To the best of our knowledge, there have been no studies that have considered the effects of antidepressants on CREM-deficient mice. Nevertheless, CREM is known to play an important function in a variety of physiological responses including cardiac function (Muller et al., 2003). Although it remains speculation, malfunction of cardiac rhythm may put these mice in a more vulnerable state for hypertension induced by fluoxetine, a side effect associated with SSRIs known from animal studies (Hong et al., 2017) and reported in clinics (Jayarajan et al., 2014). If so, this may have a direct influence on BDNF levels reported to be increased in response to hypertensive stimuli (Vermehren-Schmaedick et al., 2013; Erdos et al., 2015).

Surprisingly, we also found that male CREM−/− mice were characterized by significant increases in NTF3 to an extent similar to those observed in single $Creb1^{TPH2CreERT2}$ male mutants. Here, in contrast to the effects observed in BDNF, it seems that both single deletions of either CREB or CREM were sufficient to unleash enhanced expression of NTF3 after fluoxetine, while this effect was not observed in double mutants lacking both CREB and CREM. One may only speculate that compensatory processes evoked in both single mutations may lead to this unforeseen effect; however, this phenomenon remains difficult to explain.

Overall, these results summarized in **Table 1** provide further evidence for the important function of CREB in serotonergic neurons in antidepressant drug action through regulation of BDNF. Confirming our initial findings, we revealed a pivotal role for CREB in these neurons observed after acute response to fluoxetine (Rafa-Zablocka et al., 2017). It is rather hard to compare the data obtained in this work after chronic,

TABLE 1 | Expression of mRNA encoding for BDNF and BDNF protein in w/t, $Creb1^{TPH2CreERT2}$ and $Creb1^{TPH2CreERT2}$Crem−/− mice after fluoxetine treatment.

			Hippocampus		Prefrontal cortex	
Sex	**Treatment**	**Genotype**	**BDNF mRNA**	**BDNF protein**	**BDNF mRNA**	**BDNF protein**
MALE	+SAL	$Creb1^{TPH2CreERT2}$	–	–	–	–
		$Creb1^{TPH2CreERT2}$Crem−/−	–	–	–	–
	+FLX	Wild type	–	↑	–	–
		$Creb1^{TPH2CreERT2}$	–	–	–	–
		$Creb1^{TPH2CreERT2}$Crem−/−	–	↑ns	–	↑ns
FEMALE	+SAL	$Creb1^{TPH2CreERT2}$	–	–	–	–
		$Creb1^{TPH2CreERT2}$Crem−/−	–	–	–	–
	+FLX	Wild type	–	↑	–	↑
		$Creb1^{TPH2CreERT2}$	–	–	–	–
		$Creb1^{TPH2CreERT2}$Crem−/−	–	↑ns	–	↑ns

Arrows represent significant changes in BDNF expression vs. saline treated w/t mice. n/s, not significant; SAL, saline; FLX, fluoxetine.

21-days paradigm of fluoxetine administration, with the behavioral response of acute, one-dose fluoxetine administration described in previous study. However, indeed one may speculate that some adaptive changes evoked by removal of CREB from 5-HT neurons may influence also the response observed after acute drug administration. In particular, lack of enhancement of BDNF expression in response to chronic fluoxetine administration may be regarded as an indirect proof that the serotonergic pathway, important for SSRI effectiveness, is somewhat impaired which may be reflected on behavioral level as well. However, it remains speculative as we do not have yet any data regarding the 5-HT neurons functioning in our model.

We believe that our results may provide a valuable contribution to the discussion of the role of CREB in antidepressant drug action and depression, noting that CREB-dependent regulation of neurotrophin responses observed after some antidepressants can be associated not only with the neuronal structures traditionally regarded as important key players in depression pathology but also directly in the neurotransmitter targets of antidepressant therapies.

AUTHOR CONTRIBUTIONS

GK designed the study. KR-Z performed the Western blot and RT-qPCR assays. KR-Z and GK performed drug injections, tissue dissections, analyzed the data, and wrote the paper. MB and GK performed immunohistochemistry. MB maintained the transgenic mouse colony and performed genotyping. IN supervised the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2018.00637/full#supplementary-material

FIGURE S1 | Expression of BDNF in female w/t, Creb1^{TPH2CreERT2}, and Creb1^{TPH2CreERT2}Crem−/− mice after fluoxetine treatment as visualized by immunohistochemistry. Immunofluorescent staining revealed no visible differences between non-treated w/t and mutant mice **(A–C)**; on the other hand enhanced expression of BDNF-positive cells was noted in w/t mice after fluoxetine **(D)**, an effect no longer observed in transgenic animals **(E,F)**. Immunofluorescent staining performed on paraffin-embedded 7 μM microtome cortical slices of female w/t, Creb1^{TPH2CreERT2} and Creb1^{TPH2CreERT2}Crem−/− mice with anti-BDNF antibody (green). −FLX, saline treatment; +FLX, fluoxetine treatment. Scale bar for all pictures: 25 μm.

REFERENCES

Agostinho, F. R., Reus, G. Z., Stringari, R. B., Ribeiro, K. F., Pfaffenseller, B., Stertz, L., et al. (2011). Olanzapine plus fluoxetine treatment increases Nt-3 protein levels in the rat prefrontal cortex. *Neurosci. Lett.* 497, 99–103. doi: 10.1016/j.neulet.2011.04.039

Autry, A. E., and Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol. Rev.* 64, 238–258. doi: 10.1124/pr.111.005108

Baj, G., D'alessandro, V., Musazzi, L., Mallei, A., Sartori, C. R., Sciancalepore, M., et al. (2012). Physical exercise and antidepressants enhance BDNF targeting in hippocampal CA3 dendrites: further evidence of a spatial code for BDNF splice variants. *Neuropsychopharmacology* 37, 1600–1611. doi: 10.1038/npp.2012.5

Berton, O., Mcclung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* 311, 864–868. doi: 10.1126/science.1120972

Bhatnagar, S., Nowak, N., Babich, L., and Bok, L. (2004). Deletion of the 5-HT3 receptor differentially affects behavior of males and females in the Porsolt forced swim and defensive withdrawal tests. *Behav. Brain Res.* 153, 527–535. doi: 10.1016/j.bbr.2004.01.018

Bilgic, A., Toker, A., Isik, U., and Kilinc, I. (2017). Serum brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 levels in children with attention-deficit/hyperactivity disorder. *Eur. Child Adolesc. Psychiatry* 26, 355–363. doi: 10.1007/s00787-016-0898-2

Blendy, J. A. (2006). The role of CREB in depression and antidepressant treatment. *Biol. Psychiatry* 59, 1144–1150. doi: 10.1016/j.biopsych.2005.11.003

Blendy, J. A., Kaestner, K. H., Weinbauer, G. F., Nieschlag, E., and Schutz, G. (1996). Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature* 380, 162–165. doi: 10.1038/380162a0

Briand, L. A., Lee, B. G., Lelay, J., Kaestner, K. H., and Blendy, J. A. (2015). Serine 133 phosphorylation is not required for hippocampal CREB-mediated transcription and behavior. *Learn. Mem.* 22, 109–115. doi: 10.1101/lm.037044.114

Canals, J. M., Marco, S., Checa, N., Michels, A., Perez-Navarro, E., Arenas, E., et al. (1998). Differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 after excitotoxicity in a rat model of Huntington's disease. *Neurobiol. Dis.* 5, 357–364. doi: 10.1006/nbdi.1998.0211

Celada, P., Siuciak, J. A., Tran, T. M., Altar, C. A., and Tepper, J. M. (1996). Local infusion of brain-derived neurotrophic factor modifies the firing pattern of dorsal raphe serotonergic neurons. *Brain Res.* 712, 293–298. doi: 10.1016/0006-8993(95)01469-1

Chan, C. B., and Ye, K. (2017). Sex differences in brain-derived neurotrophic factor signaling and functions. *J. Neurosci. Res.* 95, 328–335. doi: 10.1002/jnr.23863

Chmielarz, P., Kusmierczyk, J., Parlato, R., Schutz, G., Nalepa, I., and Kreiner, G. (2013). Inactivation of glucocorticoid receptor in noradrenergic system influences anxiety- and depressive-like behavior in mice. *PLoS One* 8:e72632. doi: 10.1371/journal.pone.0072632

Conti, A. C., Cryan, J. F., Dalvi, A., Lucki, I., and Blendy, J. A. (2002). cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs.

J. Neurosci. 22, 3262–3268. doi: 10.1523/JNEUROSCI.22-08-03262.2002

Cunha, A. B., Frey, B. N., Andreazza, A. C., Goi, J. D., Rosa, A. R., Goncalves, C. A., et al. (2006). Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neurosci. Lett.* 398, 215–219. doi: 10.1016/j.neulet.2005.12.085

De Cesare, D., and Sassone-Corsi, P. (2000). Transcriptional regulation by cyclic AMP-responsive factors. *Prog. Nucleic. Acid. Res. Mol. Biol.* 64, 343–369. doi: 10.1016/S0079-6603(00)64009-6

Deltheil, T., Guiard, B. P., Guilloux, J. P., Nicolas, L., Delomenie, C., Reperant, C., et al. (2008). Consequences of changes in BDNF levels on serotonin neurotransmission, 5-HT transporter expression and function: studies in adult mice hippocampus. *Pharmacol. Biochem. Behav.* 90, 174–183. doi: 10.1016/j.pbb.2007.09.018

Diaz-Ruiz, C., Parlato, R., Aguado, F., Urena, J. M., Burgaya, F., Martinez, A., et al. (2008). Regulation of neural migration by the CREB/CREM transcription factors and altered Dab1 levels in CREB/CREM mutants. *Mol. Cell. Neurosci.* 39, 519–528. doi: 10.1016/j.mcn.2008.07.019

Dillon, D. G., and Pizzagalli, D. A. (2018). Mechanisms of memory disruption in depression. *Trends Neurosci.* 41, 137–149. doi: 10.1016/j.tins.2017.12.006

Disner, S. G., Beevers, C. G., Haigh, E. A., and Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nat. Rev. Neurosci.* 12, 467–477. doi: 10.1038/nrn3027

Dowlatshahi, D., Macqueen, G. M., Wang, J. F., and Young, L. T. (1998). Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet* 352, 1754–1755. doi: 10.1016/S0140-6736(05)79827-5

Duman, R. S. (2002). Pathophysiology of depression: the concept of synaptic plasticity. *Eur. Psychiatry* 17(Suppl. 3), 306–310. doi: 10.1016/S0924-9338(02)00654-5

Dusi, N., Barlati, S., Vita, A., and Brambilla, P. (2015). Brain structural effects of antidepressant treatment in major depression. *Curr. Neuropharmacol.* 13, 458–465. doi: 10.2174/1570159X1304150831121909

El-Brolosy, M. A., and Stainier, D. Y. R. (2017). Genetic compensation: a phenomenon in search of mechanisms. *PLoS Genet.* 13:e1006780. doi: 10.1371/journal.pgen.1006780

Erdos, B., Backes, I., Mccowan, M. L., Hayward, L. F., and Scheuer, D. A. (2015). Brain-derived neurotrophic factor modulates angiotensin signaling in the hypothalamus to increase blood pressure in rats. *Am. J. Physiol. Heart Circ. Physiol.* 308, H612–H622. doi: 10.1152/ajpheart.00776.2014

Finkbeiner, S., Tavazoie, S. F., Maloratsky, A., Jacobs, K. M., Harris, K. M., and Greenberg, M. E. (1997). CREB: a major mediator of neuronal neurotrophin responses. *Neuron* 19, 1031–1047. doi: 10.1016/S0896-6273(00)80395-5

Galter, D., and Unsicker, K. (2000). Sequential activation of the 5-HT1(A) serotonin receptor and TrkB induces the serotonergic neuronal phenotype. *Mol. Cell. Neurosci.* 15, 446–455. doi: 10.1006/mcne.2000.0841

Gass, P., and Riva, M. A. (2007). CREB, neurogenesis and depression. *Bioessays* 29, 957–961. doi: 10.1002/bies.20658

Gundersen, B. B., Briand, L. A., Onksen, J. L., Lelay, J., Kaestner, K. H., and Blendy, J. A. (2013). Increased hippocampal neurogenesis and accelerated response to antidepressants in mice with specific deletion of CREB in the hippocampus: role of cAMP response-element modulator tau. *J. Neurosci.* 33, 13673–13685. doi: 10.1523/JNEUROSCI.1669-13.2013

Hisaoka, K., Maeda, N., Tsuchioka, M., and Takebayashi, M. (2008). Antidepressants induce acute CREB phosphorylation and CRE-mediated gene expression in glial cells: a possible contribution to GDNF production. *Brain Res.* 1196, 53–58. doi: 10.1016/j.brainres.2007.12.019

Hodes, G. E., Hill-Smith, T. E., Suckow, R. F., Cooper, T. B., and Lucki, I. (2010). Sex-specific effects of chronic fluoxetine treatment on neuroplasticity and pharmacokinetics in mice. *J. Pharmacol. Exp. Ther.* 332, 266–273. doi: 10.1124/jpet.109.158717

Homberg, J. R., Molteni, R., Calabrese, F., and Riva, M. A. (2014). The serotonin-BDNF duo: developmental implications for the vulnerability to psychopathology. *Neurosci. Biobehav. Rev.* 43, 35–47. doi: 10.1016/j.neubiorev.2014.03.012

Hong, L. Z., Huang, K. F., Hung, S. W., and Kuo, L. T. (2017). Chronic fluoxetine treatment enhances sympathetic activities associated with abnormality of baroreflex function in conscious normal rats. *Eur. J. Pharmacol.* 811, 164–170. doi: 10.1016/j.ejphar.2017.06.021

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., et al. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* 98, 739–755. doi: 10.1016/S0092-8674(00)81509-3

Hummler, E., Cole, T. J., Blendy, J. A., Ganss, R., Aguzzi, A., Schmid, W., et al. (1994). Targeted mutation of the CREB gene: compensation within the CREB/ATF family of transcription factors. *Proc. Natl. Acad. Sci. U.S.A.* 91, 5647–5651. doi: 10.1073/pnas.91.12.5647

Jayarajan, R. N., Shere, S., Sutar, R., Karmani, S., Reddi, V. S., Kesavan, M., et al. (2014). Fluoxetine-induced pulmonary hypertension in a patient with schizophrenia. *J. Neuropsychiatry Clin. Neurosci.* 26, E12–E13. doi: 10.1176/appi.neuropsych.13100231

Jones, M. D., and Lucki, I. (2005). Sex differences in the regulation of serotonergic transmission and behavior in 5-HT receptor knockout mice. *Neuropsychopharmacology* 30, 1039–1047. doi: 10.1038/sj.npp.1300664

Keers, R., and Aitchison, K. J. (2010). Gender differences in antidepressant drug response. *Int. Rev. Psychiatry* 22, 485–500. doi: 10.3109/09540261.2010.496448

Kiryk, A., Sowodniok, K., Kreiner, G., Rodriguez-Parkitna, J., Sonmez, A., Gorkiewicz, T., et al. (2013). Impaired rRNA synthesis triggers homeostatic responses in hippocampal neurons. *Front. Cell. Neurosci.* 7:207. doi: 10.3389/fncel.2013.00207

Kreiner, G., Chmielarz, P., Roman, A., and Nalepa, I. (2013). Gender differences in genetic mouse models evaluated for depressive-like and antidepressant behavior. *Pharmacol. Rep.* 65, 1580–1590. doi: 10.1016/S1734-1140(13)71519-6

Kronenberg, G., Mosienko, V., Gertz, K., Alenina, N., Hellweg, R., and Klempin, F. (2016). Increased brain-derived neurotrophic factor (BDNF) protein concentrations in mice lacking brain serotonin. *Eur. Arch. Psychiatry Clin. Neurosci.* 266, 281–284. doi: 10.1007/s00406-015-0611-3

Laifenfeld, D., Karry, R., Grauer, E., Klein, E., and Ben-Shachar, D. (2005). Antidepressants and prolonged stress in rats modulate CAM-L1, laminin, and pCREB, implicated in neuronal plasticity. *Neurobiol. Dis.* 20, 432–441. doi: 10.1016/j.nbd.2005.03.023

Lee, B. H., and Kim, Y. K. (2010). The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. *Psychiatry Investig.* 7, 231–235. doi: 10.4306/pi.2010.7.4.231

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., et al. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15239–15244. doi: 10.1073/pnas.96.26.15239

Maier, T., Guell, M., and Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS Lett.* 583, 3966–3973. doi: 10.1016/j.febslet.2009.10.036

Maldonado, R., Smadja, C., Mazzucchelli, C., and Sassone-Corsi, P. (1999). Altered emotional and locomotor responses in mice deficient in the transcription factor CREM. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14094–14099. doi: 10.1073/pnas.96.24.14094

Mantamadiotis, T., Lemberger, T., Bleckmann, S. C., Kern, H., Kretz, O., Villalba, A. M., et al. (2002). Disruption of CREB function in brain leads to neurodegeneration. *Nat. Genet.* 31, 47–54. doi: 10.1038/ng882

Martenyi, F., Dossenbach, M., Mraz, K., and Metcalfe, S. (2001). Gender differences in the efficacy of fluoxetine and maprotiline in depressed patients: a double-blind trial of antidepressants with serotonergic or norepinephrinergic reuptake inhibition profile. *Eur. Neuropsychopharmacol.* 11, 227–232. doi: 10.1016/S0924-977X(01)00089-X

Martinowich, K., and Lu, B. (2008). Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* 33, 73–83. doi: 10.1038/sj.npp.1301571

Mattson, M. P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative

disorders. *Trends Neurosci.* 27, 589–594. doi: 10.1016/j.tins.2004.08.001

McPherson, C. S., and Lawrence, A. J. (2007). The nuclear transcription factor CREB: involvement in addiction, deletion models and looking forward. *Curr. Neuropharmacol.* 5, 202–212. doi: 10.2174/157015907781695937

Molteni, R., Cattaneo, A., Calabrese, F., Macchi, F., Olivier, J. D., Racagni, G., et al. (2010). Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans. *Neurobiol. Dis.* 37, 747–755. doi: 10.1016/j.nbd.2009.12.014

Muller, F. U., Lewin, G., Matus, M., Neumann, J., Riemann, B., Wistuba, J., et al. (2003). Impaired cardiac contraction and relaxation and decreased expression of sarcoplasmic $Ca2^{+}$-ATPase in mice lacking the CREM gene. *FASEB J.* 17, 103–105. doi: 10.1096/fj.02-0486fje

Nair, A., and Vaidya, V. A. (2006). Cyclic AMP response element binding protein and brain-derived neurotrophic factor: molecules that modulate our mood? *J. Biosci.* 31, 423–434. doi: 10.1007/BF02704114

Newton, S. S., Thome, J., Wallace, T. L., Shirayama, Y., Schlesinger, L., Sakai, N., et al. (2002). Inhibition of cAMP response element-binding protein or dynorphin in the nucleus accumbens produces an antidepressant-like effect. *J. Neurosci.* 22, 10883–10890. doi: 10.1523/JNEUROSCI.22-24-10883.2002

Nibuya, M., Morinobu, S., and Duman, R. S. (1995). Regulation of Bdnf and Trkb messenger-RNA in rat-brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* 15, 7539–7547. doi: 10.1523/JNEUROSCI.15-11-07539.1995

Nibuya, M., Nestler, E. J., and Duman, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J. Neurosci.* 16, 2365–2372. doi: 10.1523/JNEUROSCI.16-07-02365.1996

Phillips, C. (2017). Brain-derived neurotrophic factor, depression, and physical activity: making the neuroplastic connection. *Neural Plast.* 2017:7260130. doi: 10.1155/2017/7260130

Pliakas, A. M., Carlson, R. R., Neve, R. L., Konradi, C., and Nestler, E. J. (2001). Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J. Neurosci.* 21, 7397–7403. doi: 10.1523/JNEUROSCI.21-18-07397.2001

Rafa-Zablocka, K., Kreiner, G., Baginska, M., Kusmierczyk, J., Parlato, R., and Nalepa, I. (2017). Transgenic mice lacking CREB and CREM in noradrenergic and serotonergic neurons respond differently to common antidepressants on tail suspension test. *Sci. Rep.* 7:13515. doi: 10.1038/s41598-017-14069-6

Rocamora, N., Massieu, L., Boddeke, H. W., Palacios, J. M., and Mengod, G. (1994). Differential regulation of the expression of nerve growth-factor, brain-derived neurotrophic factor and neurotrophin-3 messenger-RNAS in adult-rat brain after intrahippocampal injection of quinolinic acid. *Mol. Brain Res.* 26, 89–98. doi: 10.1016/0169-328X(94)90078-7

Rossby, S. P., Manier, D. H., Liang, S., Nalepa, I., and Sulser, F. (1999). Pharmacological actions of the antidepressant venlafaxine beyond aminergic receptors. *Int. J. Neuropsychopharmacol.* 2, 1–8. doi: 10.1017/S1461145799001273

Russo-Neustadt, A., Beard, R. C., and Cotman, C. W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology* 21, 679–682. doi: 10.1016/S0893-133X(99)00059-7

Russo-Neustadt, A. A., and Chen, M. J. (2005). Brain-derived neurotrophic factor and antidepressant activity. *Curr. Pharm. Des.* 11, 1495–1510. doi: 10.2174/1381612053764788

Siuciak, J. A., Boylan, C., Fritsche, M., Altar, C. A., and Lindsay, R. M. (1996). BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. *Brain Res.* 710, 11–20. doi: 10.1016/0006-8993(95)01289-3

Smith, M. A., Makino, S., Kvetnansky, R., and Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.* 15, 1768–1777. doi: 10.1523/JNEUROSCI.15-03-01768.1995

Tiraboschi, E., Tardito, D., Kasahara, J., Moraschi, S., Pruneri, P., Gennarelli, M., et al. (2004). Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology* 29, 1831–1840. doi: 10.1038/sj.npp.1300488

Vaidya, V. A., Marek, G. J., Aghajanian, G. K., and Duman, R. S. (1997). 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J. Neurosci.* 17, 2785–2795. doi: 10.1523/JNEUROSCI.17-08-02785.1997

Vaidya, V. A., Terwilliger, R. M. Z., and Duman, R. S. (1999). Role of 5-HT2A receptors in the stress-induced down-regulation of brain-derived neurotrophic factor expression in rat hippocampus. *Neurosci. Lett.* 262, 1–4. doi: 10.1016/S0304-3940(99)00006-3

Vermehren-Schmaedick, A., Jenkins, V. K., Hsieh, H. Y., Brown, A. L., Page, M. P., Brooks, V. L., et al. (2013). Upregulation of brain-derived neurotrophic factor expression in nodose ganglia and the lower brainstem of hypertensive rats. *J. Neurosci. Res.* 91, 220–229. doi: 10.1002/jnr.23158

Warner-Schmidt, J. L., and Duman, R. S. (2006). Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus* 16, 239–249. doi: 10.1002/hipo.20156

Yamada, S., Yamamoto, M., Ozawa, H., Riederer, P., and Saito, T. (2003). Reduced phosphorylation of cyclic AMP-responsive element binding protein in the postmortem orbitofrontal cortex of patients with major depressive disorder. *J. Neural Transm.* 110, 671–680. doi: 10.1007/s00702-002-0810-8

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The Changes of Functional Connectivity Strength in Electroconvulsive Therapy for Depression: A Longitudinal Study

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Electroconvulsive therapy (ECT) is an effective treatment for depression, but the mechanism of ECT for depression is still unclear. Recently, neuroimaging studies have reported that the prefrontal cortex, hippocampus, angular gyrus, insular and other brain regions are involved in the mechanism of ECT for depression, and these regions are highly overlapped with the location of brain hubs. Here, we try to explore the effects of ECT on the functional connectivity of brain hubs in depression patients. In current study, depression patients were assessed at three time points: prior to ECT, at the completion of ECT and about 1 month after the completion of ECT. At each time point, resting-state functional magnetic resonance imaging, assessment of clinical symptoms and cognition function were performed respectively, which was compared with 20 normal controls. Functional connectivity strength (FCS) was used to identify brain hubs. The results showed that FCS of left angular gyrus in depression patients significantly increased after ECT, accompanied by improved mood. The changed FCS in depression patients recovered obviously at 1 month after the completion of ECT. It suggested that ECT could modulate functional connectivity of left angular gyrus in depression patients.

Keywords: depression, electroconvulsive therapy, fMRI, treatment, brain hub

INTRODUCTION

Depression is a major cause of disability and global disease burden worldwide, contributing significantly to decrease in quality of life and increase in suicide risk (Ustun et al., 2004). Even with adequate psychotherapeutic, psychopharmacologic or combined treatment, about one third of patients do not achieve symptom remission (Rush et al., 2006). For these severely depressed and treatment-resistant patients, electroconvulsive therapy (ECT) elicits a more rapid and effective response, leading to remission of symptoms in about 50–70% of patients (Husain et al., 2004). However, the mechanism by which ECT operates is still unclear. Exploring the therapeutic mechanism of ECT would promote the optimization of ECT and develop new treatment options for depression.

Recently, neuroimaging studies have reported that the prefrontal cortex, hippocampus, angular gyrus, insular, and other brain regions are involved in the mechanism of ECT for depression (Perrin et al., 2012; Abbott et al., 2014; van Waarde et al., 2015; Cano et al., 2016; Sartorius et al., 2016). These results have showed that ECT does not only affect one brain region, but also change the structure and function of wide brain areas, which indicates that ECT may influence the brain at the level of brain networks. For brain networks, studies reveal topological organization of human whole-brain connectivity by combining neuroimaging and graph-based network analysis (Bullmore and Sporns, 2009; He and Evans, 2010). One important and convergent finding is that there are several hubs in the brain, located in prefrontal cortex, hippocampal, inferior parietal lobule (angular gyrus and supramarginal gyrus), and insular. These brain hubs have a significantly larger number of structural and functional connections in comparison with other regions in the brain network, and play important roles in information communication and integration across a broad range of cognitive tasks, such as emotion and cognitive control (van den Heuvel and Sporns, 2013; Mears and Pollard, 2016). The locations of hubs are highly overlapped with changed regions observed in previous studies about ECT for depression, and it could be hypothesized that the changed organization of brain hubs may be crucial for the effects of ECT for depression. But it is still lack of studies to test the hypothesis.

Here, we try to explore the effects of ECT on the functional connectivity of brain hubs in depression patients by using resting-state functional magnetic resonance imaging (rsfMRI) and functional connectivity strength (FCS). RsfMRI measures spontaneous brain activity. It has emerged as an effective probe for functional connectivity in both healthy individuals and neuropsychiatric patients. FCS is a voxel-wise measurement of 'degree centrality' for the whole-brain functional connectivity based on the rsfMRI data, which could reflect the general functional connections of brain hubs. FCS has been used to observe the organization of brain hubs in neuropsychiatric patients, including depression (Lan et al., 2015; Wang et al., 2015; Zou et al., 2016).

In the present study, depression patients were assessed at three time points: prior to ECT, at completion of ECT and about 1 month after the completion of ECT. At the three time points, rsfMRI, the assessment of clinical symptoms, and measures of cognitive function were performed.

MATERIALS AND METHODS

Participants

Depression patients who were referred for ECT due to resistance to drug therapy or severe suicidal tendencies were recruited from Anhui Mental Health Center from September 2012 to December 2016. Depression was diagnosed on the basis of Diagnostic and Statistical Manual of Mental Disorders-IV criteria. Patients with substance dependence, pregnancy, life-threatening somatic disease, neurological disorders, other co-morbid mental disorders, MRI-related contraindications or head translation or rotation parameters exceeding 3 mm or 3° during MRI scanning were excluded. 26 right-handed patients were included in the final sample. All of them continued to take anti-depression drugs during ECT administration. At the same time, 9 patients also took antipsychotic medications and 3 patients took lithium. During the 1-month follow-up, patients continued to take anti-depression drugs. Patients were required to stop taking benzodiazepines 12–24 h before the first assessment point prior to ECT in the study.

Twenty normal controls were recruited who met the same exclusion criteria as the depression patients, except for a history of depression or antidepressant use. All the participants were right-handed.

The study was approved by the Anhui Medical University Ethics Committee, and all the participants in this study were undertaken with the understanding and written consents.

Procedures

Patients were assessed at 3 time points: TP1, 12–24 h before the first ECT administration; TP2, 24 h-1 week after the last ECT administration; and TP3, about 1 month after the last ECT administration. All the 26 patients completed TP1 and TP2 assessments, and 15 patients completed the T3 assessments. All patients maintained the drug treatments during the study. Healthy controls completed one assessment. The assessments included a clinical evaluation, cognitive tests and magnetic resonance imaging (MRI) scan.

ECT Administration

All the ECT administrations were conducted at Anhui Mental Health Center, where patients underwent modified bifrontal ECT with a Thymatron System IV Integrated ECT Instrument (Somatics, Inc., Lake Bluff, IL, United States). The first three ECT sessions occurred on consecutive days, and the remaining ECT sessions were conducted every other day with a break of weekends until patients achieved remission. The patient's age determined the stimulating intensity of ECT. If the patient was younger than 50, the initial percent energy dial was set as patient's age minus five (e.g., 43% for a 48 year-old patient); and if not, the initial percent energy dial setting was to the patient's age (e.g., 55% for a 55 year-old patient). Seizure activity was monitored with electroencephalography during ECT. If no seizure activity resulted from stimulation, the initial percent energy would be increased until a therapeutically satisfactory seizure activity was obtained. During each ECT administration, patients were anesthetized with propofol. Succinylcholine and atropine were used to relax muscles and suppress gland secretions.

Clinical Evaluation and Cognitive Tests

We used the 17-item Hamilton Depression Rating Scale (HAMD) to assess depressive symptoms for depression patients and health controls. Mini mental state examination (MMSE) was used to assess participants' general cognitive function. A verbal fluency test was used to assess executive functions with patients asked to say as many words as possible within 2 min (1 minutes for animals, 1 min for vegetables). The total number of words was recorded, excluding repetitions, and intrusions.

MRI Data Acquisition

All the participants in this study underwent the MRI scans at the First Affiliated Hospital of Anhui Medical University. All the participants were instructed to keep their eyes closed and to relax, to remain awake, and not to think of anything in particular during the scanning. All scans were performed with a clinical 3-T whole-body MRI scanner (Signa HDxt 3.0T, GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and used a standard echo planar imaging sequence. T1-weighted anatomic images were acquired in sagittal orientation by using a 3D inversion recovery prepared fast spoiled gradient recalled sequence (TR/TE = 8.676 ms/3.184 ms, inversion time = 800 ms, flip angle = 8 degrees, field of view = 256 × 256 mm^2, matrix size = 256 × 256, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3, sections = 188). The rsfMRI were recorded aligned along the anterior–posterior commissure line with the following parameters: repetition time/echo time ratio (TR/TE) = 2000 ms/22.5 ms, flip angle = 30 degrees, 33 slices, thickness/gap ratio = 4.0 mm/0.6 mm, voxel size = 3.4 × 3.4 × 4.6 mm^3, matrix size = 64 × 64, field of view = 220 × 220 mm^2.

Data Processing

Functional Image Preprocessing

Data Processing Assistant for Resting-State Functional MR Imaging toolkit (DPARSF) was used to preprocess functional images, which synthesizes procedures in Resting State Functional MR Imaging Toolkit (REST[1]) and statistical parametric mapping software package (SPM8[2]) (Chao-Gan and Yu-Feng, 2010; Song et al., 2011). The first 10 volumes of data were discarded to ensure stable longitudinal magnetization. The remaining volumes were corrected for slice timing and head motion. All the participants had no more than 3 mm maximum displacement in any direction of x, y, or z and 3 degrees of angular motion. The individual T1 images were co-registered to functional images and were segmented (gray matter, white matter, and cerebrospinal fluid). After that, the functional images were normalized to the standard Montreal Neurological Institute space by using T1 image unified segmentation with a 12-parameter non-linear transformation, and resampled at a resolution of 3 × 3 × 3 mm^3. Afterwards, functional images were linearly detrended and band-pass filtered (0.01–0.08 Hz) to reduce low-frequency drifts and physiological high-frequency noises. Finally, several sources of spurious covariance were linearly regressed, including the six motion parameters, and signals from white matter and cerebrospinal fluid.

Whole-Brain FCS Analysis

A voxel-wise whole-brain functional connectivity analysis was performed on the on the preprocessed rsfMRI data as follows. Firstly, the Pearson's correlations between the residual time series of all pairs of brain voxels were computed and a whole-brain connectivity matrix for each participant was constructed. Then, the individual correlation matrices were transformed to a z-score matrix using a Fisher r-to-z transformation. For a given voxel, FCS was computed as the sum of the z-values between the given voxel and all the other voxels. We restricted our analysis to correlations above a threshold of $r = 0.2$ to eliminate weak correlations possibly arising from noises. These FCS maps were smoothed with a 6 mm full-width at half-maximum (FWHM) Gaussian kernel (Buckner et al., 2009; Liang et al., 2013).

Statistical Analysis

Clinical and demographic data were analyzed by using SPSS 19.0 (SPSS, Inc., Chicago, IL, United States). Paired two-sample t-test was performed to determine the changed regions of FCS for depression patients between the first two time points-prior to ECT and at completion of ECT. This test was constrained in a gray matter (GM) mask, and multiple comparison corrections were based on Gaussian random field theory (voxel-level $p < 0.001$; cluster-level $p < 0.05$). The FCS data of the changed regions were extracted in normal controls and patients at all three time points-prior to ECT, at completion of ECT and about 1 month after the completion of ECT, to observe the dynamic change. Independent t-test was used for between group, and paired t-test was used for within-group comparisons. The comparisons between TP2 and TP3 were constrained to the patients who completed the procedure at TP3. Pearson correlation analyses were performed to assess the correlation of the changed behavior scores and changed values of FCS in patients between the three time points (the significance level: $p < 0.05$).

RESULTS

Baseline Characteristics

The 26 patients with depression at TP1 and TP2 are matched with health controls in gender ($\chi 2 = 0.033$, $p = 0.855$), age ($t = -0.796$, $p = 0.430$), and education years ($t = -0.555$, $p = 0.582$). The 15 patients at TP3 and control groups also did not differ in gender ($p = 0.738$; fisher exact probability), age ($t = -0.741$, $p = 0.464$), and education years ($t = -0.349$, $p = 0.730$).

Clinical and Cognitive Outcome

Depressive Symptom

Compared to health controls (HC), patients at TP1 had significantly higher HAMD scores ($t = 18.96$, $p < 0.001$). Patients at TP2 show a significant reduction of mean HAMD score compared with TP1 ($t = 18.84$, $p < 0.001$), suggesting excellent treatment effect of ECT on depressive symptoms. No significant difference of HAMD score was observed between patients at TP2 and TP3 ($t = 0.613$, $p = 0.550$), which showed that the treatment effect of ECT on depressive symptoms was maintained. Both the HAMD scores of patients at TP2 and TP3, however, were still higher than health controls (TP2 vs. HC: $t = 3.870$, $p < 0.001$; TP3 vs. HC: $t = 4.192$, $p < 0.001$). (See in **Figure 1A**).

General Cognition

Compared to normal controls, MMSE scores were significantly lower in patients at TP2 ($t = 3.788$, $p < 0.001$), but no significant difference was observed in patients at TP1 (IR: $t = 1.424$,

[1]http://www.restfmri.net

[2]www.fil.ion.ucl.ac.uk/spm

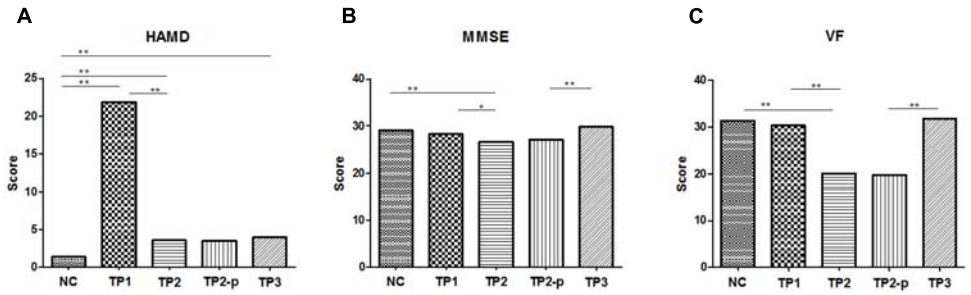

FIGURE 1 | (A) The scores of HAMD in each group. **(B)** The scores of MMSE in each group. **(C)** The scores of VF in each group. The clinical and cognitive outcome in normal controls and depression patients. NC, normal controls; TP1, patients at 12–24 h before the first ECT administration; TP2, patients at 24 h-1 week after the last ECT administration; TP2_p, patients at TP2 who completed the sacn of TP3; TP3, patients at about 1 month after the last ECT administration; HAMD, 17-item Hamilton Depression Rating Scale; MMSE, Mini mental state examination; VF, Verbal fluency. $^{*}p < 0.05$; $^{**}p < 0.01$.

$p = 0.162$) and in patients at TP3 ($t = 1.735$, $p = 0.092$). MMSE scores showed a significant decrease in patients at TP2 compared with at TP1($t = 2.476$, $p = 0.017$). At the follow-up assessments, there were significant improvements in MMSE scores at TP3 compared with at TP2 ($t = 5.074$, $p < 0.001$). (See in **Figure 1B**).

Executive Function

Compared to healthy controls, the VF score was significantly lower in patients at TP2 ($t = 5.153$, $p < 0.001$), but no significant difference was observed in patients at TP1 ($t = 0.435$, $p = 0.665$) and in patients at TP3 ($t = 0.180$, $p = 0.859$). The VF score showed a significant decrease in patients at TP2 compared with TP1 ($t = 4.087$, $p < 0.001$). This reduction was reversed at TP3 compared with at TP2 ($t = 8.465$, $p < 0.001$). (See in **Figure 1C**).

ECT Effects on FCS in Depression Patients

To explore the effects of ECT on FCS, paired two-sample *t*-test was performed between the FCS maps between depression patients at TP1 and TP2 within the gray matter mask, correcting with Gaussian random field theory (voxel-level $p < 0.001$; cluster-level $p < 0.05$). The result showed that FCS was significantly increased in left angular gyrus in patients at TP2, compared to patients at TP1. (See in **Figure 2A** and **Table 1**).

Then, to observe the dynamic changes of FCS data, the FCS values of the above cluster were extracted in health controls and patients at TP1, TP2, and TP3. Compared to healthy controls, no significant difference of FCS value was observed in left angular gyrus in patients at TP1 ($t = 0.158$, $p = 0.875$) and in patients at TP3 ($t = 0.983$, $p = 0.333$), but FCS of left angular gyrus in patients at TP2 was significantly higher than in healthy controls ($t = 4.001$, $p < 0.001$). FCS of of left angular gyrus wase significantly decreased in patients at TP3 compared with patients at TP2 ($t = 3.046$, $p = 0.009$). (See in **Figure 2B**).

The Correlation Between the Changes of Depressive Symptom and FCS

No significant correlation was observed between the changes in the HAMD score and FCS value of the left angular gyrus for patients at TP1 and TP2 ($r = 0.163$, $p = 0.427$), as well as patients at TP2 and TP3 ($r = 0.255$, $p = 0.358$).

The Correlation Between the Changes of Cognitive Outcomes and FCS

There was no significant correlation between the changes in cognitive outcomes (MMSE or VF) and FCS value of left angular gyrus for patients at TP1 and TP2 (MMSE: $r = -0.105$, $p = 0.609$; VF: $r = -0.134$, $p = 0.515$), as well as patients at TP2 and TP3 (MMSE: $r = -0.274$, $p = 0.323$; VF: $r = -0.009$, $p = 0.975$).

DISCUSSION

In this study, we observed changes of FCS in depression patients receiving ECT using a longitudinal protocol, and our main findings are 2-fold. First, ECT significantly increased the FCS of left angular gyrus in depression patients, accompanied by improved mood and impaired cognitive function. Second, the changed FCS in depression patients recovered obviously at 1 month after the completion of ECT, as well as the impaired cognitive function, but the improved mood did not worsen again. It has been demonstrated that left angular gyrus is a brain hub which has larger number of structural and functional connections with other brain regions (van den Heuvel and Sporns, 2013; Mears and Pollard, 2016), and FCS reflects general functional connections of brain hubs which has been temporarily changed after ECT in depression patients. These results have suggested that ECT could modulate the function of the brain hub (left angular gyrus) in depression patients.

Generalized epileptic seizure during ECT may contribute to the increased FCS in depression patients after ECT. When ECT is conducted, two electrodes are placed on patients' skull, and electricity of 100–500 mC through electrodes were administrated to patients. The electricity would induce generalized epileptic seizure lasting tens of seconds (Lisanby, 2007). During generalized epileptic seizure, the neurons in the brain are activated simultaneously by the spread of electricity. So generalized epileptic seizure is abnormal synchronous neuronal activity in the brain (Grasse et al., 2013). In our study,

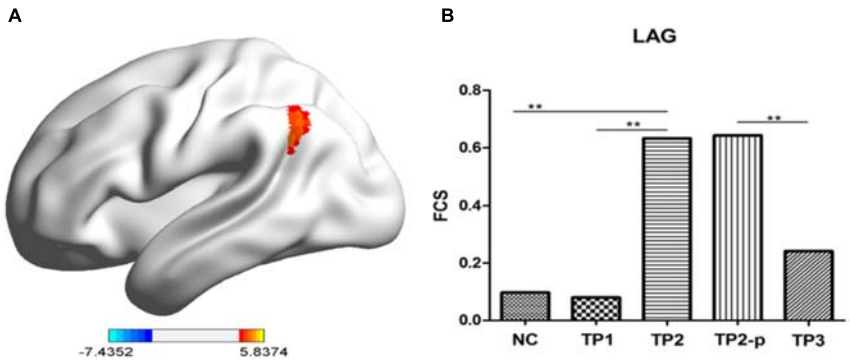

FIGURE 2 | (A) The stereogram of LAG. **(B)** The FCS value of LAG in each group. The Functional connectivity strength (FCS) values of left angular gyrus in normal controls and depression patients. NC, normal controls; TP1, patients at 12–24 h before the first ECT administration; TP2, patients at 24 h-1 week after the last ECT administration; TP2_p, patients at TP2 who completed the sacn of TP3; TP3, patients at about 1 month after the last ECT administration; LAG, left angular gyrus. $^{*}p < 0.05$; $^{**}p < 0.01$.

TABLE 1 | Regions showing significant changes in Functional connectivity strength (FCS) between depression patients at TP1 and TP2.

Brain regions	BA	Voxel number	*t* score	MNI coordinates (*x*, *y*, *z*)
Left angular gyrus	39	88	5.2981	−39, −60, 33

BA, Brodmann area; One voxel, 3 × 3 × 3 mm3; t, statistical value of the peak voxel; MNI coordinates, Montreal Neurological Institute coordinates of the peak voxel.

FCS is an index of general functional connectivity, and it is fundamentally the correlation of Blood-oxygen-level dependent (BOLD) signals, and reflects the synchronization of neuronal activity among different brain regions. Generalized epileptic seizure may increase functional connectivity in the brain due to the synchronous neuronal activity, especially in the brain hubs, which has more structure and functional connections with other brain regions. So ECT could increase functional connectivity among brain hubs and other brain regions and caused increased FCS of left angular gyrus in depression patients. After the completion of ECT, synchronous neuronal activity during generalized epileptic seizure is not induced in depression patients, which would lead to gradually decrease of FCS in left angular gyrus.

Many studies have suggested that abnormality of left angular gyrus is closely related to depression. One study has reported that the volume of left angular gyrus is associated with suicide attempts in depression patients (Lee et al., 2016). It has been found that there is abnormal functional connectivity between left angular gyrus and precentral gyrus in depression patients (Wu et al., 2016). Recently, a whole brain functional connectome study has suggested that the FCS of left angular gyrus is lower in depression patients than in healthy controls (Lai et al., 2017). As a brain hub, angular gyrus process information by connecting distinct, functional specialized regions. These regions include amygdala, ventromedial prefrontal cortex, subgenual anterior cingulate cortex, and other areas which are related to emotion regulation (Achard et al., 2006). The lower FCS of left angular gyrus means less connections with other areas, which may influence emotion regulation and cause depression. In current study, the results have shown that the FCS of left angular gyrus was lower but not reach significant in depression patients before ECT, comparing to the normal controls. It has also shown that FCS of left angular gyrus in depression patients increased significantly after the completion of ECT. The increased FCS of left angular gyrus may improve the ability of emotion regulation and lead to relief of depressive symptoms. And also, it should be noticed that the FCS of depression patients at 1 month after the completion of ECT decreased significantly comparing to the ending of ECT, without deterioration of depression. FCS is an index which reflects general functional connectivity between one brain region and the whole brain. For further studies, we need to explore the exact brain areas which contribute to the changes of FCS in left angular gyrus after ECT, and it may be conductive to clarify the relation between FCS in left angular gyrus and depression symptoms.

In this study, we also observed the change of cognitive function in depression patients. It has shown that general cognition and verbal fluency is impaired significantly after ECT, and recover one month after the completion of ECT. These results are consistent with previous study (Semkovska and McLoughlin, 2010). Many studies have shown that there is impaired cognition includling memory, verbal fluency and others, and it would be improved after successful psychotherapeutic, psychopharmacologic treatment. But if depression patients recive ECT, symptoms would be improved accompanied by reversible deterioration of cognition (Semkovska and McLoughlin, 2010). The reason is still unknow. Many studies have demonstrated left angular gyrus is involved in many cognitive tasks including executive function (Staresina and Davachi, 2006; Cabeza et al., 2008; Gauthier et al., 2009; Reineberg et al., 2015). The FCS of left angular gyrus in depression patients has increased after ECT, and it reversed in patients one month after ECT, which has shown the same tendency with general cognition, and verbal fluency. But no significant correlation between the changes in cognitive outcomes and FCS value of left angular gyrus were observed. So the relationship between the changed FCS of left angular gyrus and cognitive changes needs further studies to explore.

The limitations of this study also should be addressed. First, the patients recruited in the study took some medicines including anti-depressants and benzodiazepine. It should be noticed that these medicines may impact the fMRI results. Second, only bifrontal ECT was used in our study, and our results should be validated in other ECT protocols, such as bilateral ECT, right unilateral ECT and focal electrically administered seizure therapy.

In summary, current study have found that ECT significantly increase the FCS of left angular gyrus in depression patients, and the changed FCS in depression patients recovered obviously at 1 month after the completion of ECT. These results suggest that ECT could change functional connectivity of left angular gyrus in depression patients.

AUTHOR CONTRIBUTIONS

WX, ZX, and DZ recruited the patients. GJ, LW, and PH recruited the normal controls. ECT was performed by YC. XH and YY scanned the participants. QW, TB, and YC completed the clinical evaluation and cognitive tests, analyzed the data, and wrote the manuscript. YT and KW designed the study.

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REFERENCES

Abbott, C. C., Jones, T., Lemke, N. T., Gallegos, P., McClintock, S. M., Mayer, A. R., et al. (2014). Hippocampal structural and functional changes associated with electroconvulsive therapy response. *Transl. Psychiatry* 4:e483. doi: 10.1038/tp.2014.124

Achard, S., Salvador, R., Whitcher, B., Suckling, J., and Bullmore, E. (2006). A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. *J. Neurosci.* 26, 63–72. doi: 10.1523/JNEUROSCI.3874-05.2006

Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., Liu, H., Hedden, T., et al. (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J. Neurosci.* 29, 1860–1873. doi: 10.1523/JNEUROSCI.5062-08.2009

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. doi: 10.1038/nrn2575

Cabeza, R., Ciaramelli, E., Olson, I. R., and Moscovitch, M. (2008). The parietal cortex and episodic memory: an attentional account. *Nat. Rev. Neurosci*. 9, 613–625. doi: 10.1038/nrn2459

Cano, M., Cardoner, N., Urretavizcaya, M., Martinez-Zalacain, I., Goldberg, X., Via, E., et al. (2016). Modulation of limbic and prefrontal connectivity by electroconvulsive therapy in treatment-resistant depression: a preliminary study. *Brain stimul.* 9, 65–71. doi: 10.1016/j.brs.2015.08.016

Chao-Gan, Y., and Yu-Feng, Z. (2010). DPARSF: a matlab toolbox for "pipeline" data analysis of resting-state fMRI. *Front. Syst. Neurosci.* 4:13. doi: 10.3389/fnsys.2010.00013

Gauthier, C. T., Duyme, M., Zanca, M., and Capron, C. (2009). Sex and performance level effects on brain activation during a verbal fluency task: a functional magnetic resonance imaging study. *Cortex* 45, 164–176. doi: 10.1016/j.cortex.2007.09.006

Grasse, D. W., Karunakaran, S., and Moxon, K. A. (2013). Neuronal synchrony and the transition to spontaneous seizures. *Exp. Neurol.* 248, 72–84. doi: 10.1016/j.expneurol.2013.05.004

He, Y., and Evans, A. (2010). Graph theoretical modeling of brain connectivity. *Curr. Opin. Neurol*. 23, 341–350.

Husain, S. S., Kevan, I. M., Linnell, R., and Scott, A. I. (2004). Electroconvulsive therapy in depressive illness that has not responded to drug treatment. *J. Affect. Disord*. 83, 121–126. doi: 10.1016/j.jad.2004.05.006

Lai, C. H., Wu, Y. T., and Hou, Y. M. (2017). Functional network-based statistics in depression: theory of mind subnetwork and importance of parietal region. *J. Affect. Disord*. 217, 132–137. doi: 10.1016/j.jad.2017.03.073

Lan, C. C., Tsai, S. J., Huang, C. C., Wang, Y. H., Chen, T. R., Yeh, H. L., et al. (2015). Functional connectivity density mapping of depressive symptoms and loneliness in non-demented elderly male. *Front. Aging Neurosci.* 7:251. doi: 10.3389/fnagi.2015.00251

Lee, Y. J., Kim, S., Gwak, A. R., Kim, S. J., Kang, S. G., Na, K. S., et al. (2016). Decreased regional gray matter volume in suicide attempters compared to suicide non-attempters with major depressive disorders. *Compr. Psychiatry* 67, 59–65. doi: 10.1016/j.comppsych.2016.02.013

Liang, X., Zou, Q., He, Y., and Yang, Y. (2013). Coupling of functional connectivity and regional cerebral blood flow reveals a physiological basis for network hubs of the human brain. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1929–1934. doi: 10.1073/pnas.1214900110

Lisanby, S. H. (2007). Electroconvulsive therapy for depression. *N. Engl. J. Med*. 357, 1939–1945. doi: 10.1056/NEJMct075234

Mears, D., and Pollard, H. B. (2016). Network science and the human brain: using graph theory to understand the brain and one of its hubs, the amygdala, in health and disease. *J. Neurosci. Res*. 94, 590–605. doi: 10.1002/jnr.23705

Perrin, J. S., Merz, S., Bennett, D. M., Currie, J., Steele, D. J., et al. (2012). Electroconvulsive therapy reduces frontal cortical connectivity in severe depressive disorder. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5464–5468. doi: 10.1073/pnas.1117206109

Reineberg, A. E., Andrews-Hanna, J. R., Depue, B. E., Friedman, N. P., and Banich, M. T. (2015). Resting-state networks predict individual differences in common and specific aspects of executive function. *Neuroimage* 104, 69–78. doi: 10.1016/j.neuroimage.2014.09.045

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., et al. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am. J. Psychiatry* 163, 1905–1917. doi: 10.1176/ajp.2006.163.11.1905

Sartorius, A., Demirakca, T., Bohringer, A., Clemm von Hohenberg, C., Aksay, S. S., Bumb, J. M., et al. (2016). Electroconvulsive therapy increases temporal gray matter volume and cortical thickness. *Eur. Neuropsychopharmacol.* 26, 506–517. doi: 10.1016/j.euroneuro.2015.12.036

Semkovska, M., and McLoughlin, D. M. (2010). Objective cognitive performance associated with electroconvulsive therapy for depression: a systematic review and meta-analysis. *Biol. psychiatry* 68, 568–577. doi: 10.1016/j.biopsych.2010.06.009

Song, X. W., Dong, Z. Y., Long, X. Y., Li, S. F., Zuo, X. N., Zhu, C. Z., et al. (2011). REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS One* 6:e25031. doi: 10.1371/journal.pone.0025031

Staresina, B. P., and Davachi, L. (2006). Differential encoding mechanisms for subsequent associative recognition and free recall. *J. Neurosci*. 26, 9162–9172. doi: 10.1523/JNEUROSCI.2877-06.2006

Ustun, T. B., Ayuso-Mateos, J. L., Chatterji, S., Mathers, C., and Murray, C. J. (2004). Global burden of depressive disorders in the year 2000. *Br. J. Psychiatry* 184, 386–392. doi: 10.1192/bjp.184.5.386

van den Heuvel, M. P., and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci*. 17, 683–696. doi: 10.1016/j.tics.2013.09.012

van Waarde, J. A., Scholte, H. S., van Oudheusden, L. J., Verwey, B., Denys, D., and van Wingen, G. A. (2015). A functional MRI marker may predict the outcome of electroconvulsive therapy in severe and treatment-resistant depression. *Mol. Psychiatry* 20, 609–614. doi: 10.1038/mp.2014.78

Wang, L., Xia, M., Li, K., Zeng, Y., Su, Y., Dai, W., et al. (2015). The effects of antidepressant treatment on resting-state functional brain networks in patients with major depressive disorder. *Hum. Brain Mapp.* 36, 768–778. doi: 10.1002/hbm.22663

Wu, X., Lin, P., Yang, J., Song, H., Yang, R., and Yang, J. (2016). Dysfunction of the cingulo-opercular network in first-episode medication-naive patients with major depressive disorder. *J. Affect. Disord.* 200, 275–283. doi: 10.1016/j.jad.2016.04.046

Zou, K., Gao, Q., Long, Z., Xu, F., Sun, X., Chen, H., et al. (2016). Abnormal functional connectivity density in first-episode, drug-naive adult patients with major depressive disorder. *J. Affect. Disord.* 194, 153–158. doi: 10.1016/j.jad.2015.12.081

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Abnormal Global Functional Connectivity Patterns in Medication-Free Major Depressive Disorder

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Mounting studies have applied resting-state functional magnetic resonance imaging (rs-fMRI) to study major depressive disorder (MDD) and have identified abnormal functional activities. However, how the global functional connectivity patterns change in MDD is still unknown. Using rs-fMRI, we investigated the alterations of global resting-state functional connectivity (RSFC) patterns in MDD using weighted global brain connectivity (wGBC) method. First, a whole brain voxel-wise wGBC map was calculated for 23 MDD patients and 34 healthy controls. Two-sample *t*-tests were applied to compare the wGBC and RSFC maps and the significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$. MDD patients showed significantly decreased wGBC in left temporal pole (TP) and increased wGBC in right parahippocampus (PHC). Subsequent RSFC analyses showed decreased functional interaction between TP and right posterior superior temporal cortex and increased functional interaction between PHC and right inferior frontal gyrus in MDD patients. These results revealed the abnormal global FC patterns and its corresponding disrupted functional connectivity in MDD. Our findings present new evidence for the functional interruption in MDD.

Keywords: major depressive disorder, fMRI, resting-state, global brain connectivity, functional connectivity

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INTRODUCTION

Major depressive disorder (MDD) is a highly prevalent and worldwide psychiatric disorder causing severe societal and familial burdens (Mathers and Loncar, 2006). Brain structural changes, including gray matter volume of insula, amygdala, hippocampus, frontal and temporal cortex (Bora et al., 2012; Wang et al., 2017b), and surface morphological properties of hippocampus and amygdala have been widely reported in MDD patients (Chen et al., 2016). In addition, altered structural covariance between angular gyrus and amygdala, posterior cingulate cortex in MDD is also observed (Chen et al., 2017; Wu et al., 2017). Using resting-state functional magnetic resonance imaging (rs-fMRI), abnormal local brain activities in precuneus, cerebellum, lingual gyrus and inter-regional functional connectivity between subgenal anterior cingulate cortex and temporal cortex, between insula and thalamus, inferior parietal cortex, and between intraparietal sulcus and superior temporal gyrus (STG) were also identified (Wu et al., 2016a; Wang et al., 2017a,c; Sun et al., 2018; Wang J. et al., 2018). Moreover, using graph-theory method, disrupted whole brain

functional topological organization of network has also been found (Gong and He, 2015). All these studies suggested that structure and function have changed in MDD. However, how and where the global functional connectivity patterns changed in MDD remains unclear.

A large number of literatures have revealed that brain function was constrained by its connectivity patterns (Passingham et al., 2002; Fan et al., 2014, 2016; Zhang et al., 2014; Wu et al., 2016b; Yang et al., 2016). A recently developed weighted global brain connectivity (wGCB) method can search for the global functional connectivity patterns based on resting-state functional connectivity (RSFC) MRI (Cole et al., 2010). Resting-state fMRI is a non-invasive way to study the functional interactions between different brain areas (Biswal et al., 1995; Wang et al., 2012, 2015a; Xu et al., 2015; Zhang et al., 2016; Wang L. et al., 2018). Resting-state fMRI has been widely adopted to characterize functional connectivity patterns to identify intrinsic functional modules (Fox et al., 2006; Buckner et al., 2009; Power et al., 2011; Yeo et al., 2011; Cole et al., 2014; Wang et al., 2015b, 2016b, 2017d; Mears and Pollard, 2016). It has also been applied to explore the abnormal functional couplings between brain areas to delineate brain intrinsic functional changes in disorders (Wang et al., 2016a; Wu et al., 2016c; Liu et al., 2018). wGCB can reveal global changes in the connectivity of a brain region by searching for globally connected or disconnected brain regions using a data-driven manner (Cole et al., 2010). Unlike the traditional seed-based or independent component analysis methods which can merely identify same spatial patterns of connectivity across subjects, the wGBC is less likely to be affected by within-region and between-subject spatial variations in connectivity patterns (Cole et al., 2011). Moreover, compared to unweighted GBC, wGBC does not need to threshold the connectivity strengths and can reveal globally connected regions with many low-strength connections removed by unweighted GBC thresholding. Therefore, wGCB provides a new way to study altered global functional connectivities to identify pathophysiology of MDD.

In this study, using resting-state fMRI, we examined the potentially abnormal global brain connectivity patterns and corresponding functional connectivity changes in 23 MDD patients and 34 gender-, age-, and education level-matched healthy controls (HC). First, we computed a voxel-wise wGCB maps for both MDD and HC to identify the abnormal global functional connectivities in MDD. Subsequently, we calculated the RSFC of the brain regions with changed wGCB to further reveal altered functional interactions in MDD.

MATERIALS AND METHODS

Subjects

Twenty-three right-handed medication-free MDD patients and 34 right-handed HC subjects were recruited at the Department of Psychiatry at the Affiliated Brain Hospital of Guangzhou Medical University. The detailed information for MDD and HC subjects can be found in **Table 1**. MDD diagnosis was performed based on the Structured Clinical Interview of DSM-IV (SCID) criteria with 24-item Hamilton Depression Rating Scale. The HC subjects were recruited with SCID Non-Patient Edition. All the included MDD and HC subjects were out of serious medical, surgical illness, history of seizures, substance abuse, head trauma, and contraindications for MRI. All the subjects signed the written informed consent. All the experiments were approved by the ethics committees of the Affiliated Brain Hospital of Guangzhou Medical University.

Resting-State fMRI Data Acquisition

Resting-state fMRI data were acquired using an eight-channel 3.0-Tesla Philips MR scanner (Achieva X-series, the Netherlands) in the Department of Radiology, the Affiliated Brain Hospital of Guangzhou Medical University, China. The foam padding and earplugs were used to reduce head motion and to muffle scanner noise, respectively. During scanning, all the subjects were asked to stay awake, close their eyes, and think nothing. Resting-state fMRI data were scanned using an echo planar imaging sequence with the following parameters: repetition time = 2000 ms, echo time = 30 ms, flip angle = 90^0, field of view = 220 × 220 mm^2, matrix = 64 × 64, 33 slices, slice thickness = 4 mm with 0.6 mm gap, and 240 volumes. The resting-state fMRI data have been used in our previous studies (Wu et al., 2016a; Wang et al., 2017a).

Resting-State fMRI Data Pre-processing

The resting-state data were pre-processed using SPM8 toolkit[1]. The pre-processing includes the following steps. discarding the first 10 volumes; slice timing; head motion correction; normalizing to MNI space; regressing motion parameters, white matter, cerebrospinal fluid, and global signals; and filtering with a temporal band-path of 0.01–0.1 Hz. To exclude the head motion effects, resting-state fMRI images with head-movement exceeded 1.5 mm of translation or 1.5 degrees of rotation in any direction were discarded if. In addition, "Scrubbing" method was also used to further reduce the effects of head motion if the frame displacement (FD) > 0.5 (Power et al., 2012). In our study, no

[1]https://www.fil.ion.ucl.ac.uk/spm/

TABLE 1 | Demographics and clinical characteristics of the subjects used in present study.

Subjects	MDD	HC	*p*-value
Number of subjects	23	34	
Gender (male: female)	9/14	15/19	0.71
Age (mean ± SD)	30.48 ± 7.13	29.71 ± 7.09	0.69
Years of education (mean ± SD)	13.35 ± 3.89	14.18 ± 2.17	0.31
HDRS scores (mean ± SD)	34.30 ± 7.58		
Age of onset (years)	27 ± 7.44		
Duration of illness(months)	43.04 ± 58.18		
Episodes (*n*, patients)			
First	17		
Recurrence	6		
Family history of MDD (*n*, patients)	5		

A Pearson chi-squared test was used for gender comparison. Two-sample t-tests were used for age, education comparisons. HDRS, Hamilton Depression Rating Scale scores; MDD, major depression disorder; HC, healthy control.

frame was deleted because all subjects' FD values were smaller than 0.3. For RSFC analyses, the resting-state data were first smoothed (6 mm FWHM) after normalization and then for the following pre-processing. We did not regress out the global signal to obtain reliable results because global signal regression will exaggerate anti-correlation.

wGCB Analysis

The calculation of voxel-wise wGCB map was constrained by a gray matter mask with gray matter probability value > 0.2 (Wu et al., 2016a). The wGBC was calculated as the following steps. First, each voxel of the gray mask was selected as the seed voxel. Next, Pearson's correlations coefficient was calculated between each seed voxel and each of the whole brain voxel and transformed to z value using Fisher's z transformation. Then, all the correlations were averaged and transformed back to r value for this voxel presenting the average connectivity (Cole et al., 2010). Using the same procedure, a whole brain wGCB map was obtained for each subject and smoothed using a 6 mm FWHM Gaussian kernel before statistical analyses. The distributions of wGCB in HC and MDD subjects were identified using one-sample t-tests and the significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$.

To determine the group differences in wGCB, the two-sample t-test was used to compare the wGCB maps between HC and MDD patients with age, gender, and education as covariates. The significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$ after using a cluster-level Monte Carlo simulation with 5000 times.

Functional Connectivity Analyses

Whole brain RSFC analyses were used to determine the changed functional connectivity of the brain regions with changed wGCB between MDD and HC. Functional connectivity was computed and transformed to z value using Fisher's z transformation for each subject. A two-sample t-test was used to compare the functional connectivity maps between HC and MDD patients with age, gender, and education as covariates. The significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$.

RESULTS

Demographics and Clinical Characteristics

A chi-squared test and two-sample t-tests found that there were no significant differences in gender ($p = 0.71$), age ($p = 0.69$), and education level ($p = 0.31$) between HC and MDD groups.

wGCB Distribution in MDD and HC

Spatial distribution patterns of wGCB in MDD and HC found that the high wGCB were primarily located in the STG, lateral occipital gyrus, fusiform gyrus, intraparietal sulcus, medial temporal lobe, middle cingulate cortex, caudate, medial frontal cortex, lateral prefrontal cortex, inferior frontal cortex, and insula (**Figure 1**).

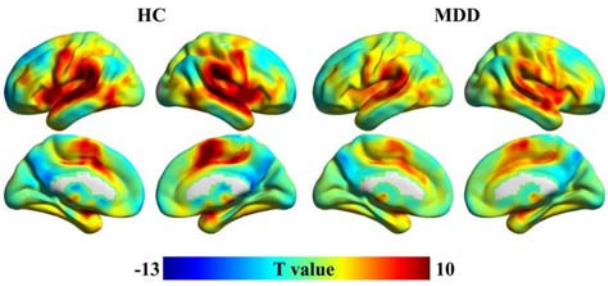

FIGURE 1 | One-sample t-tests were used to identify the distribution of weighted global brain connectivity (wGCB) in major depressive disorder (MDD) and healthy controls (HC). The high wGCB were primarily detected in the superior temporal gyrus, lateral occipital gyrus, fusiform gyrus, intraparietal sulcus, medial temporal lobe, middle cingulate cortex, caudate, medial frontal cortex, lateral prefrontal cortex, inferior frontal cortex, and insula.

Changed wGCB in MDD

A two-sample t-test (the significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$) was applied to compare the wGBC and found significantly decreased wGCB in left temporal pole (TP) (peak MNI coordinate: [−48 18 −3], 91 voxels) and significantly increased wGBC in right parahippocampus (PHC) (peak MNI coordinate: [21 −12 −21], 55 voxels) in MDD patients (**Figure 2**).

Altered Functional Connectivities

A two-sample t-test (the significant level was set at $p < 0.05$, cluster-level correction with voxel-level $p < 0.001$) was applied to compare the whole brain RSFC maps and identified significantly

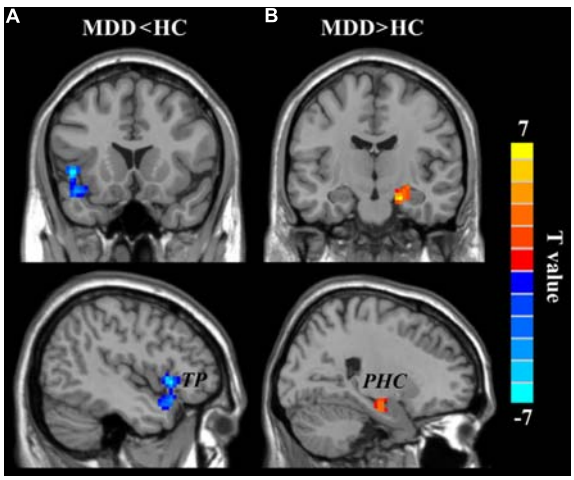

FIGURE 2 | The changed weighted global brain connectivity (wGCB) in major depressive disorder (MDD) patients. Two-sample t-test was used to compare the wGCB maps between healthy controls (HC) and MDD patients and identified **(A)** decreased wGCB in left temporal pole (TP) and **(B)** increased wGCB in right parahippocampus (PHC). The significance was determined using a cluster-level Monte Carlo simulation (5000 times) corrected threshold of $p < 0.05$ (cluster-forming threshold at voxel-level $p < 0.001$).

decreased functional connection between left TP and right posterior superior temporal gyrus (STG: peak MNI coordinate: [63 −15 3], 139 voxels) and significantly increased functional connection between right PHC and right inferior frontal gyrus (IFG: peak MNI coordinate: [42 3 24], 100 voxels) in MDD compared to HC (**Figure 3**).

DISCUSSION

In this study, we studied the changed global functional connectivity in MDD using wGCB method. wGCB analysis revealed decreased global functional connectivities in left TP and increased global functional connectivities in right PHC in MDD. The following functional connectivity analyses found decreased functional connectivity between left TP and right STG and increased functional connectivity between right PHC and right IFG in MDD. These findings suggested that abnormal emotion regulation and memory circuits play an important role in neuropathology of MDD.

Temporal pole and STG have been widely reported to be implicated in emotional processing and social cognition (Olson et al., 2007; Olson et al., 2013). TP is traditionally considered to participate in multimodal sensory integration (Skipper et al., 2011; Visser et al., 2012), but more and more studies have demonstrated that TP is also implicated in various high order cognitive functions, including face recognition (Olson et al., 2007), memory (Munoz-Lopez et al., 2010), and language processing (Hickok and Poeppel, 2007). The lateral TP which mainly connected with amygdala and orbital frontal cortex plays an important role in emotion regulation and theory of mind and is taken as a structure of emotional and social brain (Frith and Frith, 2010). The STG has also been reported to take part in emotional processing and social perception, especially the representation of emotional information during the initial stages of emotional regulation (Allison et al., 2000; Olsson and Ochsner, 2008). Structural and functional abnormalities of TP and STG in MDD were observed. Increased cortical thickness and decreased gray matter density of TP were identified in MDD (Fallucca et al., 2011; Peng et al., 2011; Igata et al., 2017). Abnormal functional activation of TP in MDD during sad emotion processing is also found (Beauregard et al., 2006; Keedwell et al., 2009). In STG, decreased gray matter volume and abnormal activity during sad response in MDD are also found (Fitzgerald et al., 2008; Takahashi et al., 2010). These studies indicated that TP and STG are two important nodes of affective network in emotion regulation. The decreased functional connectivity between TP and STG found in our study suggested that disconnectivity results in dysfunction of initial regulation of negative emotion in MDD patients.

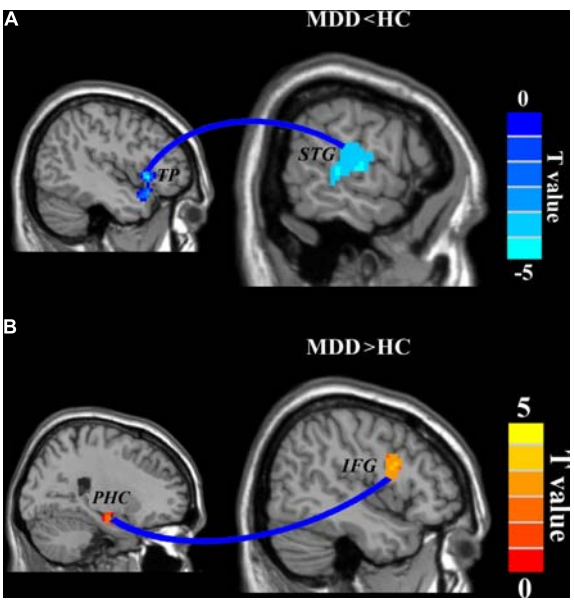

FIGURE 3 | Disrupted functional connectivities in major depressive disorder (MDD) patients. Two-sample *t*-tests were used to identify the significant differences in functional connectivity between MDD and healthy control groups. **(A)** Significantly decreased functional connectivity between left temporal pole and right superior temporal gyrus and **(B)** significantly increased functional connectivity between right parahippocampus and right inferior frontal gyrus were found. The significance was determined using a cluster-level Monte Carlo simulation (5000 times) corrected threshold of $p < 0.05$ (cluster-forming threshold at voxel-level $p < 0.001$).

The PHC which is an interface area between the hippocampus and the neocortex mainly takes part in memory function (Squire et al., 2004). PHC is also involved in recognition of emotional faces or scenes (Fitzgerald et al., 2008; Nummenmaa et al., 2008; Sabatinelli et al., 2011). The IFG plays a role in mood regulation (Baker et al., 1997; Northoff et al., 2000), associative emotional memory (Bookheimer, 2002; Price, 2003), and integrating emotional information and regulating the intensity of emotional responses (Cabeza and Nyberg, 2000; Fuster, 2001). PHC has been widely reported with decreased gray matter volume (Bora et al., 2012; Zhou et al., 2016), abnormal involvement during emotion and memory processing (Surguladze et al., 2005; Garrett et al., 2011; Palmer et al., 2014; Zamoscik et al., 2014), and damaged functional connectivity (Zeng et al., 2012). In our study, we found increased wGCB of PHC which is contrast to the gray matter volume changes. The inconsistency mainly results from gray matter volume and wGCB characterizing different properties of PHC. Structural and functional measurements of PHC may provide complementary evidence to better elucidate the role of PHC in MDD. During emotion processing in bipolar disorder during mania, hypoactivation of the IFG was observed during processing of negative faces (Altshuler et al., 2005), fear perception (Killgore et al., 2008), and negatively captioned pictures (Malhi et al., 2004). Functional disconnections of IFG have also been found in many previous studies (Murray et al., 2011; Tao et al., 2013). Moreover, reduced right IFG gray matter volume was found in MDD patients (Sabatinelli et al., 2011). All these studies suggested important roles of PHC and IFG in the pathology of MDD. In our study, we found increased functional connectivity between IFG and PHC in MDD patients compared to healthy controls. The hyperconnectivity between IFG and PHC indicated MDD patients need more efforts to inhibit negative emotion.

There are some limitations in this study. First, correlation analyses did not find significant associations between changed neuroimaging indices and HRSD scores. Thus, the conclusion needs to be further validated. Second, our samples are very small and these findings also need to be validated in a larger sample.

In conclusion, we used wGCB and functional connectivity analyses revealed abnormal global connectivity patterns in TP and PHC, and abnormal functional interactions between TP and STG, and between PHC and IFG. All these brain areas are parts of affective network and emotion regulation network. Our findings suggested that abnormal functional connectivity patterns of the two networks contribute to the pathology of MDD. The current findings will provide an important reference for future MDD therapy, including deep brain stimulation and transcranial magnetic stimulation.

AUTHOR CONTRIBUTIONS

JX and JS designed this study and revised the manuscript. HW collected the data. LZ analyzed the data and wrote the manuscript. All the authors discussed the results.

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REFERENCES

Allison, T., Puce, A., and McCarthy, G. (2000). Social perception from visual cues: role of the STS region. *Trends Cogn. Sci.* 4, 267–278. doi: 10.1016/S1364-6613(00)01501-1

Altshuler, L., Bookheimer, S., Proenza, M. A., Townsend, J., Sabb, F., Firestine, A., et al. (2005). Increased amygdala activation during mania: a functional magnetic resonance imaging study. *Am. J. Psychiatry* 162, 1211–1213. doi: 10.1176/appi.ajp.162.6.1211

Baker, S. C., Frith, C. D., and Dolan, R. J. (1997). The interaction between mood and cognitive function studied with PET. *Psychol. Med.* 27, 565–578. doi: 10.1017/S0033291797004856

Beauregard, M., Paquette, V., and Levesque, J. (2006). Dysfunction in the neural circuitry of emotional self-regulation in major depressive disorder. *Neuroreport* 17, 843–846. doi: 10.1097/01.wnr.0000220132.32091.9f

Biswal, B., Yetkin, F. Z., Haughton, V. M., and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn. Reson. Med.* 34, 537–541. doi: 10.1002/mrm.1910340409

Bookheimer, S. (2002). Functional MRI of language: new approaches to understanding the cortical organization of semantic processing. *Annu. Rev. Neurosci.* 25, 151–188. doi: 10.1146/annurev.neuro.25.112701.142946

Bora, E., Fornito, A., Pantelis, C., and Yucel, M. (2012). Gray matter abnormalities in major depressive disorder: a meta-analysis of voxel based morphometry studies. *J. Affect. Disord.* 138, 9–18. doi: 10.1016/j.jad.2011.03.049

Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., Liu, H., Hedden, T., et al. (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J. Neurosci.* 29, 1860–1873. doi: 10.1523/JNEUROSCI.5062-08.2009

Cabeza, R., and Nyberg, L. (2000). Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J. Cogn. Neurosci.* 12, 1–47. doi: 10.1162/08989290051137585

Chen, T., Kendrick, K. M., Wang, J., Wu, M., Li, K., Huang, X., et al. (2017). Anomalous single-subject based morphological cortical networks in drug-naive, first-episode major depressive disorder. *Hum. Brain Mapp.* 38, 2482–2494. doi: 10.1002/hbm.23534

Chen, V. C., Shen, C. Y., Liang, S. H., Li, Z. H., Tyan, Y. S., Liao, Y. T., et al. (2016). Assessment of abnormal brain structures and networks in major depressive disorder using morphometric and connectome analyses. *J. Affect. Disord.* 205, 103–111. doi: 10.1016/j.jad.2016.06.066

Cole, M. W., Anticevic, A., Repovs, G., and Barch, D. (2011). Variable global dysconnectivity and individual differences in schizophrenia. *Biol. Psychiatry* 70, 43–50. doi: 10.1016/j.biopsych.2011.02.010

Cole, M. W., Bassett, D. S., Power, J. D., Braver, T. S., and Petersen, S. E. (2014). Intrinsic and task-evoked network architectures of the human brain. *Neuron* 83, 238–251. doi: 10.1016/j.neuron.2014.05.014

Cole, M. W., Pathak, S., and Schneider, W. (2010). Identifying the brain's most globally connected regions. *Neuroimage* 49, 3132–3148. doi: 10.1016/j.neuroimage.2009.11.001

Fallucca, E., MacMaster, F. P., Haddad, J., Easter, P., Dick, R., May, G., et al. (2011). Distinguishing between major depressive disorder and obsessive-compulsive disorder in children by measuring regional cortical thickness. *Arch. Gen. Psychiatry* 68, 527–533. doi: 10.1001/archgenpsychiatry.2011.36

Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., et al. (2016). The human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb. Cortex* 26, 3508–3526. doi: 10.1093/cercor/bhw157

Fan, L., Wang, J., Zhang, Y., Han, W., Yu, C., and Jiang, T. (2014). Connectivity-based parcellation of the human temporal pole using diffusion tensor imaging. *Cereb. Cortex* 24, 3365–3378. doi: 10.1093/cercor/bht196

Fitzgerald, P. B., Laird, A. R., Maller, J., and Daskalakis, Z. J. (2008). A meta-analytic study of changes in brain activation in depression. *Hum. Brain Mapp.* 29, 683–695. doi: 10.1002/hbm.20426

Fox, M. D., Corbetta, M., Snyder, A. Z., Vincent, J. L., and Raichle, M. E. (2006). Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10046–10051. doi: 10.1073/pnas.0604187103

Frith, U., and Frith, C. (2010). The social brain: allowing humans to boldly go where no other species has been. *Philos. Trans. R. Soc. London B Biol. Sci.* 365, 165–176. doi: 10.1098/rstb.2009.0160

Fuster, J. M. (2001). The prefrontal cortex—an update: time is of the essence. *Neuron* 30, 319–333. doi: 10.1016/S0896-6273(01)00285-9

Garrett, A., Kelly, R., Gomez, R., Keller, J., Schatzberg, A. F., and Reiss, A. L. (2011). Aberrant brain activation during a working memory task in psychotic major depression. *Am. J. Psychiatry* 168, 173–182. doi: 10.1176/appi.ajp.2010.09121718

Gong, Q., and He, Y. (2015). Depression, neuroimaging and connectomics: a selective overview. *Biol. Psychiatry* 77, 223–235. doi: 10.1016/j.biopsych.2014.08.009

Hickok, G., and Poeppel, D. (2007). The cortical organization of speech processing. *Nat. Rev. Neurosci.* 8, 393–402. doi: 10.1038/nrn2113

Igata, R., Katsuki, A., Kakeda, S., Watanabe, K., Igata, N., Hori, H., et al. (2017). PCLO rs2522833-mediated gray matter volume reduction in patients with drug-naive, first-episode major depressive disorder. *Transl. Psychiatry* 7, e1140. doi: 10.1038/tp.2017.100

Keedwell, P., Drapier, D., Surguladze, S., Giampietro, V., Brammer, M., and Phillips, M. (2009). Neural markers of symptomatic improvement during antidepressant therapy in severe depression: subgenual cingulate and visual cortical responses to sad, but not happy, facial stimuli are correlated with changes in symptom score. *J. Psychopharmacol.* 23, 775–788. doi: 10.1177/0269881108093589

Killgore, W. D., Gruber, S. A., and Yurgelun-Todd, D. A. (2008). Abnormal corticostriatal activity during fear perception in bipolar disorder. *Neuroreport* 19, 1523–1527. doi: 10.1097/WNR.0b013e328310af58

Liu, C., Wang, J., Hou, Y., Qi, Z., Wang, L., Zhan, S., et al. (2018). Mapping the changed hubs and corresponding functional connectivity in idiopathic restless legs syndrome. *Sleep Med.* 45, 132–139. doi: 10.1016/j.sleep.2017.12.016

Malhi, G. S., Lagopoulos, J., Ward, P. B., Kumari, V., Mitchell, P. B., Parker, G. B., et al. (2004). Cognitive generation of affect in bipolar depression: an fMRI study. *Eur. J. Neurosci.* 19, 741–754. doi: 10.1111/j.0953-816X.2003.03159.x

Mathers, C. D., and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 3:e442. doi: 10.1371/journal.pmed.0030442

Mears, D., and Pollard, H. B. (2016). Network science and the human brain: using graph theory to understand the brain and one of its hubs, the amygdala, in health and disease. *J. Neurosci. Res.* 94, 590–605. doi: 10.1002/jnr.23705

Munoz-Lopez, M. M., Mohedano-Moriano, A., and Insausti, R. (2010). Anatomical pathways for auditory memory in primates. *Front. Neuroanat.* 4:129. doi: 10.3389/fnana.2010.00129

Murray, E. A., Wise, S. P., and Drevets, W. C. (2011). Localization of dysfunction in major depressive disorder: prefrontal cortex and amygdala. *Biol. Psychiatry* 69, e43–e54. doi: 10.1016/j.biopsych.2010.09.041

Northoff, G., Richter, A., Gessner, M., Schlagenhauf, F., Fell, J., Baumgart, F., et al. (2000). Functional dissociation between medial and lateral prefrontal cortical spatiotemporal activation in negative and positive emotions: a combined fMRI/MEG study. *Cereb. Cortex* 10, 93–107. doi: 10.1093/cercor/10.1.93

Nummenmaa, L., Hirvonen, J., Parkkola, R., and Hietanen, J. K. (2008). Is emotional contagion special? An fMRI study on neural systems for affective and cognitive empathy. *Neuroimage* 43, 571–580. doi: 10.1016/j.neuroimage.2008.08.014

Olson, I. R., McCoy, D., Klobusicky, E., and Ross, L. A. (2013). Social cognition and the anterior temporal lobes: a review and theoretical framework. *Soc. Cogn. Affect. Neurosci.* 8, 123–133. doi: 10.1093/scan/nss119

Olson, I. R., Plotzker, A., and Ezzyat, Y. (2007). The enigmatic temporal pole: a review of findings on social and emotional processing. *Brain* 130, 1718–1731. doi: 10.1093/brain/awm052

Olsson, A., and Ochsner, K. N. (2008). The role of social cognition in emotion. *Trends Cogn. Sci.* 12, 65–71. doi: 10.1016/j.tics.2007.11.010

Palmer, S. M., Crewther, S. G., Carey, L. M., and Team, S. P. (2014). A meta-analysis of changes in brain activity in clinical depression. *Front. Hum. Neurosci.* 8:1045. doi: 10.3389/fnhum.2014.01045

Passingham, R. E., Stephan, K. E., and Kotter, R. (2002). The anatomical basis of functional localization in the cortex. *Nat. Rev. Neurosci.* 3, 606–616. doi: 10.1038/nrn893

Peng, J., Liu, J., Nie, B., Li, Y., Shan, B., Wang, G., et al. (2011). Cerebral and cerebellar gray matter reduction in first-episode patients with major depressive disorder: a voxel-based morphometry study. *Eur. J. Radiol.* 80, 395–399. doi: 10.1016/j.ejrad.2010.04.006

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., and Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 59, 2142–2154. doi: 10.1016/j.neuroimage.2011.10.018

Power, J. D., Cohen, A. L., Nelson, S. M., Wig, G. S., Barnes, K. A., Church, J. A., et al. (2011). Functional network organization of the human brain. *Neuron* 72, 665–678. doi: 10.1016/j.neuron.2011.09.006

Price, J. L. (2003). Comparative aspects of amygdala connectivity. *Ann. N. Y. Acad. Sci.* 985, 50–58. doi: 10.1111/j.1749-6632.2003.tb07070.x

Sabatinelli, D., Fortune, E. E., Li, Q., Siddiqui, A., Krafft, C., Oliver, W. T., et al. (2011). Emotional perception: meta-analyses of face and natural scene processing. *Neuroimage* 54, 2524–2533. doi: 10.1016/j.neuroimage.2010.10.011

Skipper, L. M., Ross, L. A., and Olson, I. R. (2011). Sensory and semantic category subdivisions within the anterior temporal lobes. *Neuropsychologia* 49, 3419–3429. doi: 10.1016/j.neuropsychologia.2011.07.033

Squire, L. R., Stark, C. E., and Clark, R. E. (2004). The medial temporal lobe. *Annu. Rev. Neurosci.* 27, 279–306. doi: 10.1146/annurev.neuro.27.070203.144130

Sun, H., Luo, L., Yuan, X., Zhang, L., He, Y., Yao, S., et al. (2018). Regional homogeneity and functional connectivity patterns in major depressive disorder, cognitive vulnerability to depression and healthy subjects. *J. Affect. Disord.* 235, 229–235. doi: 10.1016/j.jad.2018.04.061

Surguladze, S., Brammer, M. J., Keedwell, P., Giampietro, V., Young, A. W., Travis, M. J., et al. (2005). A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. *Biol. Psychiatry* 57, 201–209. doi: 10.1016/j.biopsych.2004.10.028

Takahashi, T., Yucel, M., Lorenzetti, V., Walterfang, M., Kawasaki, Y., Whittle, S., et al. (2010). An MRI study of the superior temporal subregions in patients with current and past major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 98–103. doi: 10.1016/j.pnpbp.2009.10.005

Tao, H., Guo, S., Ge, T., Kendrick, K. M., Xue, Z., Liu, Z., et al. (2013). Depression uncouples brain hate circuit. *Mol. Psychiatry* 18, 101–111. doi: 10.1038/mp.2011.127

Visser, M., Jefferies, E., Embleton, K. V., and Lambon Ralph, M. A. (2012). Both the middle temporal gyrus and the ventral anterior temporal area are crucial for multimodal semantic processing: distortion-corrected fMRI evidence for a double gradient of information convergence in the temporal lobes. *J. Cogn. Neurosci.* 24, 1766–1778. doi: 10.1162/jocn_a_00244

Wang, C., Wu, H., Chen, F., Xu, J., Li, H., Li, H., et al. (2017a). Disrupted functional connectivity patterns of the insula subregions in drug-free major depressive disorder. *J. Affect. Disord.* 234, 297–304. doi: 10.1016/j.jad.2017.12.033

Wang, J., Wei, Q., Bai, T., Zhou, X., Sun, H., Becker, B., et al. (2017b). Electroconvulsive therapy selectively enhanced feedforward connectivity from fusiform face area to amygdala in major depressive disorder. *Soc. Cogn. Affect. Neurosci.* 12, 1983–1992. doi: 10.1093/scan/nsx100

Wang, J., Wei, Q., Yuan, X., Jiang, X., Xu, J., Zhou, X., et al. (2017c). Local functional connectivity density is closely associated with the response of electroconvulsive therapy in major depressive disorder. *J. Affect. Disord.* 225, 658–664. doi: 10.1016/j.jad.2017.09.001

Wang, J., Xie, S., Guo, X., Becker, B., Fox, P. T., Eickhoff, S. B., et al. (2017d). Correspondent functional topography of the human left inferior parietal lobule at rest and under task revealed using resting-state fMRI and coactivation based parcellation. *Hum. Brain Mapp.* 38, 1659–1675. doi: 10.1002/hbm.23488

Wang, J., Fan, L., Wang, Y., Xu, W., Jiang, T., Fox, P. T., et al. (2015a). Determination of the posterior boundary of Wernicke's area based on multimodal connectivity profiles. *Hum. Brain Mapp.* 36, 1908–1924. doi: 10.1002/hbm.22745

Wang, J., Yang, Y., Fan, L., Xu, J., Li, C., Liu, Y., et al. (2015b). Convergent functional architecture of the superior parietal lobule unraveled with multimodal neuroimaging approaches. *Hum. Brain Mapp.* 36, 238–257. doi: 10.1002/hbm.22626

Wang, J., Fan, L., Zhang, Y., Liu, Y., Jiang, D., Zhang, Y., et al. (2012). Tractography-based parcellation of the human left inferior parietal lobule. *Neuroimage* 63, 641–652. doi: 10.1016/j.neuroimage.2012.07.045

Wang, J., Tian, Y., Wang, M., Cao, L., Wu, H., Zhang, Y., et al. (2016a). A lateralized top-down network for visuospatial attention and neglect. *Brain Imaging Behav.* 10, 1029–1037.

Wang, J., Zhang, J., Rong, M., Wei, X., Zheng, D., Fox, P. T., et al. (2016b). Functional topography of the right inferior parietal lobule structured by anatomical connectivity profiles. *Hum. Brain Mapp.* 37, 4316–4332. doi: 10.1002/hbm.23311

Wang, J., Wei, Q., Wang, L., Zhang, H., Bai, T., Cheng, L., et al. (2018). Functional reorganization of intra- and internetwork connectivity in major depressive disorder after electroconvulsive therapy. *Hum. Brain Mapp.* 39, 1403–1411. doi: 10.1002/hbm.23928

Wang, L., Xu, J., Wang, C., and Wang, J. (2018). Whole brain functional connectivity pattern homogeneity mapping. *Front. Hum. Neurosci.* 12:164. doi: 10.3389/fnhum.2018.00164

Wu, H., Sun, H., Wang, C., Yu, L., Li, Y., Peng, H., et al. (2017). Abnormalities in the structural covariance of emotion regulation networks in major depressive disorder. *J. Psychiatr. Res.* 84, 237–242. doi: 10.1016/j.jpsychires.2016.10.001

Wu, H., Sun, H., Xu, J., Wu, Y., Wang, C., Xiao, J., et al. (2016a). Changed hub and corresponding functional connectivity of subgenual anterior cingulate cortex in major depressive disorder. *Front. Neuroanat.* 10:120. doi: 10.3389/fnana.2016.00120

Wu, Y., Wang, J., Zhang, Y., Zheng, D., Zhang, J., Rong, M., et al. (2016b). The neuroanatomical basis for posterior superior parietal lobule control lateralization of visuospatial attention. *Front. Neuroanat.* 10:32. doi: 10.3389/fnana.2016.00032

Wu, Y., Zhang, Y., Liu, Y., Liu, J., Duan, Y., Wei, X., et al. (2016c). Distinct changes in functional connectivity in posteromedial cortex subregions during

the progress of Alzheimer's disease. *Front. Neuroanat.* 10:41. doi: 10.3389/fnana.2016.00041

Xu, J., Wang, J., Fan, L., Li, H., Zhang, W., Hu, Q., et al. (2015). Tractography-based parcellation of the human middle temporal gyrus. *Sci. Rep.* 5:18883. doi: 10.1038/srep18883

Yang, Y., Fan, L., Chu, C., Zhuo, J., Wang, J., Fox, P. T., et al. (2016). Identifying functional subdivisions in the human brain using meta-analytic activation modeling-based parcellation. *Neuroimage* 124, 300–309. doi: 10.1016/j.neuroimage.2015.08.027

Yeo, B. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M., et al. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J. Neurophysiol.* 106, 1125–1165. doi: 10.1152/jn.00338.2011

Zamoscik, V., Huffziger, S., Ebner-Priemer, U., Kuehner, C., and Kirsch, P. (2014). Increased involvement of the parahippocampal gyri in a sad mood predicts future depressive symptoms. *Soc. Cogn. Affect. Neurosci.* 9, 2034–2040. doi: 10.1093/scan/nsu006

Zeng, L. L., Shen, H., Liu, L., Wang, L., Li, B., Fang, P., et al. (2012). Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis. *Brain* 135, 1498–1507. doi: 10.1093/brain/aws059

Zhang, W., Wang, J., Fan, L., Zhang, Y., Fox, P. T., Eickhoff, S. B., et al. (2016). Functional organization of the fusiform gyrus revealed with connectivity profiles. *Hum. Brain Mapp.* 37, 3003–3016. doi: 10.1002/hbm.23222

Zhang, Y., Fan, L., Zhang, Y., Wang, J., Zhu, M., Zhang, Y., et al. (2014). Connectivity-based parcellation of the human posteromedial cortex. *Cereb. Cortex* 24, 719–727. doi: 10.1093/cercor/bhs353

Zhou, H., Li, R., Ma, Z., Rossi, S., Zhu, X., and Li, J. (2016). Smaller gray matter volume of hippocampus/parahippocampus in elderly people with subthreshold depression: a cross-sectional study. *BMC Psychiatry* 16:219. doi: 10.1186/s12888-016-0928-0

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Assessing Panic: Bridging the Gap Between Fundamental Mechanisms and Daily Life Experience

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Panic disorder (PD) is one of the most common psychiatric disorders. Recurrent, unexpected panic attacks (PAs) are the primary symptom and strongly impact patients' quality of life. Clinical manifestations are very heterogeneous between patients, emphasizing the need for a dimensional classification integrating various aspects of neurobiological and psychological circuits in line with the Research Domain Criteria (RDoC) proposed by the US National Institute of Mental Health. To go beyond data that can be collected in the daily clinical situation, experimental panic provocation is widely used, which has led to important insights into involved brain regions and systems. Genetic variants can determine the sensitivity to experimental models such as carbon dioxide (CO_2) exposure and can increase the risk to develop PD. Recent developments now allow to better assess the dynamic course of PAs outside the laboratory in patients' natural environment. This can provide novel insights into the underlying mechanisms and the influence of environmental factors that can alter gene regulation by changing DNA methylation. In this mini review, we discuss assessment of PAs in the clinic, in the laboratory using CO_2 exposure, genetic associations, and the benefits of real-life assessment and epigenetic research.

Keywords: panic attacks, CO_2 exposure, genetics, DNA methylation, ambulatory assessment

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INTRODUCTION

Panic attacks (PA) are periods of intense fear concomitant with other symptoms such as breathing difficulties and palpitations. PAs are most commonly associated with panic disorder (PD), but are also severity specifiers for all mental disorders in the Diagnostic and Statistical Manual of Mental disorders (DSM-5) (American Psychiatric Association, 2013). Currently, diagnoses of psychiatric disorders are based on the presence of symptoms specified in the DSM. Individuals with very heterogeneous clinical manifestations can meet the required minimum number of symptoms for a disorder and thus receive the same diagnosis and treatment. However, the same treatment might not be equally efficient in these patients. The National Institute of Mental Health [NIMH] (2010) initiated a new research framework, the Research Domain Criteria (RDoC), to classify mental disorders based on dimensions of dysfunction in neurobiological and psychological circuits. By integrating diverse approaches such as molecular circuits and genetics, the translation of fundamental findings to the clinic can be facilitated. It can lead to a better understanding of which functions underlie a specific behavior or symptom, and what degree of dysregulation is associated

with a shift from mental health to subclinical presentation and, ultimately, full disorder level. Eventually, this could support establishing a graded classification that opens new approaches for more precise treatment strategies. This mini review provides an overview of methodologies used to assess PAs in the clinic and in the laboratory focusing on carbon dioxide (CO_2) exposure, and discusses added value and approaches for real-life assessment and epigenetic research.

PANIC ATTACKS IN THE CLINIC

The diagnostic criteria for PAs and PD are specified in the DSM (American Psychiatric Association, 2013) and serve as guideline for clinicians. Currently, PAs are defined as sudden episodes of intense fear or discomfort, reaching a peak within minutes and accompanied by at least four of 13 symptoms: palpitations, sweating, trembling or shaking, breathlessness, feeling of choking, chest pain, nausea, dizziness, chills or heat sensations, paresthesia, derealization or depersonalization, fear of losing control or going crazy, and fear of dying. PD is characterized by recurrent unexpected PAs, followed by at least 1 month of persistent concerns about the occurrence of future attacks or their consequences, and/or strong maladaptive behavioral changes related to the attacks such as avoiding places and situations. Assessment of these symptoms and behavior is classically based on patients' self-reports (interviews and rating scales covering presence and intensity of symptoms, frequency of PAs, impairments in daily life), sometimes completed by clinicians' behavioral observations. Furthermore, a complete psychiatric and medical examination are done to exclude that PAs occur due to, e.g., specific phobia, substance abuse or another medical condition such as thyroid dysfunction. Additional assessment of family history, recent life events, and stress can provide a glance at factors associated with PAs and PD. However, this is insufficient to increase our understanding of molecular alterations and disturbances that make someone vulnerable to develop disorders.

PANIC ATTACKS IN THE LABORATORY

CO_2 Exposure as Experimental Model

To gain insights into the pathophysiology of PAs many studies have made use of experimental panic provocation. Among the many agents used in the laboratory particularly CO_2 exposure is one of the best validated and most widely used models (Leibold et al., 2015). Commonly, CO_2 is administered through a nasal-oral facemask and participants rate their symptoms using questionnaires. Particularly 35% CO_2 effectively induces DSM specified symptoms (Griez, 1984) that are in striking resemblance to real-life PA ones (Griez et al., 1987; Schruers et al., 2004), indicating that an acute disturbance of the acid-base homeostasis might be the mechanism underlying PAs as we previously proposed (Esquivel et al., 2009). PD patients react significantly stronger to CO_2 than patients of many other disorders (Verburg et al., 1994; Perna et al., 1999; Kent et al., 2001) with the exception of post-traumatic stress disorder patients who are also reactive to CO_2 (Muhtz et al., 2011; Kellner et al., 2018). Additionally, social anxiety disorder patients show an intermediate reactivity between healthy individuals and PD patients (Schutters et al., 2012). This suggests that some altered mechanisms are common among these disorders.

Physiology of Panic Attacks

Given the pronounced physiological symptoms of PAs, research has aimed at obtaining detailed insights into bodily changes during attacks. Breathing 35% CO_2 led to an increase in heart rate in some studies (Poonai et al., 2000; Richey et al., 2010), while others observed an overall decrease (Argyropoulos et al., 2002; Kaye et al., 2004; Wetherell et al., 2006). General autonomic arousal is suggested by strong increases in systolic (Wetherell et al., 2006; Richey et al., 2010; Leibold et al., 2013) and diastolic blood pressure (Richey et al., 2010; Leibold et al., 2013). High blood pressure can activate a negative feedback loop, the baroreflex, which causes heart rate to decrease to subsequently lead to a reduction in blood pressure. This might explain the reported heart rate reduction in some studies. Some studies reported increases in heart rate; these varying results might be due to differences in methodology (temporal resolution, age and sex of participants, averaging time points).

CO_2 is a major activator of breathing in mammals and exerts its effects mainly through activation of central chemoreceptors (Rassovsky and Kushner, 2003). Inhaling 35% CO_2 strongly increased minute ventilation and tidal volume (Bystritsky and Shapiro, 1992). No difference in the degree of increase could be found between PD patients and healthy individuals, which, however, could have been masked by relatively long analysis epochs of 15 s. Regarding breathing, a distinct respiratory subtype was described that is characterized by a high number of respiratory symptoms during PAs and a higher response to CO_2 (Freire and Nardi, 2012). Non-medicated patients also had a higher variability in respiration rate and partial CO_2 pressure compared to medicated patients and healthy individuals in the 10 min after the inhalation. Sample sizes were small, but these results might be indicative of a less effective homeostatic control in response to a stimulus such as CO_2 that acutely disturbs the pH balance in PD patients (Niccolai et al., 2008).

Neurobiology of Panic Attacks

In recent years, the field of neurobiology has made significant progress to unravel brain regions and networks involved in PD. PD is classified as anxiety disorder, but research indicates that anxiety, fear, and panic are distinct entities. In a seminal human functional imaging study (Mobbs et al., 2007), individuals underwent a virtual predator paradigm. At a large distance, brain activation was mostly observed in the prefrontal cortex, which is associated with complex risk assessment and approach behavior. With decreasing distance brain activity shifted to the brainstem, a subcortical region associated with faster and more primitive responses such as fight-or-flight behavior. The predominant emotions that can be mapped to this "defensive distance" to a threat are anxiety and fear, respectively (Blanchard and Blanchard, 1990; McNaughton and Corr, 2004). Extending

this model, conceptually, the smallest possible distance to a threat is one coming from within the body.

A threat from within the body can be caused by an acute disbalance in the pH homeostasis. CO_2 is a stimulus that can cause such a brief disturbance. We propose that this triggers panic and is primarily coupled to activation of the primordial brainstem. If PAs are linked to primordial brain regions and fundamental mechanisms, it can be expected that every individual reacts to CO_2. In line with this, we showed that also healthy individuals experience the fear and symptoms associated with PAs, when the dosage is increased compared to PD patients (Schruers et al., 2011; Leibold et al., 2013). This suggests that PD patients are hypersensitive to CO_2. It remains to be determined which basic mechanism shifts physiological CO_2-sensitivity to pathological as in PD. A starting point is provided by a functional imaging study by our group (Goossens et al., 2014); in line with our proposition of the involvement of primordial brain regions, we demonstrated that CO_2 activates the brainstem, in PD to a greater extent than in healthy participants. This indicates that brainstem abnormalities might underlie the hyperreactivity to CO_2 in patients. Previously, the amygdala was proposed as center of the network involved in PD (Gorman et al., 2000), and in determining fear behavior to CO_2 in animal work (Ziemann et al., 2009). However, this concept recently got challenged by research in Urbach-Wiethe patients, who unexpectedly experienced experimental PAs to a CO_2 inhalation despite having a bilaterally damaged amygdala (Feinstein et al., 2013). Our study provides additional evidence that other brain regions such as the brainstem could have a key role. The question remains which neurotransmitter systems and molecules are involved in detecting and reacting to CO_2/pH changes. Among the many neurons sensitive to CO_2/pH (Dean et al., 1990; Mulkey et al., 2004; Richerson, 2004; Williams et al., 2007; Biancardi et al., 2008; Ziemann et al., 2009; da Silva et al., 2010, 2011), in context of PD, obvious candidates are proteins, neurons, and systems related to clinically effective medication. For example, Selective Serotonin Reuptake Inhibitors (SSRIs) target the serotonin transporter (5-HTT), suggesting a role of the 5-HT system in PD. As system functioning is affected by the expression of proteins, genetic research has drawn more attention in recent years.

THE ROLE OF GENETICS

A wealth of data suggests a role of the genome in the pathophysiology of PD. Family studies consistently revealed that the risk for PD is considerably increased in first-degree relatives (Crowe et al., 1983; Harris et al., 1983; Noyes et al., 1986; Maier et al., 1993; Mendlewicz et al., 1993; Weissman, 1993; Goldstein et al., 1994). Additionally, the concordance rate for PD is higher for monozygotic, genetically identical twins than for dizygotic twins, suggesting that the disorder is genetically driven and not by shared environmental factors (Torgersen, 1983; Kendler et al., 1993; Skre et al., 1993; Perna et al., 1997). Further, genetics also affects CO_2-hypersensitivity, as demonstrated by first-degree family member studies, having an intermediate CO_2 response between patients and unrelated healthy controls (Perna et al., 1995; van Beek and Griez, 2000), and twin studies (Bellodi et al., 1998; Battaglia et al., 2007, 2008; Roberson-Nay et al., 2013).

To determine distinct genes that drive the risk for PD, hypothesis-free association and candidate approaches, the latter based on prior evidence for a role in the disease, have been used. Several hundred genes have been studied to date, extensively reviewed previously (Maron et al., 2010). Here, we very briefly focus on the 5-HT system and the amiloride-sensitive cation channel 2 (ACCN2), for which rodent and human work provided support. A length polymorphism in the promoter region of the 5-HTT, the serotonin transporter gene-linked polymorphic region (5-HTTLPR), affects the 5-HTT expression level (Lesch et al., 1996). The short S-allele has a two- to threefold lower expression of the 5-HTT than the long L-allele, associated with a differential 5-HT signaling. While positive associations were reported regarding differential allele frequencies in patients compared to controls (Maron et al., 2005; Talati et al., 2017), other studies (Hamilton et al., 1999; Watanabe et al., 2017), including a meta-analysis (1,025 patients, 1,568 controls) controlling for ethnicity and comorbid agoraphobia could not confirm any association (Blaya et al., 2007). However, some evidence suggests that the S-allele might be associated with more severe symptom severity (Lonsdorf et al., 2009) and the SS genotype with poor health-related quality of life (Kang et al., 2016). Regarding CO_2-reactivity, healthy L-allele carriers of the 5-HTTLPR have a heightened fear response (Schmidt et al., 2000), which increases dose-dependently (Schruers et al., 2011). However, in PD patients, CO_2-reactivity was not affected by genotype (Perna et al., 2004). Yet, in the same study, it was observed that female L-allele carriers did respond better to SSRI treatment. Further, one study reported that no association could be found with the 5-HTTLPR but instead with other variants in the gene encoding the 5-HTT (Strug et al., 2010), suggesting that research should cover the entire gene and not solely focus on the promoter region. Mixed results were also found regarding genes expressing enzymes and receptors related to the 5-HT system. A recent meta-analysis (Howe et al., 2016) reported no association for tryptophan hydroxylase 2 (TPH2), the rate-limiting step in the synthesis of brain 5-HT, the 5HT1a receptor, 5-HT1b receptor, 5-HT2b receptor, and the 5-HT3a receptor. A nominal association was found in females regarding the 5-HT2a receptor and monoamine oxidase A (MAO-A), an enzyme involved in the breakdown of 5-HT, which however did not withstand multiple testing correction.

Furthermore, a role of the ACCN2 system is suggested by both rodent and human work. An association was reported between a variant in the ACCN2 gene and PD, but replication failed in an independent larger sample. However, a very heterogeneous sample with only a subset of PD patients might have masked the effects (Hettema et al., 2008). A later large case-control study reported that a variant was associated with the diagnosis of PD (Smoller et al., 2014). The functional consequences of this variant are unknown; authors speculated that it alters the sensitivity to detect and react to a reduced pH. This is a reasonable speculation as it was shown in rodents that the expressed ion channel is essential for CO_2-induced fear-related behavior (Ziemann et al., 2009). In further support, we provided evidence that the variant

is also associated with a differential response to CO_2 in patients and healthy individuals (Leibold et al., 2017).

Despite the progress in the past few decades, success in genetic research in anxiety disorders such as PD has proven difficult. Mental disorders like PD are very heterogeneous and complex, which make it necessary to study larger samples. One alternative approach is to focus on endophenotypes such as CO_2-hyperreactivity or neurobiological responsiveness that are considered to depend on less genes than a complex disorder and can therefore be better linked to genes (Gottesman and Gould, 2003), thereby increasing the chances of detecting links between genetic variants and disease susceptibility.

Imaging Genetics

Modern neuroimaging techniques allow studying effects of genetic variants on neuronal activation to further examine the pathogenesis of PD.

A common approach is to present pictures with emotional value and to compare induced brain activation, as measured by functional magnetic resonance imaging, to the one caused by viewing neutral stimuli. With regard to the 5-HT system, in a small study with PD patients, no effect of the 5-HTTLPR was found on brain activation in response to fearful or angry facial stimuli (Domschke et al., 2006). However, patients carrying the S-allele had an increased amygdala activation to happy faces. Contrary, in healthy individuals, fearful (Hariri et al., 2002), and aversive stimuli (Heinz et al., 2005) evoked increased amygdala activation in s-allele carriers. In addition, a greater coupling between the amygdala and the ventrolateral prefrontal cortex was found in these individuals, possibly linked to an altered capacity to regulate emotional states (Heinz et al., 2005). Furthermore, in PD patients homozygous for a specific 5-HT1A variant, presentation of fearful faces reduced the activity of the right ventromedial prefrontal cortex, the right orbitofrontal cortex, and the right anterior cingulate cortex (Domschke et al., 2006). No effect on amygdala activation was found. In a classical fear conditioning paradigm, in which repeated pairing of an initially neutral stimulus with an aversive event leads to a fear response to the previously neutral stimulus, PD patients with the "protective" low activity MAO-A allele had an increased anterior cingulate cortex activation to presentation of the paired neutral stimulus during the fear acquisition phase (Reif et al., 2014). In addition, carriers of the low activity allele also benefited more from cognitive behavioral therapy, as shown by a higher response percentage in this group. Regarding ACCN2, in addition to detecting that variants in this gene are associated with PD, an association of the PD-associated allele and bilaterally heightened amygdala volume and activity to visual presentation of emotional faces was also observed in healthy individuals (Smoller et al., 2014).

Overall, these studies suggest that processing emotional stimuli might be affected by genetic variants and that altered activation in distinct brain regions might be linked to PD and the vulnerability to develop the disorder. Success of treatment strategies such as cognitive behavioral therapy could, also in part, depend on these factors. Delineating the mechanisms could lead to improved and more individualized treatments.

FROM LABORATORY TO REAL-LIFE ASSESSMENT

Ambulatory Assessment

A general limitation of experimental models is the laboratory setting that does not reflect the natural environment. The highest ecological validity would be provided by studying PAs in a natural setting, i.e., in real-life outside the laboratory. This so-called ambulatory assessment consists of repeated within-day measurements of an individual's symptoms, behavior or physiology to monitor dynamic changes over time in relationship to the occurrence of PAs. Often a notification at specified or random time points throughout the day is given, requesting to fill out questions about presence and intensity of symptoms at that or near that moment. This momentary, real-time assessment is believed to reduce retrospective recall bias. However, to date, studies are relatively scarce, which is likely due to technical limitations such as a short battery life and very limited storage capacity in the past.

The conducted ambulatory assessment studies showed that palpitations, dizziness, dyspnea, nausea and sweating are among the most often experienced symptoms of real-life PAs (Margraf et al., 1987; Hoehn-Saric et al., 2004). Of these, dyspnea, palpitations, dizziness and chest pain were the most intense one in another study (Meuret et al., 2011). Comparing ambulatory ratings with retrospective questionnaires and structured diagnostic interviews revealed that patients had a distorted recollection and reported a greater number of symptoms, particularly fear of going crazy, faintness, trembling/shaking, and fear of dying. However, the ranking of symptom frequency remained similar (Margraf et al., 1987).

Spontaneous PAs occurred most often at home (Margraf et al., 1987) or when being with family or alone (Meuret et al., 2011); situational ones most often in a car or public places (Margraf et al., 1987). This can lead to significant behavioral changes. However, unexpectedly, patients with agoraphobia were not found to less often visit public places (Dijkman-Caes et al., 1993). Mean activity levels were higher in patients with a higher number of PAs, but there was no clear pattern if activity increased before or after attacks (Sakamoto et al., 2008). Activity was also significantly higher when patients had no comorbid agoraphobia (Clark et al., 1990).

Given the pronounced physiological symptoms during PAs, attempts have been made to delineate cardio-respiratory changes. Early and newer studies observed an increase in heart rate in some self-reported PAs during 6- (Hoehn-Saric et al., 2004) to 24 h recordings (Barr Taylor et al., 1982; Freedman et al., 1985; Cameron et al., 1987), which could not be attributed to physical activity alone (Barr Taylor et al., 1982). Subjective severity of attacks correlated with heart rate. Contrary, in another study consisting of 175 PAs in 27 patients, heart rate did not increase during spontaneous attacks, but did in anticipation of or during experiencing situational attacks (i.e., in feared situations) (Margraf et al., 1987), which is likely due to the anxiety component. Regarding breathing, PAs were associated with increased tidal volume (Martinez et al., 1996; Meuret et al., 2011),

which positively correlated with the level of anxiety and fear of dying (Meuret et al., 2011). Transcutaneous arterial CO_2 levels were reported to decrease (Hibbert and Pilsbury, 1988), which in a later study was observed in only one out of 24 situationally provoked PAs (Garssen et al., 1996). Furthermore, in a rare case study in a dialysis patient, a spontaneous PA occurred with a strong reduction in arterial CO_2 and pH increase (Salkovskis et al., 1986). Focusing on PD and trait pathophysiological characteristics as a whole did not reveal any differences between patients and controls (Pfaltz et al., 2009), also not during various levels of activity (Pfaltz et al., 2010), suggesting that changes might be limited to more intense phases.

Considering potential interventions, it is particularly interesting to determine factors that precede the onset of PAs. PD patients seem not to be aware of any symptoms before PAs occur (Kenardy and Taylor, 1999), and no increase in anticipatory anxiety was observed (measurement intervals of at least 2 h) (Helbig-Lang et al., 2012). PA expectancy (morning measurement) was also not associated with a higher likelihood of having a PA (Meuret et al., 2011); it only predicted subsequent anxiety (Rodebaugh et al., 2002). Subdividing PAs into unexpected and expected attacks showed that only expected attacks were preceded by an increased level of danger, anxiety, helplessness and a few symptoms (Kenardy and Taylor, 1999). On the physiological level, strong autonomic irregularities do occur, as early as 47 min before PAs (Meuret et al., 2011). More specifically, heart rate strongly increased the minute before the onset and was positively associated with fear of losing control. Skin temperature already increased in the hour preceding the attack, similarly, to a previous report that only measured the minutes around the attack and also observed an elevation (Freedman et al., 1985). These observations suggest that physiological changes rather than subjective emotions are highly valuable to predict the impending occurrence of attacks and to intervene in the future.

Environment and Epigenetic Modifications

Increasing evidence suggest that neurotransmitter system functioning, such as of the 5-HT system, could be sensitive to environmental stimuli like stress (Homberg and van den Hove, 2012). A mechanism of how environmental factors could affect system functioning is by altering DNA methylation (Guo et al., 2011a), the best studied type of epigenetic modifications. These modifications are the regulatory interface of genes and vital for normal brain processes, including complex cognitive-affective functioning. Dysfunction can contribute to mental disorders. DNA methylation refers to the covalent binding of a methyl group to a cytosine's pyrimidine ring at position 5 (5-methylcytosine). This occurs more frequently at cytosines located next to a guanine nucleotide, forming a cytosine-phosphate-guanine (CpG) unit. Methylated CpG sites, which are overrepresented in regulatory promoter regions of genes, generally attract chromatin modifiers, disrupt binding of gene transcription factors and attract proteins that silence gene transcription (Klose and Bird, 2006). DNA methylation is carried out by DNA methyltransferases (DNMTs), families of specific enzymes (Hermann et al., 2004). DNMT1 mainly functions as maintenance transferase to retain the current methylation pattern, while DNMT3a and DNMT3b catalyze *de novo* methylation. The role of the DNMT2 family remains unclear. DNA methylation is fairly stable, yet dynamic to environmental factors (Guo et al., 2011a; Szyf, 2013), suggesting that DNA methylation might be a mechanism of how environment factors affect gene expression (Guo et al., 2011a). Demethylation takes place through several potential repair mechanisms. After oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, subsequent base excision repair pathway (Guo et al., 2011b) or DNMT3a and DNMT3b activity might lead to the removal of the hydroxymethyl group (Chen et al., 2012). Additionally, histone acetylation might actively demethylate DNA (Cervoni and Szyf, 2001). Histones are proteins around which DNA is packed into larger nucleosomes. Acetylation takes place on the lysine residue within the N-terminal amino acid tail of histones, which affects chromatin structure and thereby gene accessibility.

DNA methylation can be affected by genetic variants (Wagner et al., 2014) and environmental stimuli (Guo et al., 2011a; Szyf, 2013). Monozygotic twins who are genetically identical can thus still have different gene expressions and therefore differently functioning biological systems. This emphasizes the need for an integrative analysis of genes, epigenetics, and environment. To date, few epigenetic studies have been done in PD and most of these examined candidate genes. The MAO-A gene was found to be hypomethylated in a mixed sex patient group (Ziegler et al., 2016) and in another study in females (Domschke et al., 2012). In the former study, hypomethylation negatively correlated with PD severity and normalized with effective cognitive behavioral therapy. *In vitro* studies showed that a decreased methylation is associated with an increased MAO-A expression (Checknita et al., 2015). Regarding other neurotransmitter systems, hypomethylation of three CpG sites in the glutamate decarboxylases 1 gene, expressing the rate-limiting enzyme in GABA synthesis, has been found in PD patients (Domschke et al., 2013). The assumed increased GABA level associated with hypomethylation might represent a compensatory mechanism that mediates the effects of negative life events. An additional gene, in which hypomethylation was found in PD patients is the gene expressing the corticotropin releasing hormone receptor 1 (CRHR1a) (Schartner et al., 2017). This receptor is essential in the hypothalamic-pituitary-adrenal axis and also affects stress responses by innervating the locus coeruleus. As some studies reported that the majority of PD patients experienced a major life event in the months before the initial PAs (Uhde et al., 1985), the receptor may be the link between stressful life events, stress responses and the risk for PD. Further, in a small case-control study minor methylation variation was found regarding the norepinephrine transporter gene (SLC6a2) (Bayles et al., 2013). In contrast to these neurotransmitter system-related studies, another research line focuses on the role of the immune system in PD. The transcription factor gene Forkhead-Box-Protein P3 (FoxP3) appeared to be hypermethylated in female PD patients (Prelog et al., 2016). This hypermethylation is assumed to be associated with reduced regulatory T-cells gene transcription,

which leads to increased T-cell activation and thus inflammation. This could contribute to the higher rates of inflammatory disorders in PD patients.

Very recently, the first two epigenome-wide association studies (EWAS) were published. Shimada-Sugimoto et al. (2017) found 40 CpG sites that were significantly associated with PD. Most of them were hypomethylated and pathway analysis revealed genes in epidermis development, cell cycle regulation, and lymphocyte activation. Examination of some candidate genes such as MAO-A, GAD1 and SLC6a2 did not show any association with PD. However, smoking and medication were not considered, which are known to affect methylation. The second EWAS study consisted of a larger sample, and an independent replication study (Iurato et al., 2017). In females, after multiple testing correction, a differential methylation was found in the Homo sapiens headcase homolog (HECA) gene, a regulator in the cell cycle and with a potential role in cancer. Its link with PD has yet to be determined. Inclusion of 15 candidate genes showed significant associations with the 5-HT1a receptor in women and 5-HT2a receptor in men, but not GAD1 and CRHR1A as in previous studies. As smoking was not included as potential confounder, these results warrant replication.

THE NEED FOR AN INTERDISCIPLINARY APPROACH AND FUTURE PERSPECTIVES

Experimental panic provocation studies have led to important insights into the nature of PAs in the last few decades. Ambulatory assessment studies are likely to provide further novel knowledge.

Both approaches address questions from different perspectives and bridging the gap between them is expected to open new opportunities, extending our understanding of PAs.

In experimental studies, the controlled environment and immediate effects of CO_2 to induce PAs allows studying involved fundamental mechanisms in a temporally controlled manner. For example, (epi)genetic research and pharmacological manipulations can determine whether specific genes and neurotransmitter systems play a role in the sensitivity to CO_2 and thus presumably PAs. This can strongly contribute to develop new and better treatment options. A major advantage of experimental studies compared to other types such as clinical or epidemiological studies is that induced effects are larger accompanied with smaller variation, making smaller samples sufficient to detect relevant effects. While it has been shown that the fear and symptoms triggered by a CO_2 inhalation are a good representation of a real-life PA (Schruers et al., 2004), a drawback is that attacks do not occur spontaneously in a natural environment but are provoked in a laboratory. The setting, procedure, and presence of the experimenter can affect individuals' responses, raising the question to which extent findings can be generalized to daily life.

In contrast to looking at acute effects in the laboratory, assessment of naturally occurring PAs in PD patients' daily lives provides the powerful opportunity to unravel the dynamic course over time. This approach has a higher ecological validity than laboratory models. A drawback is that prolonged recordings are required for comprehensive monitoring, which has been limited by the rather small storage capacity and short battery life of previous devices. The discomfort associated with carrying large setups also restricted sampling to small study samples in the past, but recent advances have led to small devices such as smartwatches that can be worn without being perceived as bothersome. Modern devices also have built-in actigraphy to quantify rest and activity patterns and to record body position, thereby providing more detailed situational data. Incorporating these and other measurements such as physiological parameters, and thereby moving from a main focus on patient ratings (e.g., feelings and symptoms) to automatic assessment, could help to develop interventions targeting the phase preceding attacks and to empower patients to use the device's feedback for self-directed changes in behavior. This approach puts the individual patient into the center and stimulates informed decisions. Likewise, ambulatory assessment is also suited to test the efficacy of treatments in real life. In the long-term, monitoring after successful treatment could identify individuals at risk to relapse and initiate early prevention. Using advanced devices measuring a broad spectrum of parameters, from physiology to context, can help to determine how symptoms are interrelated and interact with natural environmental factors that are controlled in a laboratory setting.

Environmental factors individuals are exposed to in real-life can affect molecular mechanisms such as epigenetic modifications and thus gene expression. Therefore, it is important to strive for an integrative, interdisciplinary approach of daily-life and fundamental research. For instance, determining which environmental factors are related to the disease and what their functional effects are on a molecular level could provide highly valuable insights into the pathophysiology of PAs. A major challenge is to identify the relevant environmental factors. In this respect, cumulative environmental risk scores combined with genotypes and epigenetic pattern could provide vastly informative data about which interactions are relevant and potentially identify risk groups to develop the disorder. Nowadays it is possible to cost-effectively determine epigenetic pattern in high-resolution, i.e., site-specific DNA methylation. However, these analyses require multiple testing correction for each CpG site, letting many sites fail to reach significance. An approach to tackle this issue could be the development of a novel correction methods similar to the ones applied in genome-wide association studies (Li and Ji, 2005), taking into account the often highly correlated methylation levels of adjacent CpG sites to reduce the number of tests.

Moreover, most epigenetic PD studies to date were association studies, which do not allow drawing any conclusions about the exact role of DNA methylation. One approach to imply a causal role is the two-step Mendelian randomization method, in which first the causal impact of a risk factor on DNA methylation is examined, followed by testing the causal effect of DNA methylation on the outcome (Relton and Davey Smith, 2012). Experimentally, large longitudinal studies can determine whether methylation changes over time are associated with developing

the disorder. In this context, rodent models could be a highly beneficial addition as they allow molecular manipulations that exceed ethical possibilities in humans. For instance, studying behavioral consequences to central infusion of pharmacological compounds that target molecules of the epigenetic machinery and thereby lead to overwriting DNA methylation patterns could strengthen causality assumptions (Weaver et al., 2004). At the same time, environmental factors can be controlled to assess their effects. We and others have analyzed rodents' behavioral performance under CO_2 exposure (Ziemann et al., 2009; Johnson et al., 2012; Leibold et al., 2016). Adding for example cardio-respiratory monitoring as outcome variables, which can also be done in humans, further enhances the similarity between experiments and increases the translational value (Leibold et al., 2016). We recently provided a quantitative comparison between rodents, healthy volunteers, and PD patients and showed that the physiological response in mice corresponds well to the one in both human groups (Leibold et al., 2016). Such a model overcomes the challenge to align observed behavioral performances in rodents with behavioral self-reports in humans and maximizes translation of data between species. This can significantly drive forward research and eventually application of discoveries. For instance, once causality is suggested, efforts could focus on developing pharmacological compounds targeting epigenetic enzymes to normalize methylation pattern in rodents. Compensatory genes could be activated to reduce symptoms or even obtain a biochemical balance to eventually let patients fully recover. A caveat is that complex disorders are most likely caused by a network disturbance rather than a single gene, but such a starting point might inspire new directions and eventually brings us closer to better treatments.

CONCLUSION

The manifestation of PAs can vary widely between people. To better understand the dimensional physiology and pathology and to facilitate a new classification in line with the RDoC project a more interdisciplinary approach of genome × epigenome × environment interactions is needed. Overall, integrating fundamental and real-life research can greatly advance the field, determine biomarkers to identify individuals at risk, and support developing more effective treatment strategies.

AUTHOR CONTRIBUTIONS

NL and KS equally contributed to manuscript writing and revision and approved the submitted version.

REFERENCES

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders-5*. Arlington, VA: American Psychiatric Publishing. doi: 10.1176/appi.books.9780890425596

Argyropoulos, S. V., Bailey, J. E., Hood, S. D., Kendrick, A. H., Rich, A. S., Laszlo, G., et al. (2002). Inhalation of 35% CO(2) results in activation of the HPA axis in healthy volunteers. *Psychoneuroendocrinology* 27, 715–729. doi: 10.1016/S0306-4530(01)00075-0

Barr Taylor, C., Telch, M. J., and Havvik, D. (1982). Ambulatory heart rate changes during panic attacks. *J. Psychiatr. Res.* 17, 261–266. doi: 10.1016/0022-3956(82)90004-8

Battaglia, M., Ogliari, A., Harris, J., Spatola, C. A., Pesenti-Gritti, P., Reichborn-Kjennerud, T., et al. (2007). A genetic study of the acute anxious response to carbon dioxide stimulation in man. *J. Psychiatr. Res.* 41, 906–917. doi: 10.1016/j.jpsychires.2006.12.002

Battaglia, M., Pesenti-Gritti, P., Spatola, C. A., Ogliari, A., and Tambs, K. (2008). A twin study of the common vulnerability between heightened sensitivity to hypercapnia and panic disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B, 586–593. doi: 10.1002/ajmg.b.30647

Bayles, R., Baker, E. K., Jowett, J. B., Barton, D., Esler, M., El-Osta, A., et al. (2013). Methylation of the SLC6a2 gene promoter in major depression and panic disorder. *PLoS One* 8:e83223. doi: 10.1371/journal.pone.0083223

Bellodi, L., Perna, G., Caldirola, D., Arancio, C., Bertani, A., and Di Bella, D. (1998). CO_2-induced panic attacks: a twin study. *Am. J. Psychiatr.* 155, 1184–1188. doi: 10.1176/ajp.155.9.1184

Biancardi, V., Bicego, K. C., Almeida, M. C., and Gargaglioni, L. H. (2008). Locus coeruleus noradrenergic neurons and CO_2 drive to breathing. *Pflugers Arch.* 455, 1119–1128. doi: 10.1007/s00424-007-0338-8

Blanchard, R. J., and Blanchard, D. C. (1990). *An Ethoexperimental analysis of Defense, Fear and Anxiety*. Dunedin: Otago University Press.

Blaya, C., Salum, G. A., Lima, M. S., Leistner-Segal, S., and Manfro, G. G. (2007). Lack of association between the Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Panic Disorder: a systematic review and meta-analysis. *Behav. Brain Funct.* 3:41. doi: 10.1186/1744-9081-3-41

Bystritsky, A., and Shapiro, D. (1992). Continuous physiological changes and subjective reports in panic patients: a preliminary methodological report. *Biol. Psychiatry* 32, 766–777. doi: 10.1016/0006-3223(92)90080-J

Cameron, O. G., Lee, M. A., Curtis, G. C., and Mccann, D. S. (1987). Endocrine and physiological changes during "spontaneous" panic attacks. *Psychoneuroendocrinology* 12, 321–331. doi: 10.1016/0306-4530(87)90061-8

Cervoni, N., and Szyf, M. (2001). Demethylase activity is directed by histone acetylation. *J. Biol. Chem.* 276, 40778–40787. doi: 10.1074/jbc.M103921200

Checknita, D., Maussion, G., Labonte, B., Comai, S., Tremblay, R. E., Vitaro, F., et al. (2015). Monoamine oxidase A gene promoter methylation and transcriptional downregulation in an offender population with antisocial personality disorder. *Br. J. Psychiatry* 206, 216–222. doi: 10.1192/bjp.bp.114.144964

Chen, C. C., Wang, K. Y., and Shen, C. K. (2012). The mammalian de novo DNA methyltransferases DNMT3A and DNMT3B are also DNA 5-hydroxymethylcytosine dehydroxymethylases. *J. Biol. Chem.* 287, 33116–33121. doi: 10.1074/jbc.C112.406975

Clark, D. B., Taylor, C. B., Hayward, C., King, R., Margraf, J., Ehlers, A., et al. (1990). Motor activity and tonic heart rate in panic disorder. *Psychiatry Res.* 32, 45–53. doi: 10.1016/0165-1781(90)90134-Q

Crowe, R. R., Noyes, R., Pauls, D. L., and Slymen, D. (1983). A family study of panic disorder. *Arch. Gen. Psychiatry* 40, 1065–1069. doi: 10.1001/archpsyc.1983.01790090027004

da Silva, G. S., Giusti, H., Benedetti, M., Dias, M. B., Gargaglioni, L. H., Branco, L. G., et al. (2011). Serotonergic neurons in the nucleus raphe obscurus contribute to interaction between central and peripheral ventilatory responses to hypercapnia. *Pflugers Arch.* 462, 407–418. doi: 10.1007/s00424-011-0990-x

da Silva, G. S., Li, A., and Nattie, E. (2010). High $CO2/H^+$ dialysis in the caudal ventrolateral medulla (Loeschcke's area) increases ventilation in wakefulness. *Respir. Physiol. Neurobiol.* 171, 46–53. doi: 10.1016/j.resp.2010.01.014

Dean, J. B., Bayliss, D. A., Erickson, J. T., Lawing, W. L., and Millhorn, D. E. (1990). Depolarization and stimulation of neurons in nucleus tractus solitarii by carbon dioxide does not require chemical synaptic input. *Neuroscience* 36, 207–216. doi: 10.1016/0306-4522(90)90363-9

Dijkman-Caes, C. I., De Vries, M. W., Kraan, H. F., and Volovics, A. (1993). Agoraphobic behavior in daily life: effects of social roles and demographic characteristics. *Psychol. Rep.* 72, 1283–1293. doi: 10.2466/pr0.1993.72.3c.1283

Domschke, K., Braun, M., Ohrmann, P., Suslow, T., Kugel, H., Bauer, J., et al. (2006). Association of the functional -1019C/G 5-HT1A polymorphism with prefrontal cortex and amygdala activation measured with 3 T fMRI in panic disorder. *Int. J. Neuropsychopharmacol.* 9, 349–355. doi: 10.1017/S1461145705005869

Domschke, K., Tidow, N., Kuithan, H., Schwarte, K., Klauke, B., Ambree, O., et al. (2012). Monoamine oxidase A gene DNA hypomethylation - a risk factor for panic disorder? *Int. J. Neuropsychopharmacol.* 15, 1217–1228. doi: 10.1017/S146114571200020X

Domschke, K., Tidow, N., Schrempf, M., Schwarte, K., Klauke, B., Reif, A., et al. (2013). Epigenetic signature of panic disorder: a role of glutamate decarboxylase 1 (GAD1) DNA hypomethylation? *Prog. Neuropsychopharmacol. Biol. Psychiatry* 46, 189–196. doi: 10.1016/j.pnpbp.2013.07.014

Esquivel, G., Schruers, K. R., Maddock, R. J., Colasanti, A., and Griez, E. J. (2009). Acids in the brain: a factor in panic? *J. Psychopharmacol.* 24, 639–647. doi: 10.1177/0269881109104847

Feinstein, J. S., Buzza, C., Hurlemann, R., Follmer, R. L., Dahdaleh, N. S., Coryell, W. H., et al. (2013). Fear and panic in humans with bilateral amygdala damage. *Nat. Neurosci.* 16, 270–272. doi: 10.1038/nn.3323

Freedman, R. R., Ianni, P., Ettedgui, E., and Puthezhath, N. (1985). Ambulatory monitoring of panic disorder. *Arch. Gen. Psychiatry* 42, 244–248. doi: 10.1001/archpsyc.1985.01790260038004

Freire, R. C., and Nardi, A. E. (2012). Panic disorder and the respiratory system: clinical subtype and challenge tests. *Rev. Bras. Psiquiatr.* 34(Suppl. 1), S32–S41. doi: 10.1016/S1516-4446(12)70053-3

Garssen, B., Buikhuisen, M., and Van Dyck, R. (1996). Hyperventilation and panic attacks. *Am. J. Psychiatry* 153, 513–518. doi: 10.1176/ajp.153.4.513

Goldstein, R. B., Weissman, M. M., Adams, P. B., Horwath, E., Lish, J. D., Charney, D., et al. (1994). Psychiatric disorders in relatives of probands with panic disorder and/or major depression. *Arch. Gen. Psychiatry* 51, 383–394. doi: 10.1001/archpsyc.1994.03950050043005

Goossens, L., Leibold, N., Peeters, R., Esquivel, G., Knuts, I., Backes, W., et al. (2014). Brainstem response to hypercapnia: a symptom provocation study into the pathophysiology of panic disorder. *J. Psychopharmacol.* 28, 449–456. doi: 10.1177/0269881114527363

Gorman, J. M., Kent, J. M., Sullivan, G. M., and Coplan, J. D. (2000). Neuroanatomical hypothesis of panic disorder, revised. *Am. J. Psychiatry* 157, 493–505. doi: 10.1176/appi.ajp.157.4.493

Gottesman, I. I., and Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* 160, 636–645. doi: 10.1176/appi.ajp.160.4.636

Griez, E. (1984). Experimental models of anxiety. Problems and perspectives. *Acta Psychiatr. Belg.* 84, 511–532.

Griez, E. J., Lousberg, H., Van Den Hout, M. A., and Van Der Molen, G. M. (1987). CO_2 vulnerability in panic disorder. *Psychiatry Res.* 20, 87–95. doi: 10.1016/0165-1781(87)90001-1

Guo, J. U., Ma, D. K., Mo, H., Ball, M. P., Jang, M. H., Bonaguidi, M. A., et al. (2011a). Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat. Neurosci.* 14, 1345–1351. doi: 10.1038/nn.2900

Guo, J. U., Su, Y., Zhong, C., Ming, G. L., and Song, H. (2011b). Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* 145, 423–434. doi: 10.1016/j.cell.2011.03.022

Hamilton, S. P., Heiman, G. A., Haghighi, F., Mick, S., Klein, D. F., Hodge, S. E., et al. (1999). Lack of genetic linkage or association between a functional serotonin transporter polymorphism and panic disorder. *Psychiatr. Genet.* 9, 1–6. doi: 10.1097/00041444-199903000-00001

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science* 297, 400–403. doi: 10.1126/science.1071829

Harris, E. L., Noyes, R. Jr., Crowe, R. R., and Chaudhry, D. R. (1983). Family study of agoraphobia. Report of a pilot study. *Arch. Gen. Psychiatry* 40, 1061–1064. doi: 10.1001/archpsyc.1983.01790090023003

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., et al. (2005). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nat. Neurosci.* 8, 20–21. doi: 10.1038/nn1366

Helbig-Lang, S., Lang, T., Petermann, F., and Hoyer, J. (2012). Anticipatory anxiety as a function of panic attacks and panic-related self-efficacy: an ambulatory assessment study in panic disorder. *Behav. Cogn. Psychother.* 40, 590–604. doi: 10.1017/S1352465812000057

Hermann, A., Gowher, H., and Jeltsch, A. (2004). Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci.* 61, 2571–2587. doi: 10.1007/s00018-004-4201-1

Hettema, J. M., An, S. S., Neale, M. C., Van Den Oord, E. J., Kendler, K. S., and Chen, X. (2008). Lack of association between the amiloride-sensitive cation channel 2 (ACCN2) gene and anxiety spectrum disorders. *Psychiatr. Genet.* 18, 73–79. doi: 10.1097/YPG.0b013e3282f08a2a

Hibbert, G., and Pilsbury, D. (1988). Hyperventilation in panic attacks. Ambulant monitoring of transcutaneous carbon dioxide. *Br. J. Psychiatry* 153, 76–80. doi: 10.1192/bjp.153.1.76

Hoehn-Saric, R., Mcleod, D. R., Funderburk, F., and Kowalski, P. (2004). Somatic symptoms and physiologic responses in generalized anxiety disorder and panic disorder: an ambulatory monitor study. *Arch. Gen. Psychiatry* 61, 913–921. doi: 10.1001/archpsyc.61.9.913

Homberg, J. R., and van den Hove, D. L. (2012). The serotonin transporter gene and functional and pathological adaptation to environmental variation across the life span. *Prog. Neurobiol.* 99, 117–127. doi: 10.1016/j.pneurobio.2012.08.003

Howe, A. S., Buttenschon, H. N., Bani-Fatemi, A., Maron, E., Otowa, T., Erhardt, A., et al. (2016). Candidate genes in panic disorder: meta-analyses of 23 common variants in major anxiogenic pathways. *Mol. Psychiatry* 21, 665–679. doi: 10.1038/mp.2015.138

Iurato, S., Carrillo-Roa, T., Arloth, J., Czamara, D., Diener-Holzl, L., Lange, J., et al. (2017). DNA Methylation signatures in panic disorder. *Transl. Psychiatry* 7:1287. doi: 10.1038/s41398-017-0026-1

Johnson, P. L., Samuels, B. C., Fitz, S. D., Lightman, S. L., Lowry, C. A., and Shekhar, A. (2012). Activation of the orexin 1 receptor is a critical component of CO_2-mediated anxiety and hypertension but not bradycardia. *Neuropsychopharmacology* 37, 1911–1922. doi: 10.1038/npp.2012.38

Kang, E., Choe, A. Y., Kim, B., Lee, J. Y., Choi, T. K., Na, H. R., et al. (2016). Serotonin transporter and COMT polymorphisms as independent predictors of health-related quality of life in patients with panic disorder. *J. Korean Med. Sci.* 31, 757–763. doi: 10.3346/jkms.2016.31.5.757

Kaye, J., Buchanan, F., Kendrick, A., Johnson, P., Lowry, C., Bailey, J., et al. (2004). Acute carbon dioxide exposure in healthy adults: evaluation of a novel means of investigating the stress response. *J. Neuroendocrinol.* 16, 256–264. doi: 10.1111/j.0953-8194.2004.01158.x

Kellner, M., Muhtz, C., Nowack, S., Leichsenring, I., Wiedemann, K., and Yassouridis, A. (2018). Effects of 35% carbon dioxide (CO_2) inhalation in patients with post-traumatic stress disorder (PTSD): a double-blind, randomized, placebo-controlled, cross-over trial. *J. Psychiatr. Res.* 96, 260–264. doi: 10.1016/j.jpsychires.2017.10.019

Kenardy, J., and Taylor, C. B. (1999). Expected versus unexpected panic attacks: a naturalistic prospective study. *J. Anxiety Disord.* 13, 435–445. doi: 10.1016/S0887-6185(99)00013-4

Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C., and Eaves, L. J. (1993). Panic disorder in women: a population-based twin study. *Psychol. Med.* 23, 397–406. doi: 10.1017/S003329170002849X

Kent, J. M., Papp, L. A., Martinez, J. M., Browne, S. T., Coplan, J. D., Klein, D. F., et al. (2001). Specificity of panic response to CO^2 inhalation in panic disorder: a comparison with major depression and premenstrual dysphoric disorder. *Am. J. Psychiatry* 158, 58–67. doi: 10.1176/appi.ajp.158.1.58

Klose, R. J., and Bird, A. P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* 31, 89–97. doi: 10.1016/j.tibs.2005.12.008

Leibold, N. K., Van Den Hove, D. L., Esquivel, G., De Cort, K., Goossens, L., Strackx, E., et al. (2015). The brain acid-base homeostasis and serotonin: a perspective on the use of carbon dioxide as human and rodent experimental model of panic. *Prog. Neurobiol.* 129, 58–78. doi: 10.1016/j.pneurobio.2015.04.001

Leibold, N. K., Van Den Hove, D. L., Viechtbauer, W., Buchanan, G. F., Goossens, L., Lange, I., et al. (2016). CO_2 exposure as translational cross-species experimental model for panic. *Transl. Psychiatry* 6:e885. doi: 10.1038/tp.2016.162

Leibold, N. K., Van Den Hove, D. L. A., Viechtbauer, W., Kenis, G., Goossens, L., Lange, I., et al. (2017). Amiloride-sensitive cation channel 2 genotype affects

the response to a carbon dioxide panic challenge. *J. Psychopharmacol.* 21, 1294–1301. doi: 10.1177/0269881116686880

Leibold, N. K., Viechtbauer, W., Goossens, L., De Cort, K., Griez, E. J., Myin-Germeys, I., et al. (2013). Carbon dioxide inhalation as a human experimental model of panic: the relationship between emotions and cardiovascular physiology. *Biol. Psychol.* 94, 331–340. doi: 10.1016/j.biopsycho.2013.06.004

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Li, J., and Ji, L. (2005). Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 95, 221–227. doi: 10.1038/sj.hdy.6800717

Lonsdorf, T. B., Ruck, C., Bergstrom, J., Andersson, G., Ohman, A., Schalling, M., et al. (2009). The symptomatic profile of panic disorder is shaped by the 5-HTTLPR polymorphism. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 1479–1483. doi: 10.1016/j.pnpbp.2009.08.004

Maier, W., Lichtermann, D., Minges, J., Oehrlein, A., and Franke, P. (1993). A controlled family study in panic disorder. *J. Psychiatr. Res.* 27(Suppl. 1), 79–87. doi: 10.1016/0022-3956(93)90019-X

Margraf, J., Taylor, B., Ehlers, A., Roth, W. T., and Agras, W. S. (1987). Panic attacks in the natural environment. *J. Nerv. Ment. Dis.* 175, 558–565. doi: 10.1097/00005053-198709000-00008

Maron, E., Hettema, J. M., and Shlik, J. (2010). Advances in molecular genetics of panic disorder. *Mol. Psychiatry* 15, 681–701. doi: 10.1038/mp.2009.145

Maron, E., Lang, A., Tasa, G., Liivlaid, L., Toru, I., Must, A., et al. (2005). Associations between serotonin-related gene polymorphisms and panic disorder. *Int. J. Neuropsychopharmacol.* 8, 261–266. doi: 10.1017/S1461145704004985

Martinez, J. M., Papp, L. A., Coplan, J. D., Anderson, D. E., Mueller, C. M., Klein, D. F., et al. (1996). Ambulatory monitoring of respiration in anxiety. *Anxiety* 2, 296–302.

McNaughton, N., and Corr, P. J. (2004). A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neurosci. Biobehav. Rev.* 28, 285–305. doi: 10.1016/j.neubiorev.2004.03.005

Mendlewicz, J., Papadimitriou, G., and Wilmotte, J. (1993). Family study of panic disorder: comparison with generalized anxiety disorder, major depression and normal subjects. *Psychiatr. Genet.* 3, 73–78. doi: 10.1097/00041444-199322000-00002

Meuret, A. E., Rosenfield, D., Wilhelm, F. H., Zhou, E., Conrad, A., Ritz, T., et al. (2011). Do unexpected panic attacks occur spontaneously? *Biol. Psychiatry* 70, 985–991. doi: 10.1016/j.biopsych.2011.05.027

Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., Weiskopf, N., Seymour, B., et al. (2007). When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* 317, 1079–1083. doi: 10.1126/science.1144298

Muhtz, C., Yassouridis, A., Daneshi, J., Braun, M., and Kellner, M. (2011). Acute panicogenic, anxiogenic and dissociative effects of carbon dioxide inhalation in patients with post-traumatic stress disorder (PTSD). *J. Psychiatr. Res.* 45, 989–993. doi: 10.1016/j.jpsychires.2011.01.009

Mulkey, D. K., Stornetta, R. L., Weston, M. C., Simmons, J. R., Parker, A., Bayliss, D. A., et al. (2004). Respiratory control by ventral surface chemoreceptor neurons in rats. *Nat. Neurosci.* 7, 1360–1369. doi: 10.1038/nn1357

National Institute of Mental Health [NIMH] (2010). *Research Domain Criteria (RDoC) [Online]*. Available at: http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml [accessed September 16, 2015].

Niccolai, V., Van Duinen, M. A., and Griez, E. J. (2008). Objective and subjective measures in recovery from a 35% carbon dioxide challenge. *Can. J. Psychiatry* 53, 737–744. doi: 10.1177/070674370805301105

Noyes, R. Jr., Crowe, R. R., Harris, E. L., Hamra, B. J., Mcchesney, C. M., and Chaudhry, D. R. (1986). Relationship between panic disorder and agoraphobia: a family study. *Arch. Gen. Psychiatry* 43, 227–232. doi: 10.1001/archpsyc.1986.01800030037004

Perna, G., Bussi, R., Allevi, L., and Bellodi, L. (1999). Sensitivity to 35% carbon dioxide in patients with generalized anxiety disorder. *J. Clin. Psychiatry* 60, 379–384. doi: 10.4088/JCP.v60n0606

Perna, G., Caldirola, D., Arancio, C., and Bellodi, L. (1997). Panic attacks: a twin study. *Psychiatry Res.* 66, 69–71. doi: 10.1016/S0165-1781(97)85177-3

Perna, G., Cocchi, S., Bertani, A., Arancio, C., and Bellodi, L. (1995). Sensitivity to 35% CO2 in healthy first-degree relatives of patients with panic disorder. *Am. J. Psychiatry* 152, 623–625. doi: 10.1176/ajp.152.4.623

Perna, G., Di Bella, D., Favaron, E., Cucchi, M., Liperi, L., and Bellodi, L. (2004). Lack of relationship between CO_2 reactivity and serotonin transporter gene regulatory region polymorphism in panic disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 129B, 41–43. doi: 10.1002/ajmg.b.30035

Pfaltz, M. C., Michael, T., Grossman, P., Blechert, J., and Wilhelm, F. H. (2009). Respiratory pathophysiology of panic disorder: an ambulatory monitoring study. *Psychosom. Med.* 71, 869–876. doi: 10.1097/PSY.0b013e3181b492ff

Pfaltz, M. C., Michael, T., Grossman, P., Margraf, J., and Wilhelm, F. H. (2010). Instability of physical anxiety symptoms in daily life of patients with panic disorder and patients with posttraumatic stress disorder. *J. Anxiety Disord.* 24, 792–798. doi: 10.1016/j.janxdis.2010.06.001

Poonai, N., Antony, M. M., Binkley, K. E., Stenn, P., Swinson, R. P., Corey, P., et al. (2000). Carbon dioxide inhalation challenges in idiopathic environmental intolerance. *J. Allergy Clin. Immunol.* 105, 358–363. doi: 10.1016/S0091-6749(00)90088-5

Prelog, M., Hilligardt, D., Schmidt, C. A., Przybylski, G. K., Leierer, J., Almanzar, G., et al. (2016). Hypermethylation of FOXP3 promoter and premature aging of the immune system in female patients with panic disorder? *PLoS One* 11:e0157930. doi: 10.1371/journal.pone.0157930

Rassovsky, Y., and Kushner, M. G. (2003). Carbon dioxide in the study of panic disorder: issues of definition, methodology, and outcome. *J. Anxiety Disord.* 17, 1–32. doi: 10.1016/S0887-6185(02)00181-0

Reif, A., Richter, J., Straube, B., Hofler, M., Lueken, U., Gloster, A. T., et al. (2014). MAOA and mechanisms of panic disorder revisited: from bench to molecular psychotherapy. *Mol. Psychiatry* 19, 122–128. doi: 10.1038/mp.2012.172

Relton, C. L., and Davey Smith, G. (2012). Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int. J. Epidemiol.* 41, 161–176. doi: 10.1093/ije/dyr233

Richerson, G. B. (2004). Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat. Rev. Neurosci.* 5, 449–461. doi: 10.1038/nrn1409

Richey, J. A., Schmidt, N. B., Hofmann, S. G., and Timpano, K. R. (2010). Temporal and structural dynamics of anxiety sensitivity in predicting fearful responding to a 35% CO_2 challenge. *J. Anxiety Disord.* 24, 423–432. doi: 10.1016/j.janxdis.2010.02.007

Roberson-Nay, R., Moruzzi, S., Ogliari, A., Pezzica, E., Tambs, K., Kendler, K. S., et al. (2013). Evidence for distinct genetic effects associated with response to 35% CO^2. *Depress. Anxiety* 30, 259–266. doi: 10.1002/da.22038

Rodebaugh, T. L., Curran, P. J., and Chambless, D. L. (2002). Expectancy of panic in the maintenance of daily anxiety in panic disorder with agoraphobia: a longitudinal test of competing models. *Behav. Ther.* 33, 315–336. doi: 10.1016/S0005-7894(02)80031-4

Sakamoto, N., Yoshiuchi, K., Kikuchi, H., Takimoto, Y., Kaiya, H., Kumano, H., et al. (2008). Panic disorder and locomotor activity. *Biopsychosoc. Med.* 2:23. doi: 10.1186/1751-0759-2-23

Salkovskis, P. M., Jones, D. R., and Clark, D. M. (1986). Respiratory control in the treatment of panic attacks: replication and extension with concurrent measurement of behaviour and pCO_2. *Br. J. Psychiatry* 148, 526–532. doi: 10.1192/bjp.148.5.526

Schartner, C., Ziegler, C., Schiele, M. A., Kollert, L., Weber, H., Zwanzger, P., et al. (2017). CRHR1 promoter hypomethylation: an epigenetic readout of panic disorder? *Eur. Neuropsychopharmacol.* 27, 360–371. doi: 10.1016/j.euroneuro.2017.01.005

Schmidt, N. B., Storey, J., Greenberg, B. D., Santiago, H. T., Li, Q., and Murphy, D. L. (2000). Evaluating gene x psychological risk factor effects in the pathogenesis of anxiety: a new model approach. *J. Abnorm. Psychol.* 109, 308–320. doi: 10.1037/0021-843X.109.2.308

Schruers, K., Esquivel, G., Van Duinen, M., Wichers, M., Kenis, G., Colasanti, A., et al. (2011). Genetic moderation of CO_2-induced fear by 5-HTTLPR genotype. *J. Psychopharmacol.* 25, 37–42. doi: 10.1177/0269881110372543

Schruers, K. R. J., Van De Mortel, H., Overbeek, T., and Griez, E. (2004). Symptom profiles of natural and laboratory panic attacks. *Acta Neuropsychiatr.* 16, 101–106. doi: 10.1111/j.0924-2708.2004.0084.x

Schutters, S. I., Viechtbauer, W., Knuts, I. J., Griez, E. J., and Schruers, K. R. (2012). 35% CO_2 sensitivity in social anxiety disorder. *J. Psychopharmacol.* 26, 479–486. doi: 10.1177/0269881111430750

Shimada-Sugimoto, M., Otowa, T., Miyagawa, T., Umekage, T., Kawamura, Y., Bundo, M., et al. (2017). Epigenome-wide association study of DNA methylation in panic disorder. *Clin. Epigenetics* 9:6. doi: 10.1186/s13148-016-0307-1

Skre, I., Onstad, S., Torgersen, S., Lygren, S., and Kringlen, E. (1993). A twin study of DSM-III-R anxiety disorders. *Acta Psychiatr. Scand.* 88, 85–92. doi: 10.1111/j.1600-0447.1993.tb03419.x

Smoller, J. W., Gallagher, P. J., Duncan, L. E., Mcgrath, L. M., Haddad, S. A., Holmes, A. J., et al. (2014). The human ortholog of acid-sensing ion channel gene ASIC1a is associated with panic disorder and amygdala structure and function. *Biol. Psychiatry* 76, 902–910. doi: 10.1016/j.biopsych.2013.12.018

Strug, L. J., Suresh, R., Fyer, A. J., Talati, A., Adams, P. B., Li, W., et al. (2010). Panic disorder is associated with the serotonin transporter gene (SLC6A4) but not the promoter region (5-HTTLPR). *Mol. Psychiatry* 15, 166–176. doi: 10.1038/mp.2008.79

Szyf, M. (2013). DNA methylation, behavior and early life adversity. *J. Genet. Genomics* 40, 331–338. doi: 10.1016/j.jgg.2013.06.004

Talati, A., Odgerel, Z., Wickramaratne, P. J., Norcini-Pala, A., Skipper, J. L., Gingrich, J. A., et al. (2017). Associations between serotonin transporter and behavioral traits and diagnoses related to anxiety. *Psychiatry Res.* 253, 211–219. doi: 10.1016/j.psychres.2017.03.033

Torgersen, S. (1983). Genetic factors in anxiety disorders. *Arch. Gen. Psychiatry* 40, 1085–1089. doi: 10.1001/archpsyc.1983.01790090047007

Uhde, T. W., Boulenger, J. P., Roy-Byrne, P. P., Geraci, M. F., Vittone, B. J., and Post, R. M. (1985). Longitudinal course of panic disorder: clinical and biological considerations. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 9, 39–51.

van Beek, N., and Griez, E. (2000). Reactivity to a 35% CO_2 challenge in healthy first-degree relatives of patients with panic disorder. *Biol. Psychiatry* 47, 830–835. doi: 10.1016/S0006-3223(99)00265-6

Verburg, C., Griez, E., and Meijer, J. (1994). A 35% carbon dioxide challenge in simple phobias. *Acta Psychiatr. Scand.* 90, 420–423. doi: 10.1111/j.1600-0447.1994.tb01617.x

Wagner, J. R., Busche, S., Ge, B., Kwan, T., Pastinen, T., and Blanchette, M. (2014). The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biol.* 15:R37. doi: 10.1186/gb-2014-15-2-r37

Watanabe, T., Ishiguro, S., Aoki, A., Ueda, M., Hayashi, Y., Akiyama, K., et al. (2017). Genetic polymorphism of 1019C/G (rs6295) promoter of serotonin 1A receptor and Catechol-O-Methyltransferase in panic disorder. *Psychiatry Investig.* 14, 86–92. doi: 10.4306/pi.2017.14.1.86

Weaver, I. C., Cervoni, N., Champagne, F. A., D'alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7, 847–854. doi: 10.1038/nn1276

Weissman, M. M. (1993). Family genetic studies of panic disorder. *J. Psychiatr. Res.* 27, 69–78. doi: 10.1016/0022-3956(93)90018-W

Wetherell, M. A., Crown, A. L., Lightman, S. L., Miles, J. N., Kaye, J., and Vedhara, K. (2006). The four-dimensional stress test: psychological, sympathetic-adrenal-medullary, parasympathetic and hypothalamic-pituitary-adrenal responses following inhalation of 35% CO_2. *Psychoneuroendocrinology* 31, 736–747. doi: 10.1016/j.psyneuen.2006.02.005

Williams, R. H., Jensen, L. T., Verkhratsky, A., Fugger, L., and Burdakov, D. (2007). Control of hypothalamic orexin neurons by acid and CO_2. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10685–10690. doi: 10.1073/pnas.0702676104

Ziegler, C., Richter, J., Mahr, M., Gajewska, A., Schiele, M. A., Gehrmann, A., et al. (2016). MAOA gene hypomethylation in panic disorder-reversibility of an epigenetic risk pattern by psychotherapy. *Transl. Psychiatry* 6:e773. doi: 10.1038/tp.2016.41

Ziemann, A. E., Allen, J. E., Dahdaleh, N. S., Drebot, I. I., Coryell, M. W., Wunsch, A. M., et al. (2009). The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell* 139, 1012–1021. doi: 10.1016/j.cell.2009.10.029

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BDNF-Related Imbalance of Copine 6 and Synaptic Plasticity Markers Couples With Depression-Like Behavior and Immune Activation in CUMS Rats

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Chronic stress is a contributing risk factor in the pathogenesis of depression. Although the mechanisms are multifaceted, the relationship can be ascribed partly to stress-related alterations in immune activation and brain plasticity. Considering the increasing evidence regarding the role of Copine 6 in the regulation of synaptic plasticity, the aim of the present study is to investigate Copine 6 expression in the hippocampus and the prefrontal cortex (PFC) in a stress-induced depression rat model. The behavior of the rats was evaluated via the open field test, saccharin preference test, elevated plus maze test, tail suspension test, Morris water maze, and forced swimming test. The plasma concentrations of C-reactive protein (CRP) and interleukin-6 (IL-6) were measured, and the protein expressions of brain-derived neurotrophic factor (BDNF), Copine 6, and synaptic plasticity markers in the hippocampus and the PFC were also detected. The results showed that chronic unpredictable mild stress (CUMS) induces depression-like behavior in rats, accompanied by increased plasma concentrations of CRP and IL-6. Moreover, the protein expressions of BDNF, Copine 6, and synapsin I were decreased in both the hippocampus and the PFC of CUMS rats, and the protein expression of synaptotagmin I was decreased in the hippocampus. Furthermore, Pearson's test revealed a potential relationship between the depression-like behavior, the plasma CRP concentration, and the protein expressions of BDNF, Copine 6, synapsin I, or synaptotagmin I in the hippocampus or the PFC. Together with our previous results, the current findings suggest that apart from immune activation, the BDNF-related imbalance of Copine 6 expression in the brain might play a crucial role in stress-associated depression-like behaviors and synaptic plasticity changes.

Keywords: depression, chronic unpredictable mild stress (CUMS), saccharin preference, Copine 6, BDNF, synaptotagmin I, synapsin I

INTRODUCTION

Depression is one of the most prevalent psychiatric disorders. According to the data provided by the World Health Organization, depression affects approximately 350 million individuals in the world. Currently, most available antidepressant medications are aimed to increase the content of monoamine neurotransmitters in the synaptic cleft, based on the serendipitous discoveries of the clinical efficacy of two classes of antidepressants in the 1950s (Lopez-Munoz and Alamo, 2009). However, it has been reported that these drugs are not effective in all depressed patients and, even if they are, take weeks to months to produce a response (Berton and Nestler, 2006). Although vortioxetine has been reported as a novel antidepressant with multimodal activity and a faster response (Sanchez et al., 2015), targeting the 5-HT3, 5-HT7, 5-HT1, 5-HT1B, and 5-HT1A receptors and the serotonin (5-HT) transporter, this medication requires 8 weeks to reduce the Montgomery–Asberg depression rating scale (MADRS) total score (Mahableshwarkar et al., 2015) and has an adverse event profile similar to that of other selective serotonin reuptake inhibitors (SSRIs) (Zhang et al., 2015). Similarly, the non-competitive, glutamatergic *N*-methyl-D-aspartate receptor antagonist (R,S)-ketamine, exerts rapid and sustained antidepressant effects after a single dose in patients with depression, but its use is also associated with undesirable side effects (Zanos et al., 2016). Therefore, the mechanisms underlying the pathogenesis of depression need to be explored further and new targets for the development of next-generation, rapid-acting antidepressants must be identified (Malinow, 2016; Zanos et al., 2016).

Increasing evidence from animal and human studies show that stressful life events are among the most potent factors that trigger depressive episodes (Swaab et al., 2005), and more attention has been given to the neurobiological mechanisms underlying the association between stress and depression (Muller et al., 2011). The results of our previous studies have demonstrated that chronic unpredictable mild stress (CUMS) induces depression-like behavior and hyperactivity of the hypothalamic–pituitary–adrenal axis in rats, accompanied by imbalances in the leptin signaling pathway and hypothalamic synaptic plasticity (Ge et al., 2013). Moreover, feeding regulation-associated factors (Ge et al., 2015b) and metabolic disease, including subclinical hypothyroidism (Ge et al., 2016) and non-alcoholic fatty liver disease (Chen et al., 2017) also contribute to depression-like behaviors. Thus, the pathogenesis of depression is complicated by multiple risk factors.

Brain-derived neurotrophic factor (BDNF) is a critical effector of depression-like behaviors and antidepressant responses. Involved in neuronal development and neurotransmitter release, synaptic vesicle-associated proteins are indispensable to the integrity of synaptic structure and function. Synaptic vesicle-associated proteins are implicated in the regulation of BDNF-induced axonal growth and neurotransmitter release (Kao et al., 2017; Marte et al., 2017), and increasing evidence has demonstrated the relationship between synaptic dysfunction and depression (Duman and Aghajanian, 2012). Synapsin I is a regulator of synaptic transmission and is believed to affect axonal elongation and branching (Chin et al., 1995), and synaptotagmin I is an integral protein required for vesicle fusion and neurotransmitter release (Greengard et al., 1993). Differential expression of these two proteins may contribute to the molecular basis of stress-induced changes in synaptic plasticity in the hypothalamus (Ge et al., 2013), hippocampus, and cortex (Wu et al., 2007). The interaction between the synaptic vesicle-associated proteins and BDNF might trigger the imbalance of synaptic plasticity that occurs in depression (Kao et al., 2017; Marte et al., 2017).

Copines are a family of cytosolic proteins with the ability to bind to phospholipids in a calcium-dependent manner, which make them interesting candidates for actors in synaptic plasticity. Copine 6 is a neuron-specific member of the copine family. Shortly after Copine 6 was first described, Nakayama et al. reported a correlation between Copine 6 expression and neuronal activity, as indicated by its increased expression either upon long term potentiation (LTP) induction or kainate injection (Nakayama et al., 1998). Subsequently, further studies focused on its role in neuropsychiatric actions. Copine 6 transcripts and protein are expressed in the postnatal brain with peak expression in the hippocampus (Xu et al., 2015), and by regulating hippocampal synaptic plasticity, Copine 6 plays a crucial role in learning and memory (Reinhard et al., 2016). Knockout of Copine 6 induced a deficiency of hippocampal LTP and learning and memory in mice (Xu et al., 2015). More recently, a significantly increased expression of Copine 6 in hippocampal slices was observed after treatment with BDNF (Burk et al., 2018). Considering the crucial role of the hippocampus in the regulation of mood and behaviors, we decided to focus on Copine 6 expression in the hippocampus and the prefrontal cortex (PFC) of CUMS rats.

Dysfunction of the immune/inflammatory response is another contributor to depression (Hodes et al., 2015). Meta-analyses have indicated that C-reactive protein (CRP), interleukin-6 (IL-6), and TNF-α are the most robust evidence-based inflammatory markers associated with depression (Dowlati et al., 2010). Although Chocano-Bedoya et al. (2014) reported no significant correlation between IL-6 or CRP and depression, a growing number of studies have demonstrated that increased plasma IL-6 and CRP levels are positively associated with depression (Liu et al., 2014; Wium-Andersen et al., 2014), and can even predict subsequent depressive symptoms (Valkanova et al., 2013). Consistent with our previous study (Ge et al., 2015b), plasma concentrations of IL-6 and CRP were significantly increased in depressed rats induced by intraperitoneal injection of nesfatin-1.

To gain further insights into the association of stress with depression and to explore the possible change of Copine 6 expression in stressed rats, we replicated the CUMS rat model and observed their depression-like behaviors. Stress-induced alterations of Copine 6 and BDNF expression in the hippocampus and the PFC were detected via western blot. Changes in synaptic plasticity were investigated through the protein expressions of synapsin I and synaptotagmin I after CUMS in the rats' hippocampus and PFC, and the plasma

concentrations of IL-6 and CRP were detected using ELISA commercial kits.

MATERIALS AND METHODS

Animals

Twenty male Sprague-Dawley rats, aged 2 months, were purchased from Anhui Experimental Animal Center of China. The rats were divided randomly into control and CUMS groups and maintained under a 12:12 h light/dark cycle (lights on 07:00 h). The ambient temperature was maintained at 21–22°C with 50–60% relative humidity. Rats in the control group were housed 5 per cage with free access to food and water, while rats in the CUMS group were raised solitarily and received stress according to the CUMS procedure. All experimental procedures in this study were approved by the Animal Care and Use Committee at Anhui Medical University, which complies with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985).

CUMS Procedure

The CUMS paradigm consisted of daily exposure to alternating stressors along with occasional overnight stressors for four consecutive weeks. The stressors consisted of (Xu et al., 2015) (1) 24 h of social crowding (10 rats per cage); (2) a 20-min warm swim at 30°C; (3) 24 h in a cage tilted at 30° from the horizontal; (4) a 5-min cold swim at 8–10°C; (5) 24 h in a wet cage; (6) a 2-min tail pinch; and (7) 24 h of food and water deprivation. The different stressors were distributed randomly over an interval of at least 7 days.

Behavioral Tests

Behavioral tests were performed in a soundproof room with a neutral environment. All the tests were carried out between 08:30 and 12:30 and were matched between the groups. The observers were blind to the treatment. The behavioral tests were monitored and recorded by a digital camera interfaced to a computer running the ANY-maze video imaging software (Stoelting Co., Wood Dale, IL, United States).

Saccharin preference and open-field tests were conducted every week during CUMS. The Morris water maze, elevated plus maze, tail suspension, and forced swimming tests were conducted after CUMS was completed. The schedule is shown in **Figure 1**.

Saccharin Preference Test (SPT)

The SPT is commonly used to assess anhedonic behavior in rodents. After a 12-h period of food and water deprivation, all the rats were given free access to two bottles, one containing plain water and one containing a 1% saccharin solution, After 6 h, the volumes of water and saccharin consumed were measured. The saccharin preference index (SPI), which is the percentage of the saccharin solution that was ingested, was used as a measure of sensitivity to hedonia in rats.

Open Field Test (OFT)

The OFT, which provides simultaneous measures of locomotion, exploration, and anxiety, was carried out according to our previous studies (Ge et al., 2014). The apparatus consisted of a black square arena 100 cm × 100 cm with a black wall 30 cm high. The floor was marked with a grid dividing the floor into 16 equal-sized squares. During a 5 min observation period, rats were placed at one corner of the apparatus facing the wall. The distance, duration, and frequency in the center, and the frequencies of rearing, grooming, and defecation were recorded.

The Elevated Plus Maze (EPM) Test

The EPM test was designed according to the description in our previous studies (Ge et al., 2014) with little modification. Briefly, the maze (made of Plexiglas) consisted of a plus-shaped apparatus, with two opposite closed arms (45 cm × 11 cm) enclosed with walls (22 cm in height) and two opposite open arms (45 cm × 11 cm, without walls). The whole apparatus had a central arena (11 cm × 11 cm) and was elevated 80 cm above the floor. Each rat was placed in the central arena of the maze facing an open arm and was allowed to explore the maze for 5 min. The distance traveled in the open arms and the closed arms was analyzed.

Morris Water Maze (MWM) Test

The MWM test was used to test spatial learning and memory. The pool (1.6 m in diameter) was filled with opaque water and surrounded by complex maze cues. The escape platform (9 cm in diameter) was placed in the center of a designated quadrant with its top positioned 1.3 cm below the water surface. In the place navigation test, each rat received four trials per day of training for 3 days. The rat was allowed 60 s to find the platform, and stayed there for 20 s. If a rat failed to find the hidden platform within 60 s, it was guided to the platform and allowed to remain there for 20 s. A probe test was conducted on day 4, in which the hidden platform was removed and the rat was allowed to swim for 60 s. The escape latency to find the hidden platform in the place navigation test and the duration of time spent by the rats in the target quadrant in the probe trial were analyzed.

Tail Suspension Test (TST)

The TST was carried out according to the method described in our previous studies (Ge et al., 2013). Briefly, rats were suspended by bands around their tails and hung from a mounted hook 50 cm above the floor for 6 min. Time spent immobile during the last 4 min was measured. Immobility time was defined as a lack of all movement except for whisker movement and respiration.

Forced Swimming Test (FST)

The FST was carried out according to the method described in our previous study (Ge et al., 2013). The behavioral cylinder was 60 cm high and 25 cm in diameter, maintained at 24–25°C, and filled with 30 cm of water. The FST paradigm includes two steps: an initial 15 min pretest followed by a 5 min test 24 h later. The rats were considered to be immobile when they did not make any active movements. Struggling was considered to occur when the rats made active movements with their forepaws in and out of

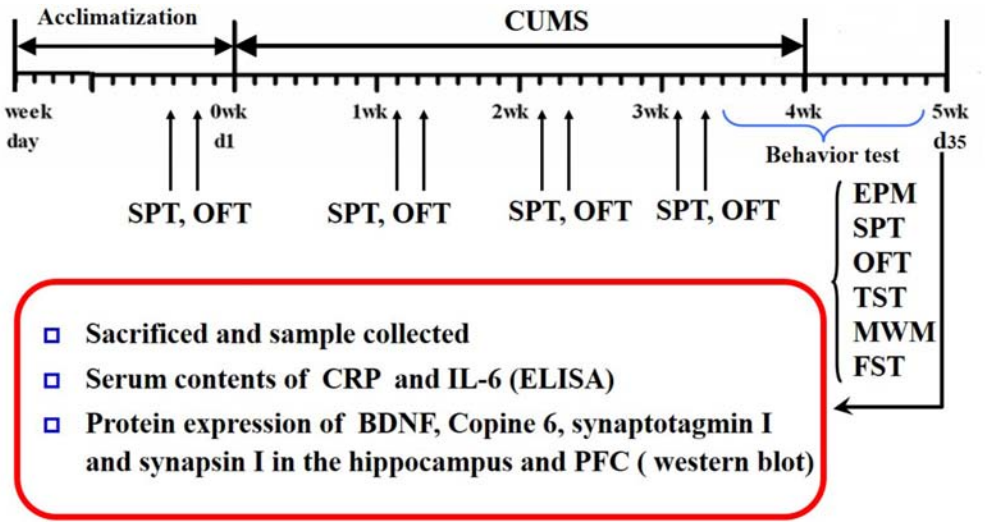

FIGURE 1 | Schedule of the experimental design. CUMS, chronic unpredictable mild stress; OFT, open field test; SPT, saccharin preference test; EPM, elevated plus maze; YM, Y-maze; MWM, Morris water maze; TST, tail suspension test; FST, forced swimming test; CRP, C-reactive protein; BDNF, brain-derived neurotrophic factor; PFC, prefrontal cortex.

the water along the side of the swim chamber. Swimming was considered to occur when the rats made active swimming or circular movements.

Western Blot Assays

The hippocampus and the PFC from four rats in each group were rapidly dissected, frozen in liquid nitrogen, and stored at −80°C. The tissues were homogenized in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.4, 0.1% SDS, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, and 1 mM Na_3VO_4). Before homogenization, a protease inhibitor cocktail (Roche, Indianapolis, IN, United States) and the phosphatase inhibitor PhosSTOP (Roche, Indianapolis, IN, United States) were added. Protein quantitation was conducted using a Lowry Protein Assay Kit (Meiji Biotech. Co., LTD., Shanghai, China). The same quantity (∼50 μg) of protein from each animal was loaded and separated by 15% SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane (Amersham Biosciences, United Kingdom). The membrane was blocked with 5% skim milk for 1 h; incubated with antibodies targeting BDNF (1:1000; ImmunoWay, Newark, DE, United States), Copine 6 (1:1,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, United States), synapsin I (1:1000; ImmunoWay, Newark, DE, United States), synaptotagmin I (1:1000; ImmunoWay, Newark, DE, United States), or β-actin (1:1000; Zhongshan Biotechnology, INC, Beijing, China) at 4°C overnight; and then incubated with a horseradish peroxidase-conjugated secondary antibody (1:2000) at 37°C for 2 h. The blots were developed with the Easy Enhanced Chemiluminescence Western Blot Kit (Pierce Biotechnology, Rockford, IL, United States). Protein bands were scanned and analyzed using Image J software (NIH), and the protein expression was normalized to β-actin.

Measurement of the Plasma Concentrations of IL-6 and CRP

Twenty-four hours after the last behavioral test, the rats were deeply anesthetized with chloral hydrate, and blood was taken from the abdominal aorta. The plasma was collected, and the concentrations of IL-6 and CRP were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Yuanye Biotech. Co., LTD., Shanghai, China) according to the manufacturer's instructions.

Statistical Analyses

All statistical analyses were performed using SPSS (version 12.0.1, SPSS Inc., Chicago, IL, United States). Data are expressed as the means ± SEM and $P < 0.05$ was considered statistically significant. The effect of time and stress on the bodyweight-gain and the behavior of the rats in the OFT and the SPT were analyzed using repeated measures ANOVA. The effect of training and stress on escape latency in the MWM test was also analyzed using repeated measures ANOVA. The difference in other parameters between the control and CUMS groups was tested by using Student's *t*- test. The correlation analysis was performed by Pearson's correlation test.

RESULTS

Slow Increase of Body-Weight Induced by CUMS

Figure 2 shows the changes in body-weight gain during the four consecutive weeks in the two groups. The result of repeated measures ANOVA showed that time [($F(3,54) = 147.068$, $P < 0.001$)] but not stress [($F(1,18) = 1.83$, $P = 0.189$)] had

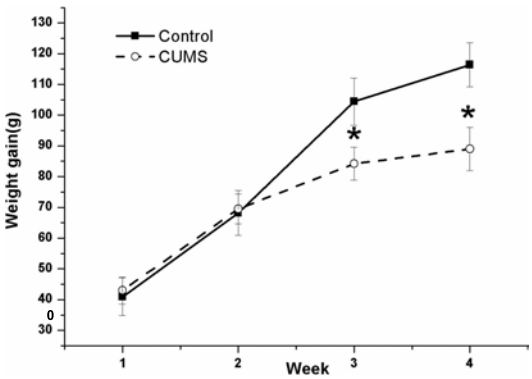

FIGURE 2 | Body-weight gain in the control and CUMS rats. The data are presented as the mean ± SEM, with 10 rats in each group. The net body-weight gain was lower in the CUMS group than in the control group during the latter 2-week stress period. *$P < 0.05$, **$P < 0.001$; compared with control group.

a significant effect on body-weight gain, with an interactive effect between time and stress [($F(3,54) = 11.031$, $P < 0.001$)]. The net bodyweight-gain was lower in the CUMS group than in the control group during the latter 2-week stress period.

Decrease of Locomotor Activity and Exploration Behavior Induced by CUMS

Figure 3 shows the performance of rats in the OFT and the EPM test. In the OFT, the result of repeated measures ANOVA showed that both time [$F(4,72) = 6.358$, $P < 0.001$] and stress [$F(1,18) = 7.753$, $P = 0.012$] had significant effects on the total moving distance (**Figure 3A**), without an interactive effect between time and stress [$F(4,72) = 0.806$, $P = 0.525$]. Consistently, both time [$F(4,72) = 7.488$, $P < 0.001$] and stress [$F(1,18) = 8.389$, $P = 0.010$] had significant effects on the center duration (**Figure 3B**), without an interactive effect between time and stress [$F(4,72) = 1.218$, $P = 0.311$]. Both time [$F(4,72) = 36.089$, $P < 0.001$] and stress [$F(1,18) = 32.058$, $P < 0.001$] had significant effects on the rearing number (**Figure 3C**), without an interactive effect between time and stress [$F(4,72) = 1.436$, $P = 0.231$]. However, an effect of time [$F(4,72) = 7.267$, $P < 0.001$] but not stress [$F(1,18) = 1.745$, $P = 0.149$] was identified for the number of grooming movements (**Figure 3D**), without an interactive effect between time and stress [$F(4,72) = 0.015$, $P = 0.903$]. In contrast, it was demonstrated that stress [$F(1,18) = 9.749$, $P = 0.006$] but not time [$F(4,72) = 2.222$, $P = 0.075$] had a significant effect on the defecation number (**Figure 3E**), without an interactive effect between time and stress [$F(4,72) = 1.094$, $P = 0.366$].

In the EPM test, the CUMS rats traveled a smaller distance than the control rats did, with significant differences between the groups in the total distance (**Figure 3F**), the distance in the closed arm (**Figure 3F**), and the duration in the junction (**Figure 3H**). There were no significant changes in the distance in the open arm (**Figure 3F**) or in the frequency (**Figure 3G**) or duration (**Figure 3H**) in both the open and closed arm between groups.

Anhedonia, Despair-Behavior, Impaired Learning, and Memory Ability Induced by CUMS

As shown in **Figure 4A**, the tendency of the saccharin preference index in the CUMS group is different from that in the control group. Because they are almost identical in the second week (0.876 ± 0.028 of control rats vs. 0.875 ± 0.029 of CUMS rats), the data were analyzed separately. From week 0 to week 2 of the CUMS, results of repeated measures ANOVA showed that time [$F(2,36) = 3.718$, $P = 0.034$] but not stress [$F(1,18) = 0.152$, $P = 0.701$] had a significant effect on the saccharin preference index, without an interactive effect between time and stress [$F(2,36) = 0.636$, $P = 0.535$]. However, as for the duration from the second week to the fourth week of stress exposure, both time [$F(2,36) = 3.973$, $P = 0.028$] and stress [$F(1,18) = 8.460$, $P = 0.009$] affected the saccharin preference index, with an interactive effect between time and stress [$F(2,36) = 4.427$, $P = 0.019$].

In the FST (**Figure 4B**) and the TST (**Figure 4C**) after 4 weeks of CUMS, the CUMS rats spent a longer time immobile.

In the MWM task, the escape latency of both groups in the consecutive 3-day place navigation test declined gradually (**Figure 4D**). Although the CUMS rats seemed to spend slightly more time in finding the hidden platform than the control rats did, the results of repeated measures ANOVA showed that training [$F(2,36) = 29.181$, $P < 0.001$] but not the stress [$F(1,18) = 1.273$, $P = 0.274$] had a significant effect on escape latency, and no interactive effect was found between time and stress [$F(2,36) = 0.068$, $P = 0.934$]. However, in the probe phase of the MWM test, the duration in the target quadrant of the CUMS rats was shorter than that in the control rats (**Figure 4E**).

Imbalanced Expression of BDNF, Copine 6, Synapsin I, and Synaptotagmin I in the Hippocampus and the PFC Induced by CUMS

Figure 5 shows the protein expressions of BDNF, Copine 6, synapsin I, and synaptotagmin I in the hippocampus and the PFC of the rats in both groups. In the hippocampus, the expression of all four of these four proteins was decreased in the CUMS group. Apart from the predicable positive correlation between synapsin I and synaptotagmin I ($r = 0.828$, $P = 0.011$, **Figure 6A**), a positive relationship was also found between the expression of BDNF and Copine 6 ($r = 0.732$, $P = 0.039$, **Figure 6B**) and synaptotagmin I ($r = 0.886$, $P = 0.003$, **Figure 6C**). Additionally, the hippocampal protein expression of Copine 6 was positively correlated with that of synaptotagmin I ($r = 0.847$, $P = 0.008$, **Figure 6D**) and synapsin I ($r = 0.931$, $P = 0.001$, **Figure 6E**).

Consistently, there was a remarkable decrease in the BDNF, Copine 6, and synapsin I protein expressions in the PFC of CUMS rats, although the expression of synaptotagmin I was not significantly changed.

Pearson's test revealed a positive relationship between the saccharin preference index after four consecutive weeks of CUMS and the hippocampal expression of BDNF ($r = 0.800$, $P = 0.017$, **Figure 7A**). A negative relationship was seen between immobility

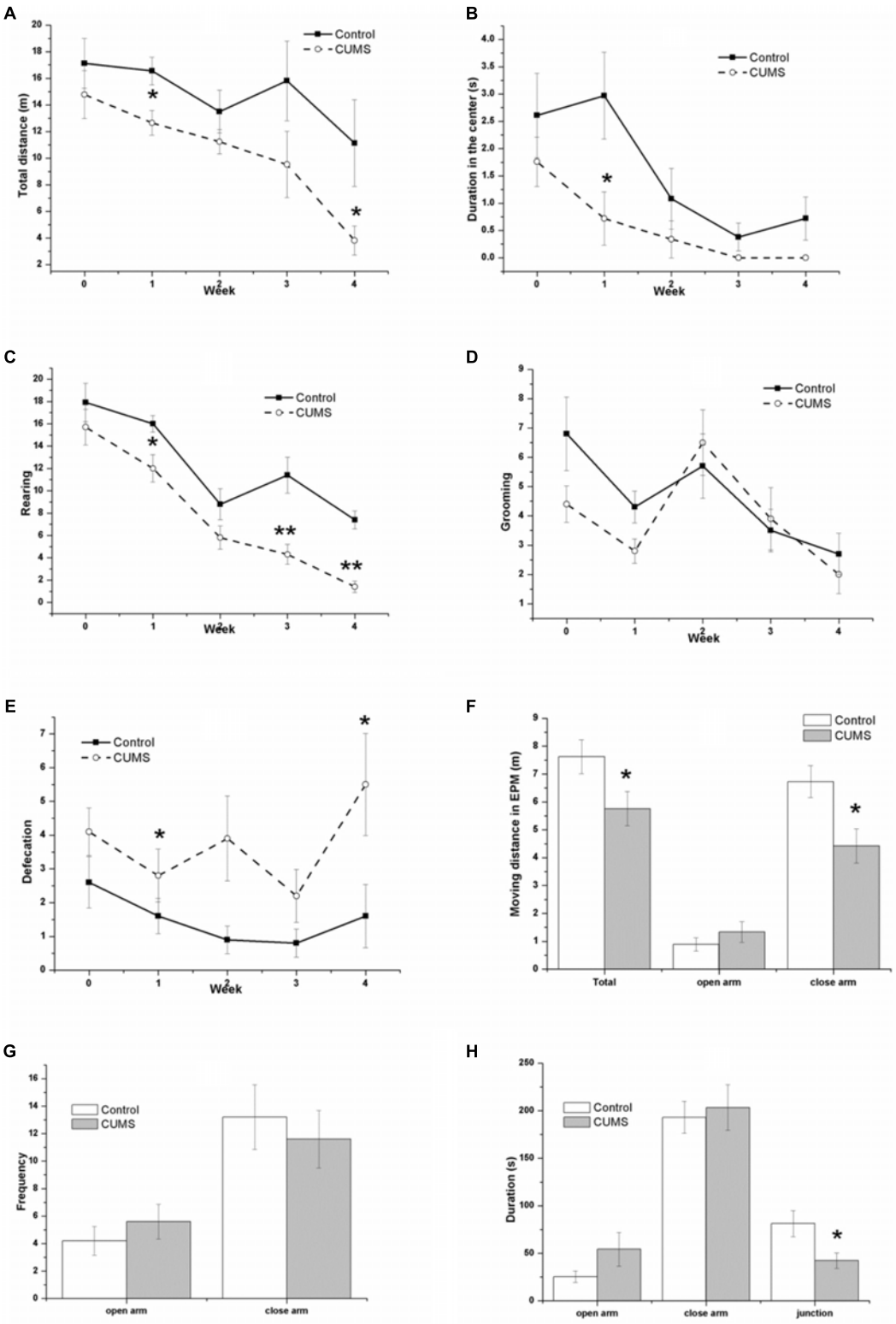

FIGURE 3 | Behavior of the control and CUMS rats in the OFT and the EPM tests. The data are presented as the mean ± SEM, with 10 rats in each group. In the OFT **(A–E)**, repeated measures ANOVA show that both time and stress had a significant effect on total moving distance **(A)**, center duration **(B)**, and rearing number **(C)**. The time effect on the grooming number **(D)** and the stress effect on the defecation number **(E)** are also shown. In the EPM test **(F–H)**, the total distance **(F)**, distance in the closed arm **(F)**, and duration in the junction **(H)** in the CUMS group were less than those in the control group, although there is no significant difference in the distance **(F)**, frequency **(G)**, and duration **(H)** in the open arm between groups, $^{*}P < 0.05$, $^{**}P < 0.001$; compared with control group.

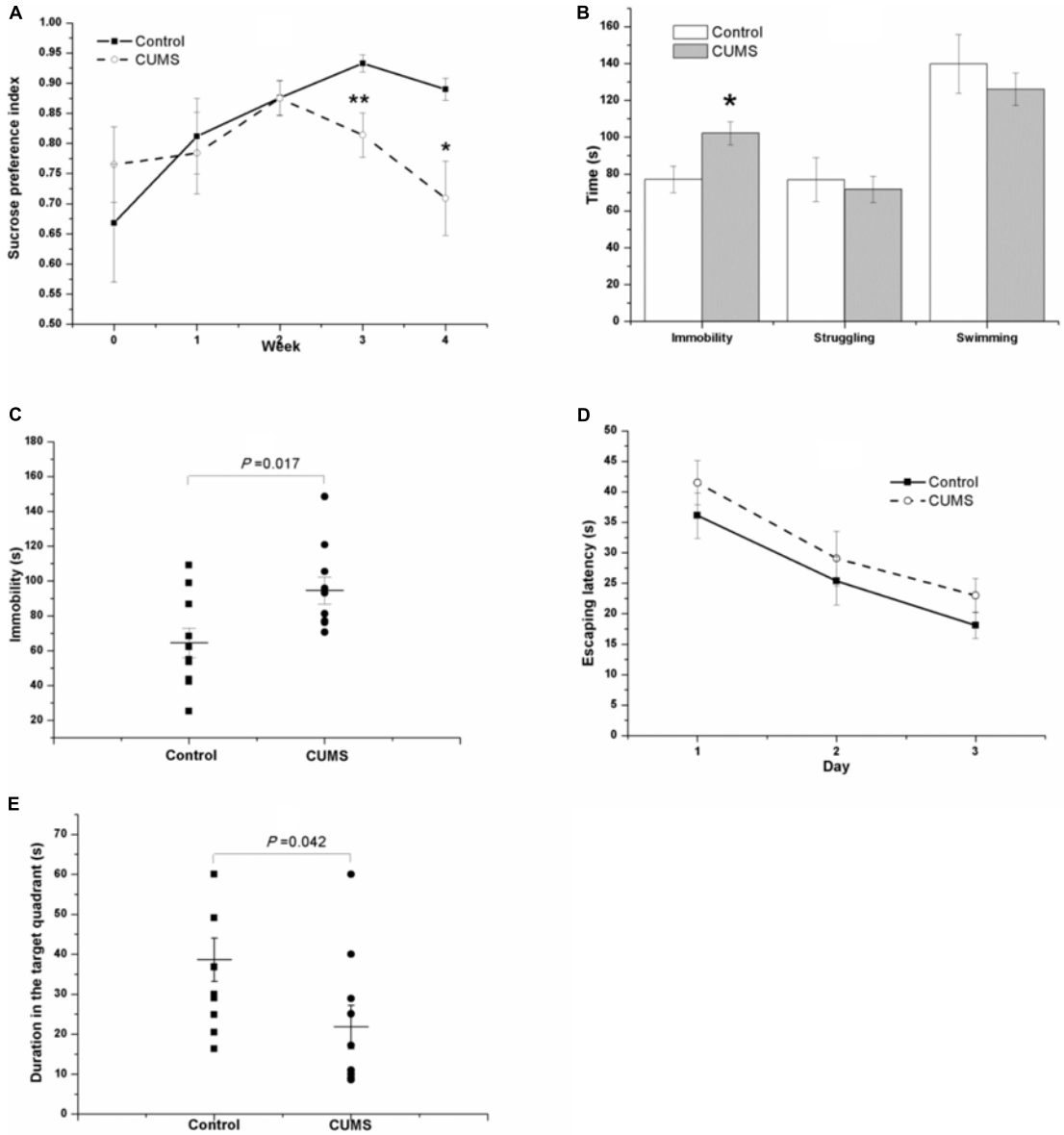

FIGURE 4 | Behavior of the control and CUMS rats in the SPT, TST, FST, and MWM test. The data are presented as the mean ± SEM, with 10 rats in each group. In the SPT **(A)**, the results of repeated measures ANOVA show an effect of time but not stress on the saccharin preference index from week 0 to stress exposure week 2. From the second to the fourth stress exposure weeks, both time and stress had a significant effect on the saccharin preference index. Compared with the control rats, the CUMS rats spent more time immobile in the FST **(B)** and TST **(C)** but less time in the probe phase of the MWM test **(E)**. In the place navigation of the MWM test, the escape latency of both groups in the consecutive three days' place navigation test gradually declined **(D)**, with no significant difference between groups.

in the FST and the expression of Copine 6 ($r = -0.789$, $P = 0.020$, **Figure 7B**) and synapsin I ($r = -0.839$, $P = 0.009$, **Figure 7C**) in the hippocampus and the expression of BDNF in the PFC ($r = -0.710$, $P = 0.048$, **Figure 7D**). Likewise, immobility in the TST was also found to be negatively related to the hippocampal expression of synapsin I ($r = -0.848$, $P = 0.008$, **Figure 7E**) and the expression of BDNF in the PFC ($r = -0.750$, $P = 0.032$, **Figure 7F**). However, immobility in the TST was not remarkably related to the expression of Copine 6 protein in the hippocampus ($r = -0.682$, $P = 0.063$).

Increase of Plasma IL-6 and CRP Concentrations Induced by CUMS

After a 4-week period of CUMS, the plasma concentrations of IL-6 (**Figure 8A**) and CRP (**Figure 8B**) were both remarkably increased, compared with those of the control rats. Interestingly, the results of Pearson's test showed that the plasma concentrations of CRP were positively related to the immobility in the FST ($r = 0.501$, $P = 0.024$, **Figure 8C**) but negatively correlated to the protein expressions of BDNF ($r = -0.716$, $P = 0.046$, **Figure 8D**) and synaptotagmin I ($r = -0.788$, $P = 0.020$,

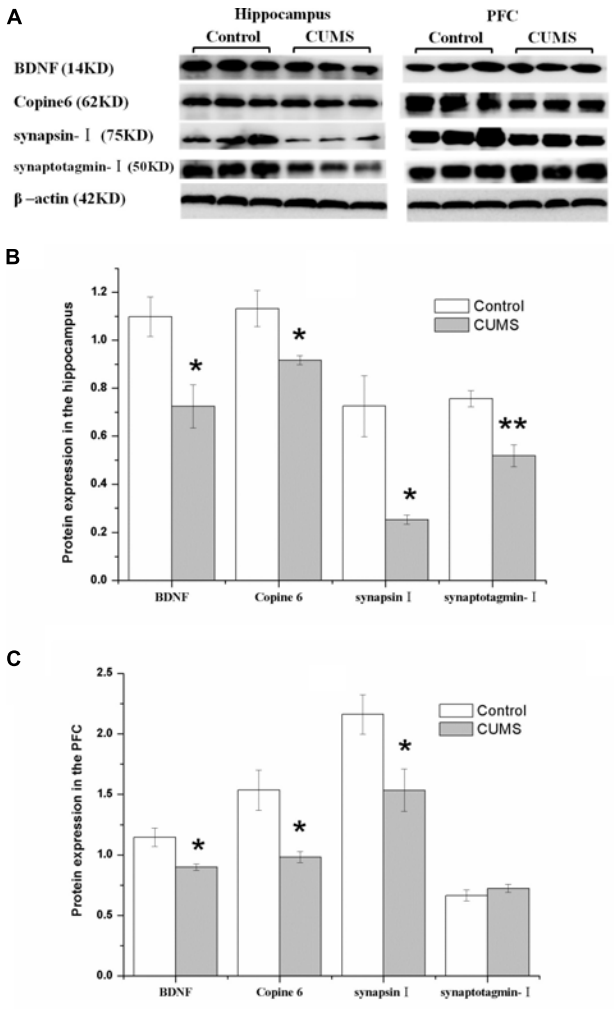

FIGURE 5 | Protein expressions of BDNF, Copine 6, synapsin I, and synaptotagmin I in the hippocampus and the PFC of the control and CUMS rats. Typical graph **(A)** and statistical analyses **(B,C)** of the western blot results. Data in **(B,C)** are presented as the mean ± SEM ($n = 4$ for each group). In the hippocampus **(A,B)**, the expression of all four proteins was all decreased in the CUMS group. In the PFC **(A,C)**, remarkable decreases in the BDNF, Copine 6 and synapsin I protein expressions were observed, with an insignificant change in synaptotagmin I expression. $^{*}P < 0.05$, $^{**}P < 0.001$; compared with control group.

Figure 8E) in the hippocampus, or to the protein expression of BDNF in the PFC ($r = -0.765$, $P = 0.027$, **Figure 8F**).

DISCUSSION

In the present study, we duplicated a CUMS-induced depression rat model, observed the behavior of the animals, and explored the possible mechanisms underlying the changed behavioral performance. Our results verified the depression-like behaviors induced by CUMS and demonstrated that the protein expressions of BDNF, Copine 6, and synapsin I were significantly decreased in both the hippocampus and the PFC of CUMS rats, together with decreased hippocampal expression of synaptotagmin I. Moreover, the plasma levels of IL-6 and CRP were remarkably increased after 4 weeks of CUMS. Furthermore, our results demonstrated the potential relationships among depression-like behavior, imbalanced protein expression in the related brain areas, and increased immune activity. These alterations are in accordance with the network hypothesis of depressive disorders that proposes that the compromised functionality of relevant neural networks may underlie the development of depressive symptomatology (Djordjevic et al., 2012).

Consistent with our previous findings (Ge et al., 2013), the present study shows that chronic unpredictable stress induces depression-like behavioral deficits in rats, including anhedonia, reduced locomotor activity and exploration behavior, and "behavioral despair," as indicated by the decreased saccharin preference index, decreased locomotion and rearing in the OFT, and increased immobility in both the FST and the TST. In accordance with the finding that an anxiety-/depression-like phenotype is associated with a cognitive deficit (Darcet et al., 2014), CUMS rats showed an impairment in learning and memory ability, as indicated by the shorter duration in the target quadrant in the probe test of the MWM test, although the increased escape latency was only a tendency without a significant difference between groups.

Although little is known about the molecular components and mechanisms involved in the stress response, increasing evidence suggests that BDNF-associated synaptic dysfunction is a key pathophysiological hallmark in depression. Prolonged stress has been associated with region-specific changes in the expression of BDNF, synaptotagmin I, and synapsin I (Smith et al., 1995; Thome et al., 2001), and decreased BDNF has been associated with age-related hippocampal dysfunction, memory impairment, and increased risk for depression (Erickson et al., 2012; Platenik et al., 2014). In consistence, our results showed that the protein expressions of BDNF and synapsin I were decreased in the hippocampus and the PFC of CUMS rats, along with a decline in the synaptotagmin I expression in the hippocampus. BDNF plays a crucial role in the regulation of synaptic function in a site-specific manner (Leal et al., 2014; Chang et al., 2018), and synapsin I plays a pivotal role in BDNF signal transduction during axonal growth (Marte et al., 2017). In our previous study (Ge et al., 2015a), decreased protein expressions of BDNF and synapsin I were also found in a subclinical hypothyroidism model with a positive relationship. These findings suggest a remarkable linkage between BDNF and synaptic structure and function. In our present study, Pearson's test uncovered a positive relationship between the hippocampal expression of synaptotagmin I and BDNF, providing new evidence for the close link between BDNF and the synaptotagmin family in regulating synaptic plasticity. Although the exact mechanism remains unknown, BDNF may serve as a homeostatic regulator, eliciting neuroprotective functions when neurons are damaged in disease conditions (Lu et al., 2013). Synapsins could act by regulating the ratio of lipids in intracellular membranes, thereby promoting lipid raft formation, and regulating BDNF-mediated synaptic potentiation and axon elongation (Kao et al., 2017). Thus, a rational way to conduct a future experiment would

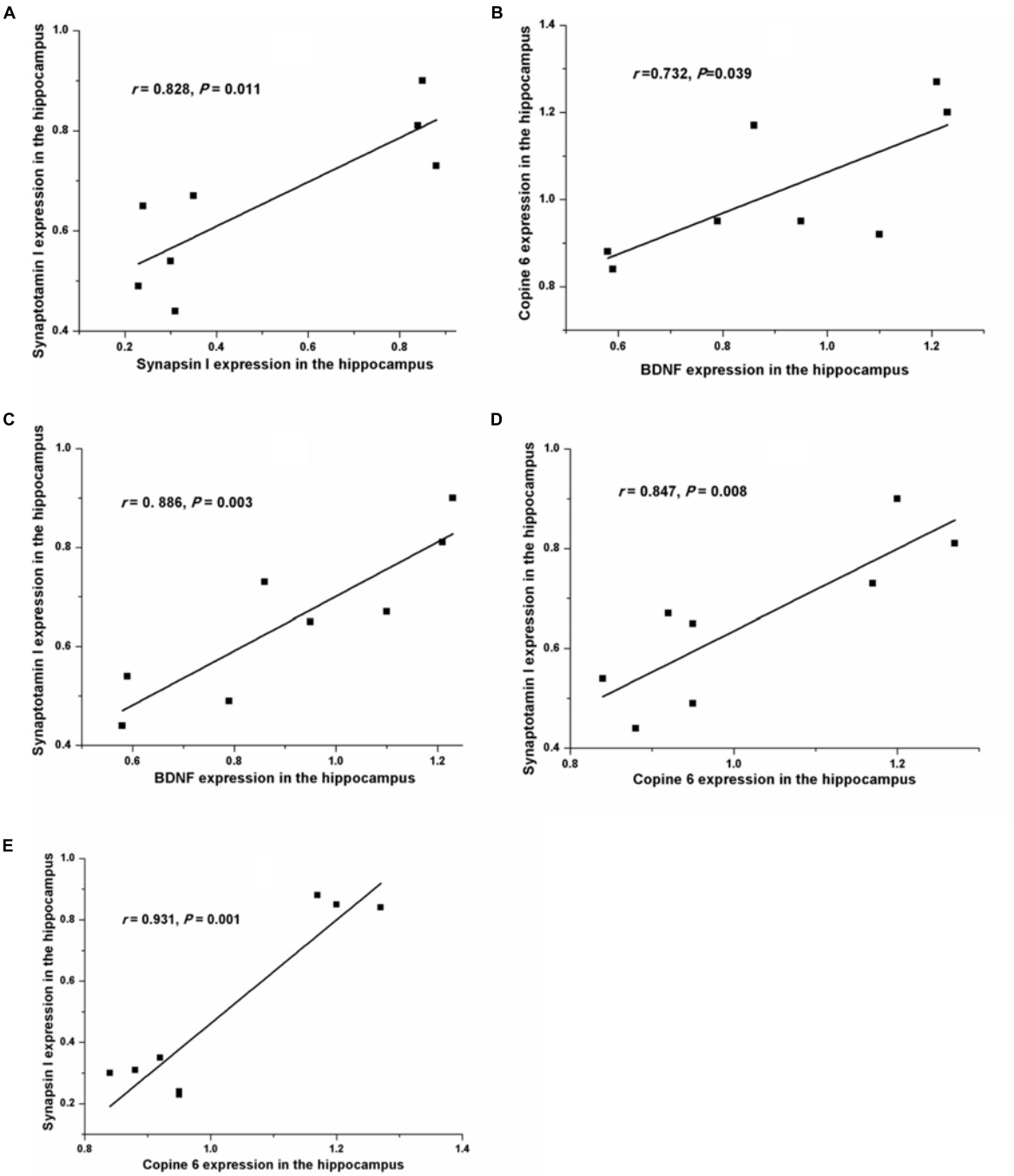

FIGURE 6 | Correlation analysis of the protein expressions of BDNF, Copine 6, synapsin I, and synaptotagmin I in the hippocampus and the PFC. In the hippocampus, apart from the positive correlation between synapsin I and synaptotagmin I **(A)** expression, BDNF expression was positively related to the expression of Copine 6 **(B)** and synaptotagmin I **(C)**. Additionally, the protein expression of Copine 6 was positively correlated with that of synaptotagmin I **(D)** and synapsin I **(E)**.

include a combination of activating the BDNF pathway and using a more reliable and sensitive method to measure the resulting synaptic changes.

Copine 6 is a brain-specific, calcium-dependent, phospholipid-binding protein with a scarcely described function (Cowland et al., 2003; Walf and Frye, 2007). Based on its ability to bind, activate, and recruit the Rho GTPase Rac1 to cell membranes, Copine 6 plays a vital role in the regulation of hippocampal synaptic plasticity (Reinhard et al., 2016); consistent with this idea, Copine 6-knockout mice present impairments in learning and memory abilities. The decreased expression of Copine 6 in the hippocampus and

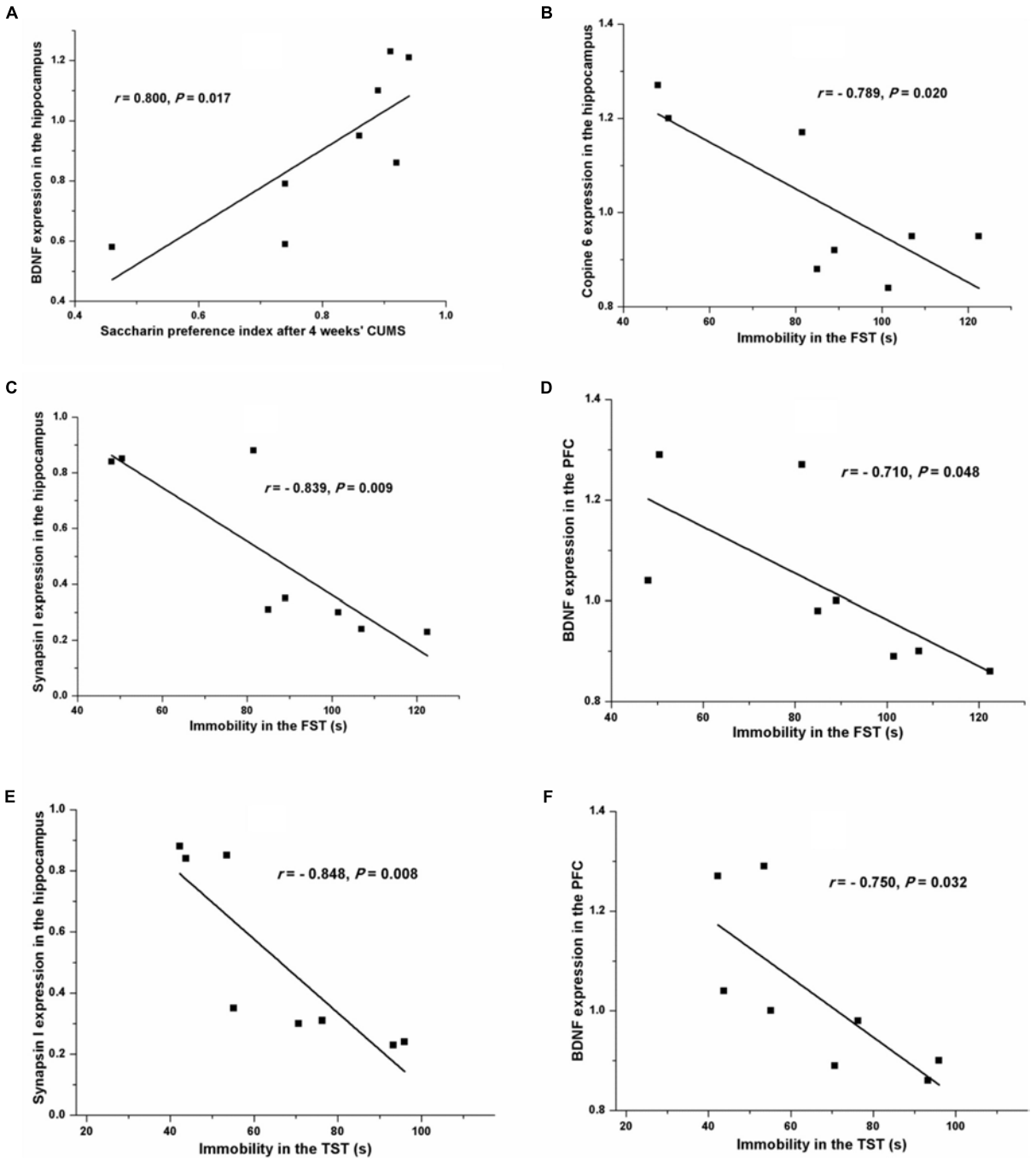

FIGURE 7 | Correlation analysis of the depression-like behavior and the protein expressions of BDNF, Copine 6, or synapsin I in the hippocampus and the PFC. A positive relationship was found between the saccharin preference index after four consecutive weeks of CUMS and the hippocampal expression of BDNF **(A)**. A negative relationship was seen between immobility in the FST and the expression of Copine 6 **(B)** and synapsin I **(C)** in the hippocampus and the expression of BDNF in the PFC **(D)**. Immobility in the TST was negatively related to the hippocampal expression of synapsin I **(E)** and the expression of BDNF in the PFC **(F)**.

the PFC of rats with non-alcoholic fatty liver disease was demonstrated in our previous study (Chen et al., 2017). In consistence, the present results showed a decreased expression of Copine 6 in the hippocampus and the PFC of CUMS rats. Moreover, in accordance with the report that BDNF treatment increases the expression of Copine 6 in hippocampal slices (Burk et al., 2018), a positive relationship was found between the hippocampal expression of BDNF and Copine 6 in the present study. Additionally, the protein expression of Copine 6 in the hippocampus was also positively correlated with that of

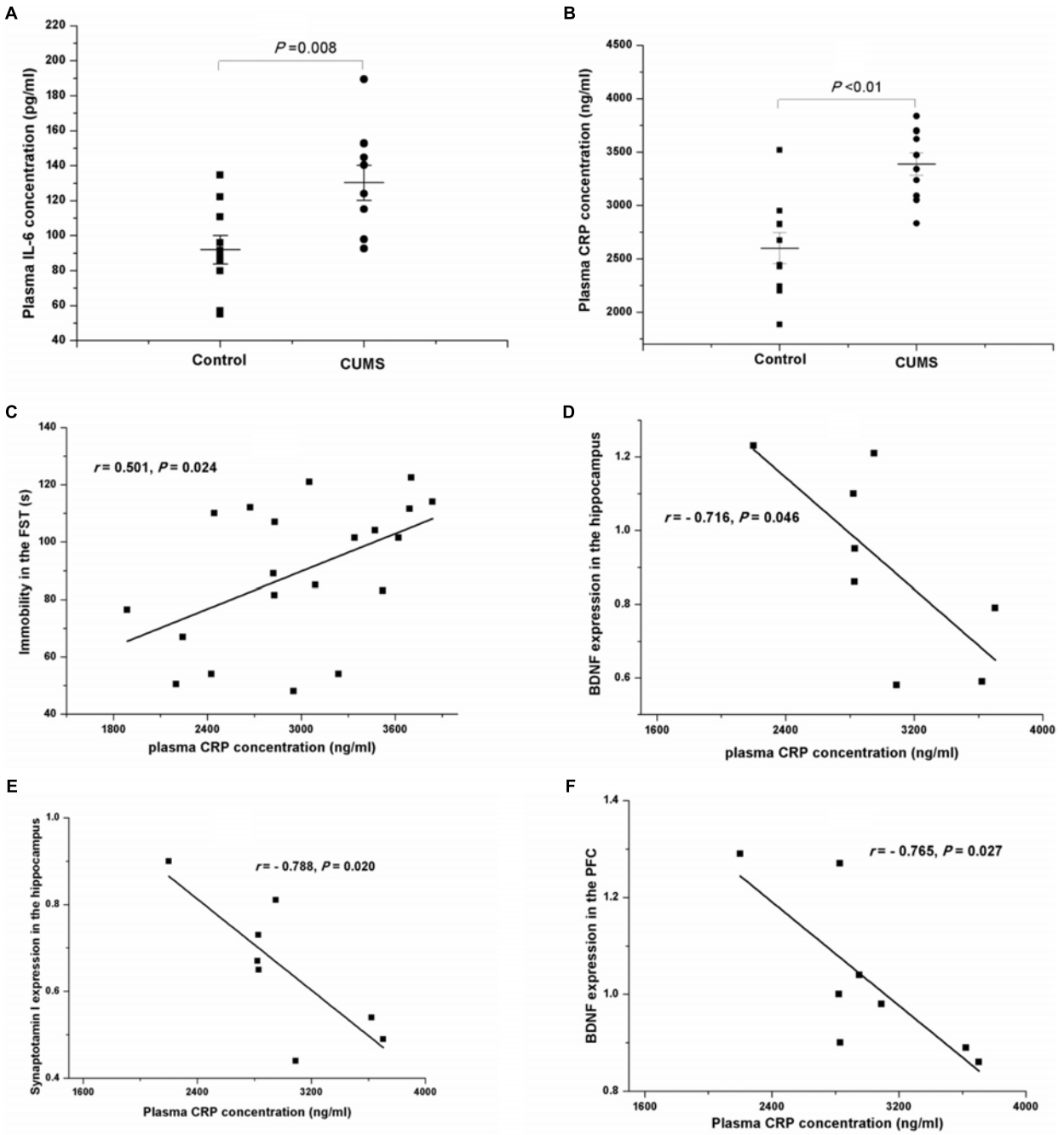

FIGURE 8 | Plasma IL-6 and CRP concentrations of the control and CUMS rats, and correlation analysis of the plasma CRP concentration and immobility in the FST and the protein expressions in the hippocampus and the PFC. The data are presented as the mean ± SEM in **(A,B)**, with 10 rats in each group. The plasma IL-6 **(A)** and CRP **(B)** concentrations were both notably increased in the CUMS group. The plasma CRP concentration was positively related to immobility in the FST **(C)**, and negatively related to the protein expressions of BDNF **(D)** and synaptotagmin I **(E)** in the hippocampus and BDNF in the PFC **(F)**.

synaptotagmin I and synapsin I. These findings indicate again the important role of Copine 6 in the regulation of hippocampal synaptic plasticity. Although the mechanism remains to be explored, it is rational to propose a role of BDNF in connecting the changes in hippocampal Copine 6 expression and synaptic plasticity.

Additionally, Pearson's test showed the potential connection between depression-like behavior and the changes in the protein expression in the hippocampus and the PFC. The saccharin preference index, which is used as an indicator of anhedonia, was positively related to the hippocampal BDNF expression. Moreover, despair behavior, as indicated by immobility in the FST

or the TST, was negatively related to the expression of synapsin I in the hippocampus and the expression of BDNF in the PFC. However, the hippocampal expression of Copine 6 was negatively related to immobility in the FST but not in the TST. These results might be ascribed to the hypothesis that different behaviors are controlled by different brain areas.

A growing body of evidence suggests the potential relationships between immune hyperactivity and the severity of the symptoms of depression (Liu et al., 2014; Wium-Andersen et al., 2014). Increased plasma concentrations of IL-6 and CRP have been detected in depressed patients and animals (Voorhees et al., 2013; Liu et al., 2014; Wium-Andersen et al., 2014). In accordance with these findings, our results show increased plasma IL-6 and CRP levels in the CUMS rats, and a positive relationship between plasma CRP concentration and immobility in the FST. Moreover, Pearson's test showed that plasma CRP concentrations were negatively related to the protein expression of BDNF in the hippocampus and the PFC. These findings further support the crucial role of immune activity in the pathogenesis of stress-induced depression.

Taken together, our study replicated depression-like behavior in a rat CUMS model, and decreased the expression of BDNF and its related synaptic plasticity change in the hippocampus and the PFC, accompanied by hyperactivity of the immune response. Moreover, our study showed a BDNF-related decrease of Copine 6 protein expression in the hippocampus and the PFC of CUMS rats, providing a preliminary evidence for the important role of Copine 6 in the regulation of neuropsychiatric behaviors and synaptic plasticity. These findings might shed light on the pathogenesis of stress-associated depression.

AUTHOR CONTRIBUTIONS

J-fG designed the study, and wrote the protocol and the first draft of the manuscript. Y-xH and CT managed the literature searches and the statistical analyses. Y-xH, X-rG, L-lW, and F-hJ performed animal model experiments. KF, X-xC, CW, and ZC performed the gene expression experiments and wrote parts of the manuscript. All authors contributed to and have approved the final manuscript.

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REFERENCES

Berton, O., and Nestler, E. J. (2006). New approaches to antidepressant drug discovery: beyond monoamines. *Nat. Rev. Neurosci.* 7, 137–151. doi: 10.1038/nrn1846

Burk, K., Ramachandran, B., Ahmed, S., Hurtado-Zavala, J. I., Awasthi, A., Benito, E., et al. (2018). Regulation of dendritic spine morphology in hippocampal neurons by copine-6. *Cereb Cortex.* 28, 1087–1104. doi: 10.1093/cercor/bhx009

Chang, S., Trimbuch, T., and Rosenmund, C. (2018). Synaptotagmin-1 drives synchronous Ca(2 +)-triggered fusion by C2B-domain-mediated synaptic-vesicle-membrane attachment. *Nat. Neurosci.* 21, 33–40. doi: 10.1038/s41593-017-0037-5

Chen, Z., Xu, Y.-Y., Wu, R., Han, Y.-X., Yu, Y., Ge, J.-F., et al. (2017). Impaired learning and memory in rats induced by a high-fat diet: involvement with the imbalance of nesfatin-1 abundance and copine 6 expression. *J. Neuroendocrinol.* 29, 1–12. doi: 10.1111/jne.12462

Chin, L. S., Li, L., Ferreira, A., Kosik, K. S., and Greengard, P. (1995). Impairment of axonal development and of synaptogenesis in hippocampal neurons of synapsin I-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 92, 9230–9234. doi: 10.1073/pnas.92.20.9230

Chocano-Bedoya, P. O., Mirzaei, F., O'Reilly, E. J., Lucas, M., Okereke, O. I., Hu, F. B., et al. (2014). C-reactive protein, interleukin-6, soluble tumor necrosis factor alpha receptor 2 and incident clinical depression. *J. Affect. Disord.* 163, 25–32. doi: 10.1016/j.jad.2014.03.023

Cowland, J. B., Carter, D., Bjerregaard, M. D., Johnsen, A. H., Borregaard, N., and Lollike, K. (2003). Tissue expression of copines and isolation of copines I and III from the cytosol of human neutrophils. *J. Leukoc. Biol.* 74, 379–388. doi: 10.1189/jlb.0203083

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A. M., Guilloux, J. P., and David, D. J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Front. Behav. Neurosci.* 8:136. doi: 10.3389/fnbeh.2014.00136

Djordjevic, J., Djordjevic, A., Adzic, M., and Radojcic, M. B. (2012). Effects of chronic social isolation on wistar rat behavior and brain plasticity markers. *Neuropsychobiology* 66, 112–119. doi: 10.1159/000338605

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457. doi: 10.1016/j.biopsych.2009.09.033

Duman, R. S., and Aghajanian, G. K. (2012). Synaptic dysfunction in depression: potential therapeutic targets. *Science* 338, 68–72. doi: 10.1126/science.1222939

Erickson, K. I., Miller, D. L., and Roecklein, K. A. (2012). The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist* 18, 82–97. doi: 10.1177/1073858410397054

Ge, J. F., Gao, W. C., Cheng, W. M., Lu, W. L., Tang, J., Peng, L., et al. (2014). Orcinol glucoside produces antidepressant effects by blocking the behavioural and neuronal deficits caused by chronic stress. *Eur. Neuropsychopharmacol.* 24, 172–180. doi: 10.1016/j.euroneuro.2013.05.007

Ge, J. F., Qi, C. C., and Zhou, J. N. (2013). Imbalance of leptin pathway and hypothalamus synaptic plasticity markers are associated with stress-induced depression in rats. *Behav. Brain Res.* 249, 38–43. doi: 10.1016/j.bbr.2013.04.020

Ge, J. F., Xu, Y. Y., Li, N., Zhang, Y., Qiu, G. L., Chu, C. H., et al. (2015a). Resveratrol improved the spatial learning and memory in subclinical hypothyroidism rat induced by hemi-thyroid electrocauterization. *Endocr. J.* 62, 927–938. doi: 10.1507/endocrj.EJ15-0253

Ge, J. F., Xu, Y. Y., Qin, G., Peng, Y. N., Zhang, C. F., Liu, X. R., et al. (2015b). Depression-like behavior induced by Nesfatin-1 in rats: involvement of increased immune activation and imbalance of synaptic vesicle proteins. *Front. Neurosci.* 9:429. doi: 10.3389/fnins.2015.00429

Ge, J. F., Xu, Y. Y., Qin, G., Cheng, J. Q., and Chen, F. H. (2016). Resveratrol ameliorates the anxiety- and depression-like behavior of subclinical hypothyroidism rat: possible involvement of the HPT Axis, HPA Axis, and Wnt/beta-catenin pathway. *Front. Endocrinol.* 7:44. doi: 10.3389/fendo.2016.00044

Greengard, P., Valtorta, F., Czernik, A. J., and Benfenati, F. (1993). Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* 259, 780–785. doi: 10.1126/science.8430330

Hodes, G. E., Kana, V., Menard, C., Merad, M., and Russo, S. J. (2015). Neuroimmune mechanisms of depression. *Nat. Neurosci.* 18, 1386–1393. doi: 10.1038/nn.4113

Kao, H. T., Ryoo, K., Lin, A., Janoschka, S. R., Augustine, G. J., and Porton, B. (2017). Synapsins regulate brain-derived neurotrophic factor-mediated synaptic potentiation and axon elongation by acting on membrane rafts. *Eur. J. Neurosci.* 45, 1085–1101. doi: 10.1111/ejn.13552

Leal, G., Comprido, D., and Duarte, C. B. (2014). BDNF-induced local protein synthesis and synaptic plasticity. *Neuropharmacology* 76(Pt C), 639–656. doi: 10.1016/j.neuropharm.2013.04.005

Liu, Y., Al-Sayegh, H., Jabrah, R., Wang, W., Yan, F., and Zhang, J. (2014). Association between C-reactive protein and depression: modulated by gender and mediated by body weight. *Psychiatry Res.* 219, 103–108. doi: 10.1016/j.psychres.2014.05.025

Lopez-Munoz, F., and Alamo, C. (2009). Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. *Curr. Pharm. Des.* 15, 1563–1586. doi: 10.2174/138161209788168001

Lu, B., Nagappan, G., Guan, X., Nathan, P. J., and Wren, P. (2013). BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat. Rev. Neurosci.* 14, 401–416. doi: 10.1038/nrn3505

Mahableshwarkar, A. R., Jacobsen, P. L., Chen, Y., Serenko, M., and Trivedi, M. H. (2015). A randomized, double-blind, duloxetine-referenced study comparing efficacy and tolerability of 2 fixed doses of vortioxetine in the acute treatment of adults with MDD. *Psychopharmacology* 232, 2061–2070. doi: 10.1007/s00213-014-3839-0

Malinow, R. (2016). Depression: ketamine steps out of the darkness. *Nature* 533, 477–478. doi: 10.1038/nature17897

Marte, A., Messa, M., Benfenati, F., and Onofri, F. (2017). Synapsins are downstream players of the BDNF-mediated axonal growth. *Mol. Neurobiol.* 54, 484–494. doi: 10.1007/s12035-015-9659-3

Muller, H. K., Wegener, G., Popoli, M., and Elfving, B. (2011). Differential expression of synaptic proteins after chronic restraint stress in rat prefrontal cortex and hippocampus. *Brain Res.* 1385, 26–37. doi: 10.1016/j.brainres.2011.02.048

Nakayama, T., Yaoi, T., Yasui, M., and Kuwajima, G. (1998). N-copine: a novel two C2-domain-containing protein with neuronal activity-regulated expression. *FEBS Lett.* 428, 80–84. doi: 10.1016/S0014-5793(98)00497-9

Platenik, J., Fisar, Z., Buchal, R., Jirak, R., Kitzlerova, E., Zverova, M., et al. (2014). GSK3beta, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 50, 83–93. doi: 10.1016/j.pnpbp.2013.12.001

Reinhard, J. R., Kriz, A., Galic, M., Angliker, N., Rajalu, M., Vogt, K. E., et al. (2016). The calcium sensor Copine-6 regulates spine structural plasticity and learning and memory. *Nat. Commun.* 7:11613. doi: 10.1038/ncomms11613

Sanchez, C., Asin, K. E., and Artigas, F. (2015). Vortioxetine, a novel antidepressant with multimodal activity: review of preclinical and clinical data. *Pharmacol. Ther.* 145, 43–57. doi: 10.1016/j.pharmthera.2014.07.001

Smith, M. A., Makino, S., Kvetnansky, R., and Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.* 15, 1768–1777. doi: 10.1523/JNEUROSCI.15-03-01768.1995

Swaab, D. F., Bao, A. M., and Lucassen, P. J. (2005). The stress system in the human brain in depression and neurodegeneration. *Ageing Res. Rev.* 4, 141–194. doi: 10.1016/j.arr.2005.03.003

Thome, J., Pesold, B., Baader, M., Hu, M., Gewirtz, J. C., Duman, R. S., et al. (2001). Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus. *Biol. Psychiatry* 50, 809–812. doi: 10.1016/S0006-3223(01)01229-X

Valkanova, V., Ebmeier, K. P., and Allan, C. L. (2013). CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *J. Affect. Disord.* 150, 736–744. doi: 10.1016/j.jad.2013.06.004

Voorhees, J. L., Tarr, A. J., Wohleb, E. S., Godbout, J. P., Mo, X., Sheridan, J. F., et al. (2013). Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. *PLoS One* 8:e58488. doi: 10.1371/journal.pone.0058488

Walf, A. A., and Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* 2, 322–328. doi: 10.1038/nprot.2007.44

Wium-Andersen, M. K., Orsted, D. D., and Nordestgaard, B. G. (2014). Elevated C-reactive protein, depression, somatic diseases, and all-cause mortality: a mendelian randomization study. *Biol. Psychiatry* 76, 249–257. doi: 10.1016/j.biopsych.2013.10.009

Wu, L. M., Han, H., Wang, Q. N., Hou, H. L., Tong, H., Yan, X. B., et al. (2007). Mifepristone repairs region-dependent alteration of synapsin I in hippocampus in rat model of depression. *Neuropsychopharmacology* 32, 2500–2510. doi: 10.1038/sj.npp.1301386

Xu, Y. Y., Ge, J. F., Qin, G., Peng, Y. N., Zhang, C. F., Liu, X. R., et al. (2015). Acute, but not chronic, stress increased the plasma concentration and hypothalamic mRNA expression of NUCB2/nesfatin-1 in rats. *Neuropeptides* 54, 47–53. doi: 10.1016/j.npep.2015.08.003

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* 533, 481–486. doi: 10.1038/nature17998

Zhang, J., Mathis, M. V., Sellers, J. W., Kordzakhia, G., Jackson, A. J., Dow, A., et al. (2015). The US food and drug administration's perspective on the new antidepressant vortioxetine. *J. Clin. Psychiatry* 76, 8–14. doi: 10.4088/JCP.14r09164

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Identification of Proteins Differentially Expressed in the Striatum by Melatonin in a Middle Cerebral Artery Occlusion Rat Model—a Proteomic and *in silico* Approach

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Ischemic stroke is characterized by permanent or transient obstruction of blood flow, which initiates a cascading pathological process, starting from acute ATP loss to subsequent membrane depolarization, glutamate excitotoxicity, and calcium overload. Melatonin is a potent antioxidant that exerts protective effects in different experimental stroke models. In this study, melatonin effects were demonstrated by a proteomic and *in silico* approach. The proteomic study identified differentially expressed proteins by 2D gel electrophoresis in the striatum 24 h after middle cerebral artery occlusion. Proteomic analysis revealed several proteins with aberrant expression and was validated by western blot and immunofluorescence analysis. Homology modeling was performed to build 3D structures for γ-enolase, thioredoxin (TRX), and heat shock 60 (HSP60) by the template crystal structures using a protein data bank as a sequence database. The structure refinement of each model was achieved by energy minimization via molecular dynamic simulation, and the generated models were further assessed for stability by Procheck and ProSA. The models were processed for docking analysis using AutoDock Vina, and post-docking analysis was determined by discovery studio. The proteomic study showed decreased expression of γ-enolase, TRX, and protein phosphatase 2A subunit B and increased expression of collapsin response mediator protein 2 and HSP60 in the striatum after ischemic injury. Treatment with melatonin modulated the expression profiles of these proteins. This study demonstrated the neuroprotective role of melatonin in the ischemic striatum using a proteomic and *in silico* approach. Collectively, melatonin may act in a multimechanistic way by modulating the expression of several proteins in the ischemic striatum.

Keywords: melatonin, striatum, ischemic stroke, docking, neuroprotection

INTRODUCTION

Ischemic stroke is the most frequent cause of mortality depending upon race and demographic location (Mozaffarian et al., 2015). Stroke is also the significant cause of human sufferings, and the tissue plasminogen activator is the only drug approved for reversal of stroke torment by recanalizing the obstructed vessel (vascular strategy for combating stroke). A consensus based on experimental results recommends that recanalization (vascular therapy) is not sufficient to attenuate ischemic damage; however, a neuroprotective strategy is more productive, and it can impede the progression of ischemic lesion, counteract many biochemical steps of ischemic cascade, and provide an appropriate therapeutic choice. Therefore, the potential neuroprotective effects of melatonin were determined using a proteomic approach in this study.

Among various endogenous and synthetic neuroprotective compounds (including estrogens and progesterone), melatonin is the most extensively studied neuroprotective compound. As a natural indole hormone produced by the pineal gland and many other tissues such as retina, gut, and glial cells in mammals, melatonin is an effective antioxidant, besides a regulator of circadian and circannual cycles through G protein-coupled receptors, i.e., melatonin type 1 and 2 receptors (Venegas et al., 2012; Vriend and Reiter, 2015; Lacoste et al., 2015). Melatonin readily crosses the blood-brain barrier (BBB) because of its amphiphilic character, and melatonin receptors are widely distributed in the central nervous system (Lacoste et al., 2015). Moreover, melatonin has a broad interacting profile with intracellular proteins, including quinone reductase 2 (melatonin type 3 receptor) (Tan et al., 2007). Therefore, the biological activities of melatonin cannot be attributed to a single pathway or receptor but involve many targets including transduction pathways. Thus, receptor-dependent and independent actions, the low toxicity profile, and excellent clinical safety records make melatonin an ideal candidate as a neuroprotective agent (Ramos et al., 2017).

The neuroprotective effects of melatonin on ischemic stroke have been extensively studied in *in vitro* and *in vivo* models. The protection of melatonin against ischemic cell death could be attributed to a variety of cellular and molecular mechanisms, including its antioxidant and anti-inflammatory activities (Mauriz et al., 2013; García et al., 2014). A high concentration of melatonin directly eradicates free radicals, but a relatively low concentration of melatonin activates antioxidant enzymes (Rodriguez et al., 2004). Several signaling pathways, including the pro-survival phosphor-inositol 3 kinase (PI3K)/protein kinase B and mitogen-activated protein kinases (MAPK) and oxidative stress-related nuclear factor (erythroid-derived 2)-like 2 (NRF2), sirtuin 1, and endothelin-1, might be involved in the role of melatonin in brain ischemia (Andrabi et al., 2015). Many studies have demonstrated that melatonin counteracts the deleterious effects of ischemic stroke in animal models by promoting BBB integrity and neurogenesis (Lee et al., 2014; Alluri et al., 2016). Furthermore, melatonin diminishes the infarct volume, reduces brain water content, and improves neurologic scores in focal cerebral ischemia.

The extent of neuronal injury triggered by middle cerebral artery occlusion (MCAO) depends upon the duration of occlusion (Fluri et al., 2015). Permanrent MCAO induces the most uniform infarction that frequently involves the neocortex and striatum. Comparatively, blood flow is lower to the striatum than to the cortex. The striatum is supplied by tiny unidirectional vessels from MCA, and there is no collateral connection to this subcortical area from the surrounding vasculatures (Fluri et al., 2015). Thus, MCA occlusion completely cuts off blood supply to this important region in the brain, and the striatum may be severely hit by ischemic stroke.

In silico and proteomic studies help understand the biochemical mechanism and thus can unknot the complex signaling network, which controls cellular function including cell survival and death. This study aimed to delineate significant targets of melatonin in the ischemic striatum. We hypothesized that melatonin modulates the expression of proteins in the striatum and may thus potentially ameliorate the molecular and organ/tissue damage associated with ischemic stroke.

MATERIALS AND METHODS

Animals and Drug Treatment

Male SD rats (weight, 230–250 g; age, 7–9 weeks) (n = 100) were used in this study, and they were obtained from the local breeding facility at Gyeongsang National University. The experimental procedures were carried out according to the protocol approved by the animal ethics committee (Approval ID: 125-IACUC) of Gyeongsang National University, Republic of Korea. The rats were divided into 4 groups. We were not blinded to the allocation of rats; instead, we randomly divided these rats into the following groups according to the criteria that the similar weight animals are kept in the same group under the same experimental condition: (1) Vehicle-treated control rats (Sham); (2) Middle cerebral artery occlusion rats (MCAO); (3) Melatonin-treated rats undergoing MCAO (Mela + MCAO); 4. Melatonin-treated sham rats (Mela+Sham).

A single dose of melatonin (Sigma, St. Louis, MO, United States) (5 mg/kg) or vehicle was administered intraperitoneally 30 min before ischemia. This dose of melatonin has shown maximum neuroprotective effects in focal cerebral ischemia based on pharmacokinetic and dose-response studies (Lee et al., 2005). In total, 10 rats died during experimental procedures, including 6 from the MCAO group, 3 from the Mela + MCAO group, and 1 from the sham-operated group.

Middle Cerebral Artery Occlusion Surgery

Middle cerebral artery occlusion was operated using a previously described method (Shah et al., 2016; Park et al., 2018). Briefly, all main arteries involved in blood occlusion were exposed, including the common carotid artery, external carotid artery, and internal carotid artery. The thinner occipital artery and superior thyroid artery (originated from the external carotid) were ligated with 6-0 silk sutures. The external carotid artery was knotted with 6-0 silk sutures, and a nylon filament with a blunt rounded

tip of about 30 mm in length was inserted into the internal carotid artery versus the external carotid artery. The filament was advanced until a resistance was felt, demonstrating that the middle cerebral artery was occluded. The sham-operated animals were subjected to the same procedures except for the filament insertion. At 24 h after onset of permanent occlusion, animals were decapitated, and brain tissues were collected.

Proteomics of the Striatal Tissues

Proteomic analysis was carried out using a previously described method (Shah et al., 2014). Briefly, the right striatum was isolated from all experimental groups and was homogenized in a buffer solution (8 M urea, 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate [CHAPS], ampholytes, and 40 mM Tris–HCl), followed by centrifugation. The resulted supernatant was discarded; the pallet was dissolved in the lysis buffer, and the protein concentration was determined by Bradford method (Bio-Rad, Hercules, CA, United States) according to the manufacturer's protocol. Immobilized pH gradient (IPG) gel strips (range pH 4–7 and 6–9, Bio-Rad) were incubated in the rehydration buffer (8 M urea, 2% CHAPS, 20 mM dithiothreitol [DTT], 0.5% IPG buffer, and bromophenol blue) for 13 h at room temperature. The assayed protein samples were loaded on IPG strips (pH 4-7 and 6-9) via the sample cup and proceed for first dimension isoelectric focusing (IEF) using Ettan IPGphor 3 (GE Healthcare, Bio-Rad) with the following protocol: l,250 V (15 min), 10,000 V (3 h), and then 10,000–50,000 V. At end of the first dimension IEF, the strips were incubated in the equilibration buffer (6 M urea, 30% glycerol, 2% sodium dodecyl sulfate [SDS], 50 mM Tris-HCl, and bromophenol blue) containing DTT and iodoacetamide. The strips were then loaded onto gradient gels (7.5–17.5%), and the second-dimension electrophoresis was performed on a Protein-II XI electrophoresis equipment (Bio-Rad) at 5 mA per gel for 2 h, followed by 10 mA per gel at 10°C until the bromophenol blue dye migrated off the bottom of the gel. The steps used for staining the gel included fixation (12% acetic acid, 50% methanol), impregnation in a silver solution (0.2% silver nitrate, 0.75 ml/L formaldehyde), and developing (0.2% sodium carbonate, 0.5 ml/L formaldehyde). Gel images were acquired, and differentially expressed protein spots were excised and destained. Gel particles were digested in the trypsin-containing buffer, and the extracted peptides were analyzed using a Voyager-DETM STR biospectrometry workstation (Applied Biosystem, Forster City, CA, United States) for peptide mass fingerprinting. Database searches were carried out using MS-Fit and ProFound software. SWISS-PROT and NCBI were used as protein sequence databases.

Western Blot

For western blot analysis, samples were homogenized in the lysis buffer (1 M Tris–HCI, 5 M sodium chloride, 0.5% sodium deoxycholate, 10% sodium dodecyl sulfate, 1% sodium azide, and 10% NP-40) with phenylmethylsulfonyl fluoride as protein inhibitor. The homogenate was sonicated and centrifuged, and protein concentration was then determined by Bicinchoninic Acid kit (Pierce, Rockford, IL, United States) according to the manufacturer's guideline. An equal amount of proteins (30 μg per sample) were electrophoresed on 10% SDS-PAGE gels, followed by transferring the protein to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, United States). The PVDF membranes were blocked with skim milk at room temperature to minimize non-specific antibody binding and were then incubated with primary antibodies overnight at 4°C. Subsequently, the membranes were incubated with appropriate secondary antibodies, and protein bands were detected using ECL detection reagents according to the manufacturer's instruction (Amersham Pharmacia Biotech, Piscataway, NJ, United States). The antibodies used included anti-γ-enolase, anti-heat shock protein 60 (HSP60), anti-thioredoxin (TRX), and anti-β-Actin from Santa Cruz Biotechnology (Santa Cruz, CA, United States) and anti-collapsin response mediator protein 2 (CRMP2) and anti-protein phosphatase 2A subunit B (PP2A) from cell signaling technology.

Tissue Collection for Morphology Analysis

Five rats were used for morphology analysis in each group. Brain tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and 4 μm coronary sections were cut using a rotary microtome. The following staining techniques were used in this study.

Cresyl Violet Staining

Tissue sections on coated slides were de-paraffinized with three different absolute xylenes and were rehydrated with ethyl alcohol (from 100% [absolute] to 70%). The slides were rinsed with distilled water and immersed in 0.01 M phosphate-buffered saline (PBS) for 10 min. Cresyl violet acetate (0.5% [w/v]; Sigma) was dissolved in distilled water, and a few drops of glacial acetic acid were then added. Brain sections were stained with cresyl violet solution for approximately 20 min. The slides were rinsed with distilled water and then dehydrated in ethyl alcohol (70, 95, and 100%). The slides were cleared with xylene and mounted with glass coverslips. The slides were imaged with an Olympus microscope, and the images were analyzed by ImageJ, a computer-based program. In total, 5 images per slide were acquired for each group, and specifically, neuropil and neuronal size and shape were focused in these images. The TIF images were optimized to the same threshold intensity for pyknotic, red, and ghost neurons in all groups.

Immunofluorescence Analysis

After deparaffinization, the slides were autoclaved in 0.1 M sodium citrate (pH 6) for antigen retrieval, washed with PBS, and incubated with 5% normal serum depending upon the sources of the secondary antibodies used. The slides were incubated with mouse polyclonal γ-enolase, HSP60, TRX, Ionized calcium binding adaptor molecule 1 (Iba-1), glial fibrillary acidic protein (GFAP), and 8-oxoguanine antibodies from Santa Cruz

Biotechnology and the rabbit monoclonal CRMP2 antibody from cell signaling overnight at 4°C. Subsequently, after washing with PBS, the slides were incubated with fluorescent-labeled secondary antibodies (Santa Cruz Biotechnology) for signal amplification in a dark chamber and were then mounted with UltraCruz mounting medium (Santa Cruz Biotechnology). Immunofluorescence images (five images per slide) were captured using a confocal scanning microscope (Flouview FV 1000, Olympus, Japan). ImageJ software was used to quantitatively determine fluorescence intensity of the same region of the striatum/total area for all groups by optimizing background of images according to the threshold intensity and analyze the immunofluorescence intensity at the same threshold intensity for all groups. The fluorescence intensity is expressed as the relative integrated density of the samples relative to the control.

Immunohistochemical Analysis

After antigen retrieval, the slides were incubated with 3% hydrogen peroxidase to quench endogenous peroxidase and were subsequently blocked with 5% serum depending upon the sources of secondary antibodies used. After blocking, the slides were incubated overnight with anti-PP2A (Cell signaling technology), p-c-Jun N-terminal kinase (JNK), and caspase3 (Santa Cruz Biotechnology) antibodies, followed by treatment with appropriate biotinylated secondary antibodies for 2 h and successively with ABC reagents (Standard Vectastain ABC Elite Kit; Vector Laboratories, Burlingame, CA, United States) for 1 h at room temperature. The sections were washed with PBS and stained in 3, 3′-Diaminobenzidine tetrahydrochloride solution; they were then washed with distilled water, dehydrated in graded ethanol (70, 95, and 100%), fixed in xylene, and cover-slipped by a mounting medium. Immunohistochemical results were analyzed by a light microscope (Olympus, Japan), which was connected to a digital photomicroscopy system. Immunohistochemical TIF images (five images per slide) were captured with a light microscope. ImageJ software was used to quantitatively determine hyperactivated p-JNK, PP2A, and caspase3 in the striatum/total area by optimizing background of images according to the threshold intensity and analyze p-JNK, PP2A, and Caspase3 positive cells at the same threshold intensity for all groups. The intensity is expressed as the relative integrated density of the samples relative to the control.

Bioinformatics Resources

The amino acid sequences of target proteins (HSP60, CRMP2, γ-enolase, TRX, and PP2A) in rats were downloaded from UniProt database[1] in FASTA format. To identify the best template structure for homology modeling, the sequence of the target protein was aligned in BLASTp (Basic Local Alignment Searching Tool for protein) using RCSB as the protein sequence database. The templates were ranked according to sequence identity, sequence coverage, and *E*-value. The topmost ranked structure was taken as the best template for the model generation of corresponding proteins.

Homology modeling of target proteins was performed by an online server of SWISS-MODEL[2]. Briefly, template sequences were fed to the automated modeling program, and models were then generated (Biasini et al., 2014). The best model was selected based on the quality estimation score and overall structure similarity. The structure refinement of these models was achieved by energy minimization via molecular dynamic (MD) simulation using GROMACSv5.0.6 with CHARMm27 force-field parameterization (Abraham et al., 2015). Briefly, for each protein, the system was prepared in a dodecahedron box, filled with the TIP3P water model. For atomic representation, the CHARMm27 force-field parameters were applied, and the system was further neutralized by adding Na^+ and/or Cl^- counter ions (Zoete et al., 2011). The well-neutralized systems were subjected to energy minimization by applying the steepest descent algorithm implanted in GROMACS v5.0.6. The energy minimization parameters were optimized to 50000 steps at 10.0 kJ/mol. The energy minimization was further verified by calculating the potential energy of the system. The generated models were further assessed for stability and overall protein quality by validation tests such as Procheck[3] and ProSA[4]. The Procheck verifies the occurrence of residues in Ramachandran plot and deals with phi and psi angles of residues (Sahoo et al., 2016), and ProSA finally confirms the validity of the model by showing a quality score plot, calculated by comparing the input model using RCSB as a reference database (Wiederstein and Sippl, 2007). After satisfying all constraints of assessment, models proceeded for docking analysis. For docking analysis, all target proteins and ligands were prepared as PDB format. Melatonin was first converted to PDBqt format using AutoDock Tools (1.5.6rc2). Both protein and ligand were then passed through AutoDockVina, which is a docking software that interprets docking results in the form of binding energies (*E*-value). The well-docked pose of ligand in each target protein was further analyzed by DSV in term of ligand pose orientation and molecular interactions.

Statistical Analysis

The 2D gel spots, western blot bands, and morphological data were analyzed using ImageJ software (Image J 1.30)[5]. Data are presented as means ± standard error of mean. Data were analyzed by one-way analysis of variance followed by post-hoc Bonferroni multiple comparison tests using the graph-pad prism-5 software. Symbol * or # represents a significant difference with a p-value of < 0.05; symbol ** or ## represents a significant difference with a p-value of < 0.01; and symbol *** or ### represents a significant difference with a p-value of < 0.001. The symbol * indicates a significant difference compared with the sham group, and # indicates a significant difference compared with the MCAO group.

[1]http://www.uniprot.org

[2]https://swissmodel.expasy.org/

[3]https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/

[4]https://prosa.services.came.sbg.ac.at/prosa.php

[5]https://imagej.nih.gov/ij/

RESULTS

Effects of Melatonin on Apoptosis and Neurodegeneration

Nissl staining was used to distinguish between necrotic and intact neurons in the striatum and to examine the neuroprotective effect of melatonin. A substantial difference was observed 24 h after permanent ischemia in the striatum between MCAO and sham-operated animals ($p < 0.001$) (**Figure 1A**). Robust neuronal changes were found in this highly prone area in the brain, and melatonin pretreatment attenuated these changes. Aberrant morphological features, including changes in neuronal size and shape (swelling and scalloped angular nature), alteration in color (cytoplasmic eosinophilia/pyknosis, nuclear basophilia), and vacuolation (swollen and shrunk appearance of neurons), were observed in the striatum in MCAO rats compared with sham-operated rats (**Figures 1A,B**). Histological analysis did not find noticeable alterations in sham-operated animals. Significantly more intact neuronal cells were found in the melatonin-treated group than in the MCAO-operated group ($p < 0.01$, **Figure 1A**). Activated JNK is linked to neuronal apoptosis by mediating caspase activation (Yang et al., 2009). JNK activation leads to more apoptosis in the ischemic striatum than in the ischemic cortex due to significant overexpression of JNK in the striatum (Okuno et al., 2004). In this study, activated JNK and caspase-3 were observed in the striatal tissue in the MCAO group compared with the sham-operated group ($p < 0.001$, **Figures 1C,D**). Notably, treatment with melatonin reversed the activation and significantly reduced the expression levels of p-JNK and caspase-3 ($p < 0.01$).

Fluoro-Jade B (FJB) is a convenient marker of neuronal degeneration. Melatonin-treated rats (Mela + MCAO) showed relatively intact neuronal morphology, which was comparable to that in sham-operated rats (**Figure 2A**). In contrast, severe neuronal degeneration (strong FJB staining) was observed in the striatum in MCAO-operated rats ($p < 0.001$; **Figure 2A**). Studies have consistently demonstrated increased free radical generation during ischemic damage, and the increased free radicals promote the breakage of BBB and facilitate cytotoxic edema. Therefore, we next determined the generation of reactive oxygen species (ROS) in the striatum using fluorescent 8-oxoguanine as an oxidative stress marker. The results showed higher expression of 8-oxoguanine in the ischemic tissues than in sham control tissues ($p < 0.01$). Notably, treatment with melatonin attenuated the ischemia-induced oxidative stress ($p < 0.05$, **Figure 2B**).

Melatonin Treatment Attenuates MCAO-Induced Reactive Gliosis

Ischemic stroke is characterized by reactive gliosis, in which astrocytes and resident glial cells are upregulated to mediate the progression of ischemic injury. The activated hypertrophic cells work as a resident machinery to generate inflammatory mediators. Because these cells are primarily involved in neuroinflammation and neurodegeneration, we investigated the neuroprotective effect of melatonin on astrocyte (GFAP-reactive cells) and microglial activation (Iba-1-reactive cells) in the ischemic striatum. Immunofluorescence analysis revealed significant increases in GFAP- and Iba-1-reactive cells in the striatum in the MCAO group compared with the sham group ($p < 0.001$, **Figure 2C**). Melatonin treatment significantly decreased the number of these hyperactive cells in the striatum ($p < 0.05$).

Differential Expression of Proteins

Electrophoretic protein maps were constructed after peptide analysis by mass spectrometry, and protein spots were clearly identified by MALDI-TOF analysis (**Figure 3A** and **Table 1**). Five proteins were selected for further analysis (**Figure 3B**). Proteins were selected based on several factors, including antibody availability, literature accessibility, and relative roles of proteins in ischemic brain injury. It is believed that γ-enolase, CRMP2, HSP60, TRX, and PP2A have vital roles in ischemic stroke because these proteins are largely involved in metabolism, hemostasis, and neuronal sprouting. The upregulated expressions of γ-enolase, CRMP2, HSP60, TRX, and PP2A, identified by MALDI-TOF analysis, were observed in the MCAO group (**Figure 3B**), but the upregulation of these proteins was significantly attenuated in the melatonin-treated group, indicating the neuroprotective effect of melatonin in the ischemic model.

Validation of Proteins Downregulated After MCAO Injury

Enolases have pivotal roles in energy metabolism, signifying the importance of enolases in stroke (Sarnat, 2013). Proteomic analysis revealed abundance of γ-enolase ($p < 0.01$), TRX ($p < 0.001$), and PP2A after MCAO injury ($p < 0.01$, **Figure 3B**). Moreover, we examined the expression levels of these proteins using western blot analysis with β-actin as a loading control, and significant differences in expression levels of these proteins were observed between MCAO and other experimental groups (**Figure 4A**). Furthermore, we performed immunofluorescence to investigate the distribution of these proteins in the ischemic striatum (**Figure 4B**). The findings further indicated that the expression levels of γ-enolase and TRX decreased in the striatum in the ischemic brain in the MCAO group ($p < 0.001$, **Figure 4B**), but melatonin treatment attenuated the decrease of these proteins in the Mela + MCAO group (**Figure 4B**, $p < 0.01$). In addition, immunohistochemical staining validated the proteomic findings. Notably, immunohistochemical staining revealed that the number of PP2A subunit B-positive cells decreased in the ischemic striatum (**Figure 4C**, $p < 0.001$), and melatonin treatment significantly recovered the PP2A expression levels in the Mela + MCAO group (**Figure 4C**, $p < 0.05$).

Validation of Proteins Upregulated After MCAO Injury

In this study, we also identified proteins with increased expression levels after MCAO injury. The proteomic analysis revealed an increased abundance of CRMP2 and HSP60 ($p < 0.001$) after MCAO (**Figure 3B**). In addition, western blotting analysis was performed with an equivalent quantity of

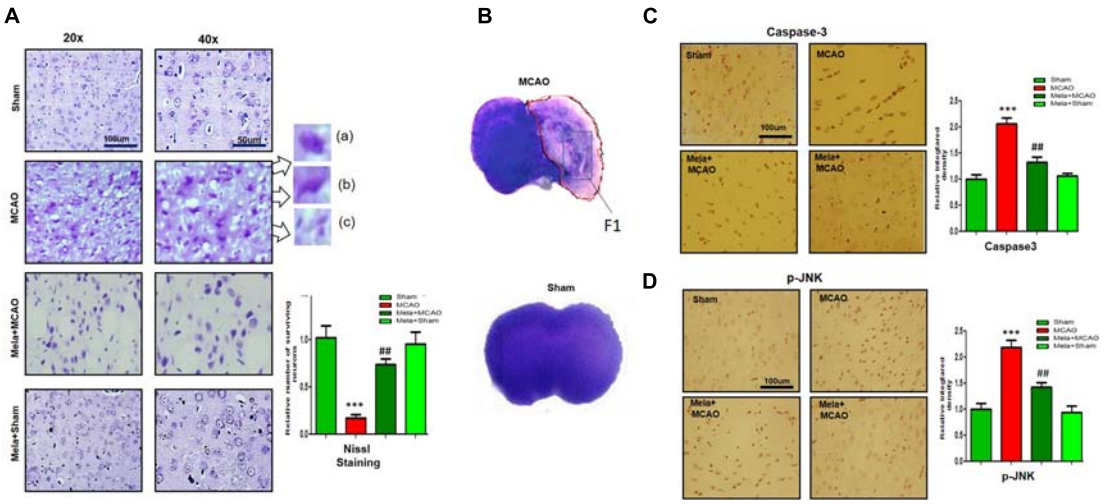

FIGURE 1 | Representative photomicrographs of cresyl violet staining show the extent of surviving neurons in the striatum **(A)**. The number of experiments performed = 3. $^{***}p < 0.001$ indicates a significant difference compared with the sham group; $^{\#\#}p < 0.01$ indicates a significant difference compared with the MCAO group. (a) Necrotic neurons with scalloped and shrunken appearance, intense cytoplasmic eosinophilia, and nucleus basophilia. Such changes are characteristics of eosinophilic necrosis (referred to as red neurons, showed by 40×). (b) Cytoplasmic fading of neurons invariably occurs at later stages of neuronal necrosis. The ghost neurons are large with no definite outline, and nuclei are shown. (c) Some of the inflammatory cells can be observed with rounded shaped oligodendrocytes and microglia near necrotic neurons. **(B)** Nissl staining of coronal sections shows hyperchromatic cortex and striatum, separated by red borderline from the corresponding contralateral brain, 24 h after permanent MCAO. The analyzed striatal region is indicated by square F. **(C,D)** Immunoreactivity of Caspase 3 and p-JNK in the striatum (N = 5 rats/group). $^{\#\#}$Represents a significant difference with a p-value of < 0.01, and *** represents a significant difference with a p-value of < 0.001. *Indicates a significant difference compared with the sham group, and $^{\#}$ indicates a significant difference compared with the MCAO group.

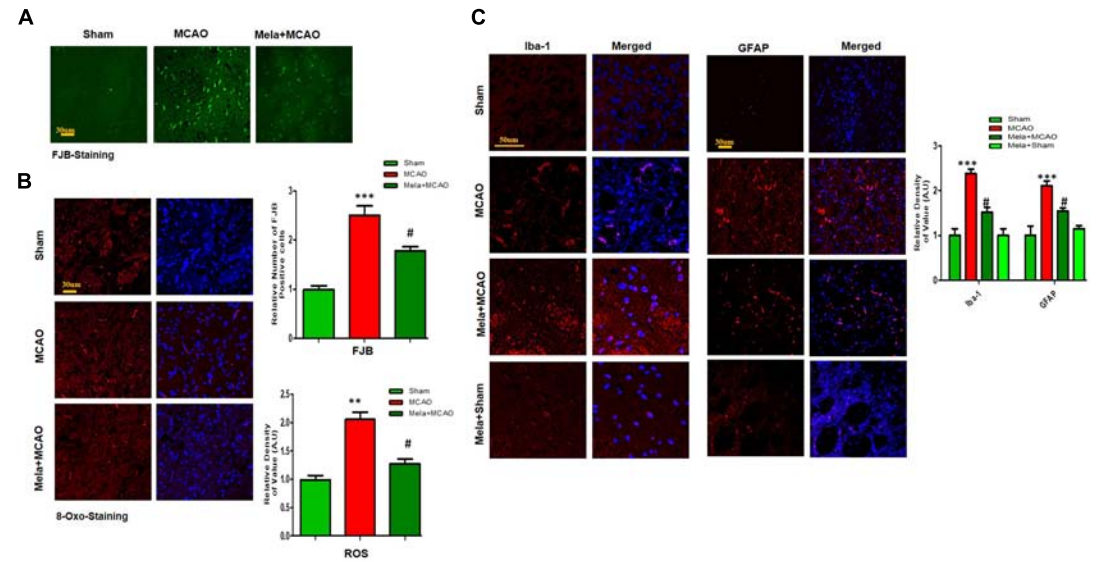

FIGURE 2 | Representative images of FJB **(A)** and 8-oxoguanine **(B)** staining. Each experiment was performed 3 times (n = 5 per group). $***$Shows a significant difference compared with sham rats; $^{\#}$ shows a significant difference compared with MCAO rats. $***p < 0.001$, $**p < 0.01$, and $^{\#}p < 0.05$. **(C)** Melatonin attenuated MCAO-triggered activation of astrocytes and microglia. Immunoreactivity of astrocytes (GFAP-positive cells) and microglia (Iba-1-positive cells) in sham, ischemic, and melatonin-operated groups is shown. Scale bar = 30 μm or 50 μm. The GFAP- and Iba-1-positive cells were visualized by TRITC. ***Represents a significant difference with a p-value of < 0.001, and $^{\#}$ represents a significant difference with a p-value of < 0.05. *Shows a significant difference compared with sham rats, and $^{\#}$ shows a significant difference compared with MCAO rats.

protein samples from different experimental groups using β-actin as a loading control (**Figure 4A**), and the results confirmed significant differences in band intensities of CRMP2 and HSP60 between MCAO and sham groups ($p < 0.001$, **Figure 4A**). Moreover, immunofluorescence analysis further validated the increased expression of these proteins after MCAO ($p < 0.001$,

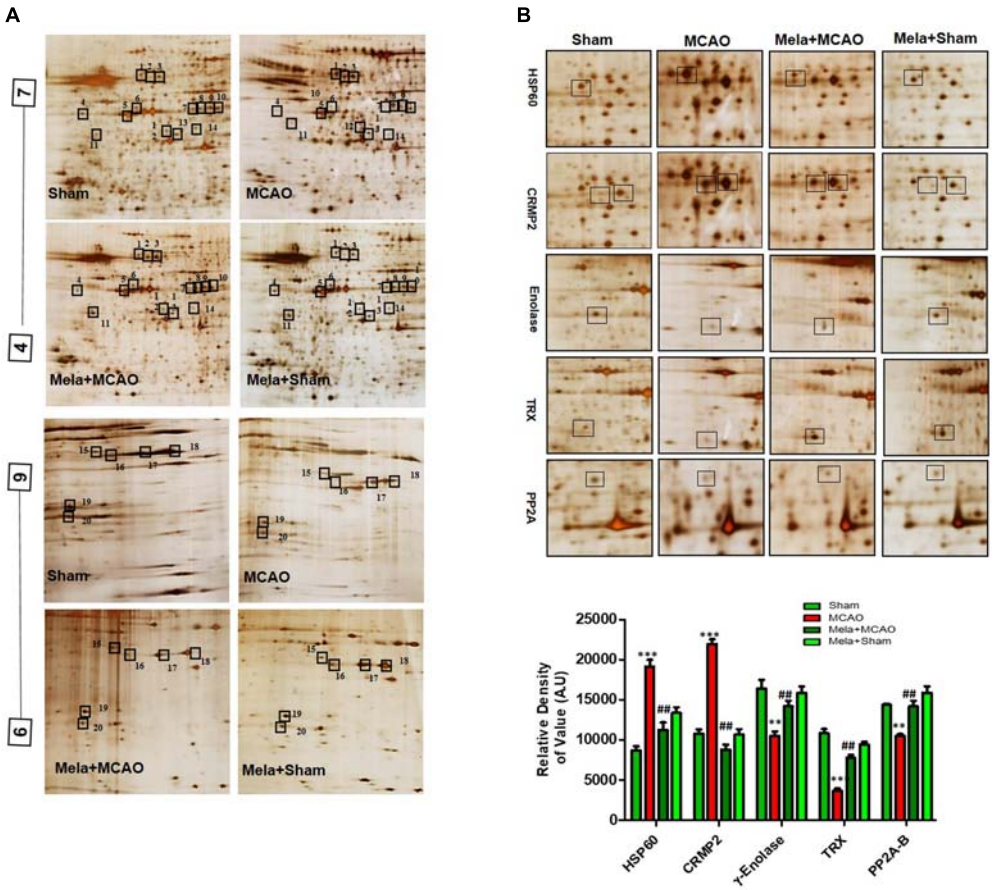

FIGURE 3 | Two-dimensional gel electrophoresis maps. **(A)** Isoelectric focusing was performed at pH 4–7 and 6-9 using IPG strips. The IPG strips were then loaded on a gradient SDS gel and stained with silver nitrate. The spots were identified using MALDI-TOF and NCBI as reference databases. Squares indicate the protein spots that were differentially expressed among different experimental groups. **(B)** Magnified protein spots of HSP60, CRMP2, γ-enolase, TRX, and PP2A. Protein spots were analyzed using ImageJ software. Densitometric analysis is expressed in arbitrary units as means ± SEM for the indicated proteins ($n = 5$ per group). Symbols ** or ## and *** or ### represent significant differences with $p < 0.01$ and $p < 0.001$, respectively.

Figure 4B). CRMP2 displayed proteolytic cleavage after MCAO, and western blotting analysis of CRMP2 showed a migration pattern of cleaved bands (**Figure 4A**). The intact mass of CRMP2 is 66 kD and can degrade to 62 and 55 kD proteins (Zhang et al., 2007). Western blotting analysis showed an elevated expression of cleaved 55 kD CRMP2 in the MCAO group.

Homology Modeling and Validation Process

The BLASTp analysis of γ–enolase, HSP60, and TRX sequences identified chain A of human γ-enolase 2 in complex with phosphonoacetohydroxamate (PDB ID: 4ZA0), chain A of mitochondrial chaperonin symmetrical 'football' complex (PDB ID: 4PJ1), and chain A crystal structure of catalytic domain of a new human thioredoxin-like protein (PDB: 1GH2) as the best templates with sequence identity of 98%, respectively (**Figure 5A**). A total of 5 models were generated for each target protein by the automated SWISS-MODEL server (Biasini et al., 2014). The chosen models were subjected to energy minimization via molecular dynamics simulation in GROMACS v5.0.6. The energy of each protein was minimized with CHARMm27 force-field parameterization and by applying the steepest descent algorithm at force 10.0 kJ/mol for 50000 steps. The potential energies of minimized γ-enolase, HSP60, and TRX were calculated as -8.0×106 kJ/mol, -2.2×106 kJ/mol, and -2.2×106 kJ/mol, respectively. The final 3D structure is shown in **Figure 5A**. The stereochemical integrity of the energy-minimized models was evaluated using Procheck. Ramachandran plot distributes the amino acid residues of γ-enolase, HSP60, and TRX models into respective regions as shown in **Figures 5B1,B3,B5**. Briefly, the amino acid residues were 90.7% and 91.1% in the favored region, 8.8 and 6.9% in the allowed region, and 0.3 and 1.9% in the generously allowed region for γ-enolase and HSP60 models, respectively. In the TRX analysis, the amino acid residues were 95.8 and 4.2% in the favored and allowed regions, respectively. These results suggest that the backbone dihedral angles of both models are reasonably accurate. Moreover, the models were further validated by ProSA server for potential errors (Wiederstein and Sippl, 2007). ProSA

TABLE 1 | List of proteins that were differentials expressed.

Spot no.	Protein name	Accession no.	M.W	PI	Mass matched	Sequence coverage (%)
1	60 kDa heat shock protein	P63039	60917	5.91	11/133	32%
2	Dihydropyrimidinase-related protein 2	P47942	83856	6.64	7/109	29%
3	Dihydropyrimidinase-related protein 2	P47942	62278	6	24/103	52%
4	γ-enolase	P07323	47141	5	14/70	34%
5	Prolactin-8A5 isoform XI	P33579	27267	5.47	7/97	22%
6	Eukaryotic initiation factor 4A-II	Q5RKI1	46373	5.33	16/82	40%
7	Succinyl-CoA ligase subunit beta	Q9Z219	50274	7.75	11/74	24%
8	Adenosine kinase	Q64640	40108	5.72	12/95	41%
9	MAP kinase kinase	Q01986	43465	6.18	12/72	30%
10	Adenosylhomocysteinase	P10760	47507	6.07	15/132	33%
11	Thioredoxin	Q920J4	3223	4.84	8./87	42%
12	Isocitrate dehydrogenase[NAD+] subunit alpha	Q99NA5	39588	6.47	8/93	31%
13	NAD-specific Isocitrate dehydrogenase[NAD+] subunit alpha	Q99NA5	39588	6.47	8/93	31%
14	Protein phosphatase 2A, regulatory subunit B	P36877	36594	5.88	9/56	29%
15	Alchol dehrdogenase	P51635	36510	6.8	9/104	28%
16	Glyceraldehyde-3- phosphate dehydrogenase	P04797	35828	8.14	16/106	46%
17	Glyceraldehyde-3- phosphate dehydrogenase	P04797	35828	8.14	20/94	62%
18	Glyceraldehyde-3- phosphate dehydrogenase	P04797	35828	8.14	16/75	55%
19	Nucleoside diphosphate kinase B	P19804	17283	6.9	8/86	49%
20	Peroxiredoxin-5	Q9R063	22165	8.94	9/114	46%

is used to determine the refinement of protein structures in term of *Z*-score and residue energies. The *Z*-score generally shows the quality of the model, and negative values of residue energies confirm the uniformity of the model. *Z*-scores of γ-enolase (−9.95), HSP60 (−10.95), and TRX (−6.5) are depicted in **Figures 5B2,B4,B6**. The findings suggest that the generated 3D models are of good quality (Wiederstein and Sippl, 2007).

Notably, the BLASTp of CRMP2 (UniProt accession number P47942) and PP2A (UniProt accession number P36877) identified mouse CRMP2 structure (PDB code: 5UQC) and human PP2A (PDB ID: 3DW8) as the best identical sequence templates (100% sequence identity) in RCSB, respectively (**Figure 5A**). Furthermore, we aligned these protein sequences by Clustal Omega (Chenna et al., 2003), and the findings revealed that entire sequences of these proteins were identical and conserved (**Figure 5C**). Therefore, we used mouse-CRMP2 and human PP2A as structure analogs of rat-CRMP2 and rat PP2A for docking studies, respectively. Moreover, the structure of melatonin was retrieved from Pubchem database[6], and its 2D structure, which was drawn in ChemSketch, was converted to 3D structure and saved as PDB file in DSV (**Figure 5D**).

Docking Studies

The ligand melatonin was docked in the catalytic active pocket of HSP60, γ-enolase, CRMP2, TRX, and PP2A. **Table 2** shows the binding energies, and **Figure 6** represents the best pose of melatonin that fits γ-enolase, HSP60, CRMP2, TRX, and PP2A after docking analysis. The docking analysis showed that melatonin fitted with γ-enolase with a bond distance of 2.46–2.54Å, indicating high polar contacts (**Figures 6A,B**). Furthermore, it was observed that four hydrogen bonds were formed between melatonin and γ-enolase. Two hydrogen bonds were formed between Lys120 and melatonin. Nitrogen in pyrrole ring is hydrogen bond donor, while oxygen of methoxy group acts as hydrogen bond acceptor. Similarly, two hydrogen bonds were formed between the amide group (consisting of both hydrogen bond donor and acceptor) and amino acids including Asp383 and Gln409 (**Figure 6B**). In addition, Van der Waal and electrostatic interactions, which further provide stability to melatonin binding in the γ-enolase active site (Bosshard et al., 2004), were also observed.

The docking results of melatonin and HSP60 are shown in **Figures 6C,D**. Four hydrogen bonds were observed between melatonin and HSP60, and three were formed by the amide group. Other residues including Arg395, Glu394, Arg197, and Thr202 had Van der Waal interactions with melatonin (**Figures 6C,D**). Post-docking interactions for CRMP2 are shown in **Figures 6E,F**. Melatonin fits with CRMP2 with a bond distance of 2.22–2.72Å. All kinds of polar and other electrostatic interactions were visualized in DSV. In addition, the docking results of TRX (**Figures 6G,H**) and PP2A (**Figures 6I,J**) are also shown in this study. All these analyses examined the most likely binding patterns of melatonin with each target protein.

DISCUSSION

In this study, we performed a comparative analysis of differentially expressed proteins in the ischemic striatum between MCAO and Mela + MCAO rats. The focus of this study was to

[6]https://pubchem.ncbi.nlm.nih.gov/search/

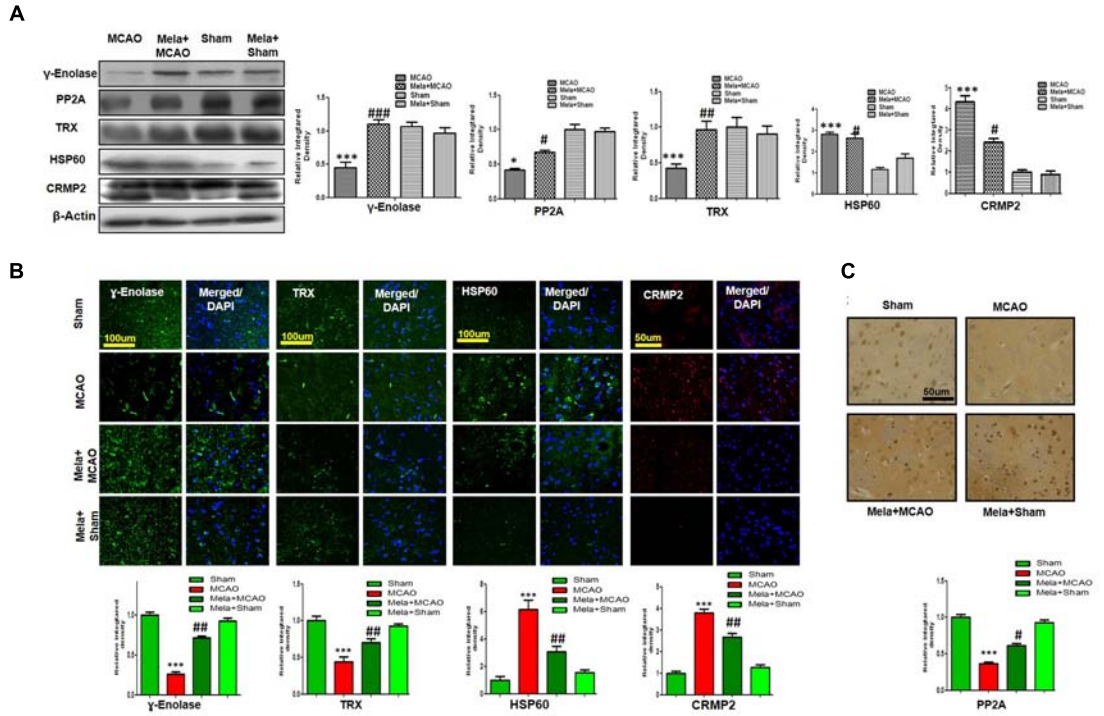

FIGURE 4 | Western blot analysis of **(A)** γ-enolase, HSP60, CRMP2, TRX, and PP2A in the striatum in the sham-operated, MCAO, and melatonin-treated MCAO groups. The bands were quantified using ImageJ and analyzed by graph-pad prism-5 software. β-actin was used as a control. Densitometric analysis is expressed in arbitrary units as means ± SEM for the indicated proteins. Symbol * or # represents a significant difference with $p < 0.05$; symbol ** or ## represents a significant difference with $p < 0.01$; symbol *** or ### indicates a significant difference with $p < 0.001$. The symbol * indicates a significant difference compared with the sham group, and # indicates a significant difference compared with the MCAO group. (B) Immunofluorescence reactivity of γ-enolase, TRX, HSP60, and CRMP2 was examined in the striatum ($N = 5$ rats/group). The data are representative of 3 independent experiments. Magnification, 40×. Scale bar = 100 or 50 μm. These proteins showed cytoplasmic localization. HSP60, TRX, and γ-enolase were visualized by FITC, and CRMP2 was visualized by Alexa-Flour 594.
(C) Immunohistochemical staining of PP2A in the striatum. Symbols ## and # represent significant differences with $p < 0.01$ and $p < 0.05$, respectively; Symbol *** or ### represents a significant difference with $p < 0.001$. The symbol * indicates a significant difference compared with the sham control group, and # indicates a significant difference compared with the MCAO group.

determine the protein profile of the rat striatal tissue after MCAO injury, to perform bioinformatics analysis, and to examine the effect of melatonin. The quantitative 2D-MS technique was used to illustrate the differential expression proteins in the striatal tissues in the MCAO adult rats.

Because no 3D structures for γ-enolase, HSP60, and TRX have been reported for rats in the PDB data bank, the homology modeling was performed to build 3D structures of these proteins (Burley, 2000). The stability of these structures was further assessed by MD simulation. MD simulation is an *in silico* modeling method for studying movements of particles (mostly atoms). MD simulation is used to infer the real behavior of atoms under the specified environmental conditions and is a well-practiced discipline in biological systems, where MD can be used to investigate the stability and physiological orientation and/or confirmation of bio-molecules. In this study, we used GROMACS v5.0.6 package to minimize the energy of each modeled structure. We found that rat CRMP2 and PP2A showed 100% sequence coverage with mouse CRMP2 (PDB# 5UQC) and human PP2A (PDB ID: 3DW8) respectively, and the sequences were further aligned by clustal omega (**Figure 2B**). Omega analysis suggests that sequences of CRMP2 and PP2A in these species are conserved. Melatonin structure was drawn in ChemSketch and converted to PDB format by Pymol. Docking analysis was performed by AutodockVina, and binding energy was evaluated. The interaction of docked pose of ligand (melatonin) in protein was further visualized by discovery studio (DS). Weak intermolecular interactions play an important role in stabilizing the ligand energetically in the target protein. Therefore, we identified the molecular interaction patterns of melatonin binding in each target protein. Molecular interactions were elaborated in terms of hydrogen bonding, Van der Waals forces, and electrostatic interactions. Our thorough analysis of computational docking indicates that melatonin binds each target protein by H-bonds and other hydrophobic interactions. Therefore, we speculate that the formation of H-bonds between the corresponding protein and melatonin supports the correspondent complex stability.

The molecular mechanism of ischemic brain damage is characterized by complicated pathophysiology. Brain ischemia leads to a cascade of events, such as glutamate excitotoxicity, energy failure, and formation of toxic radicals. The proteomic

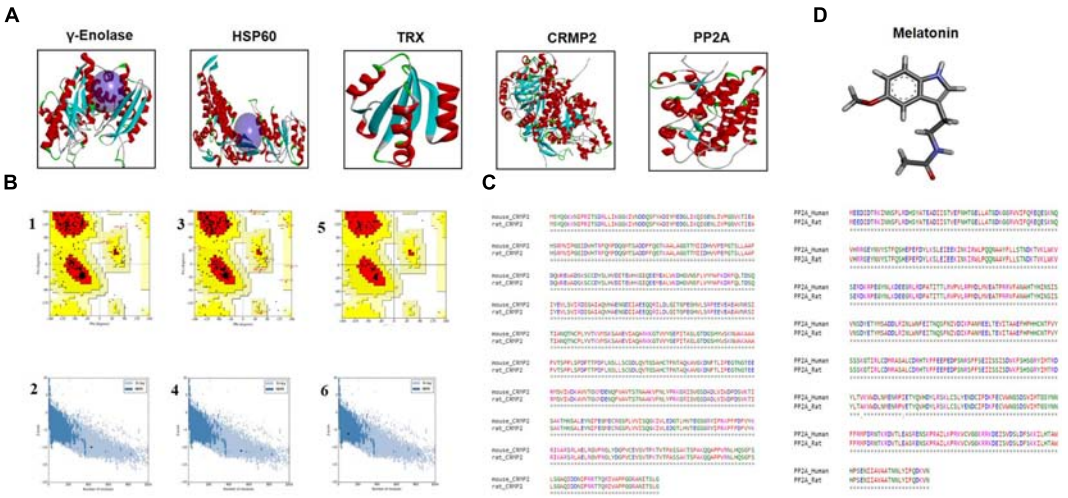

FIGURE 5 | (A) The representative tertiary structures of proteins (γ-enolase, HSP60, CRMP2, TRX, and PP2A). **(B)** Homology modeling and validation of tertiary structures. Ramachandran plots for γ-enolase (1), HSP60 (3), and TRX (5) are shown. ProSA findings of γ-enolase, HSP60, and TRX are represented by (2), (4), and (6), respectively. **(C)** Representative sequence alignment of CRMP2 (rat accession number P47942) with mouse CRMP2 (PDB code:5UQC) and PP2A (UniProt accession number P36877) with human PP2A (PDB ID: 3DW8) by clustal omega. **(D)** The ligand melatonin structure was drawn in ChemSketch and was converted to PDB format by Pymol.

study helps identify proteins that are imperative in brain injury and provides an in-depth analysis of the mechanism underlying neuronal degeneration following ischemic stroke. In this study, we identified 5 proteins that were differentially expressed in the ischemic brain between MCAO and mela + MCAO groups.

CRMPs are a group of cytoplasmic proteins (CRMP1, CRMP2, and CRMP5) and have important roles in neuronal polarization and axonal growth (Quach et al., 2015; Zhang and Koch, 2017). Expression of CRMP2 is largely depending upon the underlying neurological diseases (Czech et al., 2004; Li et al., 2018). The biological role of CRMP2 in ischemic brain injury has remained unclear. Some studies suggest increased expression of CRMP2 in the ischemic brain, but other studies indicate reduced expression of this protein (Chen et al., 2007; Shah et al., 2014; Yang et al., 2016). Moreover, consistent findings suggest that intact CRMP2 (66 kDa) is degraded into 55 kDa break down product (BDP) proteins by calpain-mediated proteolysis during brain injury (Yoshimura et al., 2005). These studies also identify different CRMP2 isoforms with varied pI values (Franzen et al., 2003). However, we identified two CRMP2 spots with the same pI values and molecular weight. The discrepancy could be attributed to species and method variability. Several studies have shown the entanglement of CRMP2 in Ca^{2+} signaling (Chi et al., 2009; Brittain et al., 2011). Sequestration of CRMP2-Ca^{2+} signaling attenuates inflammation in both stroke and neuropathic pain model (Brittain et al., 2011, 2012). Moreover, altered expression of CRMP2 has been previously demonstrated in Alzheimer's disease, Parkinson's disease, alcohol-induced neurodegeneration, and traumatic brain injury (Barzilai et al., 2000; Kobeissy et al., 2006; Matsuda-Matsumoto et al., 2007). CRMP2 is also implicated in neurogenesis and plasticity because it promotes axonal regeneration and inhibits the generation of p53-induced apoptotic genes (Suzuki et al., 2003; Llanos et al., 2006). CRMP2 is a downstream target of GSK3β, which can antagonize the polarization activity of CRMP2 by phosphorylation (Yoshimura et al., 2005). In this present study, we found two different spots for CRMP2, which were differentially regulated in the ischemic striatum and sham-operated control striatum. Moreover, melatonin treatment maintained the expression level of intact CRMP2 in the Mela + MCAO group. Western blotting analysis indicates that increased production of 55 kDa breakdown product (BDP) is associated with ischemic injury, and melatonin treatment attenuates the increase of the BDP and maintains the integrity of intact CRMP2.

Heat shock proteins are physiological sensors and are localized largely in mitochondria with a little fraction in the cytosol. Several studies suggest that overexpression of HSP60 induces mitochondrial biogenesis during ischemic damage, indicating the intrinsic protective mechanism of the brain during stressful settings (Bertoni-Freddari et al., 2006; Gutsaeva et al., 2006; Yin et al., 2008; Truettner et al., 2009). HSP60 dimerizes with HSP10, playing an integral role in protein folding and import and thus helping maintain the structural and functional integrity of

TABLE 2 | Binding energy values or binding affinity (*E*-value), expressed as Kcal/mol of Gamma enolase (γ-Enolase), heat shock protien-60 (HSP-60), collapsin response mediated protein 2 (CRMP2), Thioredoxin (TRX), and Protein phosphatase 2A (PP2A).

Protein	Energy values after docking (kcal/mol)
γ-Enolase	−5.5
HSP60	−5.9
CRMP2	−6.2
TRX	−5.4
PP2A	−6.6

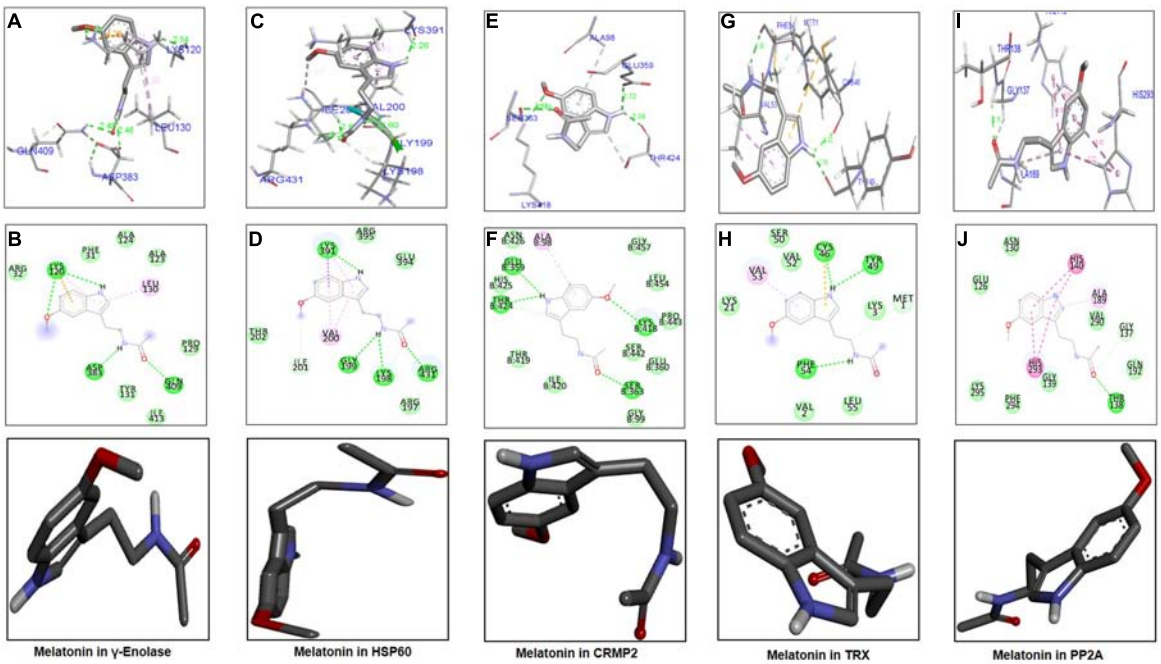

FIGURE 6 | Docking results and best pose of melatonin that fits γ-enolase, HSP60, CRMP2, TRX, PP2A. Post-docking analysis were visualized by DSV in both 2D and 3D poses. Interaction between melatonin and γ-enolase were shown by **A,B**, HSP60 by **C,D**, CRMP2 **(E,F)**, TRX **(G,H)**, and PP2A by **I,J**. 3D poses were shown by **A,C,E,G,I** and 2D by **B,D,F,H,J**.

mitochondria. Furthermore, the inflammatory role of HSP60 has been also demonstrated in various brain injuries, where HSP60 binds to toll-like receptors (TLRs) in microglia cells in the brain and protects against disturbances in the neuronal environment (Lehnardt et al., 2008). Because of the significant role in neuroinflammation, HSP60 has been considered a promising biomarker of neuronal injury (Chang et al., 2012). HSP60 has both survival and apoptotic functions largely depending on its localization (Kim et al., 2009). Coupling of HSP60 to TLR4 and Bax leads to apoptotic (NF_KB activation) and survival pathways in the cardiac tissue, respectively (Lin et al., 2007; Wang et al., 2010). Our proteomic analysis suggests overexpressed HSP60 in the ischemic brain, and the findings are consistent with previous observations (Hwang et al., 2007; Yin et al., 2008). Furthermore, melatonin treatment maintains the expression level of HSP60. In agreement to the previous findings, the induction of HSP60 in the present study indicates the intrinsic protective activity of the brain after ischemic brain damage.

γ-enolases/enolase-2 is abundantly present in mature neurons in the white and gray matters, and one recent study has regarded γ-enolases as a biochemical marker of brain injury (Sahu et al., 2017). Previous studies have demonstrated that γ-enolase is a neurotropic agent, and it can enhance neuronal survival and promote axonal growth (Hafner et al., 2012). γ-enolases are very important for energy generation during glycolysis, and deterioration in γ-enolase activity would adversely affect energy metabolism in the brain. The glycolytic functions of γ-enolases and other metabolic enzymes are significantly impaired by ischemic damage. Notably, downregulation of γ-enolase leads to neurodegeneration (Kilic et al., 2005). However, a high concentration of γ-enolase is found in various brain pathologies, and inhibition of γ-enolase attenuates inflammation-related cellular injury (Haque et al., 2017). Our study showed that NSE expression decreased 24 h after cerebral ischemia, and the results are similar to previous findings (Gim et al., 2015; Jeon et al., 2017; Park et al., 2018). Our findings are further verified by western blot analysis and confocal immunofluorescence analysis, and melatonin treatment prevents the ischemia-induced decrease of NSE. γ-enolases exert pleiotropic action and mediate neuronal repair, axonal outgrowth, and neurotrophic activity by PI3K and MAPK pathways (Hafner et al., 2012; Polcyn et al., 2017). Our findings suggest that preservation of γ-enolase during MCAO contributes to the neuroprotective effect of melatonin, but the relation between γ-enolase and melatonin is not yet well established. Therefore, future work is needed to comprehensively investigate the underlying neuroprotective mechanism.

Thioredoxin (TRX) has multi-biological functions including redox signaling. TRX participates in the eradication of ROS such as hydrogen peroxide and other toxic radicals (Landino et al., 2004; Messens and Silver, 2006). TRX is an important component of the TRX system, in which TRX and peroxiredoxin act as anti-oxidant enzymes and maintain a hemostatic-reduced environment. Downregulation of thioredoxin leads to apoptotic death by activating ASK1, and apoptosis further stimulates stress signaling kinases such as JNK. In addition, upregulation of thioredoxin impedes ASK1-induced apoptosis (Lee et al., 2008). A number of studies have consistently demonstrated that ischemic brain damage is linked to free radical

generation, which triggers neuronal damage by apoptosis and necrosis. Moreover, free radical scavengers protect neuronal cells from ischemic damage by attenuating free radical formation. In fact, the exogenous administration of these scavengers attenuates neuronal degeneration during ischemic stroke (Wang et al., 2015). During ischemic brain injury, ROS are generated via the hyperactivated electron transport chain and the NADPH oxidase system and are implicated in the detrimental effects on DNA, cell membrane, ion channels, and redox signaling (Valko et al., 2007). Protective endogenous enzymes, such as TRX, play a significant role in the prevention of the oxidative stress-induced neuronal damage (Chan, 2001). TRX isoforms are localized in both cytosol and mitochondria with varying degrees of antioxidant capacity. Several studies have demonstrated enhanced tolerability of ischemic brain with overexpressed TRX (Stroev et al., 2004). Moreover, TRX is demarcated as an oxidation, inflammation, and immune deregulatory marker due to the substantial role (Al-Gayyar et al., 2011; Zhang et al., 2012). Furthermore, oxidative stress and ROS are hallmarks of several neurodegenerative disorders, further strengthening the anti-oxidant capacity of TRX because the brain has a poor catalase and glutathione activity (Erecińska and Silver, 2001). Several studies have demonstrated that TRX release is controlled by NRF2 transcriptional factors, which activate antioxidant genes, including TRX, after translocation to the nucleus (Wu et al., 2015). Moreover, one recent study by Ali et al. (2018) indicates that melatonin activates NRF2 in the brain. Our proteomics study revealed decreased expression of TRX in the striatal tissue in MCAO-operated rats, and melatonin treatment attenuated this decline of TRX triggered by ischemic injury, indicating the antioxidant nature of melatonin. However, further studies are needed to explore the exact mechanism.

Protein phosphatase 2A (PP2A) is a serine-threonine phosphatase and has multiple biological activities from cellular metabolism to development and apoptosis. Subunit B of PP2A is ubiquitously expressed in the brain, and it regulates both axonal growth and neurogenesis. Our previous study has found downregulated PP2A in the cortex 24 h after MCAO (Shah et al., 2015). Phosphorylation and de-phosphorylation should be rigidly regulated because this process determines the fate of the signaling cascade in various disease pathologies including neurodegeneration (Sontag et al., 2004), and PP2A is an important component in the de-phosphorylation process (Gong et al., 2000; Liu et al., 2005; Gong and Iqbal, 2008). PP2A maintains tau in dephosphorylated form, which helps in axonal microtubule assembling. Moreover, PP2A downregulation leads to tau hyperphosphorylation, a characteristic hallmark in the Alzheimer's brain. In addition, PP2A inhibits the induction of stress kinases, such as JNK and p38 (Chen et al., 2009). PP2A also de-phosphorylates NMDA receptor subunits and hinders calcium currents (Ma and Sucher, 2004). Amassing of calcium inside neuronal cells leads to excitotoxicity-induced neuronal death in cerebral ischemia. Our study demonstrated decreased expression of PP2A subunit B in the ischemic striatum. Moreover, the attenuated expression of PP2A is implicated in ischemic brain injury, as demonstrated by Koh (2012). In addition, immunohistochemical staining further validates the proteomics findings that PP2A subunit B-positive cells decrease in the ischemic striatum. Moreover, treatment with melatonin restores PP2A expression. Our results are consistent to previous observations that melatonin mitigates ischemic injury-induced downregulation of PP2A in the ischemic and AD animal models, as well as in *in vitro* models (Koh, 2013; Arribas et al., 2018).

In summary, melatonin attenuates MCAO-induced neuronal damage by modulating the expression of proteins involved in energy metabolism, homeostasis, axonal growth, and oxidative response in the striatum. The study suggests that melatonin may potentially ameliorate the ischemic stroke damage by modulating expression of several proteins in the striatum. Moreover, a more in-depth understanding of the functions of proteins (CRMP2, HSP60, Enolase, TRX, and PP2A) could provide new opportunities for treating a wide range of neurodegenerative disorders, including ischemic stroke.

DATA AVAILABILITY STATEMENT

The authors here by state that the datasets generated in this study will be available upon request from the corresponding author.

AUTHOR CONTRIBUTIONS

FS designed, managed the experimental work, and wrote the manuscript. FS, TA, MF, TM, SA, and KS performed the Western blot and morphological experiments, FS, TA, P-OK, and MK arranged the data and performed the data analysis. AZ and KL performed the bioinformatics analysis. MK is a corresponding author, reviewed and approved the manuscript and holds all the responsibilities related to this manuscript. All authors reviewed the manuscript.

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REFERENCES

Abraham, M. J., Murtola, T., Schulz, R., Pall, S., Smith, J. C., Hess, B., et al. (2015). GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 1, 19–25. doi: 10.1016/j.softx.2015.06.001

Al-Gayyar, M. M., Abdelsaid, M. A., Matragoon, S., Pillai, B. A., and El-Remessy, A. B. (2011). Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity. *Br. J. Pharmacol.* 1, 170–180. doi: 10.1111/j.1476-5381.2011.01336.x

Ali, T., Rehman, S. U., Shah, F. A., and Kim, M. O. (2018). Acute dose of melatonin via Nrf2 dependently prevents acute ethanol-induced neurotoxicity in the

developing rodent brain. *J. Neuroinflammation* 15:119. doi: 10.1186/s12974-018-1157-x

Alluri, H., Wilson, R. L., Anasooya Shaji, C., Wiggins-Dohlvik, K., Patel, S., Liu, Y., et al. (2016). Melatonin preserves blood-brain barrier integrity and permeability via matrix metalloproteinase-9 inhibition. *PLoS One* 11:e0154427. doi: 10.1371/journal.pone.0154427

Andrabi, S. S., Parvez, S., and Tabassum, H. (2015). Melatonin and ischemic stroke: mechanistic roles and action. *Adv. Pharmacol. Sci.* 2015:384750. doi: 10.1155/2015/384750

Arribas, R. L., Romero, A., Egea, J., and de Los Ríos, C. (2018). Modulation of serine/threonine phosphatases by melatonin: therapeutic approaches in neurodegenerative diseases. *Br. J. Pharmacol.* doi: 10.1111/bph.14365 [Eub ahead of rint].

Barzilai, A., Zilkha-Falb, R., Daily, D., Stern, N., Offen, D., Ziv, I., et al. (2000). The molecular mechanism of dopamine-induced apoptosis: identification and characterization of genes that mediate dopamine toxicity. *J. Neural. Transm. Suppl.* 60, 59–76. doi: 10.1007/978-3-7091-6301-6_4

Bertoni-Freddari, C. A., Fattoretti, P., Casoli, T., Di Stefano, G., Solazzi, M., Perna, E., et al. (2006). Reactive structural dynamics of synaptic mitochondria in ischemic delayed neuronal death. *Ann. N. Y. Acad. Sci.* 1090, 26–34. doi: 10.1196/annals.1378.003

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., et al. (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res.* 42, 252–258. doi: 10.1093/nar/gku340

Bosshard, H. R., Marti, D. N., and Jelesarov, I. (2004). Protein stabilization by salt bridges: concepts, experimental approaches and clarification of some misunderstandings. *J. Mol. Recognit.* 17, 1–16. doi: 10.1002/jmr.657

Brittain, J. M., Duarte, D. B., Wilson, S. M., Zhu, W., Ballard, C., Johnson, P. L., et al. (2011). Suppression of inflammatory and neuropathic pain by uncoupling CRMP-2 from the presynaptic Ca(2) channel complex. *Nat. Med.* 17, 822–829. doi: 10.1038/nm.2345

Brittain, J. M., Pan, R., You, H., Brustovetsky, T., Brustovetsky, N., Zamponi, G. W., et al. (2012). Disruption of NMDAR-CRMP-2 signaling protects against focal cerebral ischemic damage in the rat middle cerebral artery occlusion model. *Channels* 6, 52–59. doi: 10.4161/chan.18919

Burley, S. K. (2000). An overview of structural genomics. *Nat. Struct. Biol.* 7, 932–934. doi: 10.1038/80697

Chan, P. H. (2001). Reactive oxygen radicals in signaling and damage in the ischemic brain. *J. Cereb. Blood Flow Metab.* 21, 2–14. doi: 10.1097/00004647-200101000-00002

Chang, C. C., Lui, C. C., Lee, C. C., Chen, S. D., Chang, W. N., Lu, C. H., et al. (2012). Clinical significance of serological biomarkers and neuropsychological performances in patients with temporal lobe epilepsy. *BMC Neurol.* 12:15. doi: 10.1186/1471-2377-12-15

Chen, A., Liao, W. P., Lu, Q., Wong, W. S. F., and Wong, P. H. (2007). Upregulation of dihydropyrimidinase-related protein 2, spectrin α II chain, heat shock cognate protein 70 pseudogene 1 and tropomodulin 2 after focal cerebral ischemia in rats—a proteomics approach. *Neurochem. Int.* 50, 1078–1086. doi: 10.1016/j.neuint.2006.11.008

Chen, L., Liu, L., Yin, J., Luo, Y., and Huang, S. (2009). Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. *Int. J. Biochem. Cell Biol.* 41, 1284–1295. doi: 10.1016/j.biocel.2008.10.029

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., et al. (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res.* 31, 3497–3500. doi: 10.1093/nar/gkg500

Chi, X. X., Schmutzler, B. S., Brittain, J. M., Wang, Y., Hingtgen, C. M., Nicol, G. D., et al. (2009). Regulation of N-type voltage-gated calcium channels (Cav2.2) and transmitter release by collapsin response mediator protein-2 (CRMP-2) in sensory neurons. *J. Cell Sci.* 122, 4351–4362. doi: 10.1242/jcs.053280

Czech, T., Yang, J. W., Csaszar, E., Kappler, J., Baumgartner, C., and Lubec, G. (2004). Reduction of hippocampal collapsin response mediated protein-2 in patients with mesial temporal lobe epilepsy. *Neurochem. Res.* 29, 2189–2196. doi: 10.1007/s11064-004-7025-3

Erecińska, M., and Silver, I. A. (2001). Tissue oxygen tension and brain sensitivity to hypoxia. *Respir. Physiol.* 128, 263–276. doi: 10.1016/S0034-5687(01)00306-1

Fluri, F., Schuhmann, M. K., and Kleinschnitz, C. (2015). Animal models of ischemic stroke and their application in clinical research. *Drug Des. Devel. Ther.* 9:3445. doi: 10.2147/DDDT.S56071

Franzen, B., Yang, Y., Sunnemark, D., Wickman, M., Ottervald, J., Oppermann, M., et al. (2003). Dihydropyrimidinase related protein-2 as a biomarker for temperature and time dependent post mortem changes in the mouse brain proteome. *Proteomics* 3, 1920–1929. doi: 10.1002/pmic.200300535

García, J. J., López-Pingarrón, L., Almeida-Souza, P., Escudero, P., García-Gil, F. A., Tan, D. X., et al. (2014). Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: a review. *J. Pineal Res.* 56, 225–237. doi: 10.1111/jpi.12128

Gim, S. A., Lee, S. R., Shah, F. A., and Koh, P. O. (2015). Curcumin attenuates the middle cerebral artery occlusion-induced reduction in γ-enolase expression in an animal model. *Lab. Anim. Res.* 31, 198–203. doi: 10.5625/lar.2015.31.4.198

Gong, C. X., and Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr. Med. Chem.* 15, 2321–2328. doi: 10.2174/092986708785909111

Gong, C. X., Lidsky, T., Wegiel, J., Zuck, L., Grundke-Iqbal, I., and Iqbal, K. (2000). Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain implications for neurofibrillary degeneration in Alzheimer's disease. *J. Biol. Chem.* 275, 5535–5544. doi: 10.1074/jbc.275.8.5535

Gutsaeva, D. R., Suliman, H. B., Carraway, M. S., Demchenko, I. T., and Piantadosi, C. A. (2006). Oxygen-induced mitochondrial biogenesis in the rat hippocampus. *Neuroscience* 137, 493–504. doi: 10.1016/j.neuroscience.2005.07.061

Hafner, A., Obermajer, N., and Kos, J. (2012). γ-Enolase C-terminal peptide promotes cell survival and neurite outgrowth by activation of the PI3K/Akt and MAPK/ERK signalling pathways. *Biochem. J.* 443, 439–450. doi: 10.1042/BJ20111351

Haque, A., Capone, M., Matzelle, D., Cox, A., and Banik, N. L. (2017). Targeting enolase in reducing secondary damage in acute spinal cord injury in rats. *Neurochem. Res.* 42, 2777–2787. doi: 10.1007/s11064-017-2291-z

Hwang, I. K., Ahn, H. C., Yoo, K., kimkim, Y., Lee, J. Y., Suh, H. W., et al. (2007). Changes in immunoreactivity of HSP60 and its neuroprotective effects in the gerbil hippocampal CA1 region induced by transient ischemia. *Exp. Neurol.* 208, 247–256. doi: 10.1016/j.expneurol.2007.08.017

Jeon, S. J., Kim, M. O., Shah, F. A., and Koh, P. O. (2017). Quercetin attenuates the injury-induced reduction of γ-enolase expression in a middle cerebralartery occlusion animal model. *Lab. Anim. Res.* 33, 308–314. doi: 10.5625/lar.2017.33.4.308

Kilic, U., Kilic, E., Reiter, R. J., Bassetti, C. L., and Hermann, D. M. (2005). Signal transduction pathways involved in melatonin-induced neuroprotection after focal cerebral ischemia in mice. *J. Pineal Res.* 38, 67–71. doi: 10.1111/j.1600-079X.2004.00178.x

Kim, S. C., Stice, J. P., Chen, L., Jung, J. S., Gupta, S., Wang, Y., et al. (2009). Extracellular heat shock protein 60, cardiac myocytes, and apoptosis. *Circ. Res.* 105, 1186–1195. doi: 10.1161/CIRCRESAHA.109.209643

Kobeissy, F. H., Ottens, A. K., Zhang, Z., Liu, M. C., Denslow, N. D., Dave, J. R., et al. (2006). Novel differential neuroproteomics analysis of traumatic brain injury in rats. *Mol. Cell. Proteomics* 5, 1887–1898. doi: 10.1074/mcp.M600157-MCP200

Koh, P. O. (2012). Melatonin attenuates decrease of protein phosphatase 2A subunit B in ischemic brain injury. *J. Pineal Res.* 52, 57–61. doi: 10.1111/j.1600-079X.2011.00918

Koh, P. O. (2013). Ferulic acid attenuates the injury-induced decrease of protein phosphatase 2A subunit B in ischemic brain injury. *PLoS One* 8:e54217. doi: 10.1371/journal.pone.0054217

Lacoste, B., Angeloni, D., Dominguez-Lopez, S., Calderoni, S., Mauro, A., Fraschini, F., et al. (2015). Anatomical and cellular localization of melatonin MT1 and MT2 receptors in the adult rat brain. *J. Pineal Res.* 58, 397–417. doi: 10.1111/jpi.12224

Landino, L. M., Skreslet, T. E., and Alston, J. A. (2004). Cysteine oxidation of tau and microtubule-associated protein-2 by peroxynitrite modulation of microtubule assembly kinetics by the thioredoxin reductase system. *J. Biol. Chem.* 279, 35101–35105. doi: 10.1074/jbc.M405471200

Lee, E. J., Lee, M. Y., Chen, H. Y., Hsu, Y. S., Wu, T. S., Chen, S. T., et al. (2005). Melatonin attenuates gray and white matter damage in a mouse model of

transient focal cerebral ischemia. *J. Pineal Res.* 38, 42–52. doi: 10.1111/j.1600-079X.2004.00173.x

Lee, M., Lee, S., and Hong, Y. (2014). Melatonin plus treadmill exercise synergistically promotes neurogenesis and reduce apoptosis in focal cerebral ischemic rats. *FASEB J.* 28:877.17.

Lee, Y. C., Chuang, C. Y., Lee, P. K., Lee, J. S., Harper, R. W., Buckpitt, A. B., et al. (2008). TRX-ASK1-JNK signaling regulation of cell density-dependent cytotoxicity in cigarette smoke-exposed human bronchial epithelial cells. *Am. J. Physiol. Lung. Cell Mol. Physiol.* 294, L921–L931. doi: 10.1152/ajplung.00250.2007

Lehnardt, S., Schott, E., Trimbuch, T., Laubisch, D., Krueger, C., Wulczyn, G., et al. (2008). A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci.* 28, 2320–2331. doi: 10.1523/JNEUROSCI.4760-07.2008

Li, X. B., Ding, M. X., Ding, C. L., Li, L. L., Feng, J., and Yu, X. J. (2018). Toll-Like receptor 4 promotes the phosphorylation of CRMP2 via the activation of Rho-kinase in MCAO rats. *Mol. Med. Rep.* 18, 342–348. doi: 10.3892/mmr.2018.8968

Lin, L., Kim, S. C., Wang, Y., Gupta, S., Davis, B., Simon, S. I., et al. (2007). HSP60 in heart failure: abnormal distribution and role in cardiac myocyte apoptosis. *Am. J. Physiol. Heart Circ. Physiol.* 293, H2238–H2247. doi: 10.1152/ajpheart.00740.2007

Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C. X. (2005). Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur. J. Neurosci.* 22, 1942–1950. doi: 10.1111/j.1460-9568.2005.04391.x

Llanos, S., Efeyan, A., Monsech, J., Dominguez, O., and Serano, M. (2006). A high-throughput loss-of-function screening identifies novel p53 regulators. *Cell Cycle* 5, 1880–1885. doi: 10.4161/cc.5.16.3140

Ma, O. K., and Sucher, N. J. (2004). Molecular interaction of NMDA receptor subunit NR3A with protein phosphatase 2A. *Neuroreport* 15, 1447–1450. doi: 10.1097/01.wnr.0000132773.41720.2d

Matsuda-Matsumoto, H., Iwazaki, T., Kashem, M. A., Harper, C., and Matsumoto, I. (2007). Differential protein expression profiles in the hippocampus of human alcoholics. *Neurochem. Int.* 51, 370–376. doi: 10.1016/j.neuint.2007.04.001

Mauriz, J. L., Collado, P. S., Veneroso, C., Reiter, R. J., and González-Gallego, J. (2013). A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. *J. Pineal Res.* 54, 1–14. doi: 10.1111/j.1600-079X.2012.01014.x

Messens, J., and Silver, S. (2006). Arsenate reduction: thiol cascade chemistry with convergent evolution. *J. Mol. Biol.* 362, 1–17. doi: 10.1016/j.jmb.2006.07.002

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. (2015). Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation* 131, e29–e322. doi: 10.1161/CIR.0000000000000152

Okuno, S., Saito, A., Hayashi, T., and Chan, P. H. (2004). The c-Jun N-terminal protein kinase signaling pathway mediates Bax activation and subsequent neuronal apoptosis through interaction with Bim after transient focal cerebral ischemia. *J. Neurosci.* 24, 7879–7887. doi: 10.1523/JNEUROSCI.1745-04.2004

Park, D. J., Shah, F. A., and Koh, P. O. (2018). Quercetin attenuates neuronal cells damage in a middle cerebral artery occlusion animal model. *J. Vet. Med. Sci.* 80, 676–683. doi: 10.1292/jvms.17-0693

Polcyn, R., Capone, M., Hossain, A., Matzelle, D., Banik, N. L., and Haque, A. (2017). Neuron specific enolase is a potential target for regulating neuronal cell survival and death: implications in neurodegeneration and regeneration. *Neuroimmunol. Neuroinflamm.* 4, 254–257. doi: 10.20517/2347-8659.2017.59

Quach, T. T., Honnorat, J., Kolattukudy, P. E., Khanna, R., and Duchemin, A. M. (2015). CRMPs: critical molecules for neurite morphogenesis and neuropsychiatric diseases. *Mol. Psychiatry* 20, 1037–1045. doi: 10.1038/mp.2015.77

Ramos, E., Patiño, P., Reiter, R. J., Gil-Martín, E., Marco-Contelles, J., Parada, E., et al. (2017). Ischemic brain injury: new insights on the protective role of melatonin. *Free Radic. Biol. Med.* 104, 32–53. doi: 10.1016/j.freeradbiomed.2017.01.005

Rodriguez, C., Mayo, J. C., Sainz, R. M., Antolin, I., Herrera, F., and Martin, V. (2004). Regulation of antioxidant enzymes: a significant role for melatonin. *J. Pineal Res.* 36, 1–9. doi: 10.1046/j.1600-079X.2003.00092.x

Sahoo, M., Jena, L., Rath, S. N., and Kumar, S. (2016). Identification of suitable natural inhibitor against influenza A (H1N1) neuraminidase protein by molecular docking. *Genomics Inform.* 14, 96–103. doi: 10.5808/GI.2016.14.3.96

Sahu, S., Nag, D. S., Swain, A., and Samaddar, D. P. (2017). Biochemical changes in the injured brain. *World J. Biol. Chem.* 8, 21–31. doi: 10.4331/wjbc.v8.i1.21

Sarnat, H. B. (2013). Clinical neuropathology practice guide 5-2013: markers of neuronal maturation. *Clin. Neuropathol.* 32, 340–369. doi: 10.5414/NP300638

Shah, F. A., Gim, S. A., Kim, M. O., and Koh, P. O. (2014). Proteomic identification of proteins differentially expressed in response to resveratrol treatment in middle cerebral artery occlusion stroke model. *J. Vet. Med. Sci.* 76, 1367–1374. doi: 10.1292/jvms.14-0169

Shah, F. A., Gim, S. A., Sung, J. H., Jeon, S. J., Kim, M. O., and Koh, P. O. (2016). Identification of proteins regulated by curcumin in cerebral ischemia. *J. Surg. Res.* 201, 141–148. doi: 10.1016/j.jss.2015.10.025

Shah, F. A., Park, D. J., Gim, S. A., and Koh, P. O. (2015). Curcumin treatment recovery the decrease of protein phosphatase 2A subunit B induced by focal cerebral ischemia in Sprague-Dawley rats. *Lab. Anim. Res.* 31, 134–138. doi: 10.5625/lar.2015.31.3.134

Sontag, E., Luangpirom, A., Hladik, C., Mudrak, I., Ogris, E., Speciale, S., et al. (2004). Altered expression levels of the protein phosphatase 2A are associated with Alzheimer disease pathology. *J. Neuropathol. Exp. Neurol.* 63, 287–301. doi: 10.1093/jnen/63.4.287

Stroev, S. A., Gluschenko, T. S., Tjulkova, E. I., Spyrou, G., Rybnikova, E. A., Samoilov, M. O., et al. (2004). Preconditioning enhances the expression of mitochondrial antioxidant thioredoxin-2 in the forebrain of rats exposed to severe hypobaric hypoxia. *J. Neurosci. Res.* 78, 563–569. doi: 10.1002/jnr.20282

Suzuki, Y., Nakagomi, S., Namikawa, K., Kiryu-Seo, S., Inagaki, N., Kaibuchi, K., et al. (2003). Collapsin response mediator protein- 2 accelerates axon regeneration of nerve-injured motor neurons of rat. *J. Neurochem.* 86, 1042–1050. doi: 10.1046/j.1471-4159.2003.01920.x

Tan, D. X., Manchester, L. C., Terron, M. P., Flores, L. J., Tamura, H., and Reiter, R. J. (2007). Melatonin as a naturally occurring co-substrate of quinone reductase-2, the putative MT3 melatonin membrane receptor: hypothesis and significance. *J. Pineal Res.* 43, 317–320. doi: 10.1111/j.1600-079X.2007.00513.x

Truettner, J. S., Hu, K., Liu, C. L., Dietric, W. D., and Hu, B. (2009). Subcellular stress response and induction of molecular chaperones and folding proteins after transient global ischemia in rats. *Brain Res.* 1249, 9–18. doi: 10.1016/j.brainres.2008.10.032

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39, 44–84. doi: 10.1016/j.biocel.2006.07.001

Venegas, C., Garcia, J. A., Escames, G., Ortiz, F., Lopez, A., Doerrier, C., et al. (2012). Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. *J. Pineal Res.* 52, 217–227. doi: 10.1111/j.1600-079X.2011.00931.x

Vriend, J., and Reiter, R. J. (2015). Melatonin feedback on clock genes: a theory involving the proteasome. *J. Pineal Res.* 58, 1–11. doi: 10.1111/jpi.12189

Wang, B., Tian, S., Wang, J., Han, F., Zhao, L., Wang, R., et al. (2015). Intraperitoneal administration of thioredoxin decreases brain damage from ischemic stroke. *Brain Res.* 1615, 89–97. doi: 10.1016/j.brainres.2015.04.033

Wang, Y., Chen, L., Hagiwara, N., and Knowlton, A. A. (2010). Regulation of heat shock protein 60 and 72 expression in the failing heart. *J. Mol. Cell Cardiol.* 48, 360–366. doi: 10.1016/j.yjmcc.2009.11.009

Wiederstein, M., and Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res.* 35, 407–410. doi: 10.1093/nar/gkm290

Wu, J. X., Zhang, L. Y., Chen, Y. L., Yu, S. S., Zhao, Y., and Zhao, J. (2015). Curcumin pretreatment and post-treatment both improve the antioxidative ability of neurons with oxygen-glucose deprivation. *Neural Regen. Res.* 10, 481–489. doi: 10.4103/1673-5374.153700

Yang, W., Tiffany-Castiglioni, E., Koh, H. C., and Son, I. H. (2009). Paraquat activates the IRE1/ASK1/JNK cascade associated with apoptosis in human neuroblastoma SH-SY5Y cells. *Toxicol. Lett.* 191, 203–210. doi: 10.1016/j.toxlet.2009.08.024

Yang, X., Zhang, X., Li, Y., Han, S., Howells, D. W., Li, S., et al. (2016). Conventional protein kinase Cβ-mediated phosphorylation inhibits collapsin response-mediated protein 2 proteolysis and alleviates ischemic injury in

cultured cortical neurons and ischemic stroke-induced mice. *J. Neurochem.* 137, 446–459. doi: 10.1111/jnc.13538

Yin, W., Signore, A. P., Iwai, M., Cao, G., Gao, Y., and Chen, J. (2008). Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury. *Stroke* 39, 3057–3063. doi: 10.1161/STROKEAHA.108.520114

Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A., and Kaibuchi, K. (2005). GSK-3β regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* 120, 137–149. doi: 10.1016/j.cell.2004.11.012

Zhang, J. N., and Koch, J. C. (2017). Collapsin response mediator protein-2 plays a major protective role in acute axonal degeneration. *Neural Regen. Res.* 12, 692–695. doi: 10.4103/1673-5374.206631

Zhang, X. Y., Chen, D. C., Xiu, M. H., Yang, F. D., Tan, Y. L., He, S., et al. (2012). Thioredoxin, a novel oxidative stress marker and cognitive performance in chronic and medicated schizophrenia versus healthy controls. *Schizophr. Res.* 143, 301–306. doi: 10.1016/j.schres.2012.11.017

Zhang, Z., Ottens, A. K., Sadasivan, S., Kobeissy, F. H., Fang, T., Hayes, R. L., et al. (2007). Calpain-mediated collapsin response mediator protein-1,-2, and-4 proteolysis after neurotoxic and traumatic brain injury. *J. Neurotrauma* 24, 460–472. doi: 10.1089/neu.2006.0078

Zoete, V., Cuendet, M. A., Grosdidier, A., and Michielin, O. (2011). SwissParam: a fast force field generation tool for small organic molecules. *J. Comput. Chem.* 32, 2359–2368. doi: 10.1002/jcc.21816

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OPINION
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Affective Network Neuroscience

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The last years have seen the rise of a new paradigm in human neuroimaging: network neuroscience (Bassett and Sporns, 2017). Network neuroscience conceptualizes the brain as a connectome—an intricate network map of the brain where brain regions synchronize their activity via myriads of interconnecting nerve fibers. Network neuroscience is an interdisciplinary endeavor whose potential for cognitive science, the study of individual differences, and clinical research has been highlighted in several recent articles (Braun et al., 2015; Medaglia et al., 2015; Markett et al., 2018; Tompson et al., 2018). In the following, we will argue that network neuroscience provides an innovative toolbox that can also advance our understanding of affective processes in the brain, particularly when guided by (neuro)psychological theory.

The transient synchronization of activation between remote brain areas is typically interpreted as *functional connectivity* (Friston et al., 1993), while *structural connectivity* refers to white matter fiber tracts that connect between brain areas. Even though neuroimaging techniques for both types of brain connectivity have been available for over two decades (Biswal et al., 1995; Mori et al., 1999), it took two major developments in the mid-2000s to trigger the current enthusiasm for network neuroscience. The first new development was brought to the field by functional neuroimaging. By analyzing temporal synchronizations in the blood oxygen level dependent (BOLD) signal during stimulation-free resting state, it was shown independently by various groups that the brain is organized into large-scale functional networks that can be consistently identified across participants and time (Greicius et al., 2003; Beckmann et al., 2005; Damoiseaux et al., 2006). Brain areas that synchronize their activity at rest also tend to co-activate during task (Smith et al., 2009; Di et al., 2013), which has led to several systems neuroscience accounts of how functional networks might interact to support a wide range of behavioral and cognitive functions (Dosenbach et al., 2008; Menon, 2011). The second paradigm—based on structural neuroimaging at first—started out by demonstrating the feasibility of detailing brain connectivity in the form of a connectome map (Hagmann, 2005; Sporns et al., 2005). A connectome map can be inferred from imaging data by collating a parcellation scheme of the cortical ribbon with fiber tracking procedures applied to diffusion MRI. The resulting network map can be studied with tools from mathematical graph theory, in order to reveal the principles of network-level organization of brain connectivity (Bullmore and Sporns, 2009). The relationship between functional and structural connectivity is complex and often indirect (Mišić et al., 2016). But the current understanding is that structural connections represent a communication scaffold that enables transient functional couplings of brain regions into network modules that support a wide range of behavioral and cognitive functions (Park and Friston, 2013). Modern day connectomics therefore includes a structural and a functional branch that are ideally studied together. The approach is illustrated in **Figure 1**.

The fact that the brain is a network, and that brain connectivity plays a crucial role in thought and behavior has been known since the early days of neuroscience. Previously, the study of structural and functional brain connectivity remained restricted to experimental animals, as the required methodology involved the injection of tracers or neurotoxins into brain tissue (Stephan, 2013). Due to the invasive nature, connectivity

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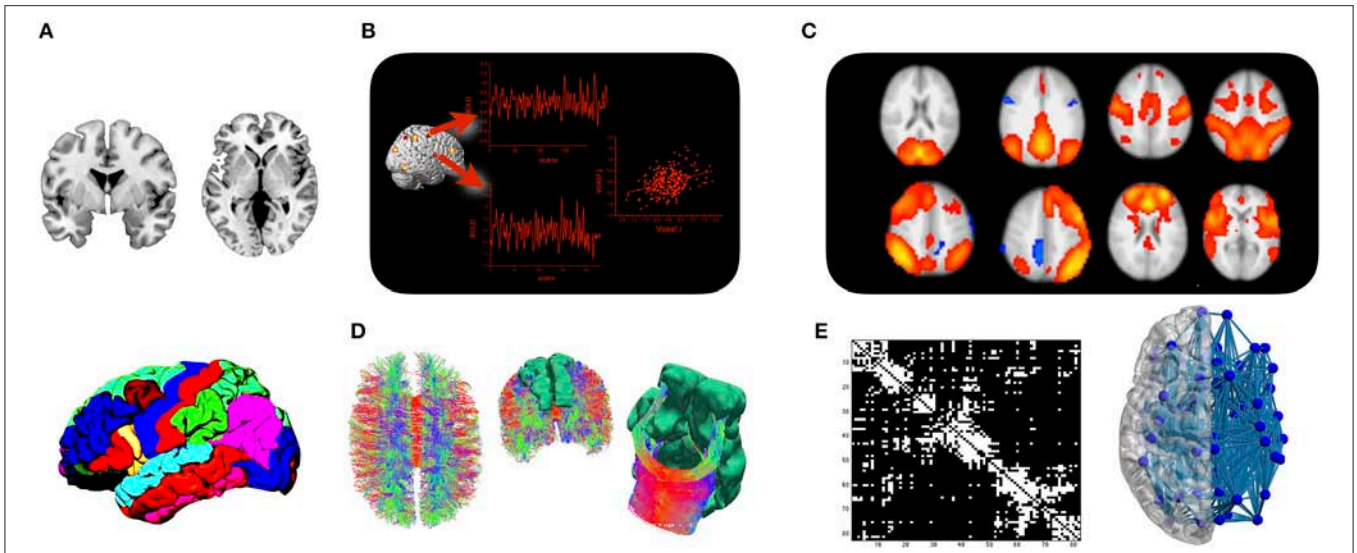

FIGURE 1 | The connectome approach: **(A)** Cortical and subcortical gray matter are parcellated into a set of regions of interest. **(B)** Mean time courses of BOLD activity are extracted from each region of interest, and functional connectivity is assessed by analyzing statistical dependencies between any two regions. **(C)** Functional connectivity is organized into a set of large-scale networks at the brain level. **(D)** Fiber tracking is applied to diffusion MRI data to assess whether regions from the parcellation are structurally connected. **(E)** Results are either displayed in a connectivity matrix whose elements indicate whether two regions are connected or not, or displayed in a connectivity plot for anatomical reference.

studies were often limited to single fiber tracts, and the assemblage of connectome maps was only possible when data were collated across many individual animals (Stephan, 2013). The current enthusiasm for network neuroscience based on non-invasive neuroimaging data reflects the fact that it allows cognitive and affective neuroscientists to catch up with connectivity analyses in human research participants. It also enables the holistic and repeated analysis of individual connectomes, particularly since it has been shown that macrolevel MRI-derived connectivity corresponds well with microlevel neuroarchitectonics (Scholtens et al., 2014). Network neuroscience represents first of all a new paradigmatic way of reasoning about the brain and second of all a fast-growing collection of methodological tools. Its full potential to the study of psychological phenotypes can be leveraged when its tools are applied to study brain connectivity in the context of psychological theory. In the following section, we will highlight the prospects of brain connectivity research in the context of three different influential theories on affect and emotion: The affective neuroscience theory (Panksepp, 1998), the reward sensitivity theory (Gilson et al., 2018), and the theory of constructed emotions (Barrett, 2017).

Affective neuroscience (AN) theory postulates seven primary emotional systems: SEEKING, LUST, CARE, PLAY on the side of positive emotions, and FEAR, ANGER/RAGE, and PANIC/SADNESS on the negative side (Panksepp, 1998, 2010; Montag and Panksepp, 2017a,b; Montag et al., 2017b; Davis and Montag, 2018). The distinct neural circuitry underlying the systems have been mainly mapped using localized electrical stimulation of the brains of experimental animals. For a detailed overview on the neuroanatomy underlying each primary emotional emotion see Panksepp (2011) and Montag and Panksepp (2016). Animals show behavioral responses consistent with basic emotions after stimulation of subcortical sites, such as the periaqueductal gray, the amygdala, or the medial forebrain bundle (Panksepp, 2010). As primary emotional systems, the seven circuits are thought to be innate and phylogenetically conserved across mammalian and non-mammalian species. An important topic for AN theory is therefore the translation of the animal data to humans. This endeavor is facilitated by the affective neuroscience personality scales (ANPS, Davis et al., 2003; Montag and Davis, 2018), a psychometric tool that has been developed on the background of AN theory and assesses individual differences in Panksepian primary emotions. A straightforward application of tools from network neuroscience entails the mapping of connectivity patterns of subcortical structures implicated by electrical stimulation, followed by correlation analysis with ANPS scores. AN theory clearly argues for a localization of the phylogenetically old primary emotional systems in the brain's oldest layers (Panksepp et al., 2017). The validity of all network neuroscience approaches depends on the careful selection of seed regions for connectivity mapping (Fornito et al., 2013). The small subcortical structures with relevance for AN theory are particularly difficult to delineate. In our own work on the ANPS, we therefore made use of a cytoarchitectonic atlas to define seed regions in the amygdala sub-nuclei. This approach ensures a more accurate and anatomically informed perspective on the human amygdala (Roy et al., 2009; Eckstein et al., 2017). We found robust correlations between functional connectivity of the basolateral section of the amygdala to parietal cortices and SADNESS (Deris et al., 2017). This study was the first to address connectivity in human participants with respect to AN theory, and demonstrates the

feasibility of this approach which is encouraging for further investigations.

Next to the study of individual differences with psychometric assessments of affective systems, it is crucial to use experimental approaches that aim at real behavior (Markett et al., 2014; Montag et al., 2017a). Several of such approaches have been proposed in the context of reward sensitivity theory (RST), a theory on approach and avoidance behavior (Gray and McNaughton, 2000). RST describes three systems in the brain that are thought to mediate between stimuli and response: the behavioral activation system (BAS) dealing with approach to appetitive stimuli, the fight-flight-freezing system (FFFS) dealing with active avoidance of threat, and the behavioral inhibition (BIS) system that mediates between the two in the case of response conflict, and deals with exploratory behavior in situations of uncertainty. RST does not resort to common language terms for emotions, but the operation of the FFFS can be equated with the emotion fear, while the operation of the BIS reflects anxiety. The dissociation between fear and anxiety is one of RST's hallmark features. The distance between a potential threat and the individual is thought to be decisive of whether the FFFS (proximal threat, fear) initiates a "get-me-out-of-here" reaction or the BIS (distant threat, anxiety) initiates a more careful assessment of the situation and strategic planning (Corr, 2013; Reuter et al., 2015). There are several behavioral assays for the study of the BIS and the FFFS: in a simulated runway-chase, participants operate a force-sensitive joystick to either escape or approach a virtual enemy (Perkins et al., 2009). Another approach includes a pac-man-style computer game where participants escape a virtual predator to avoid electric shocks (Mobbs et al., 2007). Distance to threat has been shown to map on a functional gradient in brain response, where proximal threat activates subcortical regions, such as periaqueductal gray and the (central nuclei of the) amygdala, and activation foci shift along a functional axis toward ventromedial prefrontal cortex with increasing distance to the threat (together with activation of the lateral amygdala). The defensive-distance gradient in the brain suggests an underlying network with information exchange along the functional axis. This, however, has not been formerly addressed as of yet. Network neuroscience offers tools to study modulations of functional connectivity by task context (Gerchen et al., 2014), including its dynamic changes over time (Muldoon and Bassett, 2016), and the directionality of information transfer (Gilson et al., 2018). The application of these methods with regard to predictions from RST represent excellent examples where the combination of network neuroscience and psychological theory may advance our understanding of affective systems in the human brain.

A more recent theory on affect and emotion stands as antithesis to previous accounts on primary emotions. The theory of constructed emotions (TCE, Barrett, 2017) represents a departure from the common neo-behavioristic paradigm in psychology, by moving the spotlight away from stimuli and neural systems that mediate between stimulus and response. The theory of constructed emotions follows a recent line of argumentation that the brain uses its past experience to engage in predictive modeling of the environment (Raichle, 2010). According to this perspective, emotions are constructed by the brain when it uses its model of the environment to make sense of incoming information (Barrett, 2006). TCE is quite radical in its opposition to previous accounts which has resulted in severe criticism (Panksepp, 2007). But TCE makes interesting statements on brain networks that are worth exploring. Functional connectivity mapping, for instance, has failed to delineate clear boundaries between functional systems associated with several primary emotions (Touroutoglou et al., 2015), a finding corroborated by evidence from multivariate pattern analyses (Clark-Polner et al., 2017). Functional connectivity systems for different emotions seem to converge within the insula-opercular network, a network that has been implicated in the detection of saliency (Seeley et al., 2007). TCE assumes a central role of the insula-opercular network in the conceptualization of emotions by tuning the brain's internal model of the environment to sensory signals (Barrett, 2017). Through this, TCE provides a theoretical account for other findings that have implicated the salience network in individual differences in the sensitivity to anxiety and negative affect (Markett et al., 2013, 2016). At present it is unclear, whether the TCE account can be unified with the older theories on primary emotional systems. In theory it should be possible, because primary emotional systems seem to operate at the bottom of our minds, whereas constructivist highlight neocortical processes (Panksepp, 2010).

The new field of network neuroscience with its fast growing methodological toolbox can make valuable contributions in advancing current theoretical accounts on affect and emotion. We wish to encourage further research into this direction, as well as efforts toward an *affective network neuroscience*. As any new field of study, network neuroscience is currently facing rapid methodical developments. These aim at the core challenges of the paradigmatic conceptualization of the brain as a network, such as more accurate parcellations for the cortical ribbon or better ways to measure functional connectivity, including its dynamics and directedness. Studying affect and emotion in terms of information transfer between interacting brain regions will hopefully lead to an algorithmic understanding of affective processing in the brain. This will have exciting prospects for other branches of neuroscience, e.g., for neuropsychopharmacology and molecular neurogenetics. It will also be an important step toward better treatment options for affective disorders (Richter et al., 2017) that constitute a significant public health burden with negative impact to those afflicted (Wittchen et al., 2011; Montag et al., 2017a).

AUTHOR CONTRIBUTIONS

SM and CM conceptualized the paper. OW, BB, and PJ provided critical points and revision. SM drafted the manuscript. OW, BB, PJ, and CM revised the manuscript.

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REFERENCES

Barrett, L. F. (2006). Solving the emotion paradox: categorization and the experience of emotion. *Pers. Soc. Psych. Rev.* 10, 20–46. doi: 10.1207/s15327957pspr1001_2

Barrett, L. F. (2017). The theory of constructed emotion: an active inference account of interoception and categorization. *Soc. Cogn. Affect. Neurosci.* 12, 1–23. doi: 10.1093/scan/nsx060

Bassett, D. S., and Sporns, O. (2017). Network neuroscience. *Nat. Neurosci.* 20, 353–364. doi: 10.1038/nn.4502

Beckmann, C. F., DeLuca, M., Devlin, J. T., and Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 1001–1013. doi: 10.1098/rstb.2005.1634

Biswal, B., Yetkin, F. Z., Haughton, V. M., and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar mri. *Magn. Reson. Med.* 34, 537–541. doi: 10.1002/mrm.1910340409

Braun, U., Muldoon, S. F., and Bassett, D. S. (2015). "On human brain networks in health and disease," in *eLS* (American Cancer Society), 1–9.

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. doi: 10.1038/nrn2575

Clark-Polner, E., Johnson, T. D., and Barrett, L. F. (2017). Multivoxel pattern analysis does not provide evidence to support the existence of basic emotions. *Cereb. Cortex* 27, 1944–1948. doi: 10.1093/cercor/bhw028

Corr, P. J. (2013). Approach and avoidance behaviour: multiple systems and their interactions. *Emot. Rev.* 5, 285–290. doi: 10.1177/1754073913477507

Damoiseaux, J. S., Rombouts, S. A., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., et al. (2006). Consistent resting-state networks across healthy subjects. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13848–13853. doi: 10.1073/pnas.0601417103

Davis, K. L., and Montag, C. (2018). A tribute to Jaak Panksepp (1943–2017). *Pers. Neurosci.* 1:e9. doi: 10.1017/pen.2018.5

Davis, K. L., Panksepp, J., and Normansell, L. (2003). The affective neuroscience personality scales: normative data and implications. *Neuropsychoanalysis* 5, 57–69. doi: 10.1080/15294145.2003.10773410

Deris, N., Montag, C., Reuter, M., Weber, B., and Markett, S. (2017). Functional connectivity in the resting brain as biological correlate of the affective neuroscience personality scales. *Neuroimage* 147, 423–431. doi: 10.1016/j.neuroimage.2016.11.063

Di, X., Gohel, S., Kim, E. H., and Biswal, B. B. (2013). Task vs. rest—different network configurations between the coactivation and the resting-state brain networks. *Front. Hum. Neurosci.* 7:493. doi: 10.3389/fnhum.2013.00493

Dosenbach, N. U., Fair, D. A., Cohen, A. L., Schlaggar, B. L., and Petersen, S. E. (2008). A dual-networks architecture of top-down control. *Trends Cogn. Sci.* 12, 99–105. doi: 10.1016/j.tics.2008.01.001

Eckstein, M., Markett, S., Kendrick, K. M., Ditzen, B., Liu, F., Hurlemann, R. (2017). Oxytocin differentially alters resting state functional connectivity between amygdala subregions and emotional control networks: Inverse correlation with depressive traits. *Neuroimage* 149, 458–467. doi: 10.1016/j.neuroimage.2017.01.078

Fornito, A., Zalesky, A., and Breakspear, M. (2013). Graph analysis of the human connectome: Promise, progress, and pitfalls. *Neuroimage* 80, 426–444. doi: 10.1016/j.neuroimage.2013.04.087

Friston, K. J., Frith, C. D., Liddle, P. F., and Frackowiak, R. S. (1993). Functional connectivity: the principal-component analysis of large (PET) data sets. *J. Cereb. Blood Flow Metab.* 13, 5–14. doi: 10.1038/jcbfm.1993.4

Gerchen, M. F., Bernal-Casas, D., and Kirsch, P. (2014). Analyzing task-dependent brain network changes by whole-brain psychophysiological interactions: a comparison to conventional analysis. *Hum. Brain Mapp.* 35, 5071–5082. doi: 10.1002/hbm.22532

Gilson, M., Deco, G., Friston, K. J., Hagmann, P., Mantini, D., Betti, V., et al. (2018). Effective connectivity inferred from fMRI transition dynamics during movie viewing points to a balanced reconfiguration of cortical interactions. *Neuroimage* 180(Pt B), 534–546. doi: 10.1016/j.neuroimage.2017.09.061

Gray, J. A., and McNaughton, N. (2000). *The Neuropsychology of Anxiety: An Enquiry Into the Functions of the Septo-hippocampal System*. Oxford: Oxford University Press.

Greicius, M. D., Krasnow, B., Reiss, A. L., and Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 253–258. doi: 10.1073/pnas.0135058100

Hagmann, P. (2005). *From Diffusion MRI to Brain Connectomics*. Lausanne, CH: PhD Thesis, Université de Lausanne.

Markett, S, Weber, B., Voigt, G., Montag, C., Felten, A., Elger, C. (2013). Intrinsic connectivity networks and personality: the temperament dimension harm avoidance moderates functional connectivity in the resting brain. *Neuroscience* 240, 98–105. doi: 10.1016/j.neuroscience.2013.02.056

Markett, S., Montag, C., Melchers, M., Weber, B., and Reuter, M. (2016). Anxious personality and functional efficiency of the insular-opercular network: a graph-analytic approach to resting-state fMRI. *Cogn. Affect. Behav. Neurosci.* 16, 1039–1049. doi: 10.3758/s13415-016-0451-2

Markett, S., Montag, C., and Reuter, M. (2014). In favor of behavior: on the importance of experimental paradigms in testing predictions from Gray's revised reinforcement sensitivity theory. *Front. Sys. Neurosci.* 8:184. doi: 10.3389/fnsys.2014.00184

Markett, S., Montag, C., and Reuter, M. (2018). Network neuroscience and personality. *Pers. Neurosci.* 1:e14. doi: 10.1017/pen.2018.12

Medaglia, J. D., Lynall, M.-E., and Bassett, D. S. (2015). Cognitive network neuroscience. *J. Cogn. Neurosci.* 27, 1471–1491. doi: 10.1162/jocn_a_00810

Menon, V. (2011). Large-scale brain networks and psychopathology: a unifying triple network model. *Trends Cogn. Sci.* 15, 483–506. doi: 10.1016/j.tics.2011.08.003

Mišić, B., Betzel, R. F., de Reus, M. A., van den Heuvel, M. P., Berman, M. G., McIntosh, A. R. (2016). Network-level structure-function relationships in human neocortex. *Cereb. Cortex* 26, 3285–3296. doi: 10.1093/cercor/bhw089

Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., Weiskopf, N., Seymour, B., et al. (2007). When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* 317, 1079–1083. doi: 10.1126/science.1144298

Montag, C., and Davis, K. L. (2018). Affective neuroscience theory and personality: an update. *Pers. Neurosci.* 1:e12. doi: 10.1017/pen.2018.10

Montag, C., Markowetz, A., Blaszkiewicz, K., Andone, I., Lachmann, B., Sariyska, R., et al. (2017a). Facebook usage on smartphones and gray matter volume of the nucleus accumbens. *Behav. Brain Res.* 329, 221–228. doi: 10.1016/j.bbr.2017.04.035

Montag, C., and Panksepp, J. (2016). Primal emotional-affective expressive foundations of human facial expression. *Motiv. Emot.* 40, 760–766. doi: 10.1007/s11031-016-9570-x

Montag, C., and Panksepp, J. (2017a). Primary emotional systems and personality: an evolutionary perspective. *Front. Psychol.* 8:464. doi: 10.3389/fpsyg.2017.00464

Montag, C., and Panksepp, J. (2017b). "Personality neuroscience: why it is of importance to include primary emotional systems!" in *Encyclopedia of Personality and Individual Differences*, eds V. Zeigler-Hill, and T. Shackelford (Cham: Springer). doi: 10.1007/978-3-319-28099-8

Montag, C., Widenhorn-Müller, K., Panksepp, J., and Kiefer, M. (2017b). Individual differences in affective neuroscience personality scale (ANPS) primary emotional traits and depressive tendencies. *Compr. Psychiatry* 73, 136–142. doi: 10.1016/j.comppsych.2016.11.007

Mori, S., Crain, B. J., Chacko, V. P., and Zijl, P. C. M. V. (1999). Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Ann. Neurol.* 45, 265–269. doi: 10.1002/1531-8249(199902)45:2<265::AID-ANA21>3.0.CO;2-3

Muldoon, S. F., and Bassett, D. S. (2016). Network and multilayer network approaches to understanding human brain dynamics. *Philos. Sci.* 83, 710–720. doi: 10.1086/687857

Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York, NY: Oxford University Press.

Panksepp, J. (2007). Neurologizing the psychology of affects: how appraisal-based constructivism and basic emotion theory can coexist. *Perspect. Psychol. Sci.* 2, 281–296. doi: 10.1111/j.1745-6916.2007.00045.x

Panksepp, J. (2010). Affective neuroscience of the emotional BrainMind: evolutionary perspectives and implications for understanding depression. *Dialog. Clin. Neurosci.* 12, 533–545.

Panksepp, J. (2011). Cross-species affective neuroscience decoding of the primal affective experiences of humans and related animals. *PLoS ONE* 6:e21236. doi: 10.1371/journal.pone.0021236

Panksepp, J., Lane, R. D., Solms, M., and Smith, R. (2017). Reconciling cognitive and affective neuroscience perspectives on the brain basis of emotional experience. *Neurosci. Biobehav. Rev.* 76, 187–215. doi: 10.1016/j.neubiorev.2016.09.010

Park, H.-J., and Friston, K. (2013). Structural and functional brain networks: from connections to cognition. *Science* 342:1238411. doi: 10.1126/science.1238411

Perkins, A. M., Ettinger, U., Davis, R., Foster, R., Williams, S. C., and Corr, P. J. (2009). Effects of Lorazepam and Citalopram on human defensive reactions: ethopharmacological differentiation of fear and anxiety. *J. Neurosci.* 29, 12617–12624. doi: 10.1523/JNEUROSCI.2696-09.2009

Raichle, M. E. (2010). Two views of brain function. *Trends Cogn. Sci.* 14, 180–190. doi: 10.1016/j.tics.2010.01.008

Reuter, M., Cooper, A. J., Smillie, L. D., Markett, S., and Montag, C. (2015). A new measure for the revised reinforcement sensitivity theory: psychometric criteria and genetic validation. *Front. Syst. Neurosci.* 9:38. doi: 10.3389/fnsys.2015.00038

Richter, J., Pittig, A., Hollandt, M., and Lueken, U. (2017). Bridging the gaps between basic science and cognitive-behavioral treatments for anxiety disorders in routine care. *Zeitschr Psychol.* 225, 252–267. doi: 10.1027/2151-2604/a000309

Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M. C., Uddin, L. Q., Gotimer, K., et al. (2009). Functional connectivity of the human amygdala using resting state fMRI. *Neuroimage* 45, 614–626. doi: 10.1016/j.neuroimage.2008.11.030

Scholtens, L. H., Schmidt, R., de Reus, M. A., and van den Heuvel, M. P. (2014). Linking macroscale graph analytical organization to microscale neuroarchitectonics in the Macaque connectome. *J. Neurosci.* 34, 12192–12205. doi: 10.1523/JNEUROSCI.0752-14.2014

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., et al. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13040–13045. doi: 10.1073/pnas.0905267106

Sporns, O., Tononi, G., and Kötter, R. (2005). The human connectome: A structural description of the human brain. *PLoS Comput. Biol.* 1:e42. doi: 10.1371/journal.pcbi.0010042

Stephan, K. E. (2013). The history of CoCoMac. *Neuroimage* 80, 46–52. doi: 10.1016/j.neuroimage.2013.03.016

Tompson, S., Falk, E. B., Vettel, J. M., and Bassett, D. S. (2018). Network approaches to understand individual differences in brain connectivity: opportunities for personality neuroscience. *Pers. Neurosci.* 1:e5. doi: 10.1017/pen.2018.4

Touroutoglou, A., Lindquist, K. A., Dickerson, B. C., and Barrett, L. F. (2015). Intrinsic connectivity in the human brain does not reveal networks for 'basic' emotions. *Soc. Cogn. Affect. Neurosci.* 10, 1257–1265. doi: 10.1093/scan/nsv013

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., et al. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Euro Neuro* 21, 655–679. doi: 10.1016/j.euroneuro.2011.07.018

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REVIEW
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Selected Principles of Panksepian Affective Neuroscience

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In the early nineties of the twentieth century Jaak Panksepp coined the term "Affective Neuroscience" (AN) today being accepted as a unique research area in cross-species brain science. By means of (i) electrical stimulation, (ii) pharmacological challenges, and (iii) brain lesions of vertebrate brains (mostly mammalian), Panksepp carved out seven primary emotional systems called SEEKING, CARE, PLAY, and LUST on the positive side, whereas FEAR, SADNESS, and ANGER belong to the negative affects. Abundant research into human clinical applications has supported the hypothesis that imbalances in these ancient primary emotional systems are strongly linked to psychiatric disorders such as depression. The present paper gives a concise overview of Panksepp's main ideas. It gives an historical overview of the development of Panksepp's AN thinking. It touches not only areas of neuroscience, but also shows how AN has been applied to other research fields such as personality psychology. Finally, the present work gives a brief overview of the main ideas of AN.

Keywords: Jaak Panksepp, affective neuroscience, affective neuroscience personality scales, subcortical, cross-species, affects, primary-process emotions, emotion

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A BRIEF INTRODUCTION TO JAAK PANKSEPP'S SCIENTIFIC CAREER

The scientist who coined the term *affective neuroscience*, Panksepp (1991, 1992), had the insight as a young clinical psychology student working in a mental hospital that understanding emotions was the key to developing more effective treatments for psychiatric hospital patients and all those suffering with psychopathology. This insight led to his graduate school career change from clinical psychology into what we now call neuroscience. He had also realized that the level of understanding that was needed would require brain research that could not be conducted on human beings. Hence, he began probing the neural constitution of emotions in the deep foundations of the mammalian brain. In his dissertation he was able to use electrical stimulation of the brain (ESB) to elicit two distinct emotional attack behaviors in rats: "affective attack" toward another rat and a predatory "quiet bite" attacking a mouse. He was further able to show that these rats subjectively experienced these two contrasting emotions, meaning they would work to turn off the stimulus eliciting the affective RAGE attack but would work to turn on and receive more of the stimulus eliciting the "quiet bite" attack, which was later shown to be activating the SEEKING system.

In the wake of the discovery in the early 1970s of endogenous opioids in the mouse brain, Panksepp began working on another potential emotional behavior system in the brain. J. P. Scott, a senior colleague at Bowling Green State University (BGSU), had studied the social behavior of dogs for many years (Scott and Fuller, 1965) and was currently exploring separation distress

vocalizations in puppies. Meanwhile, Jaak had recognized similarities between opioid withdrawal in drug addicts and the social distress caused by broken relationships and had also noticed that opioid addicts frequently came from marginal family social backgrounds. Panksepp hypothesized that opioids might be related to mammalian separation distress calls, and a BGSU research group soon demonstrated that low doses of morphine would soothe the separation distress vocalizations in canine puppies (Panksepp et al., 1978). In 1980, after his lab had successfully mapped distress vocalization sites in the guinea pig brain, Panksepp went on to publish his "opioid hypothesis" (Panksepp et al., 1980), namely, that brain opioids likely underlie the formation of social attachments and modulate social emotions and behaviors.

By 1982, Panksepp was convinced that there were at least four biological brain-based emotional action systems (Panksepp, 1982), which at that time he labeled Expectancy, Rage, Fear, and Panic. In a *Psychological Review* paper (Panksepp, 1992) and especially with the publication of *Affective Neuroscience* (Panksepp, 1998), Panksepp had expanded his list of primary emotions to seven well-documented primary-process emotional command systems (SEEKING/Expectancy, RAGE/Anger, FEAR/Anxiety, LUST, CARE/Nurturing, PANIC/Sadness, and PLAY/Social Joy) and introduced the use of capitalization to distinguish these primary emotional brain systems from the use of his chosen emotion labels in common language. The mapping of the seven primary emotional systems by means of electrical stimulation of the mammalian brain including pharmacological challenges and brain lesions represents the heart of what Panksepp named affective neuroscience. Of note, Panksepp concluded that there was insufficient evidence to include Social Dominance as a primary emotion, and he considered it an acquired behavior [see further thoughts on this in van der Westhuizen and Solms (2015)]. Panksepp did include a detailed treatment of the homeostatic affect HUNGER in *Affective Neuroscience* but did not consider it in the same category as emotional affects, which were more directly relevant to mammalian psychopathology and personality.

The remainder of this review essay will outline key themes of affective neuroscience as developed in the research and writings of Jaak Panksepp. Rather than continuing to review the development of his thinking throughout his scientific career, the focus here will be on selected affective neuroscience principles first featured in *Affective Neuroscience* but also elaborated in *The Archaeology of Mind* (Panksepp and Biven, 2012), *The Emotional Foundations of Personality* (Davis and Panksepp, 2018), as well as numerous theoretical review papers.

MAMMALS ARE DEEPLY AFFECTIVE

All mammals are sentient beings meaning that it feels like something to be alive and dealing with the challenges in their worlds. The philosopher, Langer, in her book, *Mind: An Essay on Human Feeling,* writes "*To feel is to do something*" (Langer, 1988, p.7). The word "emotion" is derived from the Latin verb "emovere" meaning "to move out," and that seems to be what we observe already in the evolution of early life. Primal emotions and their accompanying affects appear to have acquired the capacity to move animals to action in ways that promoted their survival. Emotions prodded animals to explore for resources (SEEKING), compete for and defend those resources (RAGE/Anger), escape from and avoid bodily danger (FEAR), and identify potential mates and reproduce (LUST). Then, mammals with their more social orientation acquired the motivational system for nurturing their offspring (CARE); the powerful separation distress system for maintaining social contact and social bonding (PANIC/Sadness); and the complex system stimulating especially young animals to regularly engage in physical activities like wrestling, running, and chasing each other (PLAY/Social Joy), which helps them bond socially and learn social limits and which seems to carry over into the "ribbing" and joking that continues to add fun in adulthood. Evolution has endowed mammalian brains with at least these seven primary-process emotional action systems, which serve as survival guides. These primary emotions arise from subcortical brain regions that are largely homologous, especially across mammals, with each emotion having a distinct brain anatomy, neuropharmacology, and physiology (for details beyond the scope of this paper, see Panksepp, 1998; Panksepp and Biven, 2012). Jaak Panksepp felt that the key affective neuroscience question was the neural constitution of raw affects (Panksepp et al., 2017, p.206), which was essential for understanding our own affects and for developing better psychiatric treatments for emotional imbalances but which would require further causal preclinical research into our ancestral subcortical primary-process emotional brain systems. For a recent published obituary on Jaak Panksepp's life please see Davis and Montag (2018).

ANCESTRAL VOICES

Mother Nature (aka evolution) speaks to all mammals in the oldest language, the language of emotional affects. The ancestral voices (to use Ross Buck's phrase) guide their choices as they navigate life. Each of the primal emotional affects is evaluative, that is, has a valence that is either pleasant or aversive and signals objects or situations to approach in the case of the pleasant ones (SEEKING, LUST, CARE, and PLAY) or to avoid in the case of the aversive ones (RAGE, FEAR, and PANIC).

Yet, experiencing a primary affect does not necessarily mean all mammals can self-reflect on their emotional experiences. That capacity may be reserved for the more cortically endowed mammals. However, "raw" primary affects are experienced as pleasant or aversive qualia, which alter behavior and provide for secondary-level learning (conditioning principles). The emotional minds of most mammals may be limited to displaying "intentions-in-action" rather than a more reflective "intentions to act." In Endel Tulving's terminology, the capacities of most mammals likely combine anoetic (without knowing) and noetic (knowing) consciousness without necessarily attaining autonoetic consciousness—being able to sufficiently hold experiences in memory to review the past and anticipate the future (Tulving, 1985). We know that animals experience

primary affects because of empirical measures: They will work vigorously to sustain affective states by learning to turn on ESB evoking positively valenced emotions and correspondingly escape or avoid the negatively valenced emotions. They will also demonstrate conditioned place preferences or aversions for situations where they have experienced such stimulation in the past (Panksepp, 2011). Further, animals will emit conditioned positive and negative vocalizations in places where they have experienced positively or negatively valenced ESB (Knutson et al., 2002). However, we are unable to measure feelings (affective qualia) directly, not even in humans.

Apart from experiencing primary-process emotional affects, what is much more difficult to study in the non-human mammalian world is Tulving's autonoetic consciousness characterized by the capacity of humans to experience affective nuances often reflecting human higher-order cognitions and language including having thoughts about thoughts. This represents a limitation of cross-species affective neuroscience. However, subtler models of affective concepts such as pessimism in dogs (Mendl et al., 2010), optimism in rats (Rygula et al., 2012, 2015), and regret in rats (Steiner and Redish, 2014) are appearing in cross-species studies.

PRIMORDIAL EMOTIONS ARE INNATE BUT ARE ALSO ADAPTIVE LEARNING AND MEMORY SYSTEMS

The primary-process emotions require no learning. It is not necessary to teach a child to become angry, fearful, or to panic after having lost sight of parents in a crowd. Nor do we need to teach children how to play. These evolved foundational tools for living are somehow automatically built into our heritage. However, these evolutionarily/genetically endowed primary-process emotional brain systems are not fixed functions but are able to learn and adapt to novel environmental experiences throughout the life of an individual. Indeed, as introduced above, the valenced affects associated with each of the primary emotions serve as endogenous rewards and punishments for behaviors that activate emotions. For example, receiving painful stings from hornets flying out of the nest you accidently disturbed fills you with fear, and without thinking about it you immediately react by running away from the menace. Having reached a safe distance, you feel relief that you seem to be out of danger, and likely begin examining the tiny wounds, which are beginning to swell slightly as the pain intensifies, and you may begin to clarify (at a safe distance) the details of the hornet nest's appearance and location. This event will be forever embedded in your memory, and you will have learned to avoid repeating this experience by remaining more vigilant when outdoors walking through unfamiliar terrain.

Each primary-process emotional command system likely encompasses a separate reward or punishment system. These learning systems can be thought of as secondary-processes integrating new experiences into the primary framework allowing for previously neutral environmental stimuli to elicit the emotion and for novel reactions to become associated with such stimuli. For an example of novel reactions to an emotional arousal, over the ages, humans when threatened have learned to reach for their swords, and more recently their pistols instead of clenching their fists. However, more evidence is needed regarding the extent to which these different emotional systems encompass different learning and memory parameters.

While deep brain stimulation (DBS) allows researchers to demonstrate that brain stimulation at specific sites evokes distinct emotional behaviors that are accompanied by corresponding affects, there remains the question of whether the emotional affects elicited at these sites are similarly distinct. We do know that rats can learn to discriminate between DBS in the hypothalamus and septal regions of the brain (Stutz et al., 1974). We also know that animals can distinguish between the emotional states induced by the addictive drugs morphine and cocaine (Overton, 1991). We also know that DBS in humans at homologous brain sites seems to evoke homologous affects (see Panksepp, 1985 for a review). However, much more research needs to be done before there is any clear assurance regarding the number of distinct primary affects or the role of electrical or pharmacological stimulants in generating those affects.

PRIMARY-PROCESS EMOTIONS SURVIVE DECORTICATION

Primary affects are constituted at the subcortical level. That is true for primary emotional affects such as ANGER and FEAR, or homeostatic affects such as HUNGER and THIRST. There is ample evidence that primary-process emotional brain systems do not require the neocortex. In rats, decortication does not block the rewarding effects of subcortical ESB (Huston and Borbely, 1973, 1974). In humans, strong emotions decrease cortical activation (Damasio et al., 2000) and cortical damage often leads to increased emotionality, which is consistent with the cortex generally providing the inhibition or regulation of emotions rather than activation (Liotti and Panksepp, 2004).

Further support in humans for subcortical emotions without cortex is offered by Merker (2007) who has reviewed the case of hydranencephalic children who are born without a cerebral cortex. He writes that even without a cerebral cortex, these children "express pleasure by smiling and laughter, and aversion by 'fussing,' arching of the back and crying (in many gradations), their faces being animated by these emotional states" (Merker, 2007, p.79). He further comments that their "[emotional] behaviors are accompanied by situationally appropriate signs of pleasure or excitement on the part of the child" (Merker, 2007, p.79). These children clearly show that appropriate emotional responses even in humans do not require the participation of the neocortex. As further evidence of an independent subcortical brain that can function without a neocortex, Merker also reviewed Penfield and Jasper (1954) in which brain surgery under local anesthesia was performed on conscious patients with a history of severe epileptic seizures. These surgeons removed sizeable sectors of cortical tissue while communicating with the patient, which "never interrupted the patient's continuity of consciousness even while the tissue was being removed" (Merker, 2007, p.65).

Along these lines, Damasio et al. (2013) reported the case of Patient B. who had contracted Herpes Simplex Type I encephalitis (HSE), which had destroyed much of his cortical brain tissue with extensive bilateral cortical damage including temporal lobe and temporal pole cortices, posterior orbitofrontal cortices, and the anterior cingulate cortices. Plus the left and right insular cortices were entirely destroyed (including the insula itself) as well as the bilateral entorhinal cortex, hippocampus proper, and amygdala.

In short, Patient B. had lost structures thought by many to be integral to emotional experience: the insula (Craig, 2011), the amygdala (LeDoux, 2012), and the anterior cingulate cortex (Bijanki et al., 2015). Yet, all indications (including the observations of strangers, the observations of the research team, psychological evaluations, and a structured questionnaire completed by his spouse comparing his emotional behavior before and after his disease) were that Patient B. retained a full range of appropriate emotions after his brain disease.

The lack of importance of the cortex for emotional behavior and displays was supported by Whishaw's (1990) review of the experimental rat decortication literature. While there were differences between whether the surgery took place neonatally or closer to maturity, the neonatal group showed few deficits. With neonatally decorticated rats, loss of cortex essentially did not disrupt survival/emotional behaviors and displays. These subjects exhibited no interruption in post-surgery sucking and grew to maturity with near normal weights. They exhibited normal posture during face washing with no deficits in grooming. They were able to reproduce with six out of eight females being able to successfully raise their litters with normal cleaning, suckling, and caring for their pups. As juveniles, they played as much as controls, and most components of aggressive and defensive behavior were present including lateral displays in response to an intruder and conditioned "freezing" after having been attacked as an intruder and later being reintroduced as the intruder to the same cage with the same resident.

Panksepp's group replicated the effect of neonatal decortication on rat juvenile play (Panksepp et al., 1994). Measures of play vigor and tests of play solicitation behaviors did not detect differences, which suggested that play motivation was intact in the decorticate rats. They did observe a decrease in frequency of pinning and shorter pin duration. However, additional control studies suggested that these changes were likely due to motor changes and reduced somatosensory sensitivity. In short, they found that the play of decorticate rats appeared normal.

Indeed, 16 graduate students were asked to observe a pair of juvenile rats for 30 min, one of which had its cortex surgically removed neonatally and the other, a control subject, that only had received sham surgery, and to decide which one had been decorticated. Most chose the control rat pup with an intact cortex (Panksepp, 2015). Overall, Panksepp concluded, like Whishaw, that "the results generally indicate little participation of the neocortex in the instigation of rough-and-tumble play" (Panksepp et al., 1994, p. 429).

By contrast this group reported that much smaller thalamic lesions had greater influence on play in rats. Lesions of the parafascicular region of the thalamus reduced pinning by 73% but also reduced play solicitation behavior, likely indicating decreased play motivation compared with controls showing the lesion effects were specific to play (Siviy and Panksepp, 1985).

The theme of small subcortical lesions having dramatic influences on emotional behavior such as losing virtually all spontaneous activity after ablating the periaqueductal gray (Bailey and Davis, 1942) was convincingly addressed by Fernandez de Molina and Hunsperger (1962). They showed that the rage responses of cats could be evoked by ESB along the basic subcortical mammalian RAGE system running from the periaqueductal gray (PAG), at the lowest level up to the medial hypothalamus and on up to the medial amygdala at the highest level in decreasing levels of importance. As such, they found that aggressive responses evoked by ESB of the amygdala were abolished by lesions at the level of the hypothalamus or PAG. Aggressive responses from the hypothalamus were dependent on the PAG but not on the amygdala. And, at the lowest level, aggressive responses evoked at the PAG level were not dependent on either of the higher two levels. Further, field studies with cats receiving small lesions to what they called the "hissing zone" of the PAG, "when confronted with a dog, no longer hissed or attacked" (Fernandez de Molina and Hunsperger, 1962, p. 201).

Clearly, our primary-process emotions and their powerful affective messages are deeply embedded in our mammalian brains. Humans and other mammals still experience these emotions without a neocortex, and the subcortical regions are organized in an evolutionary hierarchy of importance. Understanding these cross-species emotional systems may represent the greatest challenge to neuroscience. Again, in the words of Jaak Panksepp, "For A[ffective] N[euroscience] the key question is the neural constitution of raw emotional, homeostatic and sensory affects" (Panksepp et al., 2017, p. 206).

THE NEOCORTEX IS ESSENTIALLY A BLANK SLATE AT BIRTH

The Pankseppian affective neuroscience view is that "the neocortex is fundamentally tabula rasa at birth," Latin for "blank slate" (Panksepp and Biven, 2012, p.427), and it is through experience that the neocortex is "programmed" (likely through interactions with subcortical regions) to acquire its capacities that as we reach maturity come to seem like "hard-wired" brain functions. But, is any function in neocortex genetically determined? It might seem to many that the visual cortex is a possible candidate. Yet, Sadato et al. found otherwise (Sadato et al., 1998). They found that people naturally blind from an early age provided an example of occipital cortical regions that had been "programmed" to perform a non-visual function and supplanting the somatosensory area. Specifically, positron emission topography (PET) showed that when individuals who were blind from an early age were performing a braille task, the tactile processing that would normally occur in conventional somatosensory cortex had been shifted to areas in the occipital cortex that are normally assumed to process visual stimuli.

Another PET study provided evidence from blind subjects for the use of visual cortex for auditory processing. Weeks et al. (2000) used PET to determine which cortical areas were being activated during an auditory localization task. While sighted and blind subjects both showed activity in the posterior parietal cortex, only blind subjects also showed activity in the occipital cortex, a cortical area that is normally associated with visual processing. Thus, cortical plasticity seems to allow blind individuals to develop enhanced tactile and auditory capabilities by redirecting neocortical regions typically thought of as visual processing regions to enhance other sensory functions.

Experimental research from Mriganka Sur's group at MIT (von Melchner et al., 2000), supported the "reprogramming" findings documented in blind subjects. Using ferrets (a species born in an exceptionally immature stage), visual input was surgically redirected to auditory cortex shortly after being born, and as predicted the auditory cortex was developmentally programmed to process vision: A follow-up study using similar procedures with mice showed that rewired mice could learn visually-cued conditioned fear (Newton et al., 2004). Both sets of animals with visual input redirected to auditory cortex developed fine cortical visual abilities even though the neocortical processing was developmentally constructed rather than genetically dictated.

That we should not expect to find evolved specializations in the neocortex was further supported by the report in *Science* that a single gene, called *ARHGAP11B*, was responsible for much of the massive expansion of the human neocortex. The researchers further determined that this newly identified gene was found only in humans, Neanderthals, and Denisovans—another extinct hominid line in southern Siberia. The gene was not found in our closest living evolutionary relative, chimpanzees (Florio et al., 2015). This finding makes it increasingly unlikely that any of our neocortical higher mental abilities represent evolutionary genetically-determined specializations like are found in subcortical brain regions.

THE NEOCORTEX IS LIKELY ORGANIZED BY THE SUBCORTICAL FUNCTIONS OF THE BRAIN

The idea, that subcortical systems guide the development of cortical specializations gained support from Panksepp's group investigating the possible role of play in the development of the frontal cortex. Working on evidence that right hemisphere frontal lobe deficits were associated with Attention Deficit/Hyperactivity Disorder (ADHD) and the knowledge that the symptoms of frontal lobe damage generally resemble ADHD, it was hypothesized that right frontal lobe damage might be a useful rodent model of ADHD. They found that rat right frontal lobe lesions (performed at three or four days of age) significantly increased playfulness (as measured by pins and dorsal contacts) as well as increasing activity levels confirming the hypothesis (Panksepp et al., 2003) [Note: A replication obtained the same results from lesions to the left or right frontal lobes; please see also a new work on individual differences in tendencies toward ADHD and primary emotional systems as assessed with the Affective Neuroscience Personality Scales by Wernicke et al. (2018)].

Activity levels of both sham and lesioned animals decreased with age. However, in the rat pups with frontal lesions, 1 h per day of "play therapy" for seven consecutive days (tested at 30 days of age) significantly reduced the number of pins and overall activity, "ameliorating" the elevated play urges and activity levels. Indeed, the play therapy even significantly reduced overall activity in the sham lesion control subjects.

Additional tests in new subjects that had no lesions indicated that play experiences also increased behavioral inhibition not specific to increased fear. Combining both experiments, the authors suggested "the possibility that one of the long-term functions of social play is to promote maturation of various higher brain areas, including frontal cortical ones" (Panksepp et al., 2003, p.103–104). By some little understood means, an activated subcortical PLAY system seems to be facilitating the development of the frontal lobe behavioral inhibition function including a more mature regulation of excessive play urges perhaps also reflected in undesirable impulsivity.

Another line of research proposing that innate subcortical systems guide the development of refined neocortical capacities comes from the work of Mark Johnson's group linking infant facial preferences to adult face recognition and related capacities. Johnson's work also started with animal research: the laboratory imprinting of newly hatched domestic chicks (Horn, 1985; Horn and Johnson, 1989). Johnson and Horn (1988) reported that newly hatched chicks were *predisposed* to follow and attend to the *face* of an imprinting object with the correct arrangement of facial features including the heads of models and other bird species. They determined that the subcortical optic tectum of the chick brain, which is homologous to superior colliculus of the mammalian brain, was likely the source of this neonatal bias. However, regardless of the quality of the imprinting object, chicks were capable of developing strong preferences for the imprinting objects over novel stimuli, a learning process that was dependent on chick forebrain (homologous to mammalian cortex). The initial chick predisposition and the acquired preference were hypothesized to represent independent brain systems. That is, selective cortical lesions impaired the acquired preferences but not the predisposition (Johnson and Horn, 1986). It was further hypothesized that the subcortical predisposition system guided or "tutored" the cortical system in acquiring information about the mother hen or surrogate perhaps by a process as simple as a predisposing bias that oriented the chick toward the hen or appropriate substitute (Johnson et al., 2015, p.170–171).

This animal model was extended to primate and human research confirming a superior colliculus/pulvinar/amygdala system as the likely source of neonatal face processing predispositions with the superior colliculus and pulvinar receiving direct retinal input allowing for rapid orienting to faces and direct eye contact, and the detection and processing

of facial threat expressions. They proposed that this subcortical "fast-track" pathway facilitates detecting eye contact and the orientation toward and processing of faces from infancy through adulthood with the prefrontal cortex providing top-down modulation but also involving additional key structures such as the fusiform gyrus, superior temporal sulcus, and orbitofrontal cortex in a two-way model that added developmental complexity to the acquired coordination of social facial processing (Senju and Johnson, 2009).

The previous two research lines illustrate that basic subcortical processes likely constitute primary-process experiences that become developmentally elaborated in the neocortex (Panksepp and Biven, 2012). With maturation, these physically as well as evolutionarily separate brain regions develop a reciprocal seesaw-like relationship to weigh whether a life event should trigger or inhibit the expression of a primary emotion with imbalances in either direction potentially becoming dysfunctional (Liotti and Panksepp, 2004). It has also been reported that more ancient cortical midline structures such as the ventral medial prefrontal cortex may participate in such reciprocal relationships with more cognitive brain regions such as the lateral/dorsal prefrontal cortex (Goel and Dolan, 2003; Northoff et al., 2004). However, despite increasing cortical influence with maturation, it remains clear that subcortical emotional systems retain the capacity to shut down cortical activity during strong emotional experiences (Damasio et al., 2000).

Along these lines, there is evidence that all long-term memory has an emotional component, and with stronger emotions, the resulting memory becomes correspondingly stronger with the exception that traumatic emotional experiences can reduce memory retrieval and even cause amnesia (Alberini, 2010). Typically, stronger emotional activation is associated with stronger memory retrieval with strong positive emotional experiences such as a wedding and strong negative emotional experiences such as the funeral of a loved one both enhancing memory strength. Moreover, one of the basic functions of the primary-process emotional memory systems may be arousal and the associated drawing attention to specific events that can facilitate the formation of memories for important life events that in turn can subsequently inform how we respond to future life events.

With the ongoing guided acquisition of experience, the neocortex surely provides many refinements and expansions of our basic subcortical capabilities with the caveat that an ever vigilant subcortical brain can assert its predominance in response to genetically-linked wisdom embodied in, e.g., pent up urges to play or fear engendered by the menacing face of a predator. Hence, if an evolutionary significant cue resonates in the primary emotional systems, the subcortical energy can override our activity in the cortical thinking cap. This developed two-way (bottom-up and top-down) relationship requires the ongoing involvement of our affective states influencing our perceptions, thoughts, and urges as well as the acquired cortical capacity to modulate our emotional evolutionary foundations in order to achieve an appropriate seesaw positioning for ongoing life events. Yet, much research remains to be done to illuminate the mechanisms involved in these evolutionarily integrated processes.

THE ROUTE TO DISCOVERING PRIMARY-PROCESS EMOTIONS

At least since the ancient Greeks first speculated about the four humors and Ptolemy proposed the Sun, Moon, and planets set our behaviors in motion, humans have speculated about the basis of personality and psychopathology. More modern theories have ranged from Freud's theory of sex and aggression on personality development (Freud, 1920/1990) to the thesis that the differences in our personalities are mostly learned (Miller and Dollard, 1941).

Cattell pioneered using the lexical hypothesis—the assumption that any important human characteristic was embedded in language—and factor analysis and first reported four personality factors (Cattell, 1933), but as increasing computing power allowed for working with larger data sets, he eventually proposed as many as 19 distinct factors with 16 being used in his most widely used personality assessment, the 16PF (Cattell et al., 1970). However, others using factor analysis reported as few as three (Eysenck, 1967) and as many as 20 (Jackson, 1974) personality dimensions. Many others took up Cattell's approach considering factor analysis as a more theory-free and objective statistical approach to parsing personality into its components, an approach that may have peaked in accepting five personality dimensions (Tupes and Christal, 1992 – originally reported in 1961). The "Big Five" were massively shored up by Lewis Goldberg's seemingly exhaustive factor analytic studies (Goldberg, 1990, 1992) as well as a report confirming these five personality dimensions plus Dominance in chimpanzees (King and Figueredo, 1997). The Big Five scales are typically labeled Extraversion, Agreeableness, Conscientiousness, Emotional Stability, and Openness to Experience. Even the psychiatric world got on the Big Five bandwagon (Widiger et al., 2009; Krueger et al., 2012) identifying dysfunctional dimensions consistent with the Big Five scales.

However, the lexical Big Five personality bastion now seems to have splintered into multiple competing theories proposing six and seven personality dimensions (Saucier, 2009) and even theories with only one, two, and three dimensions (Saucier and Srivastava, 2015). Agreement on parsing the human personality has been very difficult to achieve (Davis and Panksepp, 2018).

Panksepp took a different approach to carving nature at its joints by using electrical stimulation to directly probe the brain for its secrets. He followed in the tradition of Hess (1957) who in the 1930's had evoked a cat rage response using hypothalamic electrical stimulation of the brain (ESB). The basic idea is that if the experimenter introduces a crude unstructured electrical stimulus into a particular brain region and reliably evokes (1) a consistent coherent emotional action pattern and (2) a subjective affect state that can be verified to be pleasant or aversive using self-stimulation or approach-avoidance measures in animals, the ESB has activated an innate, unconditioned, evolutionarily-organized brain circuit linked to the observed emotional behavior (For a summary of

humans reporting similar affective shifts following stimulation to homologous brain sites, see Panksepp, 1985). Mainly using ESB—but also pharmacological manipulations and localized brain lesions—Panksepp identified seven emotional brain systems as listed above: SEEKING, RAGE/Anger, FEAR, LUST, CARE, PANIC/Sadness, and PLAY.

These ESB sites are concentrated in subcortical regions of the brain, and correspond with Panksepp's primary-process emotional-action command systems. Cases of such dramatic and unambiguous emotional behaviors or affects have not been elicited from the neocortex. However, more muted displays of laughter have been reported from evolutionarily more ancient cortical areas such as the anterior cingulate cortex (Caruana et al., 2015), which is consistent with the finding that the most striking coherent results with the lowest stimulation required are obtained from evolutionarily ancient subcortical regions such as the periaqueductal gray (PAG).

PREDICTIONS: PRIMARY-PROCESS EMOTIONS AND FOUNDATIONS OF PERSONALITY AND PATHOLOGY

One of the ways to verify and extend the validity of a theory is to make predictions and determine whether the evidence confirms the theory. One of Panksepp's predictions was that the primary-process emotions provided the psychobiological foundations of personality. To begin linking the primary emotions to human personality, the Affective Neuroscience Personality Scales (ANPS) were developed (Davis et al., 2003; Davis and Panksepp, 2011). The ANPS was designed to measure self-reported activations of six primary emotions in human lives: SEEKING, RAGE/Anger, FEAR, CARE, PANIC/Sadness, and PLAY (LUST being excluded, as it might limit valid responding and elicit highly socially desirable response patterns). The ANPS has been translated and validated in 10 different languages, and comparisons of the ANPS with Big Five and Five Factor Model personality assessments have uniformly shown close associations of these six emotions to these personality measures (for an overview see Montag and Davis, 2018).

While ANPS PLAY consistently lines up with Big Five Extraversion and SEEKING with Openness to Experience, it is also clear from these studies that some five-factor personality scales are higher-order configurations of the more elemental primary-process emotions. For example, the Big Five/Five Factor Model Agreeableness scale combines the CARE system, which is associated with high levels of Agreeableness, and the RAGE/Anger system, associated with low Agreeableness levels, to conceptually create a "Love-Hate" scale. Further, the Big Five/Five Factor Model Emotional Stability scale places all three of the negatively valenced emotions (RAGE/Anger, FEAR, and PANIC/Sadness) on the low end of Emotional Stability making Emotional Stability a confusing scale to interpret but also conflating the distinctions between these problematic emotions that are so closely linked to the etiology and treatment of psychopathology. These associations are stable across cultures, and the same patterns between individual differences in primary emotional systems as assessed with the ANPS and the Big Five of Personality have been observed in many countries including studies in the USA, Germany and China, potentially hinting at "a global ancestral neuro-biological effect" (Montag and Panksepp, 2017, p.6). For further updates on this issue please see also a recent work by Montag and Panksepp (2018). Also, Montag and Davis (2018) review links between facets of the Big Five of Personality and the ANPS.

Thus, while much work remains to be done linking primary-process emotions to more tertiary language-derived personality models, the initial evidence is that there are close associations between Panksepp's primary-process emotional action systems and the standard Big Five personality assessment with the caveat that the primary emotions offer a more direct biopsychological personality view that more clearly illuminates each of the foundational elements likely constituting our personalities.

An additional note is that no consistent association has been observed in these ANPS studies between the ANPS measured primary emotions and the Big Five Conscientiousness scale. The thought is that Conscientiousness does not measure a primary-process emotion. Rather, it likely measures some aspects of the neocortical inhibition and the resulting cognitive regulation of primary emotions, thus providing tertiary-process top-down control over subcortical emotional reactions (Davis and Panksepp, 2011).

It has been long thought that psychopathology represents extreme expressions of personality characteristics (McDougall, 1908) and that personality disorders and other psychopathologies can be classified on the Big Five/Five Factor Model dimensions (Livesley et al., 1992; Costa and Widiger, 2002). Along these lines, ANPS studies have shown that Panksepp's primal emotions can be used to differentiate Bipolar I and Bipolar II disorders (Savitz et al., 2008a,b) as well as personality disorders (Karterud et al., 2016). See also a newer work investigating the ANPS in multiple sclerosis patients, hence patients with a neurological disorder (Sindermann et al., 2018).

Panksepp has also made several additional predictions about psychiatric treatments that have been tested. Panksepp predicted that autistic children might have dysfunctional brain opioid systems resulting in excess endogenous opioid levels (Panksepp and Sahley, 1987). Experimental research supported his proposal that autism could potentially be treated with low dose naltrexone (an opioid blocker) and suggested that naltrexone benefited a subset of children diagnosed as autistic with as many as 40 percent of autistic children exhibiting enhanced social integration when treated with naltrexone therapy (Bouvard et al., 1995).

Panksepp's work with opioids and their ability to reduce the psychological pain of social separation distress also led him to predict that opioids could reduce suicidal ideation. Research (that was finally able to be conducted in Israel) examined the use of low doses of buprenorphine (a "safe" opioid that has low respiratory depression effects and becomes a mu-opioid blocker at high doses) to counteract suicide in a population of subjects with chronic suicidal thoughts, many of which had already attempted suicide. A small pilot study provided encouraging

results (Panksepp and Yovell, 2014). In a larger randomized placebo controlled study using ultra low doses of buprenorphine, there were no instances of suicide. Additionally, there were significantly reduced levels of suicidal thought, and no reports of withdrawal symptoms after the buprenorphine treatment was discontinued at the end of the study (Yovell et al., 2016).

Panksepp has long seen depression as a manifestation of the PANIC/Sadness system, and interestingly, depression was in fact treated with opioids until the 1950s (Bodkin et al., 1995). Panksepp also envisioned that depression could be as much caused by low positive affect as high negative affect (Panksepp et al., 2014). Using the ANPS, Montag et al. (2017) further supported this approach by demonstrating lower SEEKING and higher SADNESS and higher FEAR in depressed patients compared to healthy controls.

A specific treatment prediction was that DBS of the mesolimbic dopamine tract (a main component of the SEEKING system) could alleviate treatment-refractory depression. A German group conducted an early small trial study. The site selected for DBS was the nucleus accumbens, which proved somewhat successful but largely limited to acute effectiveness (Schlaepfer et al., 2008). A larger study involving many of the same investigators showed that five out of ten treatment-resistant depression subjects reached a 50% reduction in their depression ratings (Bewernick et al., 2010). However, in a follow up study electrode placement was moved to the medial forebrain bundle (the most rewarding site in the SEEKING system). In this treatment cohort with treatment-resistant depression, six out of seven patients showed recovery that held through the 33 weeks of the study (Schlaepfer et al., 2013). Although an invasive procedure, this may offer a significant improvement in the quality of life for those with major depression for whom other treatments have failed for many years.

For virtually all drugs used to treat psychopathology, the initial discovery for use in psychiatry had been serendipitous. For example chlorpromazine was first introduced as a presurgical aid for anesthesia before its anti-psychotic benefits were discovered (Ban, 2007). However, a long-developing project based on affective neuroscience research with roots in selecting rats that were more playful than typical (Burgdorf et al., 2005) has yielded a novel drug for treating depression that is proving to be much more effective than anything ever previously used. Identifying juvenile rats that exhibited high levels of frequency-modulated 50 kHz ultrasonic vocalizations during a play experience and subsequently examining their brains for expressed genes, a small group of potential molecules were discovered with the potential for leading to drugs that could successfully treat depression (Burgdorf et al., 2011). Eventually, Glyx13 (this new drug that was later called rapastinel) (Moskal et al., 2017) was given to a group of subjects diagnosed with major depression with remarkable results. Glyx13 elevated moods in 2 h with the positive effects lasting at least seven days with no negative side effects reported (Preskorn et al., 2015). The drug was fast-tracked by the USA FDA, has completed Phase II trials, and is currently in Phase III trials with the FDA. While not yet fully FDA approved, it seems that—as a remarkable validation of cross-species affective neuroscience—we are on the verge for the first time of introducing a psychiatric drug that was developed through an understanding of brain processes. This approach to psychiatry has been a long time coming, but perhaps we should not be surprised by the results.

What predictions are currently being made with a cross-species affective neuroscience perspective? Is it possible that if we just look, multiple fountains of youth for patients with imbalanced emotional brain systems could be discovered in the brain of a playful rat? It might pay to stay tuned to the ongoing work of long time Jaak Panksepp colleagues Joe Moskal and Jeff Burgdorf to see what additional magic they may have up their sleeves (Burgdorf, 2018, personal communication).

SUMMARY

Jaak Panksepp's career spanned 50 years (Davis and Montag, 2018). Throughout, he worked to provide compelling evidence that animals experienced their emotional arousals: at least all mammals, probably all vertebrates, and with the likelihood that even some invertebrates such as crayfish (separated from humans by perhaps 600 million years) exhibited conditioned preferences to distinct visual environments when associated with injected addictive drugs abused by humans such as amphetamine (Panksepp and Huber, 2004). His was an evolutionary approach that confirmed Darwin's continuity thesis that humans are also animals. He became increasingly committed to the principle that this cross-species affective neuroscience approach would provide the foundational understanding of our subcortical primary-process emotional-affective nature that would be necessary for understanding the neocortical tertiary-level blending of primary and secondary-process values with neocortical analysis and linguistically-based interpretations of experience. Further, animal models taking advantage of the evolutionarily-conserved brain homologies would facilitate the development of novel psychiatric treatments of human emotional imbalances.

Yet, a more cognitive neuroscience-focused climate, convinced that subjective feelings were largely restricted to humans, produced cortical fMRI images of emotional manifestations in humans as their evidence. Panksepp frequently found himself writing that fMRI technology using measures of brain blood flow and oxygenation was better suited to analyzing the larger networks of rapidly firing neocortical cells engaged in cognitive information processing than the physically smaller regions of slower firing subcortical neurons associated with unconditional emotional behaviors and their affects. The more difficult PET imaging seemed a better choice for illuminating subcortical processes (Damasio et al., 2000). However, there have been exceptions with well-designed fMRI studies working with strong emotional arousals highlighting the importance of subcortical structures like the PAG (Mobbs et al., 2007, 2009).

Yet, there remain unresolved debates in the neuroscience community. A major difference reflects Panksepp's position that subcortical activation is sufficient to generate emotions and their affective states that is reviewed above in section "Primary-Process Emotions Survive Decortication". The cognitive neuroscience counter position is that emotional experience is a "readout"

of emotional bodily reactions, which means that cortical perceptions are also necessary. A related dispute emerged from the idea that emotions are learned and "constructed" and elaborated from pleasure and displeasure/arousal dimensions by multiple regions of the brain, whereas Panksepp is clear that primary emotions require no learning and are generated by genetically defined subcortical brain circuits (For a review of additional criticisms see Panksepp et al., 2017).

Yet, Panksepp was eager to reunify emotion theories at the end of his career. Among others, Montag and Panksepp (2016) provided an overview on how Ekman's facial expressions (the view of emotions from the outside) were linked to AN (the view of emotions from the inside). Beyond this—and as a response to constructivism in emotion theories—he wrote: "A primary-process/basic emotion view may prevail in many subcortical regions, and constructivist/dimensional approaches may effectively parse higher emotional concepts as processed by the neocortex. ....In other words, such debates may simply reflect investigators working at different levels of control" (Panksepp, 2010, p. 536).

Whatever the future of affective neuroscience in contributing to our understanding of the human mind from its creative heights to its distressing imbalances, it is heartening that *Frontiers* is publishing a series of articles featuring ongoing affective neuroscience research. Of course many research areas remain to be explored including (1) the interactions of early experience and brain development, (2) gene expression linked to primary-process experience, (3) the relationship between primary emotional experience and memory, (4) how many distinct subcortical emotional circuits exist, (5) whether the neocortex can generate new emotions independent of the subcortical brain, and (6) the capacity of bottom-up subcortical primary-process brain activity to guide cortical development and eventual top-down regulation of primary emotional expression [with additional experimental possibilities discussed in Panksepp et al. (2017)]. It will require many generations of brain scientists to provide satisfactory answers to such questions, which may someday reveal the secrets of the human psyche from personality to psychopathology and perhaps even the origins of consciousness itself.

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KD wrote the first draft of the manuscript, which was critically revised by CM.

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REFERENCES

Alberini, C. (2010). Long-term memories: the good, the bad, and the ugly. *Cerebrum* 2010:21. Available online at: http://dana.org/news/cerebrum/detail.aspx?id=29272

Bailey, P., and Davis, E. W. (1942). Effects of lesions of the periaqueductal gray matter in the cat. *Proc. Soc. Exp. Biol. Med.* 51, 305–306. doi: 10.3181/00379727-51-13950P

Ban, T. (2007). Fifty years chlorpromazine: a historical perspective. *Neuropsychiatr. Dis. Treat.* 3, 495–500.

Bewernick, B. H., Hurlemann, R., Matusch, A., Kayser, S., Grubert, C., Hadrysiewicz, B., et al. (2010). Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression. *Biol. Psychiatry* 67, 110–116. doi: 10.1016/j.biopsych.2009.09.013

Bijanki, K. R., Matsui, J. T., Mayberg, H. S., Magnotta, V. A., Arndt, S., Johnson, H. J., et al. (2015). Depressive symptoms related to low fractional anisotropy of white matter underlying the right ventral anterior cingulate in older adults with atherosclerotic vascular disease. *Front. Hum. Neurosci.* 9:408. doi: 10.3389/fnhum.2015.00408

Bodkin, J. A., Zornberg, G. L., Lukas, S. E., and Cole, J. O. (1995). Buprenorphine treatment of refractory depression. *J. Clin. Psychopharmacol.* 15, 49–57. doi: 10.1097/00004714-199502000-00008

Bouvard, M., Leboyer, M., Launay, J.-M., Recasens, C., Plumet, M.-H., Waller-Perotte, D., et al. (1995). Low-dose naltrexone effects on plasma chemistries and clinical symptoms in autism: a double-blind, placebo-controlled study. *Psychiatry Res.* 58, 191–201. doi: 10.1016/0165-1781(95)02601-R

Burgdorf, J. (2018). *Jaak Panksepp Memorial Symposium*. Washington State University (Personal Communication).

Burgdorf, J., Panksepp, J., Brudzynski, S. M., Kroes, R., and Moskal, J. R. (2005). Breeding for 50-kHz positive affective vocalization in rats. *Behav. Genet.* 35, 67–72. doi: 10.1007/s10519-004-0856-5

Burgdorf, J., Panksepp, J., and Moskal, J. R. (2011). Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci. Biobehav. Rev.* 35, 1831–1836. doi: 10.1016/j.neubiorev.2010.11.011

Caruana, F., Avanzini, P., Gozzo, F., Francione, S., Cardinale, F., and Rizzolatti, G. (2015). Mirth and laughter elicited by electrical stimulation of the human anterior cingulate cortex. *Cortex* 71, 323–331. doi: 10.1016/j.cortex.2015.07.024

Cattell, R. B. (1933). Temperament tests. II. Tests. *Br. J. Psychol.* 24, 20–49.

Cattell, R. B., Eber, H., and Tatsuoka, M. (1970). *Handbook for the Sixteen Personality Factor Questionnaire (16PF)*. Champaign, IL: Institute for Personality and Ability Testing.

Costa, P., and Widiger, T. (2002). "Introduction: personality disorders and the five-factor model of personality," in *Personality Disorders and the Five-Factor Model of Personality, 2nd Edn.*, eds P. Costa and T. Widiger (Washington, DC: American Psychological Association), 3–14.

Craig, A. D. (2011). Significance of the insula for the evolution of human awareness of feelings from the body. *Ann. N. Y. Acad. Sci.* 1225, 72–82. doi: 10.1111/j.1749-6632.2011.05990.x

Damasio, A., Damasio, H., and Tranel, D. (2013). Persistence of feelings and sentience after bilateral damage of the insula. *Cereb. Cortex* 23, 833–846. doi: 10.1093/cercor/bhs077

Damasio, A. R., Grabowski, T. J., Bechara, A., Damasio, H., Ponto, L. L. B., Parvizi, J., et al. (2000). Subcortical and cortical brain activity during the feeling of self-generated emotions. *Nat. Neurosci.* 3, 1049–1056. doi: 10.1038/79871

Davis, K., and Montag, C. (2018). A tribute to Jaak Panksepp (1943-2017). *Pers. Neurosci.* 1:e9. doi: 10.1017/pen.2018.5

Davis, K., and Panksepp, J. (2011). The brain's emotional foundations of human personality and the Affective Neuroscience Personality Scales. *Neurosci. Biobehav. Rev.* 35, 1946–1958. doi: 10.1016/j.neubiorev.2011.04.004

Davis, K., and Panksepp, J. (2018). *The Emotional Foundations of Personality*. New York, NY: W.W. Norton and Company.

Davis, K., Panksepp, J., and Normansell, L. (2003). The affective neuroscience personality scales: normative data and implications. *Neuropsychoanalysis* 5, 57–69. doi: 10.1080/15294145.2003.10773410

Eysenck, H. J. (1967). *The Biological Basis of Personality*. Springfield, IL: Charles, C. Thomas.

Fernandez de Molina, A., and Hunsperger, R. W. (1962). Organization of the subcortical system governing defence and flight reactions in the cat. *J. Physiol.* 160, 200–213. doi: 10.1113/jphysiol.1962.sp006841

Florio, M., Albert, M., Taverna, E., Namba, T., Brandl, H., Lewitus, E., et al. (2015). Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. *Science* 347, 1465–1470. doi: 10.1126/science.aaa1975

Freud, S. (1920/1990). *Beyond the Pleasure Principle*. New York, NY: W. W. Norton and Company.

Goel, V., and Dolan, R. J. (2003). Reciprocal neural response within lateral and ventral medial prefrontal cortex during hot and cold reasoning. *Neuroimage* 20, 2314–2321. doi: 10.1016/j.neuroimage.2003.07.027

Goldberg, L. R. (1990). An alternative "description of personality": the Big-Five factor structure. *J. Pers. Soc. Psychol.* 59, 1216–1229.

Goldberg, L. R. (1992), The development of markers for the Big-Five factor structure. *Psychol. Assess.* 4, 26–42.

Hess, W. R. (1957). *The Functional Organization of the Diencephalon*. New York, NY: Grune and Statton.

Horn, G. (1985). "Imprinting and the neural basis of memory," in *Brain Plasticity, Learning and Memory,* eds B. Will, P. Schmitt, and J. C. Dalrymple-Alford (New York, NY: Plenum), 13–19. doi: 10.1007/978-1-4684-5003-3_2

Horn, G., and Johnson, M. (1989). Memory systems in the chick: dissociations and neuronal analysis. *Neuropsychologia* 27, 1–22. doi: 10.1016/0028-3932(89)90086-9

Huston, J., and Borbely, A. (1973). Operant conditioning in forebrain ablated rats by use of rewarding hypothalamic stimulation. *Brain Res.* 50, 467–472. doi: 10.1016/0006-8993(73)90753-1

Huston, J., and Borbely, A. (1974). The thalamic rat: general behavior, operant learning with rewarding hypothalamic stimulation, and effects of amphetamine. *Physiol. Behav.* 12, 433–448. doi: 10.1016/0031-9384(74)90121-8

Jackson, D. N. (1974). *Personality Research Form Manual*. Goshen, NY: Research Psychologists Press, Inc. (Currently available from Sigma Assessment Systems, Inc.)

Johnson, M., and Horn, G. (1986). Dissociation of recognition memory and associative learning by a restricted lesion of the chick forebrain. *Neuropsychologia* 24,329–340. doi: 10.1016/0028-3932(86)90018-7

Johnson, M., and Horn, G. (1988). Development of filial preferences in dark-reared chicks. *Anim. Behav.* 36, 675–683. doi: 10.1016/S0003-3472(88)80150-7

Johnson, M., Senju, A., and Tomalski, P. (2015).The two-process theory of face processing: modifications based on two decades of data from infants and adults. *Neurosci. Biobehav. Rev.* 50, 169–179. doi: 10.1016/j.neubiorev.2014.10.009

Karterud, S., Pedersen, G., Johansen, M., Wilberg, T., Davis, K., and Panksepp, J. (2016). Primary emotional traits in patients with personality disorders. *Pers. Mental Health* 10, 261–273. doi: 10.1002/pmh.1345

King, J. E., and Figueredo, A. J. (1997). The five-factor model plus dominance in chimpanzee personality. *J. Res. Pers.* 31, 257–271. doi: 10.1006/jrpe.1997.2179

Knutson, B., Burgdorf, J., and Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychol. Bull.* 128, 961–977. doi: 10.1037/0033-2909.128.6.961

Krueger, R. F., Derringer, J., Markon, K. E., Watson, D., and Skodol, A. E. (2012). Initial construction of a maladaptive personality trait model and inventory for DSM-5. *Psychol. Med.* 42, 1879–1890. doi: 10.1017/S0033291711002674

Langer, S. K. (1988). *Mind: An Essay on Human Feeling (Abridged Edition)*. Baltimore, MD: Johns Hopkins University Press.

LeDoux, J. (2012). Rethinking the emotional brain. *Neuron* 73, 653–676. doi: 10.1016/j.neuron.2012.02.004

Liotti, M., and Panksepp, J. (2004). "Imaging human emotions and affective feelings: implications for biological psychiatry," in *Textbook of Biological Psychiatry,* ed J. Panksepp (Hoboken, NJ: Wiley), 33–74.

Livesley, W. J., Jackson, D. N., and Schroeder, M. L. (1992). Factorial structure of traits delineating personality disorders in clinical and general population samples. *J. Abnorm. Psychol.* 101, 432–440. doi: 10.1037/0021-843X.101.3.432

McDougall, W. (1908). *Introduction to Social Psychology*, Methuen: London.

Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E., et al. (2010). Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias. *Curr. Biol.* 20, R839–R840. doi: 10.1016/j.cub.2010.08.030

Merker, B. (2007). Consciousness without a cerebral cortex: a challenge for neuroscience and medicine. *Behav. Brain Sci.* 30, 63–81; discussion: 81–134. doi: 10.1017/S0140525X07000891

Miller, N. E., and Dollard, J. (1941). *Social Learning and Imitation*. New Haven, CT: Yale University Press.

Mobbs, D., Marchant, J., Hassabis, D., Seymour, B., Tan, G., Gray, M., et al. (2009). From threat to fear: the neural organization of defensive fear systems in humans. *J. Neurosci.* 29, 12236–12243. doi: 10.1523/JNEUROSCI.2378-09.2009

Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., Weiskopf, N., Seymour, B., et al. (2007). When fear is near: threat imminence elicits prefrontal-periaqueductal grey shifts in humans. *Science* 317, 1079–1083. doi: 10.1126/science.1144298

Montag, C., and Davis, K. (2018). Affective neuroscience theory and personality: an update. *Pers. Neurosci.* 1:e12. doi: 10.1017/pen.2018.10

Montag, C., and Panksepp, J. (2016). Primal emotional-affective expressive foundations of human facial expression. *Motiv. Emot.* 40, 760–766. doi: 10.1007/s11031-016-9570-x

Montag, C., and Panksepp, J. (2017). Primary emotional systems and personality: an evolutionary perspective. *Front. Psychol.* 8:464. doi: 10.3389/fpsyg.2017.00464

Montag, C., and Panksepp, J. (2018). "Personality Neuroscience: why it is of importance to include primary emotional systems!" in *Encyclopedia of Personality and Individual Differences*, eds V. Zeigler-Hill and T. Shackelford (Heidelberg: Springer-Verlag). Available online at: https://link.springer.com/referenceworkentry/10.1007%2F978-3-319-28099-8_1338-1

Montag, C., Widenhorn-Müller, K., Panksepp, J., and Kiefer, M. (2017). Individual differences in Affective Neuroscience Personality Scale (ANPS) primary emotional traits and depressive tendencies. *Comp. Psychiatry* 73, 136–142. doi: 10.1016/j.comppsych.2016.11.007

Moskal, J., Burgdorf, J., Stanton, P., Kroes, R., Disterhoft, J., Burch, R., et al. (2017). The development of rapastinel (formerly GLYX-13); a rapid acting and long lasting antidepressant. *Curr. Neuropharmacol.* 15, 47–56. doi: 10.2174/1570159X14666160321122703

Newton, J., Ellsworth, C., Miyakawa, T., Tonegawa, S., and Sur, M. (2004). Acceleration of visually cued conditioned fear through the auditory pathway. *Nat. Neurosci.* 7, 968–973. doi: 10.1038/nn1306

Northoff, G., Heinzel, A., Bermpohl, F., Niese, R., Pfennig, A., Pascual-Leone, A., et al. (2004). Reciprocal modulation and attenuation in the prefrontal cortex: an fMRI study on emotional-cognitive interaction. *Hum. Brain Mapp.* 21, 202–212. doi: 10.1002/hbm.20002

Overton, D. A. (1991). Historical context of state dependent learning and discriminative drug effects. *Behav. Pharmacol.* 2, 253–264. doi: 10.1097/00008877-199109000-00002

Panksepp, J. (1982).Toward a general psychobiological theory of emotions. *Behav. Brain Sci.* 5, 407–467. doi: 10.1017/S0140525X00012759

Panksepp, J. (1985). "Mood changes," in *Handbook of Clinical Neurology,* eds P. Vinken, G. Bruyn, and H. Klawans (Amsterdam: Elsevier), 271–285.

Panksepp, J. (1991). "Affective neuroscience: a conceptual framework for the neurological study of emotions," in *International Reviews of Emotion Research,* ed K. Strongman (Chichester: Wiley), 59–99.

Panksepp, J. (1992). A critical role for "affective neuroscience" in resolving what is basic about basic emotions. *Psychol. Rev.* 99, 554–560. doi: 10.1037/0033-295X.99.3.554

Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York, NY: Oxford University Press.

Panksepp, J. (2010). Affective neuroscience of the emotional BrainMind: evolutionary perspectives and implications for understanding depression. *Dialog. Clin. Neurosci.* 12, 533–545. Available online at: www.dialogues-cns.org

Panksepp, J. (2011). Cross-species affective neuroscience decoding of the primal affective experiences of humans and related animals. *PLoS ONE* 6:e21236. doi: 10.1371/journal.pone.0021236

Panksepp, J. (2015). Affective preclinical modeling of psychiatric disorders: taking imbalanced primal emotional feelings of animals seriously in our search for novel antidepressants. *Dialog. Clin. Neurosci.* 17, 363–379. Available online at: www.dialogues-cns.org

Panksepp, J., and Biven, L. (2012). *The Archeology of Mind*. New York, NY: W.W. Norton and Company.

Panksepp, J., Burgdorf, J., Gordon, N., and Turner, C. (2003). Modeling ADHD-type arousal with unilateral frontal cortex damage in rats and beneficial effects of play therapy. *Brain Cogn.* 52, 97–105. doi: 10.1016/S0278-2626(03)00013-7

Panksepp, J., Herman, B., Conner, R., Bishop, P., and Scott, J. P. (1978). The biology of social attachments: opiates alleviate separation distress. *Biol. Psychiatry* 13, 607–618.

Panksepp, J., Herman, B., Vilberg, T., Bishop, P., and DeEskinazi, F. (1980). Endogenous opioids and social behavior. *Neurosci. Biobehav. Rev.* 4, 473–487. doi: 10.1016/0149-7634(80)90036-6

Panksepp, J., Lane, R., Solms, M., and Smith, R. (2017). Reconciling cognitive and affective neuroscience perspectives on the brain basis of emotional experience. *Neurosci. Biobehav. Rev.* 76(Part B), 187–215. doi: 10.1016/j.neubiorev.2016.09.010

Panksepp, J., Normansell, L., Cox, J., and Siviy, S. (1994). Effects of neonatal decortication on the social play of juvenile rats. *Physiol. Behav.* 56, 429–443. doi: 10.1016/0031-9384(94)90285-2

Panksepp, J., and Sahley, T. (1987). "Possible brain opioid involvement in disrupted social intent and language development of autism," in *Neurobiological Issues in Autism,* eds E. Schopler and G. Mesibov (New York, NY: Plenum), 357–382.

Panksepp, J., Wright, J. S., Döbrössy, M. D., Schlaepfer, T. E., and Coenen, V. A. (2014). Affective neuroscience strategies for understanding and treating depressions: from preclinical models to novel therapeutics. *Clin. Psychol. Sci.* 2, 472–494. doi: 10.1177/2167702614535913

Panksepp, J., and Yovell, Y. (2014). Preclinical modeling of primal emotional affects (Seeking, Panic and Play): gateways to the development of new treatments for depression. *Psychopathology* 47, 383–393. doi: 10.1159/000366208

Panksepp, J. B., and Huber, R. (2004). Ethological analyses of crayfish behavior: a new invertebrate system for measuring the rewarding properties of psychostimulants. *Behav. Brain Res.* 153, 171–180. doi: 10.1016/j.bbr.2003.11.014

Penfield, W., and Jasper, H. (1954). *Epilepsy and the Functional Anatomy of the Human Brain.* Boston, MA: Little, Brown and Company.

Preskorn, S., Macaluso, M., Mehra, V., Zammit, G., Moskal, J. R., Burch, R. M., et al. (2015). Randomized proof of concept trial of GLYX-13, an N-methyl-D-aspartate receptor glycine site partial agonist, in major depressive disorder nonresponsive to a previous antidepressant agent. *J. Psychiatr. Pract.* 21, 140–149. doi: 10.1097/01.pra.0000462606.17725.93

Rygula, R., Golebiowska, J., Kregiel, J., Kubik, J., and Popik, P. (2015). Effects of optimism on motivation in rats. *Front. Behav. Neurosci.* 9:32. doi: 10.3389/fnbeh.2015.00032

Rygula, R., Pluta, H., and Popik, P. (2012). Laughing rats are optimistic. *PLoS ONE* 7:e51959. doi: 10.1371/journal.pone.0051959

Sadato, N., Pascual-Leone, A., Grafman, J., Deiber, M. P., Ibanez, V., and Hallett, M. (1998). Neural networks for braille reading by the blind. *Brain* 121, 1213–1229. doi: 10.1093/brain/121.7.1213

Saucier, G. (2009). Recurrent personality dimensions in inclusive lexical studies: indications for a big six structure. *J. Pers.* 77, 1577–1614. doi: 10.1111/j.1467-6494.2009.00593.x

Saucier, G., and Srivastava, S. (2015). "What makes a good structural model of personality? evaluating the big five and alternatives," in *APA Handbook of Personality and Social Psychology, Volume 4: Personality Processes and Individual Differences*, eds M. Mario, P. R. Shaver, C. M. Cooper, and R. J. Larsen (Washington, DC: American Psychological Association), 283–305.

Savitz, J., van der Merwe, L., and Ramesar, R. (2008a). Dysthymic and anxiety-related personality traits in bipolar spectrum illness. *J. Affect. Disord.* 109, 305–311. doi: 10.1016/j.jad.2007.12.006

Savitz, J., van der Merwe, L., and Ramesar, R. (2008b). Hypomanic, cyclothymic and hostile personality traits in bipolar spectrum illness: a family-based study. *J. Psychiatr. Res.* 42, 920–929. doi: 10.1016/j.jpsychires.2007.10.011

Schlaepfer, T. E., Bewernick, B. H., Kayser, S., Madler, B., and Coenen, V. A. (2013). Rapid effects of deep brain stimulation for treatment-resistant major depression. *Biol. Psychiatry* 73, 1204–1212. doi: 10.1016/j.biopsych.2013.01.034

Schlaepfer, T. E., Cohen, M. X., Frick, C., Kosel, M., Brodesser, D., Axmacher, N., et al. (2008). Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology* 33, 368–377. doi: 10.1038/sj.npp.1301408

Scott, J. P., and Fuller, J. L. (1965). *Genetics and the Social Behavior of the Dog.* Chicago, IL: University of Chicago Press.

Senju, A., and Johnson, M. (2009). The eye contact effect: mechanisms and development. *Trends Cogn. Sci.* 13, 127–134. doi: 10.1016/j.tics.2008.11.009

Sindermann, C., Saliger, J., Nielsen, J., Karbe, H., Markett, S., Stavrou, M., et al. (2018). Personality and primary emotional traits: disentangling multiple sclerosis related fatigue and depression. *Arch. Clin. Neuropsychol.* 33, 552-561. doi: 10.1093/arclin/acx104

Siviy, S., and Panksepp, J. (1985). Dorsomedial Diencephalic Involvement in the Juvenile Play of Rats. *Behav. Neurosci.* 99, 1103–1113. doi: 10.1037/0735-7044.99.6.1103

Steiner, A. P., and Redish, A. D. (2014). Behavioral and neurophysiological correlates of regret in rat decision-making on a neuroeconomic task. *Nat. Neurosci.* 17, 995–1002. doi: 10.1038/nn.3740

Stutz, R., Rossi, R., Hastings, L., and Brunner, R. (1974). Discriminability of intracranial stimuli: the role of anatomical connectedness. *Physiol. Behav.* 12, 69–73. doi: 10.1016/0031-9384(74)90069-9

Tulving, E. (1985). Memory and consciousness. *Can. Psychol.* 26, 1–10. doi: 10.1037/h0080017

Tupes, E., and Christal, R. (1992). Recurrent personality factors based on trait ratings. *J. Pers.* 60, 225–251. doi: 10.1111/j.1467-6494.1992.tb00973.x

van der Westhuizen, D., and Solms, M. (2015). Social dominance and the affective neuroscience personality scales. *Conscious. Cogn.* 33, 90–111. doi: 10.1016/j.concog.2014.12.005

von Melchner, L., Pallas, S. L., and Sur, M. (2000). Visual behaviour mediated by retinal projections directed to the auditory pathway. *Nature* 404, 871–876. doi: 10.1038/35009102

Weeks, R., Horwitz, B., Aziz-Sultan, A., Tian, B., Wessinger, C. M., Cohen, L. G., et al. (2000). A positron emission tomographic study of auditory localization in the congenitally blind. *J. Neurosci.* 20, 2664–2672. doi: 10.1523/JNEUROSCI.20-07-02664.2000

Wernicke, J., Li, M., Sha, P., Zhou, M., Sindermann, C., Becker, B., et al. (2018). Individual differences in tendencies to attention-deficit/hyperactivity disorder and emotionality: empirical evidence in young healthy adults from Germany and China. *ADHD Attent. Defic. Hyperact. Disord.* doi: 10.1007/s12402-018-0266-9. [Epub ahead of print].

Whishaw, I. (1990). "The decorticate rat," in *The Cerebral Cortex of the Rat*, eds B. Kolb and R. Tees (Cambridge, MA: The MIT Press), 239–267.

Widiger, T. A., Livesley, W. J., and Clark, L. A. (2009). An integrative dimensional classification of personality disorder. *Psychol. Assess.* 21, 243–255. doi: 10.1037/a0016606

Yovell, Y., Bar, G., Mashiah, M., Baruch, Y., Briskman, I., Asherov, J., et al. (2016). Ultra-low-dose buprenorphine as a time-limited treatment for severe suicidal ideation: a randomized controlled trial. *Am. J. Psychiatry* 173, 491–498. doi: 10.1176/appi.ajp.2015.15040535

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Sex-Specific Associations Between Inter-Individual Differences in Heart Rate Variability and Inter-Individual Differences in Emotion Regulation

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Neurobiological theories suggest that inter-individual differences in vagally mediated heart rate variability (vmHRV) have the potential to serve as a biomarker for inter-individual differences in emotion regulation that are due to inter-individual differences regarding the engagement of prefrontal and (para-)limbic brain regions during emotion processing. To test these theories, we investigated whether inter-individual differences in vmHRV would be associated with inter-individual differences in emotion regulation. We determined resting state vmHRV in a sample of 176 individuals that had also completed a short self-report measure of reappraisal and suppression use. Resting state vmHRV was derived from short-term (300 s) and ultra-short-term (120 s, 60 s) recordings of participants' heart rate to determine the robustness of possible findings. Irrespective of recording length, we found that an increase in resting state vmHRV was associated with an increase in self-reported reappraisal but not suppression use. However, this association was only evident among male but not female participants, indicating a sex-specific association between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported emotion regulation. These findings, which are consistent with previous ones, support theoretical claims that inter-individual differences in vmHRV serve as a biomarker for inter-individual differences in emotion regulation. Combing (ultra-)short-term measures of resting state vmHRV with short self-report measures of emotion regulation may, thus, be useful for researchers who have to investigate the neurobiological mechanisms of emotion regulation in a time- and resource-efficient manner.

Keywords: emotion regulation, suppression, reappraisal, vagus nerve, heart rate variability

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INTRODUCTION

As social beings, we interact on a daily basis with other individuals. Although these interactions are often rewarding (e.g., interacting with a caring partner), they may also turn out to be frustrating (e.g., interacting with a stubborn child). Whereas in some situations it may be appropriate to show our frustration (e.g., in an argument with our partner), in other situations it may be more appropriate to control our emotions that are fueled by our frustration (e.g., in an argument with our

child). The ability to regulate our emotions, thus, determines the course of our social interactions (Gross, 2002), which may have positive outcomes after successful emotion regulation and negative outcomes after unsuccessful emotion regulation (Butler et al., 2003; Gross and John, 2003; English et al., 2012). Unsuccessful emotion regulation may also result in clinical and subclinical levels of stress, anxiety and depression (Garnefski et al., 2001; Gross and John, 2003; Martin and Dahlen, 2005; Moore et al., 2008; Hofmann et al., 2009; Aldao and Nolen-Hoeksema, 2010), thereby increasing the risk to develop mental disorders in the aftermath of emotionally arousing interactions (Gross, 2002). However, whether we succeed or fail to regulate our emotions crucially depends on the type of strategy we employ for this type of purpose (Gross, 2002). Reappraisal is an emotion regulation strategy aimed at changing the meaning of an emotional event (antecedent-focused strategy), whereas suppression is an emotion regulation strategy aimed at changing the reaction to an emotional event (response-focused strategy). Reappraisal use appears to be more efficient in attenuating emotional distress and physiological arousal than suppression use (Gross, 1998; Butler et al., 2003; Gross and John, 2003; Moore et al., 2008; Hofmann et al., 2009; Aldao and Nolen-Hoeksema, 2010), presumably because reappraisal use is more associated with an increase in prefrontal activity and a decrease in (para-)limbic activity than suppression use (Goldin et al., 2008; Drabant et al., 2009; McRae et al., 2010; Vanderhasselt et al., 2013; Nelson et al., 2015) As a consequence, suppression use is more likely to cause symptoms and disorders of stress, anxiety and depression than reappraisal use (Gross, 2002).

Considering the importance of reappraisal and suppression use for mental health (Aldao et al., 2010; Sheppes et al., 2015), much research has been devoted to identify biomarkers that indicate deficits in emotion regulation. Although abnormal activity changes in prefrontal and (para-)limbic brain regions have been considered as biomarkers for deficits in emotion regulation (Ochsner et al., 2012; Frank et al., 2014), the recording of changes in brain activity require neuroimaging protocols that are difficult to realize without the necessary staff and equipment. Other techniques than neuroimaging ones may be more useful to identify biomarkers for emotion regulation. Electrocardiologic techniques that allow an indirect assessment of changes in brain activity via changes in cardiac activity may represent a promising alternative to neuroimaging techniques because these techniques do not require dedicated staff or equipment (Beauchaine, 2015). Whereas changes in cardiac activity can be assessed within a couple of minutes with mobile or stationary devices that do not require extensive training, changes in brain activity can only be assessed over longer time periods with stationary devices that require extensive training. Although changes in cardiac activity can be measured in several ways, measures related to parasympathic induced changes in heart rate (HR) that are mediated by the vagus nerve, which are commonly described as vagally-mediated heart rate variability (vmHRV; Shaffer and Ginsberg, 2017), are of particular relevance. Inter-individual differences in vmHRV are associated with inter-individual differences in prefrontal and (para-)limbic brain activity that are implicated in self-regulatory processes (Thayer and Lane, 2009; Thayer et al., 2012; Smith et al., 2017), indicating that inter-individual differences vmHRV may serve as a biomarker for inter-individual differences in emotion regulation (Appelhans and Luecken, 2006; Beauchaine, 2015; Holzman and Bridgett, 2017). It has already been shown that individuals with high vmHRV have fewer difficulties in emotion regulation than individuals with low HRV (Williams et al., 2015), which may explain why individuals with high vmHRV report less stress, anxiety and depression than individuals with low vmHRV (Fabes and Eisenberg, 1997; Oveis et al., 2009; Kok and Fredrickson, 2010; Kogan et al., 2013; Lischke et al., 2018a). It remains, however, unclear whether these differences in stress, anxiety and depression are due to differences in reappraisal and suppression use. It may be possible that individuals with high vmHRV experience less stress, anxiety and depression than individuals with low vmHRV because individuals with high vmHRV use more reappraisal and less suppression for emotion regulation than individuals with low vmHRV. To address this issue, we investigated whether inter-individual differences in vmHRV would be associated with inter-individual differences in reappraisal and suppression use in a large and homogenous sample of participants. Inter-individual differences in reappraisal and suppression use were determined on basis of participants' self-reports. Inter-individual differences in vmHRV were determined on basis of short-term and ultra-short-term recordings of participants' resting state HR. This allowed us to test whether associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported reappraisal or suppression use would be invariant across recording conditions, thereby indicating the robustness and stability of these associations. We expected that inter-individual differences in resting state vmHRV would be positively associated with inter-individual differences in self-reported reappraisal use and that inter-individual differences in resting state vmHRV would be, if at all, negatively associated with inter-individual differences in self-reported suppression use. We also explored whether participants' sex would moderate these associations because inter-individual differences in emotion regulation and vmHRV seem to be sex-dependent (Nolen-Hoeksema, 2012; Koenig and Thayer, 2016).

MATERIALS AND METHODS

Participants

As a previous study revealed medium sized associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reports regarding the *inability* to regulate emotions (Williams et al., 2015), we expected to find similar sized associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reports regarding the *ability* to regulate emotions. A power analysis with G*Power 3 indicated that we had to recruit 82–92 participants to have sufficient power ($1\text{-}\beta = 0.80$, $\alpha = 0.05$) to detect medium sized associations ($r = 0.030$, $f^2 = 0.015$) in our correlation and regression based

analyses (Faul et al., 2007). We only considered Caucasians with an age range of 18–35 years for recruitment, which resulted in the recruitment of 176 participants, 91 male and 85 females (see **Table 1**). All participants provided written-informed consent to the study protocol that was approved by the ethics committee of the University of Rostock and carried out in accordance with the Declaration of Helsinki.

Procedure

Participants were instructed to abstain from alcohol and drugs in the 24 h preceding the testing day. On the testing day, participants were asked to use the bathroom to control for the effects of bladder filling and gastric digestion on resting state vmHRV (Quintana and Heathers, 2014). Thereafter, participants were seated in a comfortable chair and prepared for a HR recording that was used to determine participants' resting state vmHRV. As recently recommended (Quintana et al., 2016), participants were instructed to breathe spontaneously and to keep their eyes open during the HR recording. Thereafter, participants completed self-report measures of psychopathology (Brief Symptom Inventory 18, BSI-18; Franke et al., 2017) and emotion regulation (Affective Style Questionnaire, ASQ; Hofmann and Kashdan, 2010; Graser et al., 2012).

TABLE 1 | Participant characteristics.

	Female participants (*n* = 85)		Male participants (*n* = 91)	
	M (SE M)	95% CI	*M (SE M)*	95% CI
Age	23.85 (0.42)	[23.02, 24.68]	25.81 (0.41)	[25.01, 26.62]
BMI	21.49 (0.27)	[20.96, 22.02]	23.90 (0.26)	[23.39, 24.42]
BSI-18	0.50 (0.04)	[0.42, 0.57]	0.35 (0.04)	[0.28, 0.42]
ASQ-REA	3.39 (0.07)	[3.26, 3.53]	3.80 (0.06)	[3.67, 3.93]
ASQ-SUP	2.85 (0.07)	[2.70, 2.99]	3.00 (0.07)	[2.86, 3.13]
RMSSD-300	49.56 (3.42)	[42.76, 56.35]	43.37 (2.43)	[38.53. 48.21]
RMSSD-120[a]	54.22 (4.00)	[46.27, 62.17]	45.92 (2.76)	[40.43. 51.42]
RMSSD-060[a]	55.63 (4.09)	[47.49, 63.77]	46.85 (2.72)	[41.44. 52.26]
Log-RMSSD-300	1.62 (0.03)	[1.56, 1.67]	1.57 (0.03)	[1.52, 1.63]
Log-RMSSD-120[a]	1.65 (0.03)	[1.59, 1.71]	1.60 (0.03)	[1.54, 1.65]
Log-RMSSD-060[a]	1.66 (0.03)	[1.61, 1.72]	1.61 (0.03)	[1.55, 1.66]

ASQ-REA, Affective Style Questionnaire – Reappraisal Scale (Hofmann and Kashdan, 2010; Graser et al., 2012); ASQ-SUP, Affective Style Questionnaire – Suppression Scale (Hofmann and Kashdan, 2010; Graser et al., 2012); BMI, body mass index; BSI-18, Brief Symptom Inventory 18 (Franke et al., 2017); 95% CI, 95% confidence interval; RMSSD-300, root mean square of successive differences between consecutive heart beats that had been derived from 300 s lasting heartrate recordings; RMSSD-120, root mean square of successive differences between consecutive heart beats that had been derived from 120 s lasting heartrate recordings; RMSSD-060, root mean square of successive differences between consecutive heart beats that had been derived from 60 s lasting heartrate recordings; Log-RMSSD-300, log-transformed root mean square of successive differences between consecutive heart beats that had been derived from 300 s lasting heartrate recordings; Log-RMSSD-120, log-transformed root mean square of successive differences between consecutive heart beats that had been derived from 120 s lasting heartrate recordings; Log-RMSSD-060, log-transformed root mean square of successive differences between consecutive heart beats that had been derived from 60 s lasting heartrate recordings. [a]Data was missing for one male participant due to technical difficulties.

Heart Rate Variability

Using a portable HR monitor (RS 800, Polar Electro Oy, Kempele, Finland), participants' HR was recorded for 300 s at a sampling rate of 1000 HR. Portable HR monitors are as accurate in HR recording as stationary HR monitors (Weippert et al., 2010; Quintana et al., 2012), indicating a valid and reliable recording of the HR data. Device-specific software (Polar ProTrainer 5 Polar Electro Oy, Kempele, Finland) was used to transfer the recorded data to a computer for further data processing with Kubios HRV 2.2 (Tarvainen et al., 2014). Following established guidelines (Task Force of the European Society of Cardiology, 1996), the recorded data was artifact corrected and subjected to a time-domain analysis for the determination of the root mean square of successive differences between consecutive heart beats (RMSSD). RMSSD was determined on basis of different recording lengths, including a short-term recording that lasted 300 s (RMSSD-300) and two ultra-short-term recordings that lasted 120 s (RMSSD-120) and 60 s (RMSSD-060). Although multiple HRV measures can be derived from resting state HR recordings (Shaffer and Ginsberg, 2017), no other HRV measures than RMSSD were considered for statistical analysis to avoid interpretational issues from the use of multiple (inter-correlated) HRV measures. Compared to other HRV measures, RMSSD is the most robust measure of parasympathetic induced changes in HR that are mediated by the vagus nerve (Penttila et al., 2001; Bertsch et al., 2012). RMSSD is also one of the few HRV measures that can be derived from short-term *as well as* ultra-short-term resting state HR recordings in a valid and reliable manner (Bertsch et al., 2012; Munoz et al., 2015). RMSSD was, thus, as recommended (Task Force of the European Society of Cardiology, 1996), the vmHRV measure of interest.

Psychopathology

Participants' psychopathology was assessed with the BSI-18 (Franke et al., 2017), an 18 item comprising self-report measure of current psychopathological distress [BSI-18: $\alpha = 0.84$]. Each item asked for the severity of anxious, depressive and somatoform symptoms within the last 7 days (e.g., "*Within the last 7 days, how much did you suffer from feelings of loneliness?*"). Participants had to indicate how much they were suffering from these symptoms by using a 5-point Likert that ranged from 0 ("*not at all*") to 4 ("*extremely*"). Ratings of these symptoms have been shown to be associated with formal diagnoses of mental disorders (Prinz et al., 2013), implying a valid and reliable assessment of participants' psychopathological distress.

Emotion Regulation

Participants' emotion regulation abilities were assessed with the ASQ (Hofmann and Kashdan, 2010; Graser et al., 2012), a self-report measure that differentiates between various emotion regulation strategies. Reappraisal strategies aimed at changing the meaning of an emotional event were assessed with a scale that comprised 5 items [ASQ-REA: $\alpha = 0.77$], whereas suppression

strategies aimed at changing the reaction to an emotional event were assessed with a scale that comprised 9 items [ASQ-SUP: $\alpha = 0.83$]. The items described these emotion regulation strategies in terms of statements (e.g., "*I can avoid getting upset by taking a different perspective on things*" or "*I often suppress my emotional reactions to things*"). Participants had to indicate how much they agreed with each statement by using a 5-point Likert scale that ranged from 0 ("*not true for me at all*") to 4 ("*extremely true of me*"). Agreement with these statements has been shown to be associated with the actual use of the respective emotion regulation strategies (Szasz et al., 2011, 2012), indicating a valid and reliable assessment of participants' reappraisal and suppression use.

Statistical Analysis

SPSS 22 (SPSS Inc., Chicago, IL, United States) was used for all analyses. Analyses of variances (ANOVAs) were run to investigate inter-individual differences in age, body mass index, psychopathology, self-reported emotion regulation abilities and resting state vmHRV. Correlation and regression analyses with bootstrapping (1000 samples) were run to analyze associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported reappraisal or suppression use. Precautions were taken to control for inter-individual differences in age, body mass index and psychopathology that may have affected the results of the correlation and regression analyses (Licht et al., 2008, 2009; Abhishekh et al., 2013; Koenig et al., 2014). Whereas correlation analyses were used to explore these associations in male and female participants, regression analyses were used to test whether these associations differed between male and female participants. In the respective regression models, inter-individual differences in age, sex, body mass index, psychopathology and resting state vmHRV were used as predictors and self-reported reappraisal or suppression use as criterion. All predictors, with the exception of sex, were z-transformed to control for multicollinarity (Aiken and West, 1991). The first block of predictors comprised sex, age, body mass index, psychopathology and resting state vmHRV, whereas the second block of predictors comprised the interaction of sex and resting state vmHRV (Aiken and West, 1991). Significant interactions between the predictor sex and the predictor resting state vmHRV were investigated with simple slope analyses (Aiken and West, 1991). To determine the robustness of possible associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported reappraisal or suppression use, the correlation and regression analyses were run with short-term (ST) and ultra-short-term (UT) measures of resting state vmHRV (vmHRV-ST: RMSSD-300; vmHRV-UT: RMSSD-120, RMSSD-060). Correspondence between the different resting state vmHRV measures was assessed on basis of correlation analyses that comprised bivariate correlations and intra-class correlations (ICC: absolute agreement, two-way ANOVA; Fleiss et al., 2013). To account for deviations from normality distribution, the resting state vmHRV measures were log transformed (log 10) before all analyses. The significance level for the analyses was set at $p \leq 0.05$. In addition to the significance value p, 95% confidence intervals (CI) and effect size measures (r, R^2, ΔR^2, B,η_p^2) were determined to facilitate the interpretation of (marginally) significant findings (Cohen, 1988; Cumming, 2013).

RESULTS

Participant Characteristics

A series of one-way ANOAVs was run to investigate inter-individual differences in participant characteristics (see **Table 1**). Male participants were slightly older than female participants [$F(1,174) = 11.24$, $p = 0.001$, $\eta_p^2 = 0.06$]. Male participants also had a higher body mass index than female participants [$F(1,174) = 41.32$, $p = 0.001$, $\eta_p^2 = 0.19$] but did not differ from female participants on any resting state vmHRV measure [RMSSD-300: $F(1,174) = 1.40$, $p = 0.249$, $\eta_p^2 = 0.01$; RMSSD-120: $F(1,173) = 1.78$, $p = 0.184$, $\eta_p^2 = 0.01$; RMSSD-060: $F(1,173) = 2.03$, $p = 0.156$, $\eta_p^2 = 0.01$]. Female participants reported more psychopathology [$F(1,174) = 7.87$, $p = 0.006$, $\eta_p^2 = 0.10$] and less reappraisal use than male participants [$F(1,174) = 19.28$, $p = 0.001$, $\eta_p^2 = 0.10$]. Reports of suppression use, on the contrary, did not differ between male and female participants [$F(1,174) = 2.20$, $p = 0.140$, $\eta_p^2 = 0.01$].

Associations Between Short-Term Measures of Heart Rate Variability and Self-Report Measures of Reappraisal or Suppression Use

Using resting state vmHRV-ST measures, a series of correlation analyses was run to explore sex-dependent associations between inter-individual differences in resting state vmHRV-ST and inter-individual differences in self-reported emotion regulation abilities. In male participants, inter-individual differences in resting state vmHRV-ST correlated significantly with inter-individual differences in self-reported reappraisal [RMSSD-300: $r(86) = 0.25$, 95% CI [0.05,0.44], $p = 0.019$] but not suppression [RMSSD-300: $r(86) = -0.08$, 95% CI [−0.30,0.14], $p = 0.476$] use. In female participants, there were no significant correlations between inter-individual differences in resting state vmHRV-ST and inter-individual differences in self-reported reappraisal [RMSSD-300: $r(80) = -0.11$, 95% CI [−0.32,0.11], $p = 0.337$] or suppression [RMSSD-300: $r(80) = 0.02$, 95% CI [−0.19,0.21], $p = 0.858$] use. These findings suggested that inter-individual differences in resting state vmHRV-ST may have been differentially associated with inter-individual differences in self-reported emotion regulation abilities in male and female participants (see **Figure 1**).

To test whether inter-individual differences in resting state vmHRV-ST were in fact differentially associated with inter-individual differences in self-reported emotion regulation abilities in male and female participants, a series of regression analyses was run (see **Table 2**). A regression analysis with self-reported reappraisal use as the criterion revealed a significant interaction between the predictor sex and the predictor resting state vmHRV-ST [RMSSD-300: $t(169) = 2.45$,

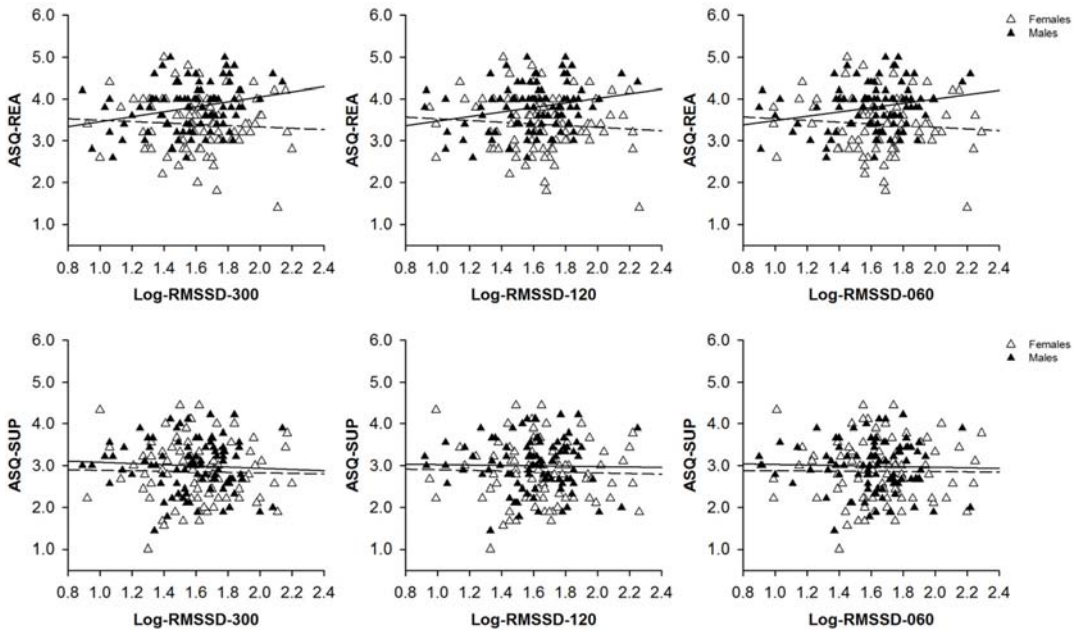

FIGURE 1 | Scatter plots with lines of best fit demonstrating bivariate correlations between self-reported reappraisal (ASQ-REA) or suppression (ASQ-SUP) use and (log-transformed) vagally mediated heartrate variability (Log-RMSSD) that was derived from 300 s (Log-RMSSD-300), 120 s (Log-RMSSD-120), or 60 s (Log-RMSSD-060) lasting resting state recordings of male (black triangulars, solid line) and female (white triangulars, dashed line) participants' heart rate.

TABLE 2 | Associations between short-term measures of heartrate variability that had been derived from 300 s lasting heartrate recordings and measures of reappraisal and suppression.

	ASQ-REA					ASQ-SUP				
	B	***SE B***	**95 % CI**	***t***	***p***	***B***	***SE B***	**95 % CI**	***t***	***p***
Step 1										
Sex	0.36	0.11	[0.15, 0.56]	3.42	0.003**	0.24	0.11	[0.05, 0.47]	2.19*	0.030**
Age	−0.02	0.04	[−0.11, 0.06]	−0.47	0.613	−0.07	0.05	[−0.17, 0.03]	−1.44	0.145
BMI	0.00	0.06	[−0.10, 0.12]	0.02	0.982	0.03	0.06	[−0.10, 0.13]	0.56	0.584
BSI-18	−0.16	0.05	[−0.27, -0.06]	−3.39	0.002**	0.21	0.06	[0.07, 0.31]	4.12	0.002**
Log-RMSSD-300	0.04	0.05	[−0.06, 0.13]	0.80	0.475	−0.01	0.05	[−0.11, 0.09]	−0.25	0.824
Step 2										
Sex	0.34	0.11	[0.13, 0.54]	3.28	0.003**	0.25	0.11	[0.05, 0.48]	2.20	0.027*
Age	−0.03	0.04	[−0.11, 0.06]	−0.64	0.503	−0.07	0.05	[−0.17, 0.03]	−1.42	0.160
BMI	0.02	0.06	[−0.09, 0.15]	0.46	0.673	0.03	0.06	[−0.10, 0.14]	0.49	0.633
BSI-18	−0.17	0.05	[−0.28, -0.06]	−3.61	0.003**	0.21	0.06	[0.07, 0.31]	4.13	0.003*
Log-RMSSD-300	−0.07	0.08	[−0.23, 0.07]	−1.14	0.343	0.00	0.07	[−0.14, 0.14]	0.05	0.949
Log-RMSSD-300 × Sex	0.22	0.10	[0.03, 0.42]	2.45	0.019*	−0.03	0.10	[−0.24, 0.17]	−0.32	0.752

*ASQ-REA: R^2 = 0.16, F(5,170) = 6.63, p = 0.001, ΔR^2 = 0.03, $\Delta F(6,169)$ = 6.01, p = 0.015, ASQ-SUP: R^2 = 0.13, F(5,170) = 4.87, p = 0.001, ΔR^2 = 0.00, $\Delta F(6,169)$ = 0.10, p = 0.751. ASQ-REA, Affective Style Questionnaire – Reappraisal Scale [42, 43]; ASQ-SUP, Affective Style Questionnaire – Suppression Scale [42, 43]; BMI, body mass index; BSI-18, Brief Symptom Inventory 18 [41]; 95% CI, 95% confidence interval; Log-RMSSD-300, log-transformed root mean square of successive differences between consecutive heart beats that had been derived from 300 s lasting heartrate recordings. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.*

p = 0.015], a significant effect of the predictor psychopathology [RMSSD-300: $t(169) = -3.61$, p = 0.001] and a significant effect of the predictor sex [RMSSD-300: $t(169)$ = 3.28, p = 0.001]. The effects of the remaining predictors, on the contrary, were all insignificant [RMSSD-300: all $t(169) \leq |-1.14|$, all $p \geq 0.343$]. A simple slope analysis indicated that resting state vmHRV-ST was a significant predictor of self-reported reappraisal use among male [RMSSD-300: B = 0.14, $SE\ B$ = 0.06, 95% CI [0.03,0.26], $t(86)$ = 2.39, p = 0.017] but not female [RMSSD-300: $B = -0.07$, $SE\ B$ = 0.07, 95% CI [−0.23,0.06], $t(80) = -0.97$, p = 0.356] participants. A regression analysis with self-reported suppression use as the criterion failed to reveal a significant interaction between the predictor sex and the predictor resting state vmHRV-ST [RMSSD-300: $t(169) = -0.32$,

TABLE 3 | Associations between short-term measures of heartrate variability that had been derived from 120 s lasting heartrate recordings and measures of reappraisal and suppression.

	ASQ-REA					ASQ-SUP				
	B	*SE B*	95% CI	*t*	*p*	*B*	*SE B*	95% CI	*t*	
Step 1										
Sex	0.35	0.11	[0.13, 0.55]	3.36	0.002**	0.24	0.11	[0.04, 0.44]	2.18	0.029*
Age	−0.02	0.05	[−0.11, 0.07]	−0.50	0.595	−0.07	0.05	[−0.17, 0.03]	−1.43	0.151
BMI	0.00	0.06	[−0.11, 0.13]	−0.04	0.969	0.03	0.06	[−0.09, 0.14]	0.57	0.575
BSI-18	−0.16	0.05	[−0.27, -0.06]	−3.47	0.003**	0.21	0.06	[0.08, 0.31]	4.12	0.001**
Log-RMSSD-120[a]	0.02	0.05	[−0.08, 0.12]	0.48	0.652	−0.01	0.05	[−0.10, 0.09]	−0.13	0.884
Step 2										
Sex	0.34	0.11	[0.13, 0.55]	3.29	0.002**	0.24	0.11	[0.04, 0.45]	2.18	0.028*
Age	−0.03	0.05	[−0.12, 0.07]	−0.61	0.537	−0.07	0.05	[−0.17, 0.03]	−1.42	0.155
BMI	0.01	0.06	[−0.10, 0.14]	0.25	0.839	0.03	0.06	[−0.09, 0.14]	0.55	0.586
BSI-18	−0.17	0.05	[−0.27, -0.06]	−3.67	0.002**	0.21	0.06	[0.08, 0.31]	4.10	0.001**
Log-RMSSD-120[a]	−0.08	0.08	[−0.23, 0.06]	−1.32	0.276	0.00	0.07	[−0.14, 0.14]	−0.03	0.981
Log-RMSSD-120[a] × Sex	0.22	0.09	[0.05, 0.41]	2.45	0.015*	−0.01	0.10	[−0.19, 0.18]	−0.09	0.932

*ASQ-REA: R^2 = 0.16, F(5,169) = 6.50, p = 0.001, ΔR^2 = 0.03, ΔF(6,168) = 6.02, p = 0.015, ASQ-SUP: R^2 = 0.12, F(5,169) = 4.79, p = 0.001, ΔR^2 = 0.00, ΔF(6,168) = 0.01, p = 0.926. ASQ-REA, Affective Style Questionnaire – Reappraisal Scale [42, 43]; ASQ-SUP, Affective Style Questionnaire – Suppression Scale [42, 43]; BMI, body mass index; BSI-18, Brief Symptom Inventory 18 [41]; 95% CI, 95% confidence interval; Log-RMSSD-120, log transformed root mean square of successive differences between consecutive heart beats that had been derived from 120 s lasting heartrate recordings. [a]Data was missing for one male participant due to technical difficulties. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.*

p = 0.751]. The effect of the other predictors, with the exception of the predictor sex [RMSSD-300: $t(169)$ = 2.20, p = 0.029] and the predictor psychopathology [RMSSD-300: $t(169)$ = 4.13, p = 0.001], were all insignificant [RMSSD-300: all $t(169) \leq |-1.42|$, all $p \geq 0.159$]. Taken together, these findings confirmed that there were sex-dependent associations between inter-individual differences in resting state vmHRV-ST and inter-individual differences in self-reported emotion regulation abilities.

Associations Between Ultra-Short-Term Measures of Heart Rate Variability and Self-Report Measures of Reappraisal or Suppression Use

Using resting state vmHRV-UT measures, another series of correlation analyses was run to explore sex-dependent associations between inter-individual differences in resting state vmHRV-UT and inter-individual differences in self-reported emotion regulation abilities. These analyses yielded similar findings like those that have been obtained in the correlation analyses that used resting state vmHRV-ST measures. In male participants, inter-individual differences in resting state vmHRV-UT were significantly correlated with inter-individual differences in self-reported reappraisal [RMSSD-120: $r(85)$ = 0.23, 95% CI [0.03,0.44], p = 0.031; RMSSD-060: $r(85)$ = 0.21, 95% CI [0.02,0.39], p = 0.05] but not suppression [RMSSD-120: $r(85)$ = −0.04, 95% CI [−0.24,0.18], p = 0.702; RMSSD-060: $r(85)$ = −0.05, 95% CI [−0.25,0.18], p = 0.679] use. In female participants, inter-individual differences in resting state vmHRV-UT correlated neither with inter-individual differences in self-reported reappraisal use [RMSSD-120: $r(80)$ = −0.12, 95% CI [−0.33,0.10], p = 0.272; RMSSD-060: $r(80)$ = −0.18, 95% CI [−0.31,0.09], p = 0.297] nor with inter-individual differences in self-reported suppression use [RMSSD-120: $r(80)$ = 0.02, 95% CI [−0.20,0.21], p = 0.884; RMSSD-060: $r(86)$ = 0.02, 95% CI [−0.18,0.22], p = 0.836]. According to these findings, inter-individual differences in resting state vmHRV-UT may have been differentially associated with inter-individual differences in self-reported emotion regulation abilities in male and female participants (see **Figure 1**).

A series of regression analyses was run to test whether inter-individual differences in resting state vmHRV-UST were in fact differentially associated with inter-individual differences in self-reported emotion regulation abilities (see **Tables 3**, **4**). These analyses revealed similar findings like those that have been found in the regression analyses that used resting state vmHRV-ST measures. A regression analysis with self-reported reappraisal use as the criterion revealed a significant interaction between the predictor sex and the predictor resting state vmHRV-UT [RMSSD-120: $t(168)$ = 2.45, p = 0.015, RMSSD-060: $t(168)$ = 2.30, p = 0.023], a significant effect of the predictor psychopathology [RMSSD-120: $t(168)$ = −3.67, p = 0.001, RMSSD-060: $t(168)$ = −3.61, p = 0.001] and a significant effect of the predictor sex [RMSSD-120: $t(168)$ = 3.29, p = 0.001, RMSSD-060: $t(168)$ = 3.25, p = 0.001]. The effects of the other predictors were all insignificant [RMSSD-120: all $t(168) \leq |-1.32|$, all $p \geq 0.189$, RMSSD-060: all $t(168) \leq |-1.23|$, all $p \geq 0.221$]. A simple slope analysis showed that resting state vmHRV-UT was a significant predictor of self-reported reappraisal use in male [RMSSD-120: B = 0.13, $SE\ B$ = 0.06, 95% CI [0.02,0.25], $t(85)$ = 2.20, p = 0.026; RMSSD-060: B = 0.12, $SE\ B$ = 0.06, 95% CI [0.01,0.24], $t(85)$ = 1.99, p = 0.035] but not female [RMSSD-120: B = −0.08, $SE\ B$ = 0.07, 95% CI [−0.23,0.07],

$t(80) = -1.11$, $p = 0.294$; RMSSD-060: $B = -0.07$, $SE\ B = 0.07$, 95% CI [−0.21, 0.05], $t(80) = -1.05$, $p = 0.276$] participants. A regression analysis with suppression use as the criterion found no significant interaction between the predictor sex and the predictor resting state vmHRV-UT [RMSSD-120: $t(168) = -0.01$, $p = 0.926$, RMSSD-060: $t(168) = -0.11$, $p = 0.912$] but a significant effect of the predictor sex [RMSSD-120: $t(168) = 2.18$, $p = 0.031$, RMSSD-060: $t(168) = 2.19$, $p = 0.030$] and a significant effect of the predictor psychopathology [RMSSD-120: $t(168) = 4.10$, $p = 0.001$, RMSSD-060: $t(168) = 4.12$, $p = 0.001$]. The effects of the remaining predictors were not significant [RMSSD-120: all $t(168) \leq |-1.42|$, all $p \geq 0.158$; RMSSD-060: all $t(168) \leq |-1.40|$, all $p \geq 0.162$]. Taken together, these findings confirmed that there were sex-dependent associations between inter-individual differences in resting state vmHRV-UT and inter-individual differences in emotion regulation abilities.

Correspondence Between (Ultra-)Short-Term Measures of Heart Rate Variability

Bivariate correlations and intra-class correlations were used to analyze the correspondence between resting state vmHRV-ST and vmHRV-UT measures (see **Table 5** and **Figure 2**). The respective correlation coefficients indicated a high correspondence between the different resting state vmHRV measures, in male [all $r \geq 0.90$, all ICC ≥ 0.95] as well as in female [all $r \geq 0.96$, all ICC ≥ 0.97] participants.

DISCUSSION

In the present study, we investigated whether inter-individual differences in resting state vmHRV would be associated with inter-individual differences in self-reported emotion regulation abilities. Across a series of correlation and regression analyses, we found sex-dependent associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported reappraisal use but not suppression use: Male participants with high resting state vmHRV reported more reappraisal but similar suppression use than male participants with low resting state vmHRV, indicating an increase in self-reported reappraisal but not suppression use with increasing resting state vmHRV. Female participants with high and low resting state vmHRV, on the contrary, did not differ in self-reported reappraisal or suppression use, implying that self-reported reappraisal and suppression use did not increase or decrease with increasing or decreasing resting state vmHRV. Of note, the aforementioned associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported emotion regulation abilities emerged not only in correlation but also in regression analyses that involved short-term as well as ultra-short term measures of resting state vmHRV, indicating the robustness of our findings.

Our findings regarding sex-differences in self-reported emotion regulation abilities are consistent with those of previous studies revealing more self-reported reappraisal and suppression use in male as compared to female participants (Graser et al., 2012; Erreygers and Spooren, 2017; Totzeck et al., 2018). Moreover, our findings complement findings of other studies indicating that female participants report and show more emotionality than male participants (Grossman and Wood, 1993; Kring and Gordon, 1998; Bradley et al., 2001), implying that sex-differences in emotion regulation may account for sex-differences in emotional sensitivity and emotional expressivity. Notwithstanding the role of sex-differences in emotion regulation, it is interesting to note that our findings converge with the findings of a study that investigated the association of inter-individual differences in HRV with inter-individual differences in self-reports regarding the *inability* rather than *ability* to regulate emotions (Williams et al., 2015). In that study, participants with high vmHRV reported fewer difficulties to understand and control emotions than participants with low vmHRV (Williams et al., 2015). As an understanding and control of emotions is more relevant for reappraisal than suppression use (Hofmann and Kashdan, 2010; Totzeck et al., 2018), the findings of that study indicate a similar association of inter-individual differences in vmHRV with inter-individual differences in emotion regulation like the one that has been found in the present study. Although the findings of these studies suggest that inter-individual differences in vmHRV are more associated with inter-individual differences in self-reported reappraisal than self-reported suppression use, it is important to note that both studies relied on self-report measures that lack ecologic validity in comparison to performance measures. Studies that used performance measures, however, revealed similar associations of inter-individual differences in vmHRV with inter-individual differences in reappraisal use (Butler et al., 2006; Vogele et al., 2010; Volokhov and Demaree, 2010; Denson et al., 2012; Berna et al., 2014; Williams et al., 2015). Moreover, these associations were not only found in studies that measured inter-individual differences in vmHRV during rest (Vogele et al., 2010; Volokhov and Demaree, 2010; Williams et al., 2015) but also in studies that measured inter-individual differences in vmHRV during performance (Butler et al., 2006; Denson et al., 2012; Berna et al., 2014). Methodological aspects regarding the measurement of inter-individual differences in HRV and emotion regulation, thus, do not affect the association between inter-individual differences in vmHRV and inter-individual differences in reappraisal use, implying that this association is remarkably robust. Inter-individual differences in vmHRV may, therefore, indeed have the potential to work as a biomarker for inter-individual differences in emotion regulation (Appelhans and Luecken, 2006; Beauchaine, 2015; Holzman and Bridgett, 2017).

With respect to the neurobiological mechanisms underlying associations between inter-individual differences in resting state vmHRV and inter-individual differences in emotion regulation, it is interesting to note that inter-individual differences regarding the activity and integrity of prefrontal and (para-)limbic brain regions are associated with inter-individual differences in emotion regulation (Ochsner et al., 2012; Frank et al., 2014; Etkin et al., 2015) as well as with inter-individual

TABLE 4 | Associations between ultra-short term measures of heartrate variability that had been derived from 60 s lasting heartrate recordings and measures of reappraisal and suppression use.

	ASQ-REA					ASQ-SUP				
	B	*SE B*	95% CI	*t*	*p*	*B*	*SE B*	95% CI	*t*	
Step 1										
Sex	0.35	0.10	[0.15, 0.54]	0.3.36	0.003**	0.24	0.11	[0.02, 0.46]	2.19	0.040*
Age	−0.02	0.05	[−0.11, 0.07]	−0.50	0.627	−0.07	0.05	[−0.17, 0.03]	−1.41	0.162
BMI	0.00	0.06	[−0.11, 0.13]	−0.02	0.991	0.03	0.06	[−0.09, 0.14]	0.58	0.577
BSI-18	−0.16	0.05	[−0.27, -0.08]	−3.46	0.003**	0.21	0.06	[0.08, 0.31]	4.13	0.002
Log-RMSSD-60	0.02	0.05	[−0.07, 0.12]	0.50	0.637	0.00	0.05	[−0.10, 0.10]	−0.01	0.994
Step 2										
Sex	0.34	0.10	[0.13, 0.53]	3.25	0.003	0.24	0.11	[0.02, 0.47]	2.19	0.041*
Age	−0.03	0.05	[−0.12, 0.07]	−0.52	0.612	−0.07	0.05	[−0.17, 0.03]	−1.40	0.158
BMI	0.02	0.06	[−0.10, 0.15]	0.30	0.791	0.03	0.06	[−0.09, 0.15]	0.55	0.595
BSI-18	−0.17	0.05	[−0.28, -0.08]	−3.61	0.002**	0.21	0.06	[0.08, 0.32]	4.12	0.002**
Log-RMSSD-60[a]	−0.08	0.07	[−0.22, 0.06]	−1.23	0.242	0.01	0.07	[−0.14, 0.13]	0.07	0.947
Log-RMSSD-60[a] × Sex	0.21	0.09	[0.04, 0.39]	2.30	0.016*	−0.01	0.09	[−0.20, 0.18]	−0.11	0.898

*ASQ-REA: R^2 = 0.16, F(5,169) = 6.50, p = 0.001, ΔR^2 = 0.03, ΔF(6,168) = 5.30, p = 0.023, ASQ-SUP: R^2 = 0.12, F(5,169) = 4.79, p = 0.001, ΔR^2 = 0.00, ΔF(6,168) = 0.01, p = 0.912. ASQ-REA, Affective Style Questionnaire – Reappraisal Scale [42, 43]; ASQ-SUP, Affective Style Questionnaire – Suppression Scale [42, 43]; BMI, body mass index; BSI-18, Brief Symptom Inventory 18 [41]; 95% CI, 95% confidence interval; Log-RMSSD-60, log transformed root mean square of successive differences between consecutive heart beats that had been derived from 60 s lasting heartrate recordings. [a]Data was missing for one male participant due to technical difficulties. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.*

TABLE 5 | Correspondence between short-term and ultra-short-term measures of heart rate variability.

	Female participants (*n* = 85)				Male participants (*n* = 90)			
	r	95% CI	ICC	95% CI	*r*	95% CI	ICC	95% CI
Log-RMSSD-300 vs. Log-RMSSD-120[a]	0.98	[0.96,0.99]	0.98	[0.96,0.99]	0.94	[0.87,0.97]	0.96	[0.94,0.98]
Log-RMSSD-300 vs. Log-RMSSD-060[a]	0.96	[0.94,0.97]	0.97	[0.93,0.99]	0.90	[0.83,0.95]	0.95	[0.91,0.97]
Log-RMSSD-120[a] vs. Log-RMSSD-060[a]	0.98	[0.96,0.99]	0.99	[0.98,0.99]	0.97	[0.95,0.98]	0.98	[0.98,0.99]

95% CI, 95% confidence interval; Log-RMSSD-300, root mean square of successive differences between consecutive heart beats that had been derived from 300 s lasting heartrate recordings; Log-RMSSD-120, root mean square of successive differences between consecutive heart beats that had been derived from 120 s lasting heartrate recordings; Log-RMSSD-60, root mean square of successive differences between consecutive heart beats that had been derived from 60 s lasting heartrate recordings. [a]Data was missing for one male participant due to technical difficulties.

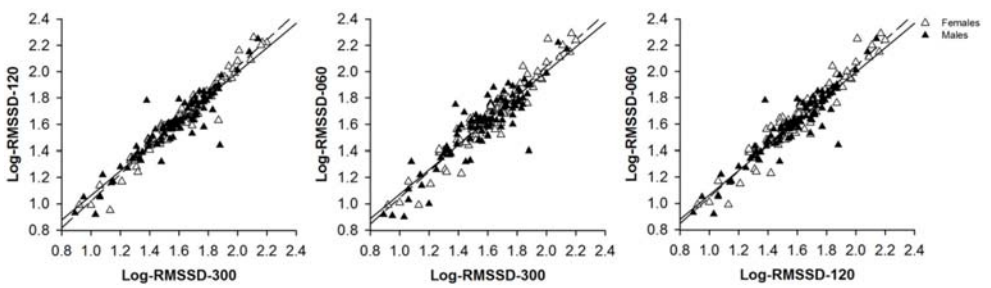

FIGURE 2 | Scatter plots with lines of best fit demonstrating bivariate correlations between (log-transformed) vagally mediated heartrate variability (Log-RMSSD) that was derived from 300 s (Log-RMSSD-300), 120 s (Log-RMSSD-120), or 60 s (Log-RMSSD-060) lasting resting state recordings of male (black triangulars, solid line) and female (white triangulars, dashed line) participants' heart rate.

differences in vmHRV (Thayer and Lane, 2009; Thayer et al., 2012; Smith et al., 2017). Of these brain regions, prefrontal ones, like, for example, the dorsolateral and ventrolateral prefrontal cortex or the anterior cingulate cortex, and (para-)limbic ones, like, for example, the amygdala and the insula, appear to be of particular relevance. Imaging studies revealed that an increase in prefrontal activity and a decrease in (para-)limbic activity is more likely to occur during reappraisal than suppression use (Goldin et al., 2008; Drabant et al., 2009; McRae et al., 2010; Vanderhasselt et al., 2013; Nelson et al., 2015), indicating a more efficient inhibition of (para-)limbic brain regions by prefrontal brain regions via an

increased coupling of these brain regions in the context of reappraisal use (Ochsner et al., 2002; Banks et al., 2007; Morawetz et al., 2017). However, imaging studies also revealed that an increase in prefrontal activity and a decrease in (para-)limbic activity is accompanied by an increase in vmHRV (Gianaros et al., 2004; Lane et al., 2009; Sakaki et al., 2016). These studies even showed that an increased coupling of prefrontal and (para-)limbic brain regions, which is thought to be crucial for successful emotion regulation following reappraisal use (Ochsner et al., 2012), is also accompanied by an increase in vmHRV (Sakaki et al., 2016). On basis of these findings, it may be assumed that inter-individual differences in vmHRV reflect inter-individual differences in reappraisal use that are mediated by inter-individual differences regarding the inhibition of (para-)limbic brain regions by prefrontal brain regions. Inter-individual differences in vmHRV may, thus, indeed serve as a biomarker for inter-individual differences in emotion regulation (Appelhans and Luecken, 2006; Beauchaine, 2015; Holzman and Bridgett, 2017).

Despite the plausibility of these assumptions, it has to be acknowledged that they are only partially supported by the findings of the present and previous studies. Although previous studies demonstrated that inter-individual differences in prefrontal-(para-)limbic engagement are associated with inter-individual differences vmHRV and that inter-individual differences in prefrontal-(para-)limbic engagement are associated with inter-individual differences in emotion regulation, these studies *either* focused on inter-individual differences in emotion regulation (Goldin et al., 2008; Drabant et al., 2009; McRae et al., 2010; Vanderhasselt et al., 2013; Nelson et al., 2015) *or* on inter-individual differences in vmHRV (Gianaros et al., 2004; Lane et al., 2009; Sakaki et al., 2016). It, thus, remains open whether inter-individual differences in prefrontal-(para-)limbic engagement in fact account for associations between inter-individual differences in vmHRV and inter-individual differences in emotion regulation. It is also unclear whether the assumed associations emerge in a similar way under active as under passive conditions because previous studies *either* employed resting state (Williams et al., 2015) *or* task based (Butler et al., 2006; Vogele et al., 2010; Volokhov and Demaree, 2010; Denson et al., 2012; Berna et al., 2014) measures in their investigations. Consequently, there is a need for a *combined* assessment of inter-individual differences in prefrontal-(para-)limbic engagement, inter-individual differences in vmHRV and inter-individual differences in emotion regulation under various conditions, passive as well as active ones. In this respect, it is noteworthy that previous studies measured inter-individual differences in vmHRV on basis of HRV measures that were derived from short-term HR recordings (Butler et al., 2006; Vogele et al., 2010; Volokhov and Demaree, 2010; Denson et al., 2012; Berna et al., 2014; Williams et al., 2015) and that the present study measured inter-individual differences in vmHRV on basis of HRV measures that were derived from short-term as well as ultra-short-term HR recordings. Although the findings of the present and previous studies suggest a high correspondence between short-term and ultra-short-term vmHRV measures (Munoz et al., 2015; Lischke et al., 2018b), it remains to be determined whether short-term measures can be substituted by ultra-short-term measures in studies like the present one (Pecchia et al., 2018). Future studies that assess the correspondence between ultra-short-term and short-term vmHRV measures in larger participant samples with more sophisticated methods than in the present study may be useful to delineate the conditions under which ultra-short-term vmHRV measures can be used as a substitute for short-term vmHRV measures. However, vmHRV measures may not be the only measure that may be useful for an assessment of inter-individual differences in emotion regulation. Previous studies revealed associations between inter-individual differences in pupil size (PLS) and inter-individual differences in emotion regulation that were mediated by inter-individual differences in prefrontal-(para-)limbic engagement (Urry et al., 2006, 2009), implying that inter-individual differences in pupil size may also work as a biomarker for inter-individual differences in emotion regulation. Moreover, previous studies also suggest that inter-individual differences in PLS co-vary with inter-individual differences in vmHRV (Park et al., 2018). It may, thus, be worthwhile to consider not only measures of vmHRV but also measures of PLS in future studies that are concerned with the identification of brain-based biomarkers of emotion regulation.

Notwithstanding that further studies are needed to replicate and extend the findings of the present study, we tentatively suggest that the neurobiological mechanisms underlying inter-individual differences in emotion regulation do not necessarily have to be investigated with techniques that require dedicated staff or equipment (Beauchaine, 2015). First of all, inter-individual differences in prefrontal-(para-)limbic control that account for inter-individual differences in emotion regulation may be assessed with measurements of cardiac activity that are less time- and resource-consuming than measurements of neural activity. Second, measurements of cardiac activity that represent inter-individual differences in vmHRV may be assessed on basis of ultra-short-term resting state vmHR recordings. There was not only a remarkable correspondence between ultra-short-term and short-term measurements of resting state vmHRV regarding the measurement of inter-individual differences in resting state vmHRV but also regarding the association between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported emotion regulation abilities. Ultra-short-term measures of resting state vmHRV have already been shown to be a valid a reliable alternative to short-term measures of resting state vmHRV (Munoz et al., 2015; Lischke et al., 2018b), indicating that ultra-short term measures may be used as time-saving alternative to short-term measures under certain conditions. Third, inter-individual differences in emotion regulation may be assessed on basis of self-report measures whose scores are associated with inter-individual differences in emotion regulation on the behavioral and neurobiological level (Drabant et al., 2009; Szasz et al., 2011; Nelson et al., 2015). Well-validated self-report measures, such as the ASQ (Hofmann and Kashdan, 2010; Graser et al., 2012) or

ERQ (Gross and John, 2003; Abler and Kessler, 2009), may be used as a time- and resource-saving alternative to more complex emotion regulation tasks (Gross, 1998; Hofmann et al., 2009). Combining (ultra-)short-term measures of resting state vmHRV with short self-report measures of emotion regulation may, therefore, be interesting for researchers who need to investigate the neurobiological mechanisms underlying inter-individual differences in emotion regulation in a time- and resource-efficient manner. However, even if time and resources are not scarce, it may be valuable to combine the aforementioned measures with other measures to fully elucidate the behavioral and neurobiological correlates of emotion regulation.

In the present study, we found sex-specific associations between inter-individual differences in resting state vmHRV and inter-individual differences in self-reported reappraisal but not suppression use. As we did not assess neural and behavioral correlates of emotion regulation in our study, we can only assume that these associations reflect inter-individual differences in prefrontal-(para-)limbic engagement during emotion regulation (Thayer and Lane, 2009; Thayer et al., 2012; Smith et al., 2017). However, similar associations between inter-individual differences in emotion regulation and inter-individual differences in vmHRV have been found in previous studies (Butler et al., 2006; Denson et al., 2012; Vogele et al., 2010; Volokhov and Demaree, 2010; Berna et al., 2014; Williams et al., 2015), supporting theoretical claims that inter-individual differences in vmHRV serve as a biomarker for inter-individual differences in emotion regulation (Appelhans and Luecken, 2006; Beauchaine, 2015; Holzman and Bridgett, 2017). Future studies are now warranted that further investigate whether measurements of cardiac activity are in fact a time- and resource-saving alternative to measurements of neural activity in the search for biomarkers indicating deficits in emotion regulation (Beauchaine, 2015).

AUTHOR CONTRIBUTIONS

AL and RP designed the study. AL, AM-M, MW, RJ, and SP collected the data. AL and RP analyzed the data. AL and RP wrote the manuscript. AH, AM-M, MW, RJ, and SP contributed to writing, reviewing and editing of the manuscript. All authors approved the final version of the manuscript.

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REFERENCES

Abhishekh, H. A., Nisarga, P., Kisan, R., Meghana, A., Chandran, S., Trichur, R., et al. (2013). Influence of age and gender on autonomic regulation of heart. *J. Clin. Monit. Comput.* 27, 259–264. doi: 10.1007/s10877-012-9424-3

Abler, B., and Kessler, H. (2009). Emotion regulation questionnaire–Eine deutschsprachige Fassung des ERQ von Gross und John. *Diagnostica* 55, 144–152. doi: 10.1026/0012-1924.55.3.144

Aiken, L. S., and West, S. G. (1991). *Multiple Regression: Testing and Interpreting Interactions*. London: Sage.

Aldao, A., and Nolen-Hoeksema, S. (2010). Specificity of cognitive emotion regulation strategies: a transdiagnostic examination. *Behav. Res. Ther.* 48, 974–983. doi: 10.1016/j.brat.2010.06.002

Aldao, A., Nolen-Hoeksema, S., and Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: a meta-analytic review. *Clin. Psychol. Rev.* 30, 217–237. doi: 10.1016/j.cpr.2009.11.004

Appelhans, B. M., and Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Rev. Gen. Psychol.* 10, 229–240. doi: 10.1037/1089-2680.10.3.229

Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J., and Phan, K. L. (2007). Amygdala-frontal connectivity during emotion regulation. *Soc. Cogn. Affect. Neurosci.* 2, 303–312. doi: 10.1093/scan/nsm029

Beauchaine, T. P. (2015). Respiratory Sinus Arrhythmia: a transdiagnostic biomarker of emotion dysregulation and psychopathology. *Curr. Opin. Psychol.* 3, 43–47. doi: 10.1016/j.copsyc.2015.01.017

Berna, G., Ott, L., and Nandrino, J. L. (2014). Effects of emotion regulation difficulties on the tonic and phasic cardiac autonomic response. *PLoS One* 9:e102971. doi: 10.1371/journal.pone.0102971

Bertsch, K., Hagemann, D., Naumann, E., Schachinger, H., and Schulz, A. (2012). Stability of heart rate variability indices reflecting parasympathetic activity. *Psychophysiology* 49, 672–682. doi: 10.1111/j.1469-8986.2011.01341.x

Bradley, M. M., Codispoti, M., Sabatinelli, D., and Lang, P. J. (2001). Emotion and motivation II: sex differences in picture processing. *Emotion* 1, 300–319. doi: 10.1037/1528-3542.1.3.300

Butler, A. C., Chapman, J. E., Forman, E. M., and Beck, A. T. (2006). The empirical status of cognitive-behavioral therapy: a review of meta-analyses. *Clin. Psychol. Rev.* 26, 17–31. doi: 10.1016/j.cpr.2005.07.003

Butler, E. A., Egloff, B., Wilhelm, F. H., Smith, N. C., Erickson, E. A., and Gross, J. J. (2003). The social consequences of expressive suppression. *Emotion* 3, 48–67. doi: 10.1037/1528-3542.3.1.48

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Earlbaum.

Cumming, G. (2013). *Understanding the New Statistics: Effect Sizes, Confidence Intervals, and Meta-Analysis*. New York, NY: Routledge. doi: 10.4324/9780203807002

Denson, T. F., Moulds, M. L., and Grisham, J. R. (2012). The effects of analytical rumination, reappraisal, and distraction on anger experience. *Behav. Ther.* 43, 355–364. doi: 10.1016/j.beth.2011.08.001

Drabant, E. M., McRae, K., Manuck, S. B., Hariri, A. R., and Gross, J. J. (2009). Individual differences in typical reappraisal use predict amygdala and prefrontal responses. *Biol. Psychiatry* 65, 367–373. doi: 10.1016/j.biopsych.2008.09.007

English, T., John, O. P., Srivastava, S., and Gross, J. J. (2012). Emotion regulation and peer-rated social functioning: a four-year longitudinal study. *J. Res. Pers.* 46, 780–784. doi: 10.1016/j.jrp.2012.09.006

Erreygers, S., and Spooren, P. (2017). Factor structure of the Affective Style Questionnaire in Flemish adolescents. *Psychol. Belg.* 57, 112–122. doi: 10.5334/pb.369

Etkin, A., Buchel, C., and Gross, J. J. (2015). The neural bases of emotion regulation. *Nat. Rev. Neurosci.* 16, 693–700. doi: 10.1038/nrn4044

Fabes, R. A., and Eisenberg, N. (1997). Regulatory control and adults' stress-related responses to daily life events. *J. Pers. Soc. Psychol.* 73, 1107–1117. doi: 10.1037/0022-3514.73.5.1107

Faul, F., Erdfelder, E., Lang, A. G., and Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi: 10.3758/BF03193146

Fleiss, J. L., Levin, B., and Paik, M. C. (2013). *Statistical Methods for Rates and Proportions*. New York, NY: John Wiley & Sons.

Frank, D. W., Dewitt, M., Hudgens-Haney, M., Schaeffer, D. J., Ball, B. H., Schwarz, N. F., et al. (2014). Emotion regulation: quantitative meta-analysis of functional activation and deactivation. *Neurosci. Biobehav. Rev.* 45, 202–211. doi: 10.1016/j.neubiorev.2014.06.010

Franke, G. H., Jaeger, S., Glaesmer, H., Barkmann, C., Petrowski, K., and Braehler, E. (2017). Psychometric analysis of the brief symptom inventory 18 (BSI-18) in a representative German sample. *BMC Med. Res. Methodol.* 17:14. doi: 10.1186/s12874-016-0283-3

Garnefski, N., Kraaij, V., and Spinhoven, P. (2001). Negative life events, cognitive emotion regulation and emotional problems. *Pers. Individ. Dif.* 30, 1311–1327. doi: 10.1016/S0191-8869(00)00113-6

Gianaros, P. J., Van Der Veen, F. M., and Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology* 41, 521–530. doi: 10.1111/1469-8986.2004.00179.x

Goldin, P. R., McRae, K., Ramel, W., and Gross, J. J. (2008). The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biol. Psychiatry* 63, 577–586. doi: 10.1016/j.biopsych.2007.05.031

Graser, J., Bohn, C., Kelava, A., Schreiber, F., Hofmann, S. G., and Stangier, U. (2012). [The "Affective Style Questionnaire (ASQ)": German Adaptation and Validity]. *Diagnostica* 58, 100–111. doi: 10.1026/0012-1924/a000056

Gross, J. J. (1998). Antecedent- and response-focused emotion regulation: divergent consequences for experience, expression, and physiology. *J. Pers. Soc. Psychol.* 74, 224–237. doi: 10.1037/0022-3514.74.1.224

Gross, J. J. (2002). Emotion regulation: affective, cognitive, and social consequences. *Psychophysiology* 39, 281–291. doi: 10.1017/S0048577201393198

Gross, J. J., and John, O. P. (2003). Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being. *J. Pers. Soc. Psychol.* 85, 348–362. doi: 10.1037/0022-3514.85.2.348

Grossman, M., and Wood, W. (1993). Sex differences in intensity of emotional experience: a social role interpretation. *J. Pers. Soc. Psychol.* 65, 1010–1022. doi: 10.1037/0022-3514.65.5.1010

Hofmann, S. G., Heering, S., Sawyer, A. T., and Asnaani, A. (2009). How to handle anxiety: the effects of reappraisal, acceptance, and suppression strategies on anxious arousal. *Behav. Res. Ther.* 47, 389–394. doi: 10.1016/j.brat.2009.02.010

Hofmann, S. G., and Kashdan, T. B. (2010). The Affective Style Questionnaire: development and psychometric properties. *J. Psychopathol. Behav. Assess.* 32, 255–263. doi: 10.1007/s10862-009-9142-4

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74(Pt A), 233–255. doi: 10.1016/j.neubiorev.2016.12.032

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., et al. (2014). Body mass index is related to autonomic nervous system activity as measured by heart rate variability-a replication using short term measurements. *J. Nutr. Health Aging* 18, 300–302. doi: 10.1007/s12603-014-0022-6

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Kogan, A., Gruber, J., Shallcross, A. J., Ford, B. Q., and Mauss, I. B. (2013). Too much of a good thing? Cardiac vagal tone's nonlinear relationship with well-being. *Emotion* 13, 599–604. doi: 10.1037/a0032725

Kok, B. E., and Fredrickson, B. L. (2010). Upward spirals of the heart: autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Kring, A. M., and Gordon, A. H. (1998). Sex differences in emotion: expression, experience, and physiology. *J. Pers. Soc. Psychol.* 74, 686–703. doi: 10.1037/0022-3514.74.3.686

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., and Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213–222. doi: 10.1016/j.neuroimage.2008.07.056

Licht, C. M., de Geus, E. J., van Dyck, R., and Penninx, B. W. (2009). Association between anxiety disorders and heart rate variability in The Netherlands Study of Depression and Anxiety (NESDA). *Psychosom. Med.* 71, 508–518. doi: 10.1097/PSY.0b013e3181a292a6

Licht, C. M., de Geus, E. J., Zitman, F. G., Hoogendijk, W. J., van Dyck, R., and Penninx, B. W. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch. Gen. Psychiatry* 65, 1358–1367. doi: 10.1001/archpsyc.65.12.1358

Lischke, A., Jacksteit, R., Mau-Moeller, A., Pahnke, R., Hamm, A. O., and Weippert, M. (2018a). Heart rate variability is associated with psychosocial stress in distinct social domains. *J. Psychosom. Res.* 106, 56–61. doi: 10.1016/j.jpsychores.2018.01.005

Lischke, A., Mau-Moeller, A., Jacksteit, R., Pahnke, R., Hamm, A. O., and Weippert, M. (2018b). Heart rate variability is associated with social value orientation in males but not females. *Sci. Rep.* 8:7336. doi: 10.1038/s41598-018-25739-4

Martin, R. C., and Dahlen, E. R. (2005). Cognitive emotion regulation in the prediction of depression, anxiety, stress, and anger. *Pers. Individ. Dif.* 39, 1249–1260. doi: 10.1016/j.paid.2005.06.004

McRae, K., Hughes, B., Chopra, S., Gabrieli, J. D., Gross, J. J., and Ochsner, K. N. (2010). The neural bases of distraction and reappraisal. *J. Cogn. Neurosci.* 22, 248–262. doi: 10.1162/jocn.2009.21243

Moore, S. A., Zoellner, L. A., and Mollenholt, N. (2008). Are expressive suppression and cognitive reappraisal associated with stress-related symptoms? *Behav. Res. Ther.* 46, 993–1000. doi: 10.1016/j.brat.2008.05.001

Morawetz, C., Bode, S., Baudewig, J., and Heekeren, H. R. (2017). Effective amygdala-prefrontal connectivity predicts individual differences in successful emotion regulation. *Soc. Cogn. Affect. Neurosci.* 12, 569–585. doi: 10.1093/scan/nsw169

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (ultra-)short recordings for heart rate variability measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Nelson, B. D., Fitzgerald, D. A., Klumpp, H., Shankman, S. A., and Phan, K. L. (2015). Prefrontal engagement by cognitive reappraisal of negative faces. *Behav. Brain Res.* 279, 218–225. doi: 10.1016/j.bbr.2014.11.034

Nolen-Hoeksema, S. (2012). Emotion regulation and psychopathology: the role of gender. *Annu. Rev. Clin. Psychol.* 8, 161–187. doi: 10.1146/annurev-clinpsy-032511-143109

Ochsner, K. N., Bunge, S. A., Gross, J. J., and Gabrieli, J. D. (2002). Rethinking feelings: an fMRI study of the cognitive regulation of emotion. *J. Cogn. Neurosci.* 14, 1215–1229. doi: 10.1162/089892902760807212

Ochsner, K. N., Silvers, J. A., and Buhle, J. T. (2012). Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Ann. N. Y. Acad. Sci.* 1251, E1–E24. doi: 10.1111/j.1749-6632.2012.06751.x

Oveis, C., Cohen, A. B., Gruber, J., Shiota, M. N., Haidt, J., and Keltner, D. (2009). Resting respiratory sinus arrhythmia is associated with tonic positive emotionality. *Emotion* 9, 265–270. doi: 10.1037/a0015383

Park, S., Won, M. J., Lee, D. W., and Whang, M. (2018). Non-contact measurement of heart response reflected in human eye. *Int. J. Psychophysiol.* 123, 179–198. doi: 10.1016/j.ijpsycho.2017.07.014

Pecchia, L., Castaldo, R., Montesinos, L., and Melillo, P. (2018). Are ultra-short heart rate variability features good surrogates of short-term ones? State-of-the-art review and recommendations. *Healthc. Technol. Lett.* 5, 94–100. doi: 10.1049/htl.2017.0090

Penttila, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Prinz, U., Nutzinger, D. O., Schulz, H., Petermann, F., Braukhaus, C., and Andreas, S. (2013). Comparative psychometric analyses of the SCL-90-R and

its short versions in patients with affective disorders. *BMC Psychiatry* 13:104. doi: 10.1186/1471-244X-13-104

Quintana, D. S., Alvares, G. A., and Heathers, J. A. (2016). Guidelines for Reporting Articles on Psychiatry and Heart rate variability (GRAPH): recommendations to advance research communication. *Transl. Psychiatry* 6:e803. doi: 10.1038/tp.2016.73

Quintana, D. S., and Heathers, J. A. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805. doi: 10.3389/fpsyg.2014.00805

Quintana, D. S., Heathers, J. A., and Kemp, A. H. (2012). On the validity of using the Polar RS800 heart rate monitor for heart rate variability research. *Eur. J. Appl. Physiol.* 112, 4179–4180. doi: 10.1007/s00421-012-2453-2

Sakaki, M., Yoo, H. J., Nga, L., Lee, T., Thayer, J. F., and Mather, M. (2016). Heart rate variability is associated with amygdala functional connectivity with MPFC across younger and older adults. *Neuroimage* 139, 44–52. doi: 10.1016/j.neuroimage.2016.05.076

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Sheppes, G., Suri, G., and Gross, J. J. (2015). Emotion regulation and psychopathology. *Annu. Rev. Clin. Psychol.* 11, 379–405. doi: 10.1146/annurev-clinpsy-032814-112739

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Szasz, P. L., Szentagotai, A., and Hofmann, S. G. (2011). The effect of emotion regulation strategies on anger. *Behav. Res. Ther.* 49, 114–119. doi: 10.1016/j.brat.2010.11.011

Szasz, P. L., Szentagotai, A., and Hofmann, S. G. (2012). Effects of emotion regulation strategies on smoking craving, attentional bias, and task persistence. *Behav. Res. Ther.* 50, 333–340. doi: 10.1016/j.brat.2012.02.010

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Task Force of the European Society of Cardiology (1996). Heart rate variability standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Totzeck, C., Teismann, T., Hofmann, S. G., von Brachel, R., Zhang, X. C., Pflug, V., et al. (2018). Affective styles in mood and anxiety disorders - Clinical validation of the "Affective Style Questionnaire" (ASQ). *J. Affect. Disord.* 238, 392–398. doi: 10.1016/j.jad.2018.05.035

Urry, H. L., van Reekum, C. M., Johnstone, T., and Davidson, R. J. (2009). Individual differences in some (but not all) medial prefrontal regions reflect cognitive demand while regulating unpleasant emotion. *Neuroimage* 47, 852–863. doi: 10.1016/j.neuroimage.2009.05.069

Urry, H. L., van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., et al. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J. Neurosci.* 26, 4415–4425. doi: 10.1523/JNEUROSCI.3215-05.2006

Vanderhasselt, M. A., Baeken, C., Van Schuerbeek, P., Luypaert, R., and De Raedt, R. (2013). Inter-individual differences in the habitual use of cognitive reappraisal and expressive suppression are associated with variations in prefrontal cognitive control for emotional information: an event related fMRI study. *Biol. Psychol.* 92, 433–439. doi: 10.1016/j.biopsycho.2012.03.005

Vogele, C., Sorg, S., Studtmann, M., and Weber, H. (2010). Cardiac autonomic regulation and anger coping in adolescents. *Biol. Psychol.* 85, 465–471. doi: 10.1016/j.biopsycho.2010.09.010

Volokhov, R. N., and Demaree, H. A. (2010). Spontaneous emotion regulation to positive and negative stimuli. *Brain Cogn.* 73, 1–6. doi: 10.1016/j.bandc.2009.10.015

Weippert, M., Kumar, M., Kreuzfeld, S., Arndt, D., Rieger, A., and Stoll, R. (2010). Comparison of three mobile devices for measuring R-R intervals and heart rate variability: polar S810i, Suunto t6 and an ambulatory ECG system. *Eur. J. Appl. Physiol.* 109, 779–786. doi: 10.1007/s00421-010-1415-9

Williams, D. P., Cash, C., Rankin, C., Bernardi, A., Koenig, J., and Thayer, J. F. (2015). Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Front. Psychol.* 6:261. doi: 10.3389/fpsyg.2015.00261

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COMTVal158Met Genotype Affects Complex Emotion Recognition in Healthy Men and Women

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The catechol-o-methyltransferase (COMT) gene has repeatedly been shown to change amygdala activity and amygdala-prefrontal connectivity during face processing. Although the COMT gene appears to induce a negativity bias during the neural processing of faces, it is currently unclear whether a similar negativity bias emerges during the behavioral processing of faces. To address this issue, we investigated differences in complex emotion recognition between participants (n = 181) that had been *a priori* genotyped for functional polymorphisms of the COMT (Val158Met) and serotonin transporter (5-HTTLPR) gene. We were, thus, able to analyze differences in face processing on basis of participants' COMT genotype while controlling for participants' 5-HTTLPR genotype. Variations of participants' COMT but not 5-HTTLPR genotype accounted for differences in participants' emotion recognition performance: Met/Met carriers and Met/Val carriers were more accurate in the recognition of negative, but not neutral or positive, expressions than Val/Val carriers. We, therefore, revealed a similar negativity bias during the behavioral processing of faces that has already been demonstrated during the neural processing of faces, indicating that genotype-dependent changes in catecholamine metabolism may affect face processing on the behavioral and neural level.

Keywords: COMT, catecholamine, 5-HTTLPR, serotonin, emotion recognition, social cognition

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INTRODUCTION

Over the last two decades, there has been a growing interest to determine the genetic basis of social behavior (Ebstein et al., 2010). Social behavior crucially depends on the processing of facial cues providing information about others' intentions, thoughts and emotions. Consequently, much research has been devoted to delineate the genetic mechanisms underlying face processing (Niedenthal and Brauer, 2012). However, most research dealt with these mechanisms on the neural not behavioral level, presumably because neural processes are more susceptible to genetic variations than behavioral processes (Hariri and Weinberger, 2003). As a result, we know a lot about the genetic modulation of neural activity during face processing, but almost nothing about the behavioral consequences of this genetic modulation.

Of the various genes implicated in face processing, the catechol-o-methyltransferase (COMT) gene appears to be of particular relevance (Montag et al., 2012). The COMT gene regulates

the extracellular degradation of catecholamines (dopamine, norepinephrine, and epinephrine). A single nucleotide polymorphism predicts the substitution of amino acid methionine (Met) for valine (Val) at codon 158 (VAL158MET), which results in a threefold to fourfold reduction of catecholamine degradation in Met as compared to Val carriers (Lachman et al., 1996). The associated differences in extracellular catecholamine levels appear to account for neural differences in face processing as suggested by imaging studies revealing increased amygdala activity (Williams et al., 2010; Lonsdorf et al., 2011) and increased amygdala-prefrontal connectivity (Surguladze et al., 2012) in response to negative expressions in Met carriers. Met carriers, thus, show an enhanced processing of negative expressions, indicating a negativity bias during face processing. However, such a negativity bias has not always been found in behavioral studies (Weiss et al., 2007; Defrancesco et al., 2011; Gohier et al., 2014). Two studies failed to find robust differences in emotion recognition between Met and Val carriers (Weiss et al., 2007; Defrancesco et al., 2011), whereas a third study revealed that Met carriers misperceived neutral expressions as negative ones (Gohier et al., 2014). Met carriers may, thus, not only show an enhanced processing of negative expressions as suggested by the imaging studies (Williams et al., 2010; Lonsdorf et al., 2011; Surguladze et al., 2012) but also a negatively tuned processing of neutral expression as suggested by one of the behavioral studies (Gohier et al., 2014). In this respect, it is important to note that this behavioral study (Gohier et al., 2014) differed markedly from the other two behavioral studies (Weiss et al., 2007; Defrancesco et al., 2011) in terms of sample size (e.g., inclusion of more than 100 participants), genotype frequencies (e.g., consideration of COMT and 5-HTTLPR polymorphisms), task design (e.g., presentation of expressions with varying emotional intensity) and data analysis (e.g., control of multiple comparisons). Methodological differences between the behavioral studies may, thus, have accounted for the inconsistent findings regarding Met carriers negativity bias during face processing. Consequently, there is a need for studies that investigate differences in emotion recognition between Met and Val carriers with more methodological rigor.

In the present study, we further investigated whether Met and Val carriers differ in emotion recognition. In contrast to previous studies (Weiss et al., 2007; Defrancesco et al., 2011; Gohier et al., 2014), we employed a task that required the recognition of complex rather than basic emotional expressions (Reading the Mind in the Eyes Test, RMET; Baron-Cohen et al., 2001). We decided to use complex expressions for our task because these expressions resemble more the type of expressions one encounters throughout social interactions than basic expressions (Zelenski and Larsen, 2000). The set of complex expressions is also much larger than the set of basic expressions (Cordaro et al., 2018), which usually comprises six different expressions (Ekman et al., 1969). Due to the large number of different expressions, our task was far more challenging than the tasks that had been employed in previous studies (Weiss et al., 2007; Defrancesco et al., 2011). We, thus, expected to detect subtle differences in emotion recognition, which may have not been the case in previous studies (Weiss et al., 2007; Defrancesco et al., 2011). The task was administered to a sample of participants that had been *a priori* genotyped for functional polymorphisms of the COMT and serotonin transporter (5-HTTLPR) gene. We *simultaneously* considered participants' COMT and 5-HTTLPR genotype in our analyses because some studies suggest that the 5-HTTLPR genotype also affects face processing (Lonsdorf et al., 2011; Surguladze et al., 2012; Gohier et al., 2014). These analyses were based on an *a priori* power analysis and corrected for multiple comparisons to guard of false positive or false negative findings, indicating that our analyses were liberal and conservative enough to detect meaningful differences in face processing. As the aforementioned studies suggest that the negativity bias in face processing is more pronounced in Met than Val carriers (Williams et al., 2010; Lonsdorf et al., 2011; Surguladze et al., 2012; Gohier et al., 2014), we expected Met carriers to recognize more negative expressions than Val carriers.

MATERIALS AND METHODS

Participants

Previous studies investigated how the COMT genotype modulated face processing in young to middle-aged participants of European descent (Weiss et al., 2007; Williams et al., 2010; Defrancesco et al., 2011; Lonsdorf et al., 2011; Surguladze et al., 2012; Gohier et al., 2014). We, thus, decided to include participants with an European background and an age range of 18–40 years in our study. Although none of the participants appeared to be of Asian descent, we did not formally check whether participants were indeed Caucasians. As the emotion recognition task required a fluent understanding of German, we excluded participants from the study whose native language was not German. In order to estimate the minimum number of participants that we needed to detect differences in emotion recognition on basis of participants' COMT and 5-HTTLPR genotype, we performed an *a priori* power analysis with the freely available program G*Power (Faul et al., 2007). Of note, as we were only interested in genotype- not allel-dependent differences in emotion processing, we solely based our power analysis on participants' genotype. G*Power indicated that we had to recruit at least 144 participants to have sufficient power ($1\text{-}\beta = 0.80$, $\alpha = 0.05$) to detect medium-sized differences in emotion recognition ($f = 0.25$) in a multi-factorial analysis of variance (ANOVA) with the within-subjects factor expression valence and the between-subjects factors genotype. Allowing for attrition, we recruited 181 participants from a database of healthy volunteers who had been *a priori* genotyped for functional polymorphisms of the COMT and 5-HTTLPR gene (Wendt et al., 2015). All participants provided written-informed consent for the study protocol, which was approved by the ethics committee of the University of Greifswald and carried out in accordance with the Declaration of Helsinki.

Genotyping

Details regarding the genotyping procedure can be found elsewhere (Wendt et al., 2015). In brief, standard procedures

were used to extract DNA from whole blood (Autopure LS System, Gentra Systems, Minneapolis, MN, United States), a 5′-exonuclease TaqMan® assay (C_25746809; Applied Biosystems, Foster City, CA, United States) was used for genotyping of the COMT VAL158MET (rs4680) polymorphism and polymerase chain reaction primers (forward 5′-TGAATGCCAGCACCTAACCCCTAA-3′, reverse 5′-GAATACTGGTAGGGTGCAAGGAGA-3; Thermo Scientific, Ulm, Germany) were used for genotyping of the triallelic 5-HTTLPR (5-HTTLPR/rs255331) polymorphism. Genotyping of the COMT VAL158MET polymorphism resulted in 54 Met/Met, 86 Met/Val and 41 Val/Val carriers, while genotyping of 5-HTTPLPR polymorphism resulted in 44 s/s, 85 s/l, and 52 l/l carriers. The distribution of the different COMT and 5-HTTLPR genotypes is illustrated in **Tables 1**, **2**.

Psychopathology

The Brief Symptom inventory (BSI-18; Franke et al., 2017) was used to asses participants' psychopathological distress at the time of the study. The BSI-18, which measures anxious, depressive and somatoform symptoms within the last 7 days, demonstrated good psychometric properties [BSI-18: $\alpha = 0.82$].

Emotion Recognition

A computerized version of the Reading the Mind in the Eyes Test (RMET; Baron-Cohen et al., 2001) was used to assess participants' emotion recognition abilities. Whereas other emotion recognition tasks, like, for example, the morphed emotion recognition test (Lischke et al., 2012), required the recognition of basic emotional expressions (e.g., fear or happiness), the RMET required the recognition of complex emotional expressions (e.g., contempt or pride). The complex expressions had to be recognized on basis of subtle cues that were provided by the eye region of faces. These eye regions were randomly presented in form of 37 different black and white pictures (1 picture was used for practice and 36 pictures were used for testing). Each eye region was shown together with four labels describing distinct emotional expressions (see **Figure 1**). One label described the depicted emotional expression (target label), whereas three other labels described emotional expressions that did not correspond to the depicted emotional expression (distractor labels). Participants had to select the label that best described the emotional expression by pressing a corresponding button as fast as possible. Similar as in previous studies (Hysek et al., 2012; Lischke et al., 2017; Pahnke et al., 2018), an established algorithm was used to determine the percentage of correctly identified positive, negative and neutral expressions on basis of participants' responses (Harkness et al., 1999).

TABLE 1 | Genotype distribution.

	COMT VAL158MET		
	Met/Met carriers	Met/Val carriers	Val/Val carriers
5-HTTLPR	***N***	***N***	***N***
s/s carriers	16	22	6
s/l carriers	23	36	26
l/l carriers	15	28	9

TABLE 2 | Participant characteristics.

	Sex (m/f)	Age (years)		Psychopathology (BSI-18-GSI)	
	N	***M***	***SEM***	***M***	***SEM***
COMT VAL158MET					
Met/Met carriers	25/29	26.28	0.44	0.35	0.38
Met/Val carriers	47/39	26.18	0.35	0.27	0.28
Val/Val carriers	24/17	27.84	0.60	0.26	0.22
5-HTTLPR					
s/s carriers	23/21	26.18	0.56	0.26	0.27
s/l carriers	41/44	27.03	0.35	0.30	0.29
l/l carriers	32/20	27.10	0.49	0.29	0.26

m, male; f, female; BSI-18-GSI, brief symptom inventory 18 global severity index (Franke et al., 2017).

Statistical Analysis

SPSS 22 (SPSS Inc., Chicago, IL, United States) was used for all analyses. Chi-square tests and 3 × 3 ANOVAs (COMT genotype × 5-HTTLPR genotype) were run to investigate genotype dependent differences in participants' age, sex and psychopathology. A 3 × 3 × 3 ANOVA (COMT genotype × 5-HTTLPR genotype × Expression Valence) was run to investigate genotype dependent differences in participants' emotion recognition. The significance level for all analyses was set at $p \leq 0.05$ (two-sided) and, if appropriate, corrected for multiple comparisons using the Bonferroni method (Shaffer, 1995). Partial eta squared (η_p^2) was reported as an effect size measure to facilitate the interpretation of significant findings (Cohen, 1988).

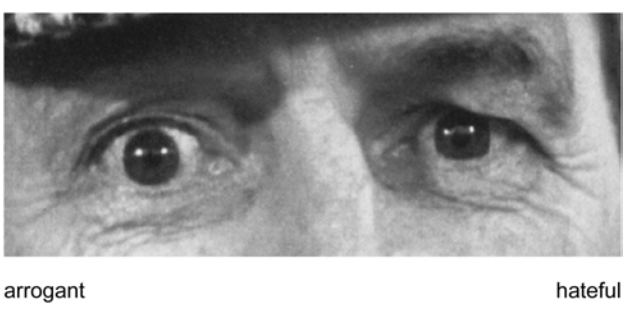

FIGURE 1 | Example of a black and white picture that was used in the Reading the Mind in the Eyes test (Baron-Cohen et al., 2001). The picture shows an eye region and labels that describe four different expressions (one target label, three distractor labels). Participants had to identify the label that correctly described the depicted expression (panicked).

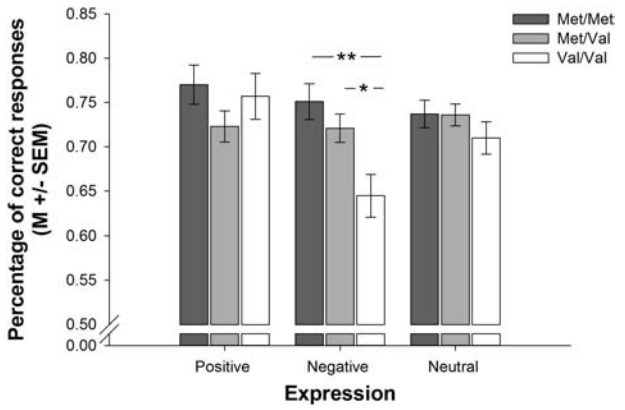

FIGURE 2 | Barplots showing differences in complex emotion recognition as a function of COMT genotype. Met/Met and Met/Val carriers were more accurate in the recognition of negative expressions than Val/Val carriers. Bars represent M +/– SEM. $*p \leq 0.05$; $**p \leq 0.001$.

RESULTS

Participant Characteristics

Chi-square tests revealed a comparable proportion of participants with differences in 5-HTTLPR genotype among participants with differences in COMT genotype [χ^2 (N = 81, df = 4) = 6.436, p = 0.169]. There were also no differences in the proportion of male and female participants across participants with different COMT or 5-HTTLPR genotypes as indicated by another series of chi-square tests [all $\chi^2 \leq 2.306$, all $p \geq 0.316$]. A 3 × 3 ANOVA (COMT genotype × 5-HTTLPR genotype) suggested age differences among participants with different COMT but not 5-HTTLPR genotypes [effect of COMT genotype: $F(2,172) = 3.065$, $p = 0.049$, $\eta_p^2 = 0.034$; all other effects and interactions involving 5-HTTLPR genotype and COMT genotype: all $F \leq 1.249$, all $p \geq 0.292$, all $\eta_p^2 \leq 0.028$]. *Post hoc* tests showed that Met/Met and Met/Val carriers were of same age [p = 1.000] but of younger age than Val/Val carriers [p = 0.111 and p = 0.052, respectively]. Consequently, age was used as a covariate in the subsequent analyses. A 3 × 3 ANCOVA (COMT genotype × 5-HTTLPR genotype) revealed no differences in psychopathological distress among participants with different COMT or 5-HTTLPR genotypes [all effects involving COMT genotype, 5-HTTLPR genotype or the interaction of COMT and 5-HTTLPR genotype: all $F \leq 2.210$, all $p \geq 0.113$, all $\eta_p^2 \leq 0.025$]. Of note, psychopathological distress was generally very low among participants with different COMT or 5-HTTLPR genotypes, indicating that participants were in good mental health at the time of the study. **Table 2** provides an overview about the aforementioned participant characteristics.

Emotion Recognition

A 3 × 3 × 3 ANCOVA (COMT genotype × 5-HTTLPR genotype × Expression Valence) indicated valence dependent differences in emotion recognition among participants with different COMT but not 5-HTTLPR genotypes [effect of COMT genotype: $F(2,171) = 3.361$, $p = 0.037$, $\eta_p^2 = 0.038$; interaction of COMT genotype and expression valence: $F(3.62,309.30) = 3.124$, $p = 0.019$, $\eta_p^2 = 0.035$; all other effects and interactions involving COMT genotype, 5-HTTLPR genotype or expression valence: all $F \leq 1.80$, all $p \geq 0.085$, all $\eta_p^2 \leq 0.040$]. Follow-up 3 × 3 ANCOVAs (COMT genotype × 5-HTTLPR genotype) revealed that these differences emerged during the processing of negative [effect of COMT genotype: $F(2,171) = 6.378$, $p = 0.002$, $\eta_p^2 = 0.069$; all other effects and interactions involving COMT genotype or 5-HTTLPR genotype: all $F \leq 0.906$, all $p \geq 0.462$, all $\eta_p^2 \leq 0.021$], but not positive [all effects and interactions involving COMT genotype or 5-HTTLPR genotype: all $F \leq 1.972$, all $p \geq 0.101$, all $\eta_p^2 \leq 0.044$] or neutral [all effects and interactions involving COMT genotype or 5-HTTLPR genotype: all $F \leq 1.020$, all $p \geq 0.363$, all $\eta_p^2 \leq 0.012$] expressions. *Post hoc* tests indicated that Met/Met and Met/Val carriers, who did not differ from one another [p = 0.722], were more accurate in the recognition of negative expressions than Val/Val carriers [p = 0.002 and p = 0.015, respectively]. **Figure 2** demonstrates the aforementioned differences in emotion recognition on basis of participants' COMT genotype.

DISCUSSION

Across a series of well-powered and comparison-corrected analyses, we were able to demonstrate that differences in participants' COMT but not 5-HTTLPR genotype accounted for differences in participants' emotion recognition performance. Met/Met and Met/Val carriers were more accurate in the recognition of negative, but not positive or neutral, expressions than Val/Val carriers, indicating a negativity bias in face processing. Of note, studies that failed to reveal a negativity bias in face processing in Met carriers did not simultaneously consider differences in participants' COMT and 5-HTTLPR genotype in their analyses (Weiss et al., 2007; Defrancesco et al., 2011). Moreover, these analyses were neither well-powered nor comparison-corrected (Weiss et al., 2007; Defrancesco et al., 2011), implying the possibility of false positive or false negative findings. The findings of these studies should, thus, be treated with caution (Weiss et al., 2007; Defrancesco et al., 2011). Another study, however, simultaneously considered variations of the COMT and 5-HTTLPR genotype in their well-powered and comparison-corrected analyses (Gohier et al., 2014). This study revealed a negativity bias in face processing among Met carriers (Gohier et al., 2014), indicating that the failure to detect such a bias in the other studies may have been due to the aforementioned methodological limitations (Weiss et al., 2007; Defrancesco et al., 2011). Give that our study shared many methodological similarities with this study, it appears plausible to assume that Met carriers show increased recognition biases and recognition accuracies for negative expressions on the behavioral level. This assumption is also compatible with other studies that revealed an increased processing of negative expressions on the neural level in Met carriers. Notably, Met carriers showed an increase in amygdala activity (Williams et al., 2010; Lonsdorf et al., 2011) and amygdala-prefrontal connectivity (Surguladze et al., 2012)

in response to basic expressions of negative valence. As the amygdala is also implicated in the recognition of complex expressions (Baron-Cohen et al., 1999; Adolphs et al., 2002), similar activity and connectivity changes may have occurred while Met carriers were processing complex expressions of negative valence. Moreover, the amygdala is highly susceptible to catecholamine transmission (Hariri et al., 2002; Onur et al., 2009), implying that genotype dependent changes in catecholamine metabolism may in fact account for Met carriers' negativity bias during face processing.

It should be noted, however, that several genes are implicated in the catecholamine metabolism via enzyme activity and/or receptor density. Polymorphisms of the dopamine beta-hydroxylase gene, for example, also account for changes in catecholamine metabolism that are associated with differences in face processing (Gong et al., 2014). Moreover, face processing is also modulated by genes that change metabolisms of other neurotransmitters than catecholamine ones. Polymorphisms of the oxytocin receptor gene, for instance, are associated with differences in face processing via changes in oxytocin metabolism (Rodrigues et al., 2009). It, thus, seems likely that multiple genes, either alone or in concert, modulated participants' face processing in the present study. Given the complexity of genetic influences on face processing, it may be too simplistic to assume that differences in participants' face processing were solely due to differences in participants' COMT genotype.

Consequently, it has to be determined in future studies whether catecholamine induced changes in amygdala activity and amygdala-prefrontal connectivity in fact account for the negativity bias in face processing that has been observed in Met as compared to Val carriers (Williams et al., 2010; Lonsdorf et al., 2011; Surguladze et al., 2012; Gohier et al., 2014). Notwithstanding that the neurobiological mechanisms underlying this negativity bias remain unclear, it seems plausible to assume that Met carriers perceive their social interactions as more negative than Val carriers because of this negativity bias. As a consequence, Met carriers may be more vulnerable to negative experiences in social interactions. These negative experiences may lead to anxious and depressive feelings, which may eventually manifest themselves in anxious and depressive symptoms or disorders. This may explain why Met carriers experience more anxiety and depression related symptoms or disorders than Val carriers (Montag et al., 2012). It may, thus, be interesting to investigate in longitudinal studies whether Met and Val carriers' performance on face processing tasks is differentially associated with Met and Val carriers' risk to develop anxiety or depression related disorders. Ideally, these studies should comprise large number of participants that have been genotyped for polymorphisms of multiple genes that have been shown to be associated with face processing on the neural and behavioral level. These studies may help to determine whether genotype dependent differences in face processing represent biomarkers with utility for the development of interventions that are concerned with the prevention or treatment of anxiety and depression related disorders. We hope that findings of the present study, which have to be replicated and extended, stimulates this type of research.

AUTHOR CONTRIBUTIONS

AL and JW designed the study. JK and JW collected the data. AL, GH, and RP analyzed the data. AL and RP wrote the manuscript. AH, GH, JK, JW, and RP contributed to writing, reviewing, and editing of the manuscript. All authors approved the final version of the manuscript.

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REFERENCES

Adolphs, R., Baron-Cohen, S., and Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. *J. Cogn. Neurosci.* 14, 1264–1274. doi: 10.1162/089892902760807258

Baron-Cohen, S., Ring, H. A., Wheelwright, S., Bullmore, E. T., Brammer, M. J., Simmons, A., et al. (1999). Social intelligence in the normal and autistic brain: an fMRI study. *Eur. J. Neurosci.* 11, 1891–1898. doi: 10.1046/j.1460-9568.1999.00621.x

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., and Plumb, I. (2001). The "reading the mind in the eyes" test revised version: a study with normal adults, and adults with asperger syndrome or high-functioning autism. *J. Child Psychol. Psychiatry* 42, 241–251. doi: 10.1111/1469-7610.00715

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Earlbaum.

Cordaro, D. T., Sun, R., Keltner, D., Kamble, S., Huddar, N., and McNeil, G. (2018). Universals and cultural variations in 22 emotional expressions across five cultures. *Emotion* 18, 75–93. doi: 10.1037/emo0000302

Defrancesco, M., Niederstatter, H., Parson, W., Oberacher, H., Hinterhuber, H., Canazei, M., et al. (2011). Influence of serotonin transporter genotype and catechol-O-methyltransferase Val158Met polymorphism on recognition of emotional faces. *J. Int. Neuropsychol. Soc.* 17, 1014–1020. doi: 10.1017/S135561771100097X

Ebstein, R. P., Israel, S., Chew, S. H., Zhong, S., and Knafo, A. (2010). Genetics of human social behavior. *Neuron* 65, 831–844. doi: 10.1016/j.neuron.2010.02.020

Ekman, P., Sorenson, E. R., and Friesen, W. V. (1969). Pan-cultural elements in facial displays of emotion. *Science* 164, 86–88. doi: 10.1126/science.164.3875.86

Faul, F., Erdfelder, E., Lang, A. G., and Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi: 10.3758/BF03193146

Franke, G. H., Jaeger, S., Glaesmer, H., Barkmann, C., Petrowski, K., and Braehler, E. (2017). Psychometric analysis of the brief symptom inventory 18 (BSI-18) in a representative German sample. *BMC Med. Res. Methodol.* 17:14. doi: 10.1186/s12874-016-0283-3

Gohier, B., Senior, C., Radua, J., El-Hage, W., Reichenberg, A., Proitsi, P., et al. (2014). Genetic modulation of the response bias towards facial displays of anger and happiness. *Eur. Psychiatry* 29, 197–202. doi: 10.1016/j.eurpsy.2013.03.003

Gong, P., Liu, J., Li, S., and Zhou, X. (2014). Dopamine beta-hydroxylase gene modulates individuals' empathic ability. *Soc. Cogn. Affect. Neurosci.* 9, 1341–1345. doi: 10.1093/scan/nst122

Hariri, A. R., Mattay, V. S., Tessitore, A., Fera, F., Smith, W. G., and Weinberger, D. R. (2002). Dextroamphetamine modulates the response of the human amygdala. *Neuropsychopharmacology* 27, 1036–1040. doi: 10.1016/S0893-133X(02)00373-1

Hariri, A. R., and Weinberger, D. R. (2003). Imaging genomics. *Br. Med. Bull.* 65, 259–270. doi: 10.1093/bmb/65.1.259

Harkness, K., Sabbagh, M., Jacobson, J., Chowdrey, N., and Chen, T. (1999). Enhanced accuracy of mental state decoding in dysphoric college students. *Cogn. Emot.* 19, 999–1025. doi: 10.1080/02699930541000110

Hysek, C. M., Domes, G., and Liechti, M. E. (2012). MDMA enhances "mind reading" of positive emotions and impairs "mind reading" of negative emotions. *Psychopharmacology* 222, 293–302. doi: 10.1007/s00213-012-2645-9

Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y. M., Szumlanski, C. L., and Weinshilboum, R. M. (1996). Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 6, 243–250. doi: 10.1097/00008571-199606000-00007

Lischke, A., Berger, C., Prehn, K., Heinrichs, M., Herpertz, S. C., and Domes, G. (2012). Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology* 37, 475–481. doi: 10.1016/j.psyneuen.2011.07.015

Lischke, A., Lemke, D., Neubert, J., Hamm, A. O., and Lotze, M. (2017). Inter-individual differences in heart rate variability are associated with inter-individual differences in mind-reading. *Sci. Rep.* 7:11557. doi: 10.1038/s41598-017-11290-1

Lonsdorf, T. B., Golkar, A., Lindstom, K. M., Fransson, P., Schalling, M., Ohman, A., et al. (2011). 5-HTTLPR and COMTval158met genotype gate amygdala reactivity and habituation. *Biol. Psychol.* 87, 106–112. doi: 10.1016/j.biopsycho.2011.02.014

Montag, C., Jurkiewicz, M., and Reuter, M. (2012). The role of the catechol-O-methyltransferase (COMT) gene in personality and related psychopathological disorders. *CNS Neurol. Disord. Drug Targets* 11, 236–250. doi: 10.2174/187152712800672382

Niedenthal, P. M., and Brauer, M. (2012). Social functionality of human emotion. *Annu. Rev. Psychol.* 63, 259–285. doi: 10.1146/annurev.psych.121208.131605

Onur, O. A., Walter, H., Schlaepfer, T. E., Rehme, A. K., Schmidt, C., Keysers, C., et al. (2009). Noradrenergic enhancement of amygdala responses to fear. *Soc. Cogn. Affect. Neurosci.* 4, 119–126. doi: 10.1093/scan/nsn049

Pahnke, R., Mau-Moeller, A., Junge, M., Wendt, J., Weymar, M., Hamm, A. O., et al. (2018). Oral contraceptives impair complex emotion recognition in healthy women. *Front. Neurosci.* doi: 10.3389/fnins.2018.01041

Rodrigues, S. M., Saslow, L. R., Garcia, N., John, O. P., and Keltner, D. (2009). Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21437–21441. doi: 10.1073/pnas.0909579106

Shaffer, J. P. (1995). Multiple hypothesis testing. *Annu. Rev. Psychol.* 46, 561–584. doi: 10.1146/annurev.ps.46.020195.003021

Surguladze, S. A., Radua, J., El-Hage, W., Gohier, B., Sato, J. R., Kronhaus, D. M., et al. (2012). Interaction of catechol O-methyltransferase and serotonin transporter genes modulates effective connectivity in a facial emotion-processing circuitry. *Transl. Psychiatry* 2:e70. doi: 10.1038/tp.2011.69

Weiss, E. M., Stadelmann, E., Kohler, C. G., Brensinger, C. M., Nolan, K. A., Oberacher, H., et al. (2007). Differential effect of catechol-O-methyltransferase Val158Met genotype on emotional recognition abilities in healthy men and women. *J. Int. Neuropsychol. Soc.* 13, 881–887. doi: 10.1017/S1355617707070932

Wendt, J., Neubert, J., Lindner, K., Ernst, F. D., Homuth, G., Weike, A. I., et al. (2015). Genetic influences on the acquisition and inhibition of fear. *Int. J. Psychophysiol.* 98(3 Pt 2), 499–505. doi: 10.1016/j.ijpsycho.2014.10.007

Williams, L. M., Gatt, J. M., Grieve, S. M., Dobson-Stone, C., Paul, R. H., Gordon, E., et al. (2010). COMT Val(108/158)Met polymorphism effects on emotional brain function and negativity bias. *Neuroimage* 53, 918–925. doi: 10.1016/j.neuroimage.2010.01.084

Zelenski, J. M., and Larsen, R. J. (2000). The distribution of basic emotions in everyday life: a state and trait perspective from experience sampling data. *J. Res. Pers.* 34, 178–197. doi: 10.1006/jrpe.1999.2275

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ORIGINAL RESEARCH
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GABA and 5-HT Systems Are Involved in the Anxiolytic Effect of Gan-Mai-Da-Zao Decoction

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The Gan-Mai-Da-Zao (GMDZ) decoction is one of the most famous Chinese medicine prescriptions to treat emotional diseases in China. Here we examined the anxiolytic-like effects of the GMDZ decoction in mice. The mice were orally administered with GMDZ decoction (1, 2, and 4 g/kg, respectively) for 7 days, diazepam (2 mg/kg, p.o.) and buspirone (5 mg/kg, p.o.) were used as positive controls. Then, elevated plus maze (EPM) test, light/dark box (LDB) test, and marble burying (MB) test, open field (OF) test and rota-rod test were performed. We found that GMDZ treatment (2 and 4 g/kg) significantly increased the percentage of open arm entries and time spent on the open arms in EPM as compared to the control. GMDZ treatment also significantly increased the time spent in the light box and the number of light box entries in LDB and reduced the number of marbles buried in MB. Similarly to those observed with diazepam and buspirone. In contrast, GMDZ did not affect the locomotor activity in the OF and motor coordination in the rota-rod test. Furthermore, the anxiolytic-like effects induced by GMDZ were inhibited by the γ-aminobutyric acid-A ($GABA_A$) receptor antagonist flumazenil and 5-hydroxytryptamine-1A ($5\text{-}HT_{1A}$) receptor antagonist WAY-100635. These results showed that GMDZ possesses anxiolytic-like effects in animal models, and its mechanism of action might be modulated by $5\text{-}HT_{1A}$ and $GABA_A$ receptors.

Keywords: Gan-Mai-Da-Zao decoction, anxiolytic, elevated plus maze, light/dark box, $GABA_A$ receptor, $5\text{-}HT_{1A}$ receptor

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INTRODUCTION

Anxiety disorders is one of the most common mental disorders that influence people of all ages in the general population and is becoming an increasing public health challenge worldwide (Mackenzie et al., 2011). The prevalence of anxiety disorders is more than 25% in the United States (Matsuzaki et al., 2012).Current therapies for anxiety disorders mainly consist of benzodiazepines and selective serotonin reuptake inhibitors (Girish et al., 2013). However, both these two types of drugs have well-known side effects (Pollack, 2002). Benzodiazepines have relation to muscle relaxation, sedation, and cognitive impairments; whereas, selective serotonin reuptake inhibitors show a delayed onset of action of several weeks (Shorter and Tyrer, 2003; Liu et al., 2015). Other problems can include the development of resistance to medicines and the risk of potential

dependence (Mansouri et al., 2014). Therefore, the development of other antianxiety drugs with fewer side effects is necessary, which becomes one of the more pressing issues in the field of mental science (Harada et al., 2018).

Complementary and alternative therapies may play an important role in the clinical treatment of anxiety disorders (Hazim et al., 2014; Thorn et al., 2016). Compared to classical anxiolytic drugs, use of complementary and alternative medicine could show fewer side-effects (Li et al., 2016; Abouhosseini Tabari et al., 2018). The Gan-Mai-Da-Zao (GMDZ) decoction is one of the most famous herbal prescriptions in Chinese medical book Jin Gui Yao Lue (Medical Treasures of the Golden Chamber), which is written by medical sage Zhang Zhongjing (Ruan, 2003). The GMDZ decoction is comprised of three herbs *Triticum, Glycyrrhiza*, and *Zizyphi Fructus*. GMDZ decoction was used by Zhang Zongjing to treat Zang Zao syndrome, which is a kind of emotional diseases with symptoms such as a racing heart and shakiness. The contents about Zang Zao syndrome in Jin Gui Yao Lue were contained in anxiety of modern clinical diseases (Jun et al., 2014). Clinical studies showed that it is an effective anxiolytic prescription with good tolerance (Duan, 1996; Chen, 2014). However, the mechanisms underlying anxiolytic effect of GMDZ decoction remains unclear.

We previously found GMDZ decoction exerted anxiolytic effect in the open field (OF) test in chronic stressed rats (Lou et al., 2010). In this study, we further examined the anxiolytic-like effects of the GMDZ decoction in mice. The anxiolytic-like effects of the decoction were examined by the elevated plus maze (EPM), light/dark box (LDB), and marble burying (MB) tests, respectively. Moreover, to detect the locomotor activity and motor coordination, the OF and rota-rod tests were also conducted. Finally, $GABA_A$ and 5-HT_{1A} receptors antagonists were used to clarify whether GABAergic and serotonergic systems were involved in GMDZ-induced anxiolytic-like effects.

MATERIALS AND METHODS

Preparation of GMDZ Decoction

The raw herbs for GMDZ decoction were purchased from Tongrentang Pharmacy. The herb materials were authenticated by Prof. Zhao B, a professor of pharmacognostical identification in Beijing University of Chinese Medicine. The voucher specimens (D1312110715) were deposited in the storage cabinet of room 226 at the Institute of Psychology, Chinese Academy of Sciences. GMDZ extract was prepared according to our previous method with minor modifications (Lou et al., 2010). Briefly, three crude herbs (*Triticum aestivum* L., *Glycyrrhiza uralensis* Fisch., and *Ziziphus jujuba* Mill.) were mixed in a ratio of 3:2:3. Then the mixture was powdered and boiled in distilled water (40 g/320 mL, reflux, 2 h × 2). After completion of extraction, it was filtered and dried under reduced pressure at a temperature below 60°C. Quality control of GMDZ was performed by high-performance liquid chromatographic (HPLC) analysis (**Figure 1**). The contents of liquiritin and ammonium glycyrrhizinate in the extract were 11.3 mg/g and 5.2 mg/g.

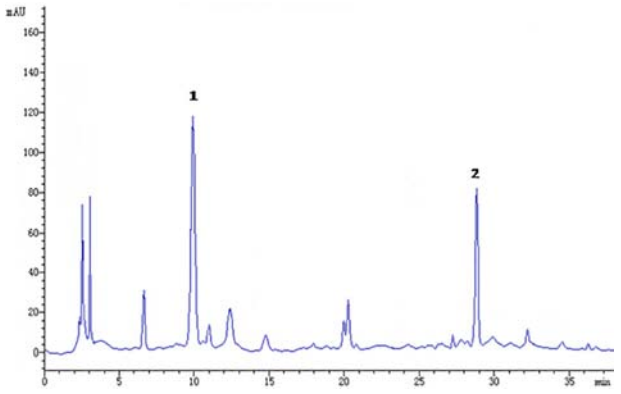

FIGURE 1 | Chromatographic profile of Gan-Mai-Da-Zao (GMDZ) decoction; 1, liquiritin; 2, ammonium glycyrrhizinate. Chromatographic separation was achieved on a Kromasil 100-5 C18 column maintained at 25°C.

The dosages were presented in terms of the dried weight of the GMDZ decoction per unit body weight of the animals (g/kg).

Animals and Treatment

Male ICR mice (18–22 g) were obtained from the Chinese Academy of Military Medical Sciences and kept in cages (25 × 15 × 14 cm) at 22 ± 1°C on a 12/12 h light/dark cycle (the light was on from 8:00 A.M. to 8:00 P.M.). The mice were housed five per cage, provided water and food *ad libitum*. All experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Institute of Psychology of the Chinese Academy of Sciences.

The present study was divided into two parts experiments. In the first experiment, mice were divided into six groups (n = 12): control, DZP, BUSP, GMDZ-1, GMDZ-2, and GMDZ-4. The diazepam (DZP, Yimin Pharmaceutical Factory, Beijing, China; 2 mg/kg) and buspirone (BUSP, PKU Healthcare corporation, Beijing, China, 5 mg/kg) were chosen as the positive control drugs, which were orally administrated for 7 days. GMDZ mice were orally administered GMDZ decoction (1, 2, and 4 g/kg, respectively) for 7 days, and control animals were orally administered the vehicle (saline) for 7 days. The behavior tests (EPM, LDB, MB, OF, and rota-rod test) were conducted 60 min after GMDZ administration or 30 min after DZP and BUSP administration at seventh day. Once the potential anxiolytic-like effect of GMDZ decoction was observed, another experiment was performed to investigate whether the $GABA_A$ or 5-HT_{1A} receptor was involved in the anxiolytic effects of GMDZ decoction. Mice were divided into 10 groups (n = 12): Control, Flu, WAY, DZP, DZP+Flu, BUSP, BUSP+WAY, GMDZ, GMDZ+Flu, and GMDZ+WAY. The mice in DZP and DZP+Flu groups were received DZP (2 mg/kg, p.o.) for 7 days, the BUSP and BUSP+WAY groups were administrated with BUSP (5 mg/kg, p.o.) for 7 days, while GMDZ, GMDZ+Flu, and GMDZ+WAY received GMDZ (4 g/kg, p.o.) for 7 days. Control, Flu, and WAY animals were orally administrated the vehicle for

7 days. On the seventh day, flumazenil (Flu, 3 mg/kg; i.p.) was intraperitoneal injected 15 min before administration of DZP (DZP+Flu group), GMDZ (GMDZ+Flu group), and vehicle (Flu group), while WAY-100635 (WAY, 1 mg/kg, i.p.) was intraperitoneal injected 15 min before administration of BUSP (BUSP+WAY group), GMDZ (GMDZ+WAY group), and vehicle (WAY group). Then three behavior tests (EPM, LDB, and MB) were conducted 60 min after GMDZ administration or 30 min after DZP and BUSP administration. The dosages of GMDZ, DZP, WAY, and Flu were based on our previously studies (Lou et al., 2010; Liu et al., 2015), and the dose of BUSP 5 mg/kg is enough to produce an anxiolytic effect (Pires et al., 2013). As most Chinese medicines are slowly absorbed drugs, the behavior tests were conducted 60 min after GMDZ administration (Liu et al., 2015). DZP, BUSP, WAY, and Flu were all dissolved in physiological saline. The GMDZ decoctions were prepared with three concentrations (0.05, 0.1, and 0.2 g/ml) so that mice in GMDZ groups were all administrated orally in a volume of 0.5 ml/25 g body weight.

Elevated Plus Maze

The maze was comprised of two open arms (30 × 5 × 0.2 cm) and two closed arms (30 × 5 × 15 cm) that directly opposed each other. The arms extended from a central platform (5 × 5 cm), and the entire apparatus was elevated 45 cm above the floor. A video camera was suspended above the maze to capture animals' location in the maze. To begin with, the mouse was placed individually in the center of the maze facing an open arm, and the time spent on and the number of entries into and the open and closed arms were detected during a 5 min test period (Liu et al., 2015). An effective entry was defined as the placement of all four paws into an arm. The percent time spent in open arm [(open arm time/total time) × 100%] and open arm entries [(open arm entries/total arm entries) × 100%] were calculated for each animal. Heat maps were generated using the Noduls software (Netherland) to create a representative image of animal movement. The apparatus was cleanly wiped with 70% alcohol after each trial.

Light/Dark Box

The light/dark apparatus is a rectangular box (45 × 21 × 21 cm) divided into two compartments, with one-third painted white and two-thirds painted black. The black compartment was closed with a lid, whereas white compartment was illuminated by two 60 W bulbs placed 30 cm above the box. These two compartments were separated by a divider with a 3.5 × 3.5 cm opening at floor level. The mouse was initially placed individually in the corner of the white compartment away from the dark compartment and observed for 5 min. The number of light box entries and time spent in the light compartment were detected (Narasingam et al., 2017). The apparatus was cleaned with 70% methanol between each test.

Marble Burying Test

A normal glass cage (27 × 16 × 13 cm) with 25 glass marbles equidistantly distributed on a 5 cm layer of sawdust was used in this experiment. The animals were placed individually in the cages for 30 min. At the end of the test, the number of marbles buried in the sawdust was measured by an observer who was blinded to group assignment and outcome assessment. A marble was considered as hidden when it was at least two-thirds covered by bedding (Pires et al., 2013). After each test, the marbles and sawdust were washed and cleaned with ethanol 70%.

Open Field Test

The OF device was comprised of a plexiglas arena (60 × 60 × 25 cm) with a white floor, which was divided into 36 squares (10 × 10 cm). For testing, a mouse was placed individually in the middle of the arena and the number of squares crossed (with four legs on each square) was recorded for 5 min (Rotheneichner et al., 2017). After each trial, the device was wiped clean with a 70% ethanol.

Rota-Rod Test

Prior to experimentation, the animals were trained to learn the ability to remain for 180 s on a diameter rod with a rotation of 17 rpm. For testing, the animals were placed on the rotating bar, which is 2.5 cm in diameter and 25 cm above the floor. The number of falls and the time of spent on the rotating bar and were detected for a period of 180 s (de Almeida et al., 2012). After each trial, the apparatus was wiped clean with a 70% ethanol.

Statistical Analysis

The results were expressed as means ± SEM. Data were evaluated by one-way analysis of variance (ANOVA) with Dunnett's tests for *post hoc* analysis. In the antagonistic experiments, two-way ANOVA followed by *post hoc* Bonferroni's test was used. All data analyses were done by using GraphPad Prism 5.0. The threshold for statistical significance was set at $p < 0.05$.

RESULTS

Effect of GMDZ Decoction in the Elevated Plus Maze

The one-way ANOVA indicated significant group effects in terms of the percent of time spent on the open arms [$F_{(5,66)} = 4.301$, $P < 0.01$; **Figures 2A,D**] and the percentage of open arm entries [$F_{(5,66)} = 3.361$, $P < 0.01$; **Figure 2B**]. GMDZ (2 and 4 g/kg) markedly increased the percentage of time spent on the open arms ($P < 0.05$ and $P < 0.01$, respectively) and the percent of open arm entries ($P < 0.05$ and $P < 0.01$, respectively) compared to control group treated with vehicle. DZP and BUSP treatment showed significant elevation in the percentage of time spent in the open arms (both $P < 0.01$) and open arm entries (both $P < 0.01$) as compared to control. No significant differences were observed in total number of entries among groups [$F_{(5,66)} = 0.545$, $P > 0.05$; **Figure 2C**]. The high dosage 4 g/kg was chosen for further antagonist experiments.

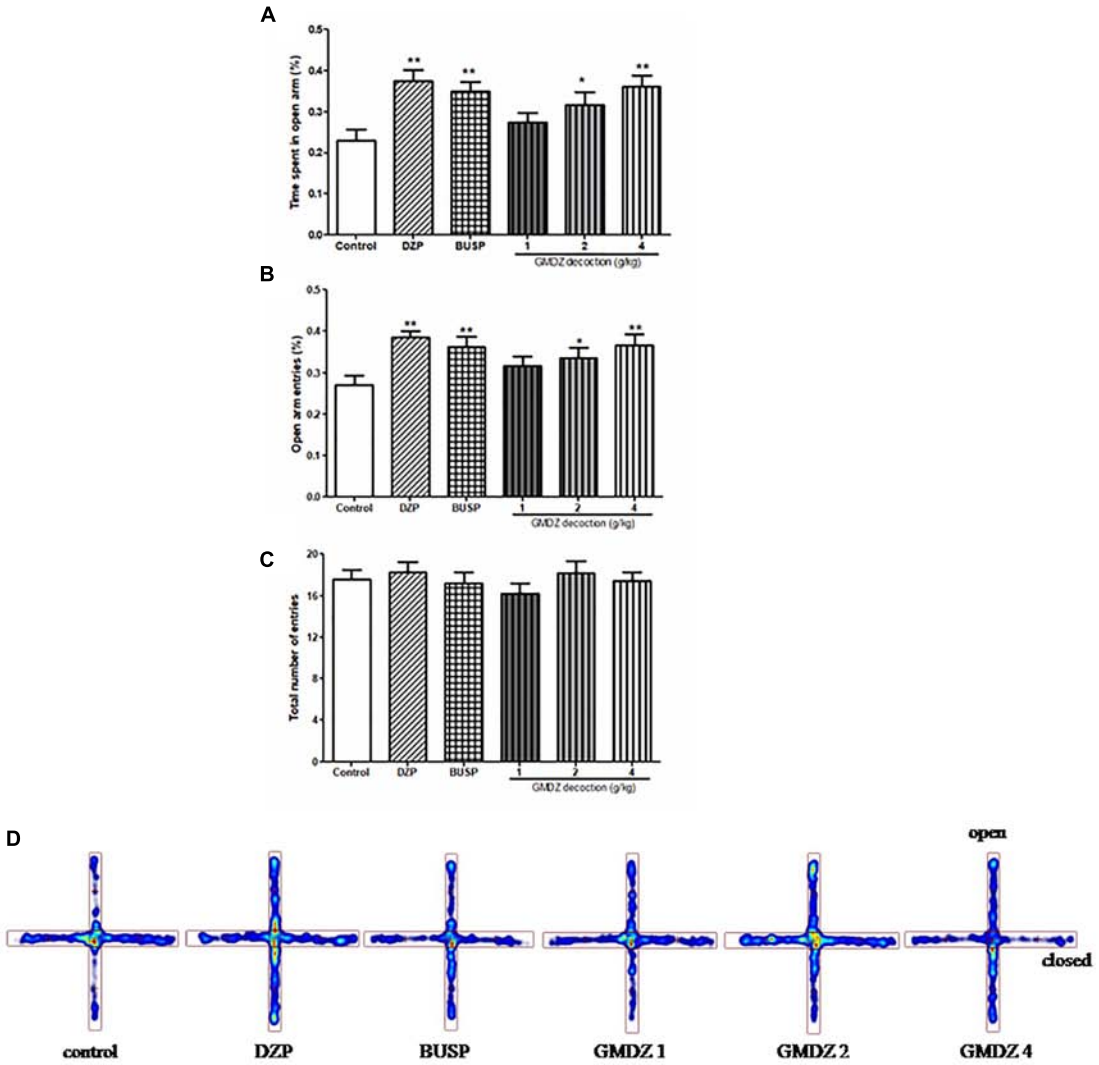

FIGURE 2 | Effect of GMDZ decoction on the EPM test **(A)** The percentage of time spent on open arms. **(B)** The percent of open arms entries. **(C)** The total arm entries. **(D)** Representative heat map analysis of one animal in each group. Value are represented as mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group. One way ANOVA with Student–Newman–Keuls *post hoc* test.

Effects of Receptor Antagonists on the Anxiolytic-Like Effect of GMDZ Decoction in the Elevated Plus Maze

Flu was co-administrated with DZP or GMDZ to determine whether the $GABA_A$-benzodiazepine receptor antagonist would alter the anxiolytic-like effect in EPM. DZP and GMDZ treatment showed a significant increase in the percentage of time spent on open arms (both $P < 0.01$) and the percent of open arms entries (both $P < 0.01$) compared to the vehicle group, while Flu alone was not markedly different from the vehicle group (both $P > 0.05$). As shown in **Figures 3A–C**, the two way ANOVA revealed that Flu could antagonize the DZP effect on the percentage of time spent on open arms [DZP (treatment): $F_{(1,44)} = 4.38$, $P < 0.05$; Flu (antagonist): $F_{(1,44)} = 4.16$, $P < 0.05$; and DZP (treatment) × Flu (antagonist) interaction: $F_{(1,44)} = 8.16$, $P < 0.01$] and the percent of open arms entries [DZP: $F_{(1,44)} = 10.01$, $P < 0.01$; Flu: $F_{(1,44)} = 5.38$, $P < 0.05$; and DZP × Flu interaction: $F_{(1,44)} = 15.7$, $P < 0.01$], and it also could inhibit the GMDZ effect in the percentage of time spent on open arms [GMDZ: $F_{(1,44)} = 4.62$, $P < 0.05$; Flu: $F_{(1,44)} = 1.93$, $P > 0.05$; and GMDZ × Flu interaction: $F_{(1,44)} = 4.15$, $P < 0.05$], and the percent of open arms entries [GMDZ: $F_{(1,44)} = 7.63$, $P < 0.01$; Flu: $F_{(1,44)} = 2.27$, $P > 0.05$; and GMDZ × Flu interaction: $F_{(1,44)} = 7.18$, $P < 0.01$]. WAY was then co-administration with BUSP or GMDZ to investigate if $5\text{-}HT_{1A}$ receptor antagonist could affect the anxiolytic-like effect. The two-way ANOVA showed BUSP and GMDZ significantly increased the percentage of time spent

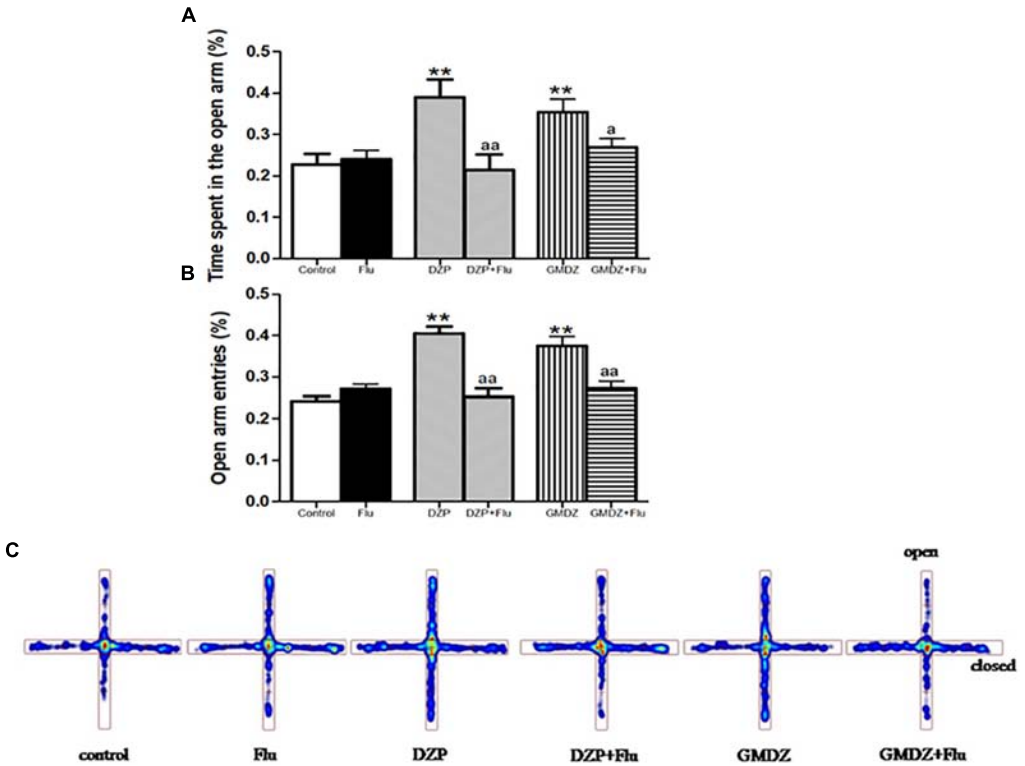

FIGURE 3 | Effects of receptor antagonists on anxiolytic-like effect of GMDZ in EPM test. **(A)** Effect of Flu on the percentage of time spent in open arms. **(B)** Effect of Flu on the percent of open arms entries. **(C)** Representative heat map analysis of one animal in each group. Value are represented as mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group; $^{a}P < 0.05$ or $^{aa}P < 0.01$ treatment vs. antagonist (interaction effect). Two-way ANOVA with Bonferroni *post hoc* test.

in open arms (both $P < 0.01$) and the percent of open arm entries (both $P < 0.01$) compared with vehicle group (**Figures 4A–C**), the WAY alone was not significantly different from vehicle (both $P > 0.05$). WAY could antagonize the BUSP effect on the percent of time spent on open arms [BUSP: $F_{(1,44)} = 8.38$, $P < 0.01$, WAY: $F_{(1,44)} = 3.21$, $P > 0.05$, and BUSP × WAY interaction: $F_{(1,44)} = 11.15$, $P < 0.01$] and the percent of open arms entries [BUSP: $F_{(1,44)} = 4.06$, $P < 0.05$, WAY: $F_{(1,44)} = 2.56$, $P > 0.05$, and BUSP × WAY interaction: $F_{(1,44)} = 4.4$, $P < 0.05$], and it also could inhibit the GMDZ effect in the percentage of time spent on open arms [GMDZ: $F_{(1,44)} = 6.84$, $P < 0.05$, WAY: $F_{(1,44)} = 2.11$, $P > 0.05$, and GMDZ × WAY interaction: $F_{(1,44)} = 5.13$, $P < 0.05$], and the number of open arms entries [GMDZ: $F_{(1,44)} = 13.18$, $P < 0.01$, WAY: $F_{(1,44)} = 3.16$, $P > 0.05$, and GMDZ × WAY interaction: $F_{(1,44)} = 5.55$, $P < 0.05$].

Effect of GMDZ Decoction in the Light/Dark Box Test

As shown in **Figures 5A,B**, the one-way ANOVA showed significant differences among six groups in the number of light box entries [$F_{(5,66)} = 4.905$, $P < 0.01$] and time spent in the light box [$F_{(5,66)} = 3.471$, $P < 0.01$]. Compared with the control group, treatment with GMDZ at dosages of 2 and 4 g/kg significantly increased the number of light box entries ($P < 0.05$ and $P < 0.01$, respectively) and time spent in the light box (both $P < 0.01$). Both DZP and BUSP treatment also significantly elevated the number of light box entries (both $P < 0.01$) and the time spent in the light box (both $P < 0.01$).

Effects of Receptor Antagonists on the Anxiolytic-Like Effect of GMDZ in the Light/Dark Box Test

As shown in **Figures 6A,B**, Flu was i.p. administrated to determine whether it could block the anxiolytic-like effect of DZP or GMDZ in LDB. The statistical analyses revealed that DZP and GMDZ significantly increased the number of light box entries (both $P < 0.01$) and time spent in the light box (both $P < 0.01$) compared with the vehicle group, while Flu alone was not markedly different from the vehicle group (both $P > 0.05$). Flu could antagonize the DZP effect on the number of light box entries [DZP: $F_{(1,44)} = 8.21$, $P < 0.01$; Flu: $F_{(1,44)} = 2.37$, $P > 0.05$; and DZP × Flu interaction: $F_{(1,44)} = 7.50$, $P < 0.01$] and time spent in the light box [DZP: $F_{(1,44)} = 10.26$, $P < 0.01$; Flu: $F_{(1,44)} = 2.61$, $P > 0.05$; and DZP × Flu interaction: $F_{(1,44)} = 3.96$, $P < 0.05$], and it also could block the GMDZ effect on the number of light box entries [GMDZ: $F_{(1,44)} = 12.07$, $P < 0.01$; Flu: $F_{(1,44)} = 2.58$, $P > 0.05$; and GMDZ × Flu interaction: $F_{(1,44)} = 4.12$, $P < 0.05$], and time spent in the light box [GMDZ: $F_{(1,44)} = 14.53$, $P < 0.01$; Flu: $F_{(1,44)} = 2.38$, $P > 0.05$; and GMDZ × Flu interaction: $F_{(1,44)} = 3.95$, $P < 0.05$]. WAY

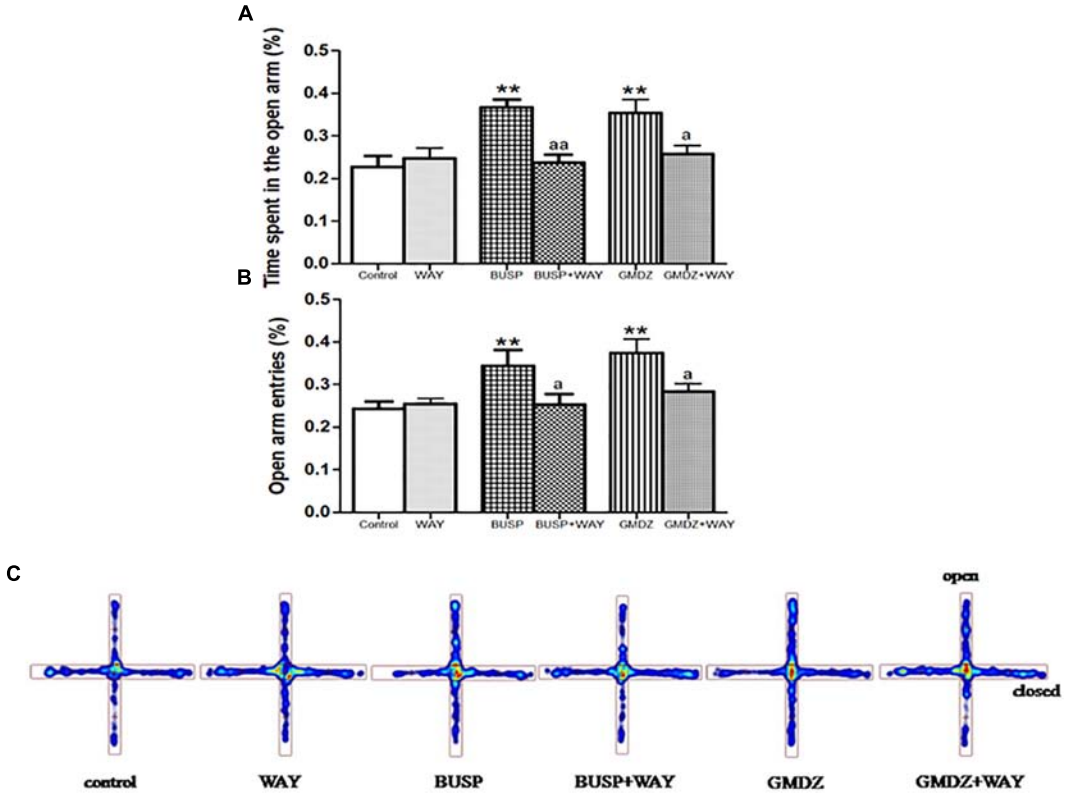

FIGURE 4 | Effects of receptor antagonists on anxiolytic-like effect of GMDZ in EPM test. **(A)** Effect of WAY on the percentage of time spent in open arms. **(B)** Effect of WAY on the percent of open arms entries. **(C)** Representative heat map analysis of one animal in each group. Value are represented as mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group; $^{a}P < 0.05$ or $^{aa}P < 0.01$ treatment vs. antagonist (interaction effect). Two-way ANOVA with Bonferroni *post hoc* test.

was pretreated with BUSP or GMDZ to investigate if 5-HT_{1A} receptor was involved in the anxiolytic-like of GMDZ. The two-way ANOVA showed BUSP and GMDZ significantly increased the number of light box entries (both $P < 0.01$) and time spent in the light box (both $P < 0.01$) as compared to vehicle group (**Figures 6C,D**), the WAY alone was not significantly different from vehicle (both $P > 0.05$). WAY could antagonize the BUSP effect on the number of light box entries [BUSP: $F_{(1,44)} = 6.37$, $P < 0.05$; WAY: $F_{(1,44)} = 3.04$, $P > 0.05$; and BUSP × WAY interaction: $F_{(1,44)} = 4.98$, $P < 0.05$] and time spent in the light box [BUSP: $F_{(1,44)} = 5.19$, $P < 0.05$; WAY: $F_{(1,44)} = 1.88$, $P > 0.05$; and BUSP × WAY interaction: $F_{(1,44)} = 4.08$, $P < 0.05$], and it also could inhibit the GMDZ effect on the number of light box entries [GMDZ: $F_{(1,44)} = 11.14$, $P < 0.01$; WAY: $F_{(1,44)} = 3.14$, $P > 0.05$; and GMDZ × WAY interaction: $F_{(1,44)} = 3.99$, $P < 0.05$], and time spent in the light box [GMDZ: $F_{(1,44)} = 9.05$, $P < 0.01$; WAY: $F_{(1,44)} = 2.22$, $P > 0.05$; and GMDZ × WAY interaction: $F_{(1,44)} = 5.43$, $P < 0.05$].

Effect of GMDZ Decoction in the Marble Burying Test

There were significant group effects in the number of marbles buried among groups as analyzed by a one-way ANOVA analysis [$F_{(5,66)} = 16.545$, $P < 0.01$, **Figure 7A**]. GMDZ at dosages of 2 and 4 g/kg significantly reduced the number of marbles buried (both $P < 0.01$), similar effect was also observed in DZP and BUSP groups (both $P < 0.01$).

Effect of Antagonist on the Anxiolytic-Like Effect of GMDZ Decoction in the Marble Burying Test

The mice were pretreated with Flu before administration of DZP or GMDZ. As shown in **Figure 7B**, DZP and GMDZ significantly increased the number of marbles buried (both $P < 0.01$) compared to the vehicle group, while the Flu alone was not significantly different from the vehicle group ($P > 0.05$). The two way ANOVA showed that pretreatment with Flu before the DZP administration could antagonize the drug effects [DZP: $F_{(1,44)} = 33.01$, $P < 0.01$; Flu: $F_{(1,44)} = 13.85$, $P < 0.05$; and DZP × Flu interaction: $F_{(1,44)} = 25.76$, $P < 0.01$], and it also could inhibit the GMDZ effect [GMDZ: $F_{(1,44)} = 50.51$, $P < 0.01$; Flu: $F_{(1,44)} = 10.62$, $P < 0.01$; and GMDZ × Flu interaction: $F_{(1,44)} = 22.45$, $P < 0.01$]. We further explored whether WAY could affect the effect of BUSP and GMDZ. The two-way ANOVA showed BUSP and GMDZ significantly increased the number of marbles buried as compared to control group (both $P < 0.01$, **Figure 7C**), the WAY alone was not significantly different from control ($P > 0.05$).The number of

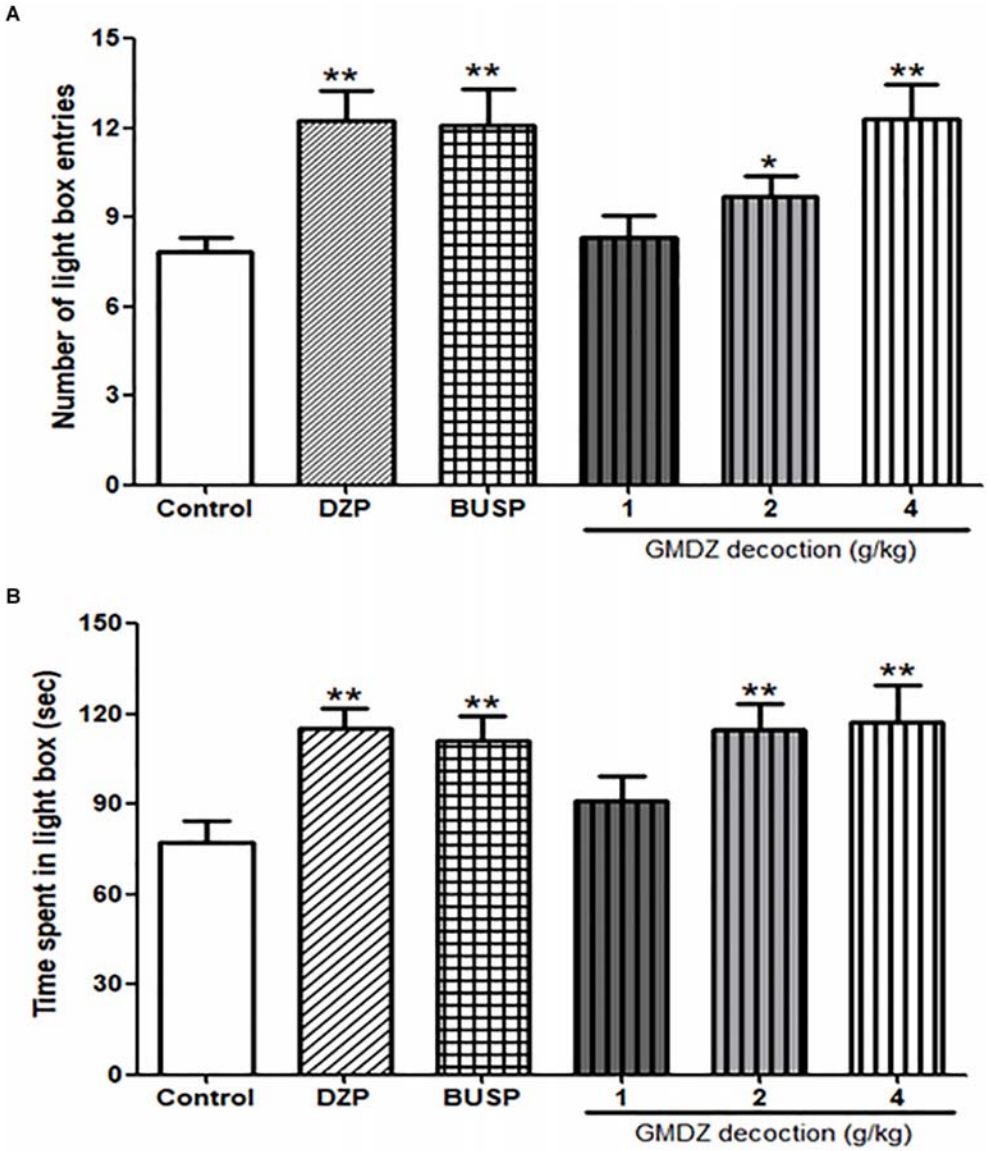

FIGURE 5 | Effect of GMDZ decoction on the LDB test. **(A)** The numbers of light box entries. **(B)** The time spent in the light box. Value are represented as mean ± SEM (n = 12). *P < 0.05 or **P < 0.01 vs. control group. One way ANOVA with Student–Newman–Keuls *post hoc* test.

marbles buried was significantly decreased when WAY was injected before BUSP treatment [BUSP: $F_{(1,44)} = 21.47$, $P < 0.01$; WAY: $F_{(1,44)} = 8.68$, $P < 0.01$; and BUSP × WAY interaction: $F_{(1,44)} = 14.42$, $P < 0.01$], and it was also reduced by WAY treatment before GMDZ administration [GMDZ: $F_{(1,44)} = 24.92$, $P < 0.01$; WAY: $F_{(1,44)} = 6.56$, $P < 0.05$; and GMDZ × WAY interaction: $F_{(1,44)} = 14.57$, $P < 0.01$].

Effects of GMDZ Decoction in the Open Field Test

As shown in **Table 1**, one-way ANOVA showed significant differences in the number of squares crossed [$F_{(5,66)} = 4.25$, $P < 0.01$]. Administration with DZP significantly reduced locomotor activity as compared to control group ($P < 0.01$). BUSP treatment slightly decrease the locomotor activity, but the difference was not statistically significant ($P > 0.05$). All GMDZ groups did not affect the locomotor activity as compared to control ($P > 0.05$).

Effect of GMDZ Decoction on the Rota-Rod Test

A one-way ANOVA revealed significant differences on the number of falls [$F_{(5,66)} = 28.113$, $P < 0.01$, **Figure 8A**] and the time spent on the rotating bar [$F_{(5,66)} = 3.763$, $P < 0.01$, **Figure 8B**]. Compared with the control group, DZP treatment significantly increased the number of falls by 2.3 fold ($P < 0.01$),

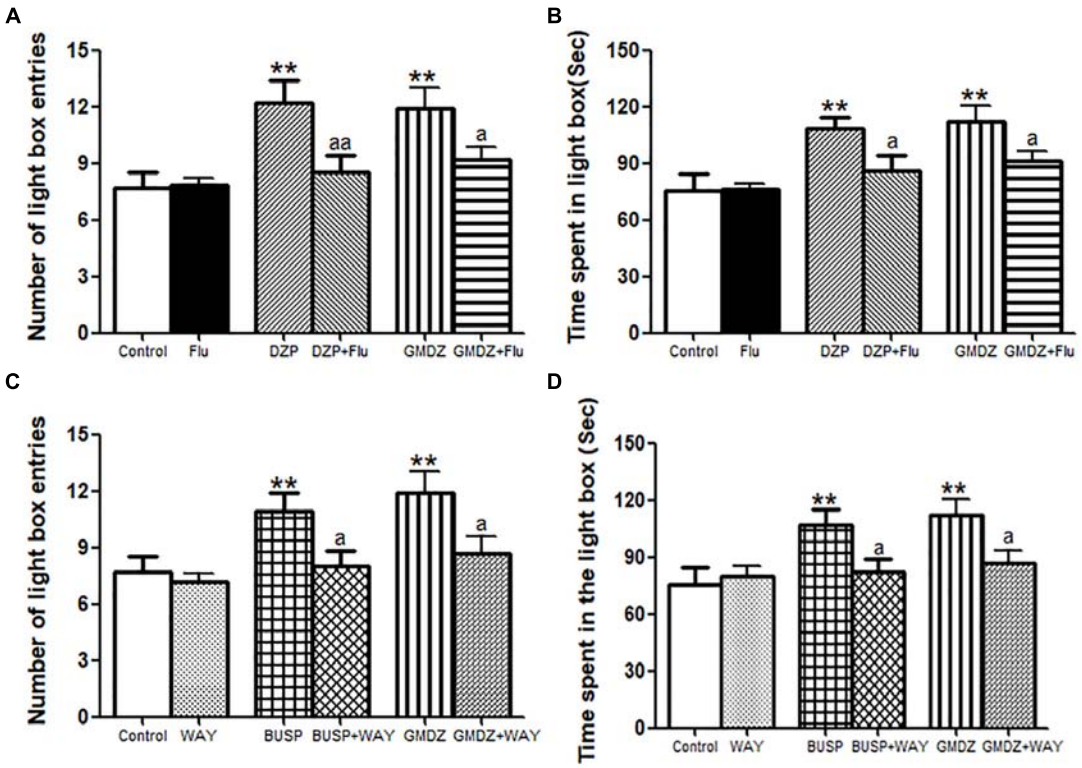

FIGURE 6 | Effect of receptor antagonists on the anxiolytic-like effect of GMDZ in the LDB test. **(A)** Effect of Flu on the numbers of light box entries. **(B)** Effect of Flu on the time spent in the light box. **(C)** Effect of WAY on the numbers of light box entries. **(D)** Effect of WAY on the time spent in the light box. Value are represented as mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group; $^{a}P < 0.05$ or $^{aa}P < 0.01$ treatment vs. antagonist (interaction effect). Two-way ANOVA with Bonferroni *post hoc* test.

while the GMDZ groups and BUSP did not affect the number of falls (all $P > 0.05$). The time spent in the rotating bar was reduced in DZP group (77%) in compared to control but did not change in the GMDZ groups and BUSP ($P > 0.05$).

DISCUSSION

Gan-Mai-Da-Zao decoction is a famous Chinese herb medicine that is widely used in East Asia for emotional diseases, e.g., depression and anxiety. Known as Ganmckdaecko-tang in Korea and Kambakutaisoto in Japan, GMDZ is an effective prescription in treating depression (Jun et al., 2014; Kim et al., 2017). GMDZ decoction may possess anxiolytic activity because it increased the number of rearings in OF test in stressed rats (Lou et al., 2010). Here we further investigated the anxiolytic-like effects of GMDZ decoction using a battery of behavioral tests (EPM, LDB, and MB). DZP and BUSP were used as reference anxiolytics. Consistent with previous research (Lou et al., 2010), this study showed that GMDZ decoction exerted anxiolytic-like actions in mice and no major side effects, and the mechanism might be related with its action on benzodiazepine and 5-HT receptors.

GABAergic and serotonergic neurotransmission are considered to play important roles in the regulation of anxiety (Narasingam et al., 2017). Previous researches showed that reduced brain levels of the inhibitory neurotransmitter GABA and its major receptor, $GABA_A$ receptor, are involved in the etiology of anxiety (Luscher et al., 2011). The classical benzodiazepines can relieve anxiety, while $GABA_A$ receptor antagonists induce anxiety (Dalvi and Rodgers, 1996; Chioca et al., 2013). Besides, altered 5-HT signal pathway contributes to the anxiety (Mi et al., 2017). The $5\text{-}HT_{1A}$ receptor is widely distributed in the frontal cortex, amygdale and hippocampus, and activation of $5\text{-}HT_{1A}$ receptor could decrease 5-HT outflow and reduce serotonergic neuron activity (Zhang et al., 2016). Thus, $5\text{-}HT_{1A}$ receptor is of great important in modulating anxiety-related behavior and might offer the potential to regulate anxiety (Jung et al., 2013). In order to investigate the mechanism underlying anxiolytic-like effect of GMDZ, a pharmacological study using $GABA_A$ receptor antagonist and $5\text{-}HT_{1A}$ receptor antagonist was conducted in this study.

The EPM has been used effectively to evaluate the efficacy of anxiety modifying interventions and investigate the neurobiological basis of anxiety disorders (Walf and Frye, 2007). Exposure to the open arms of the maze causes markedly more anxiety-related behaviors than exposure to the closed arms (Ludwig et al., 2008). An elevation in the number of open arm entries and the time spent in open arm is a very powerful indicator for the anxiolytic agent (Wilson et al., 2013; Mansouri et al., 2014). In this study, we found that GMDZ treatment

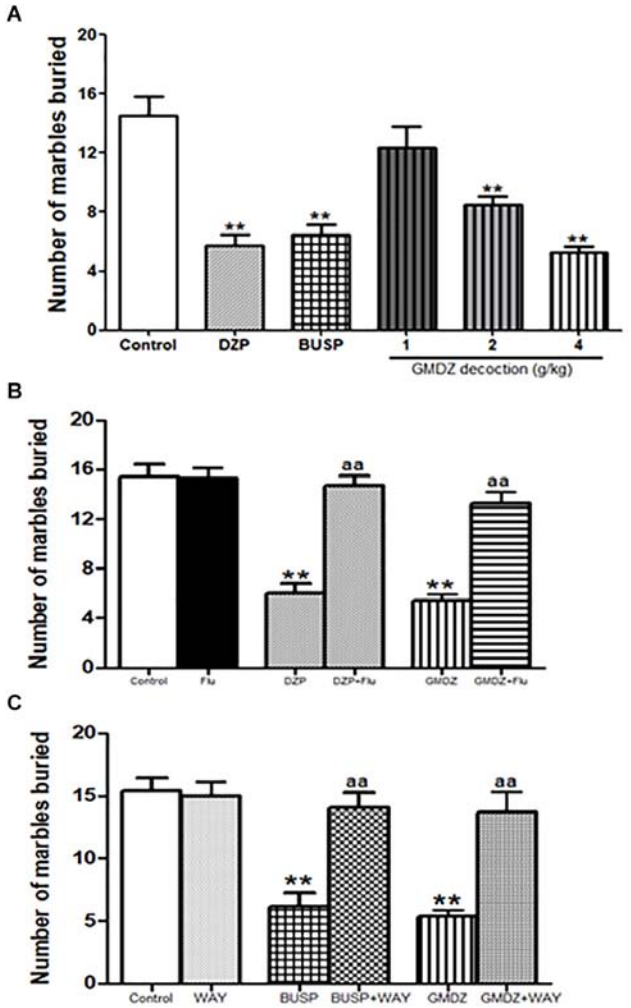

FIGURE 7 | Effect of GMDZ decoction and receptor antagonist on the number of marbles buried in the MB test. **(A)** Effect of GMDZ decoction on the MB test. **(B)** Effect of Flu on the numbers of marbles buried. **(C)** Effect of WAY on the number of marbles buried. Value are represented as mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group; One way ANOVA with Student–Newman–Keuls *post hoc* test. $^{a}P < 0.05$ or $^{aa}P < 0.01$ treatment vs. antagonist (interaction effect). Two-way ANOVA with Bonferroni *post hoc* test.

TABLE 1 | Effects of GMDZ decoction on the open field test in mice.

Groups	Dose	Number	Number of squares crossed
Control	saline	12	71.26 ± 5.39
DZP	2 mg/kg	12	46.26 ± 3.18**
BUSP	5 mg/kg	12	59.88 ± 3.84
GMDZ	1 g/kg	12	71.26 ± 4.92
GMDZ	2 g/kg	12	67.92 ± 5.95
GMDZ	4 g/kg	12	70.15 ± 4.86

*Values are expressed as mean ± SEM (n = 12). **P < 0.01 vs. control group. One way ANOVA with Student–Newman–Keuls post hoc test.*

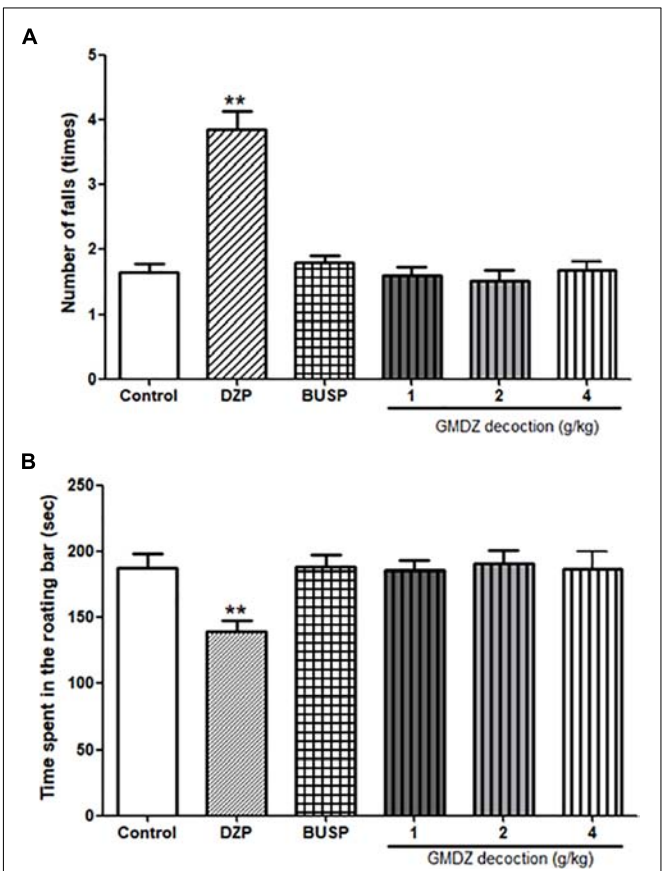

FIGURE 8 | Effect of GMDZ decoction on the rota-rod test. **(A)** Number of falls. **(B)** Time spent on the rotating bar. Value are represented mean ± SEM (n = 12). $^{**}P < 0.01$ vs. control group. One way ANOVA with Student–Newman–Keuls *post hoc* test.

significantly increased the percent of time spent in open arms and the percent of arm entries in a dose-dependent manner, indicating that GMDZ decoction can produce anxiolytic-like effects in mice. These effects could be antagonized by the selective $5HT_{1A}$ receptor antagonist, WAY and $GABA_A$ receptor antagonist, Flu.

To further demonstrate the potential anxiolytic-like effects of GMDZ decoction, the LDB test was also conducted. This experiment is based on the inherent aversion of animals to brightly illuminated areas and on the spontaneous exploratory behavior of animals respond to mild stressors, such as light and novel circumstance (Li et al., 2003; Fortes et al., 2013).Thus, the time spent in the light part of the box is the most useful marker for investigating the anxiolytic action (Moniruzzaman et al., 2018). Our data clearly showed that GMDZ decoction significantly increased the time spent in light box and the number of light box entries in a dose-dependent manner. Similarly, the anxiolytic-like effect in LDB test was also blocked by Flu and WAY.

Although the good predictive validity of the EPM and LDB tests for anxiolytic drugs, the MB test was also added to avoid false-positive results. As anxiolytic agents could decrease the number of marbles buried in this test, without inducing any changes in locomotor activity (Gaikwad and Parle, 2011).

GMDZ decoction markedly decreased the number of marbles buried in a dose-dependent manner, and the anxiolytic-like effect was also inhibited by Flu and WAY.

The EPM is based on exploration behavior, but this test is also dependent on the motor activity of the rodents. Therefore, the locomotor activity should be detected to interpret drug effects as being specific to anxiety-related behavior (Chassot et al., 2011). The total arm entries were measured as an marker of the locomotor activity in EPM (Walf and Frye, 2007). GMDZ decoction did not alter the total arm entries, suggesting that the anxiolytic-like effects in EPM were not due to a stimulation of locomotor activity. This was further confirmed by the OF test, which is widely used to evaluate the locomotor activity and anxiety. GMDZ treatment did not exhibit alterations in the number of squares crossed in the OF. These results clearly showed that GMDZ treatment does not affect the locomotor activity in mice.

Diazepam is a $GABA_A$ receptor agonist and BUSP is a partial 5-HT_{1A} receptors agonist (Belmer et al., 2018). In line with past studies (Pires et al., 2013; Liu et al., 2015), the current findings confirmed the anxiolytic effect of DZP and BUSP. As DZP and BUSP increased the percentage of time spent and the number of entries into open arms, elevated the number of light box entries and the time spent in light box, and reduce the number of marbles buried in EPM, LDB, and MB tests, respectively. Pretreated with Flu reversed the anxiolytic-like effects of DZP, whereas WAY blocked the anxiolytic-like effects of BUSP. Moreover, we found that BUSP slight decreased the locomotor activity in OF test, which might be because activation of $5HT_{1A}$ receptor can lead to neural inhibition (Muller et al., 2007). Besides its anxiolytic effect, the $5HT_{1A}$ receptor agonist has profound effect on memory or reward (Shapiro et al., 2018).

The benzodiazepines (e.g., DZP) and barbiturates can cause muscle weakness and sedation (Akindele et al., 2013). Rota-rod test was therefore conducted to evaluate the motor coordination and muscle relaxation, which could be observed by the time of permanence and number of falls on the rotating rods. The present study suggested that DZP reduced the time of permanence on rotating rod and increased the number of falls in rota-rod test, indicating a sedation effect that influenced the motor coordination. Contrary to DZP, GMDZ decoction, at the doses employed, did not exhibit significant effect on motor coordination in rota-rod test.

Gan-Mai-Da-Zao decoction had stable anxiolytic-like effects, and WAY or Flu could inhibit the anxiolytic-like effect of GMDZ in EPM, LDB, and MB. However, we did not measure the combination effects of these two antagonists. As Flu blocks GABAergic system and WAY inhibits the serotonergic system, Flu and WAY together might have greater influence on the anxiolytic effects, and it will be detected in a future study. In addition, the levels of GABA and monoamine in brain were not measured in this study, and the effects of GMDZ on changes of GABA and serotonin pathways remain to be further investigated.

CONCLUSION

In summary, the present data show that GMDZ decoction possesses strong anxiolytic-like effects in the EPM, LDB, and OF tests but did not alters locomotor activity and motor coordination. The antagonism experiments suggest that the mode of action for GMDZ is via the GABAergic and serotonergic systems.

AUTHOR CONTRIBUTIONS

J-YG designed the study and drafted the manuscript. L-JG, H-SC, and Y-XY conceived of the study. All authors read and approved the final manuscript.

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REFERENCES

Abouhosseini Tabari, M., Hajizadeh Moghaddam, A., Maggi, F., and Benelli, G. (2018). Anxiolytic and antidepressant activities of *Pelargonium roseum* essential oil on Swiss albino mice: possible involvement of serotonergic transmission. *Phytother. Res.* 32, 1014–1022. doi: 10.1002/ptr.6038

Akindele, A. J., Ajao, M. Y., Aigbe, F. R., and Enumah, U. S. (2013). Effects of *Telfairia occidentalis* (fluted pumpkin; Cucurbitaceae) in mouse models of convulsion, muscle relaxation, and depression. *J. Med. Food* 16, 810–816. doi: 10.1089/jmf.2012.0211

Belmer, A., Patkar, O. L., Lanoue, V., and Bartlett, S. E. (2018). 5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol. *Sci. Rep.* 8:2099. doi: 10.1038/s41598-018-20504-z

Chassot, J. M., Longhini, R., Gazarini, L., Mello, J. C., and de Oliveira, R. M. (2011). Preclinical evaluation of *Trichilia catigua* extracts on the central nervous system of mice. *J. Ethnopharmacol.* 137, 1143–1148. doi: 10.1016/j.jep.2011.07.032

Chen, Y. J. (2014). Combined Ganmaidazao decoction with psychological adjust to treat anxiety disorder. *Chin. J. Health Psychol.* 8, 1186–1188.

Chioca, L. R., Ferro, M. M., Baretta, I. P., Oliveira, S. M., Silva, C. R., Ferreira, J., et al. (2013). Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not $GABA_A$/benzodiazepine neurotransmission. *J. Ethnopharmacol.* 147, 412–418. doi: 10.1016/j.jep.2013.03.028

Dalvi, A., and Rodgers, R. J. (1996). GABAergic influences on plus-maze behaviour in mice. *Psychopharmacology* 128, 380–397. doi: 10.1007/s002130050148

de Almeida, A. A., Costa, J. P., de Carvalho, R. B., de Sousa, D. P., and de Freitas, R. M. (2012). Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic-like action. *Brain Res.* 1448, 56–62. doi: 10.1016/j.brainres.2012.01.070

Duan, Y. M. (1996). Clinical observation of Ganmaidazao decoction. *Trad. Chin. Med. Jilin* 6, 36–37.

Fortes, A. C., Almeida, A. A., Mendonca-Junior, F. J., Freitas, R. M., Soares-Sobrinho, J. L., and de La Roca Soares, M. F. (2013). Anxiolytic properties of

new chemical entity, 5TIO1. *Neurochem. Res.* 38, 726–731. doi: 10.1007/s11064-013-0970-y

Gaikwad, U., and Parle, M. (2011). Combination of aripiprazole and ethanol attenuates marble-burying behavior in mice. *Acta Pol. Pharm.* 68, 435–440.

Girish, C., Raj, V., Arya, J., and Balakrishnan, S. (2013). Involvement of the GABAergic system in the anxiolytic-like effect of the flavonoid ellagic acid in mice. *Eur. J. Pharmacol.* 710, 49–58. doi: 10.1016/j.ejphar.2013.04.003

Harada, H., Kashiwadani, H., Kanmura, Y., and Kuwaki, T. (2018). Linalool odor-induced anxiolytic effects in mice. *Front. Behav. Neurosci.* 12:241. doi: 10.3389/fnbeh.2018.00241

Hazim, A. I., Ramanathan, S., Parthasarathy, S., Muzaimi, M., and Mansor, S. M. (2014). Anxiolytic-like effects of mitragynine in the open-field and elevated plus-maze tests in rats. *J. Physiol. Sci.* 64, 161–169. doi: 10.1007/s12576-014-0304-0

Jun, J. H., Choi, T. Y., Lee, J. A., Yun, K. J., and Lee, M. S. (2014). Herbal medicine (Gan Mai Da Zao decoction) for depression: a systematic review and meta-analysis of randomized controlled trials. *Maturitas* 79, 370–380. doi: 10.1016/j.maturitas.2014.08.008

Jung, Y. H., Ha, R. R., Kwon, S. H., Hong, S. I., Lee, K. H., Kim, S. Y., et al. (2013). Anxiolytic effects of Julibroside C1 isolated from *Albizzia julibrissin* in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 44, 184–192. doi: 10.1016/j.pnpbp.2013.02.012

Kim, S. R., Lee, H. W., Jun, J. H., and Ko, B. S. (2017). Effects of herbal medicine (Gan Mai Da Zao Decoction) on several types of neuropsychiatric disorders in an animal model: a systematic review: herbal medicine for animal studies of neuropsychiatric diseases. *J. Pharmacopuncture* 20, 5–9. doi: 10.3831/KPI.2017.20.005

Li, J., Liu, Q. T., Chen, Y., Liu, J., Shi, J. L., Liu, Y., et al. (2016). Involvement of 5-HT_{1A} receptors in the anxiolytic-like effects of quercitrin and evidence of the involvement of the monoaminergic system. *Evid. Based Complement. Alternat. Med.* 2016:6530364. doi: 10.1155/2016/6530364

Li, S., Ohgami, Y., Dai, Y., and Quock, R. M. (2003). Antagonism of nitrous oxide-induced anxiolytic-like behavior in the mouse light/dark exploration procedure by pharmacologic disruption of endogenous nitric oxide function. *Psychopharmacology* 166, 366–372. doi: 10.1007/s00213-002-1363-0

Liu, J., Zhai, W. M., Yang, Y. X., Shi, J. L., Liu, Q. T., Liu, G. L., et al. (2015). GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. *Pharmacol. Biochem. Behav.* 128, 41–49. doi: 10.1016/j.pbb.2014.11.003

Lou, J. S., Li, C. Y., Yang, X. C., Fang, J., Yang, Y. X., and Guo, J. Y. (2010). Protective effect of gan mai da zao decoction in unpredictable chronic mild stress-induced behavioral and biochemical alterations. *Pharm. Biol.* 48, 1328–1336. doi: 10.3109/13880201003789440

Ludwig, V., Mihov, Y., and Schwarting, R. K. (2008). Behavioral and neurochemical consequences of multiple MDMA administrations in the rat: role of individual differences in anxiety-related behavior. *Behav. Brain Res.* 189, 52–64. doi: 10.1016/j.bbr.2007.12.008

Luscher, B., Shen, Q., and Sahir, N. (2011). The GABAergic deficit hypothesis of major depressive disorder. *Mol. Psychiatry* 16, 383–406. doi: 10.1038/mp.2010.120

Mackenzie, C. S., Reynolds, K., Chou, K. L., Pagura, J., and Sareen, J. (2011). Prevalence and correlates of generalized anxiety disorder in a national sample of older adults. *Am. J. Geriatr. Psychiatry* 19, 305–315. doi: 10.1097/JGP.0b013e318202bc62

Mansouri, M. T., Soltani, M., Naghizadeh, B., Farbood, Y., Mashak, A., and Sarkaki, A. (2014). A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze. *Pharmacol. Biochem. Behav.* 117, 40–46. doi: 10.1016/j.pbb.2013.12.011

Matsuzaki, M., Matsushita, H., Tomizawa, K., and Matsui, H. (2012). Oxytocin: a therapeutic target for mental disorders. *J. Physiol. Sci.* 62, 441–444. doi: 10.1007/s12576-012-0232-9

Mi, G., Liu, S., Zhang, J., Liang, H., Gao, Y., Li, N., et al. (2017). Levo-tetrahydroberberrubine produces anxiolytic-like effects in mice through the 5-HT_{1A} receptor. *PLoS One* 12:e0168964. doi: 10.1371/journal.pone.0168964

Moniruzzaman, M., Mannan, M. A., Hossen Khan, M. F., Abir, A. B., and Afroze, M. (2018). The leaves of *Crataeva nurvala* buch-ham. Modulate locomotor and anxiety behaviors possibly through GABAergic system. *BMC Complement. Altern. Med.* 18:283. doi: 10.1186/s12906-018-2338-y

Muller, C. P., Carey, R. J., Huston, J. P., and De Souza Silva, M. A. (2007). Serotonin and psychostimulant addiction: focus on 5-HT_{1A}-receptors. *Prog. Neurobiol.* 81, 133–178. doi: 10.1016/j.pneurobio.2007.01.001

Narasingam, M., Vijeepallam, K., Mohamed, Z., and Pandy, V. (2017). Anxiolytic- and antidepressant-like activities of a methanolic extract of *Morinda citrifolia* Linn. (noni) fruit in mice: involvement of benzodiazepine-$GABA_A$ergic, serotonergic and adrenergic systems. *Biomed. Pharmacother.* 96, 944–952. doi: 10.1016/j.biopha.2017.11.148

Pires, L. F., Costa, L. M., Silva, O. A., de Almeida, A. A., Cerqueira, G. S., de Sousa, D. P., et al. (2013). Anxiolytic-like effects of carvacryl acetate, a derivative of carvacrol, in mice. *Pharmacol. Biochem. Behav.* 112, 42–48. doi: 10.1016/j.pbb.2013.09.001

Pollack, M. H. (2002). New advances in the management of anxiety disorders. *Psychopharmacol. Bull.* 36, 79–94.

Rotheneichner, P., Romanelli, P., Bieler, L., Pagitsch, S., Zaunmair, P., Kreutzer, C., et al. (2017). Tamoxifen activation of cre-recombinase has no persisting effects on adult neurogenesis or learning and anxiety. *Front. Neurosci.* 11:27. doi: 10.3389/fnins.2017.00027

Ruan, J. (2003). *Chinese-English Synopsis of Prescription of the Golden Chamber*. Shanghai: Shanghai Science and Technology Publishing Company.

Shapiro, Z. R., Cerasiello, S., Hartshorne, L., and Matell, M. S. (2018). 5-HT1a receptor involvement in temporal memory and the response to temporal ambiguity. *Front. Neurosci.* 12:439. doi: 10.3389/fnins.2018.00439

Shorter, E., and Tyrer, P. (2003). Separation of anxiety and depressive disorders: blind alley in psychopharmacology and classification of disease. *BMJ* 327, 158–160. doi: 10.1136/bmj.327.7407.158

Thorn, D. A., Zhang, Y., and Li, J. X. (2016). Effects of the imidazoline I2 receptor agonist 2-BFI on the development of tolerance to and behavioural/physical dependence on morphine in rats. *Br. J. Pharmacol.* 173, 1363–1372. doi: 10.1111/bph.13435

Walf, A. A., and Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* 2, 322–328. doi: 10.1038/nprot.2007.44

Wilson, C. A., Vazdarjanova, A., and Terry, A. V. Jr. (2013). Exposure to variable prenatal stress in rats: effects on anxiety-related behaviors, innate and contextual fear, and fear extinction. *Behav. Brain Res.* 238, 279–288. doi: 10.1016/j.bbr.2012.10.003

Zhang, J., Cai, C. Y., Wu, H. Y., Zhu, L. J., Luo, C. X., and Zhu, D. Y. (2016). CREB-mediated synaptogenesis and neurogenesis is crucial for the role of 5-HT1a receptors in modulating anxiety behaviors. *Sci. Rep.* 6:29551. doi: 10.1038/srep29551

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Oral Contraceptives Impair Complex Emotion Recognition in Healthy Women

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Despite the widespread use of oral contraceptives (OCs), remarkably little is known about the effects of OCs on emotion, cognition, and behavior. However, coincidental findings suggest that OCs impair the ability to recognize others' emotional expressions, which may have serious consequences in interpersonal contexts. To further investigate the effects of OCs on emotion recognition, we tested whether women who were using OCs (n = 42) would be less accurate in the recognition of complex emotional expressions than women who were not using OCs (n = 53). In addition, we explored whether these differences in emotion recognition would depend on women's menstrual cycle phase. We found that women with OC use were indeed less accurate in the recognition of complex expressions than women without OC use, in particular during the processing of expressions that were difficult to recognize. These differences in emotion recognition did not depend on women's menstrual cycle phase. Our findings, thus, suggest that OCs impair women's emotion recognition, which should be taken into account when informing women about the side-effects of OC use.

Keywords: oral contraceptives, menstrual cycle, estrogen, progesterone, emotion recognition, social cognition

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INTRODUCTION

Although oral contraceptives (OCs) have been regarded as one of the best studied drugs in the history of medicine, remarkably little is known about the psychological and behavioral consequences of OC use (Montoya and Bos, 2017). Given that more than 100 million women worldwide use OCs for birth control (Christin-Maitre, 2013), studies investigating the effects of OCs on emotion, cognition, and behavior are highly warranted. Most relevant here, only a few studies investigated how OCs affect women's ability to recognize other's emotional expressions (Hamstra et al., 2014, 2015; Radke and Derntl, 2016). However, the ability to recognize others' emotional expressions is essential for the initiation and maintenance of interpersonal relationships, in particular intimate ones (Schmidt and Cohn, 2001). As an inaccurate recognition of others' emotional expressions may lead to interpersonal conflicts, it seems mandatory to further investigate how OC use affects women's emotion recognition abilities. Previous studies revealed inconsistent

findings regarding the effects of OCs on emotion recognition. Hamstra et al. (2014) coincidentally revealed a less accurate recognition of negative expressions in women who used OCs compared to women who did not use OCs. However, the impairments in emotion recognition partially depended on genetic variations of mineralocorticoid receptor haplotypes (Hamstra et al., 2015), complicating the interpretation of the respective findings. Radke and Derntl (2016), on the contrary, found no differences in emotion recognition between women with and without OC use. It should be noted, however, that methodological aspects of the studies, such as sample composition (e.g., inclusion of women at different menstrual cycle phases) or task characteristics (e.g., employment of tasks with suboptimal task difficulty), may have accounted for the inconsistency of findings. Consequently, it remains to be determined whether the coincidental findings of Hamstra et al. (2014, 2015) can be replicated and extended in studies that explicitly consider these methodological aspects throughout study design.

In the present study, we further investigated the effects of OC use on women's emotion recognition abilities. As women with and without OC use may show minimal differences in emotion recognition (Radke and Derntl, 2016), we used a task that was sensitive enough to detect even subtle impairments in women's emotion recognition (Reading the Mind in the Eyes Test, RMET; Baron-Cohen et al., 2001). The task required the recognition of complex emotional expressions, whereas the tasks of previous studies required the recognition of basic emotional expressions (Hamstra et al., 2014, 2015; Radke and Derntl, 2016). However, both types of tasks included expressions that had been categorized with respect to their valence. We were, thus, able to investigate whether differences in emotion recognition dependent on the valence of the expressions as suggested by Hamstra et al. (2014, 2015). As the expressions of our task had also been categorized with respect to their difficulty, we were able to investigate whether differences in emotion recognition dependent on the difficulty of the expressions as suggested by Radke and Derntl (2016). Accordingly, we performed two analyses to investigate differences in emotion recognition between women with and without OC use, one that was concerned with the valence of the expressions and another one that was concerned with the difficulty of the expressions. On basis of previous studies (Hamstra et al., 2014, 2015; Radke and Derntl, 2016), we expected that women with OC would show more impairments in emotion recognition than women without OC use, in particular during the processing of negative and difficult expressions. In addition to these hypothesis-driven analyses, we analyzed whether cyclic variations of women's estrogen and progesterone levels contributed to possible differences in emotion recognition as suggested by Hamstra et al. (2014, 2015) as well as by Radke and Derntl (2016). It should be noted, however, that the respective analyses had an exploratory character because our study was designed to investigate differences in emotion recognition that dependent on women's OC use rather than to investigate differences in emotion recognition that dependent on women's menstrual cycle phase. However, combining exploratory with hypothesis-driven analyses allowed us to investigate the effects of OC use on women's emotion recognition in greater detail than in previous studies (Hamstra et al., 2014, 2015; Radke and Derntl, 2016).

EXPERIMENTAL PROCEDURES

Participants

Using G*Power (Faul et al., 2007), we performed a power analysis to determine the number of participants that we needed to detect meaningful differences in emotion recognition between participants with and without OC use. Although Hamstra et al. (2014) found a large difference in emotion recognition between participants with and without OC use (f = 0.77–1.41), we based our power analysis on a more conservative estimate of the difference in emotion recognition (f = 0.30). To be able to detect a medium-sized difference in emotion recognition (α = 0.05, 1−β = 0.80, f = 0.30), we had to recruit a minimum of 62 participants for our hypothesis-driven analysis involving the comparison of women with and without OC use and a minimum of 75 participants for our exploratory analysis involving the comparison of women with OC use and women without OC use who were in the follicular or luteal phase of their menstrual cycle. In order to be considered for recruitment, women had to be aged between 18 and 35 years, to be native speakers and to be willing to share information regarding their menstrual cycle and their use of OCs (e.g., information regarding cycle length or OC type). Only women who had a regular cycle of 28 days and who, if at all, were not using any other hormonal contraceptives than oral ones were included in the study. Women who were in psychotherapeutic or psychopharmacological treatment were excluded from the study. Taking these inclusion and exclusion criteria into account, we recruited 95 women, 42 women with and 53 women without OC use, for our study. To determine the menstrual cycle phase of the 53 women who were not using OCs, we used a similar classification scheme as in previous studies (Derntl et al., 2008; Ertman et al., 2011). According to this classification scheme, 35 women without OC were in the follicular phase (0–14 days after menses onset) and 18 women without OC use were in the luteal phase (15–28 days after menses onset) of their menstrual cycle. More specifically, women without OC were either in the early follicular phase or in the mid-luteal phase of their cycle [follicular phase: M = 6.63, SD = 3.85, luteal phase: M = 20.11, SD = 4.08, $F(1,51) = 139.86$, $p < 0.001$, $\eta_p^2 = 0.73$]. Following an established procedure (DeBruine et al., 2005; Jones et al., 2005; Roney and Simmons, 2008), we used day-specific reference values of estrogen and progesterone levels that are typically displayed by women during the menstrual cycle (Stricker et al., 2006) to confirm that women without OC use were indeed in the follicular [estrogen (pg/ml): M = 77.82, SD = 63.96; progesterone (ng/ml): M = 0.28, SD = 0.18] or luteal [estrogen (pg/ml): M = 112.09, SD = 25.03; progesterone (ng/ml): M = 7.46, SD = 4.19] phase of their cycle [estrogen: $F(1,51) = 4.76$, $p = 0.034$, $\eta_p^2 = 0.14$; progesterone: $F(1,51) = 104.27$, $p \leq 0.001$, $\eta_p^2 = 0.67$]. Of the 42 women who were using OCs, 21 were using OCs

TABLE 1 | Oral contraceptives.

Frequency	Compounds	Generation
2	EE (0.02 mg)/DRSP (3 mg)	4
5	EE (0.02 mg)/LNG (0.100 mg)	2
3	EE (0.02, 0.03 mg)/DSG (0.15 mg)	3
2	EE (0.02, 0.03 mg)/DRSP (3 mg)	4
4	EE (0.02, 0.03 mg)/LNG (0.100, 0.125 mg)	2
2	EE (0.02, 0.03 mg)/LNG (0.100, 0.150 mg)	2
3	EE (0.02, 0.03 mg)/LNG (0.100, 0.125, 0.150 mg)	2
5	EE (0.03 mg)/CMA (2 mg)	4
1	EE (0.03 mg)/CPA (2 mg)	4
11	EE (0.03 mg)/DNG (2 mg)	1
1	EE (0.03 mg)/LNG (0.125 mg)	3
2	EE (0.035mg)/NG (0.25 mg)	3
1	EE (0.035, 0.030, 0.035 mg)/DSG (0.05, 0.100 mg, 0.15 mg)	3

EE, ethinylestradiol; CMA, chlormadinone acetate; CPA, cyproterone acetate; DNG, dienogest; DSG, desogestrel; DRSP, drospirenone; NG, norgestimate; LNG, levonorgestrel.

with androgenic properties and 21 were using OCs with anti-androgenic properties (see **Table 1**). As we were interested to investigate the global effects of OC use on emotion recognition, we recruited women who were in the inactive as well as active intake phase. All women provided written-informed consent before they participated in the study and were fully debriefed after they completed the study. The protocol of the study was approved by the ethics committee of the University of Rostock and the ethics committee of the German Society of Psychology (DGPs).

Procedure

Following a screening interview (Lischke et al., 2017), participants were invited to the laboratory where they completed a series of questionnaires regarding their menstrual cycle, contraceptive use, age, education, distress (Brief Symptom Inventory 18, BSI-18; Franke et al., 2017), and empathy (Interpersonal Reactivity Index, IRI; Davis, 1983). Thereafter, they completed the emotion recognition task (RMET; Baron-Cohen et al., 2001).

Brief Symptom Inventory 18

The BSI-18 (Franke et al., 2017) was used to asses participants' distress at the time of the study. The BSI-18, which measured anxious, depressive, and somatoform symptoms within the last 7 days, displayed good psychometric properties (BSI-18: $\alpha = 0.80$).

Interpersonal Reactivity Index

The IRI (Davis, 1983) was used to assess participants' empathetic traits. The IRI, which measured empathetic traits related to empathetic concern, empathetic contagion, and empathetic perspective taking (Davis, 1994), demonstrated good psychometric properties (IRI: $\alpha = 0.76$).

Reading the Mind in the Eyes Test

The RMET (Baron-Cohen et al., 2001) was used to assess participants' ability to recognize complex emotional expressions. These expressions had to be recognized on basis of subtle cues that were provided by the eye region of faces. The respective black and white pictures were shown in random order on a computer screen (1 practice picture, 36 test pictures). Each eye region was shown together with four labels, each describing a particular emotional expression (three distractors, one target). Participants had to indicate the label that best described the expression by pressing a corresponding button as fast as possible. On the basis of participants' responses, the percentage of correctly identified expressions and the corresponding reaction times[1] were measured. Similar as in previous studies (Guastella et al., 2010; Hysek et al., 2012; Feeser et al., 2015; Lischke et al., 2017; Lischke et al., 2018), established algorithms were used to determine these measures with respect to expressions that differed in valence (Harkness et al., 1999) and with respect to expressions that differed in difficulty (Domes et al., 2007).

Statistical Analysis

SPSS 22 (SPSS Inc., Chicago, IL, United States) was used to run two sets of analyses, a hypothesis-driven one and an exploratory one. In the hypotheses-driven analyses, chi-square tests, one-way ANOVAs, and mixed-design ANOVAs were used to compare participant characteristics and emotion recognition performance between participants with and without OC use. In the exploratory analyses, chi-square tests, two-way ANOVAs, and mixed-design ANOVAs were used to compare participant characteristics and emotion recognition performance between participants with OC use, participants without OC use who were in the follicular phase, and participants without OC use who were in the luteal phase. The significance level for all analyses was set at $p \leq 0.05$ (two-sided) and, whenever necessary, corrected for multiple comparisons using the Bonferroni method (Shaffer, 1995). However, the correction for multiple comparisons was only considered in the context of hypothesis-driven not exploratory analyses because exploratory analyses follow a liberal rather than conservative analysis strategy. In addition to the significance level, effect size measures (d, η_p^2) were reported to facilitate the interpretation of the respective findings (Cohen, 1992).

RESULTS

Differences in Participant Characteristics

Chi-square tests and one-way ANOVAs revealed no differences in demographical [age: $F(1,93) = 0.71$, $p = 0.401$, $\eta_p^2 = 0.01$;

[1]In the following, we focus on participants' recognition accuracy because the respective analyses did not reveal differences in reaction times that dependent on participants' OC use or cycle phase (data not shown), implying that there was no speed accuracy trade-off that may have accounted for the reported differences in emotion recognition between participants with OC use and participants without OC use that were in the luteal or follicular phase of their menstrual cycle.

TABLE 2 | Participant characteristics.

	OC (*n* = 42)		OC-AP (*n* = 42)		OC-AAP (*n* = 42)		FOL+LUT (*n* = 53)		FOL (*n* = 35)		LUT (*n* = 18)	
	M	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***
Age	22.55	2.45	22.81	2.66	22.29	2.26	23.08	3.42	22.86	3.49	23.50	3.33
Education												
Higher education	1		0		1		0		0		0	
Intermediate education	41		20		21		53		35		18	
Distress (BSI-18)[1]	0.47	0.32	0.43	0.30	0.52	0.33	0.53	0.39	0.60	0.42	0.40	0.32
Empathy (IRI)	50.77	7.98	50.05	8.19	46.90	8.35	48.48	8.33	51.91	7.64	48.56	8.40

OC, women with OC use; OC-AP, women with androgenic OC use; OC-AAP, women with anti-androgenic OC use; FOL+LUT, women without OC use that were in the follicular and luteal phase; FOL, women without OC use who were in the follicular phase; LUT, women without OC use who were in the luteal phase; BSI-18, Brief Symptom Inventory 18 (Franke et al., 2017); IRI, Interpersonal Reactivity Index (Davis, 1983, 1994). [1]Data were missing for one woman with OC use.

education: $\chi^2(1, N = 95) = 1.28$, $p = 0.259$], psychopathological [distress: $F(1,92) = 0.63$, $p = 0.439$, $\eta_P^2 = 0.01$] or psychological [empathy: $F(1,93) = 1.87$, $p = 0.175$, $\eta_P^2 = 0.02$] characteristics between participants with and without OC use. Participants who used androgenic or anti-androgenic OCs also did not differ from one another with respect to their demographical [age: $F(1,40) = 0.47$, $p = 0.495$, $\eta_P^2 = 0.01$; education: $\chi^2(1, N = 42) = 1.02$, $p = 0.311$] psychopathological [distress: $F(1,39) = 0.70$, $p = 0.407$, $\eta_P^2 = 0.02$], or psychological [empathy: $F(1,40) = 1.52$, $p = 0.226$, $\eta_P^2 = 0.04$] characteristics. There were also no differences in demographical [age: $F(2,92) = 0.62$, $p = 0.540$, $\eta_P^2 = 0.01$; education: $\chi^2(2, N = 95) = 1.28$, $p = 0.529$], psychopathological [distress: $F(2,91) = 2.29$, $p = 0.108$, $\eta_P^2 = 0.05$], or psychological [empathy: $F(2,92) = 1.97$, $p = 0.146$, $\eta_P^2 = 0.04$] characteristics between participants with OC use and participants without OC use who were in the follicular or luteal phase of their menstrual cycle as indicated by chi-square tests and two-way ANOVAs. Taken together, these findings suggest that we investigated a sample of participants that was very homogenous in terms of demographical, psychopathological and psychological characteristics. A detailed account of these participant characteristics is given in **Table 2**.

Valence-Dependent Differences in Emotion Recognition

A mixed-design ANOVA indicated that participants with OC use were less accurate in emotion recognition than participants without OC use [effect of group: $F(1,93) = 6.51$, $p = 0.012$, $\eta_P^2 = 0.07$; effect of valence: $F(1.70,157.71) = 8.56$, $p = 0.001$, $\eta_P^2 = 0.08$], irrespective of the expressions' valence [interaction of group and valence: $F(1.70, 157.71) = 0.29$, $p = 0.712$, $\eta_P^2 = 0.00$]. Across all participants, recognition accuracy was lower for negative than positive or neutral expressions as indicated by *post hoc* tests [negative vs. positive: $p = 0.003$, negative vs. neutral: $p = 0.002$, positive vs. neutral: $p = 0.608$]. These differences in emotion recognition are shown in **Figure 1**.

Another mixed-design ANOVA revealed that the aforementioned differences in emotion recognition between participants with and without OC use did not depend on the menstrual cycle phase of participants without OC use [effect of group: $F(1,92) = 3.28$, $p = 0.042$, $\eta_P^2 = 0.07$; effect of valence: $F(1.69,155.56) = 8.21$, $p = 0.001$, $\eta_P^2 = 0.08$; interaction of group and valence: $F(3.38,155.56) = 1.27$, $p = 0.288$, $\eta_P^2 = 0.03$]. *Post hoc* tests indicated that participants with OC use were less accurate in emotion recognition than both, participants without OC use who were in the follicular phase [$p = 0.035$, $d = 0.48$] and participants without OC use who were in luteal phase [$p = 0.038$, $d = 0.58$]. There were, however, no differences in emotion recognition between participants without OC use who were in the follicular or luteal phase [$p = 0.725$, $d = 0.10$]. Again, these differences in emotion recognition occurred irrespective of the expressions' valence because all participants were less accurate in the recognition of negative than positive or neutral expressions as indicated by *post hoc* tests [negative vs. positive: $p = 0.001$, negative vs. neutral: $p = 0.003$, positive vs. neutral: $p = 0.111$]. **Figure 1** depicts the aforementioned difference in emotion recognition between participants with and without OC use who were in the follicular or luteal phase.

Of note, there were no differences in the recognition of positive, negative, or neutral expressions between participants who used OCs with androgenic and anti-androgenic properties (see the **Supplementary Material**). This implies that participants with OC use were generally less accurate in the recognition of these expressions than participants without OC use, irrespective of the type of OCs that was used by the participants.

Difficulty-Dependent Differences in Emotion Recognition

A mixed-design ANOVA indicated differences in emotion recognition between participants with and without OC use that depended on the expressions' difficulty [effect of group: $F(1,93) = 7.52$, $p = 0.007$, $\eta_P^2 = 0.08$; effect of difficulty: $F(1,93) = 256.00$, $p = 0.001$, $\eta_P^2 = 0.73$; interaction of group and difficulty: $F(1,93) = 5.71$, $p = 0.010$, $\eta_P^2 = 0.06$]. Follow-up ANOVAs revealed that these differences only emerged during the processing of difficult not easy expressions: Whereas participants with and without OC use did not differ in recognition accuracies for easy expressions [$F(1,93) = 0.63$, $p = 0.428$, $\eta_P^2 = 0.01$], participants with OC use were less accurate in the recognition of difficult expressions than participants with OC

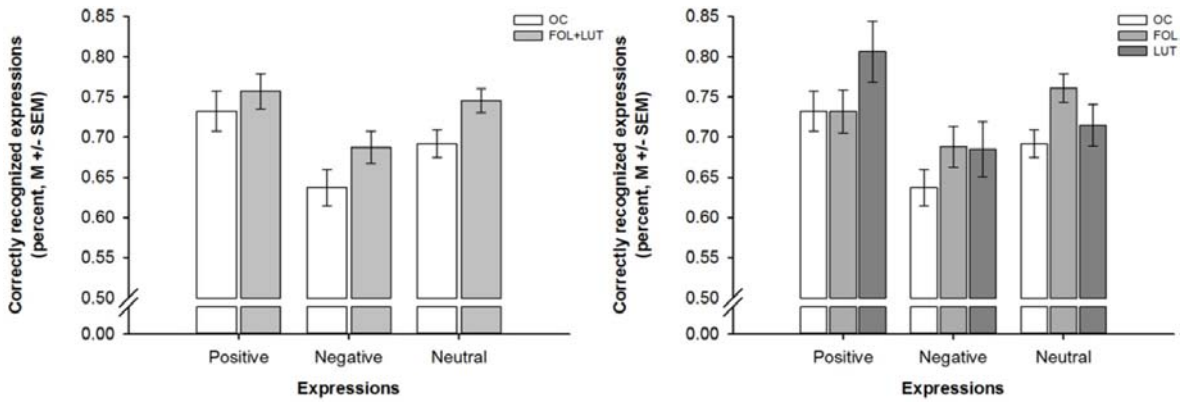

FIGURE 1 | Barplots showing valence-dependent differences in emotion recognition as a function of OC use and menstrual cycle phase. *Left panel*. Valence-dependent differences in emotion recognition between women with OC use (OC, white bars) and women without OC use who were in the follicular and luteal (FOL + LUT, light gray bars) phase. *Right panel*. Valence-dependent differences in emotion recognition between women with OC use (OC, white bars) and women without OC use who were in the follicular (FOL, medium gray bars) or luteal (LUT, dark gray bars) phase. Bars represent $M \pm$ SEM.

use [$F(1,93) = 10.59$, $p = 0.002$, $\eta_p^2 = 0.10$]. These differences in emotion recognition are depicted in **Figure 2**.

Another mixed-design ANOVA revealed that the aforementioned differences in emotion recognition between participants with and without OC use did not depend on the menstrual cycle phase of participants without OC use [effect of group: $F(1,92) = 3.74$, $p = 0.027$, $\eta_p^2 = 0.08$; effect of difficulty: $F(2,92) = 214.51$, $p = 0.001$, $\eta_p^2 = 0.70$; interaction of group and difficulty: $F(2,92) = 3.41$, $p = 0.037$, $\eta_p^2 = 0.07$]. Follow-up ANOVAs revealed again that participants with and without OC differed in recognition accuracies for difficult [$F(2,92) = 5.53$, $p = 0.005$, $\eta_p^2 = 0.11$] but not easy [$F(2,92) = 0.47$, $p = 0.628$, $\eta_p^2 = 0.01$] expressions. *Post hoc* tests indicated that participants with OC use were less accurate in the recognition of difficult expressions than both, participants without OC use who were in the follicular phase [$p = 0.002$, $d = 0.74$] and, albeit only on a trend level, participants without OC use who were in the luteal phase [$p = 0.062$, $d = 0.53$]. There were again no differences in emotion recognition between participants without OC use who were in the follicular or luteal phase [$p = 0.471$, $d = 0.22$]. These differences in emotion recognition are visualized in **Figure 2**.

Of note, participants who used OCs with androgenic and anti-androgenic properties did not differ in the recognition of easy and difficult expressions (see the **Supplementary Material**). This implies that participants with OC use were generally less accurate in the recognition of difficulty expressions than participants without OC use, irrespective of the type of OCs that was used by the participants.

DISCUSSION

In the present study, we investigated possible differences in complex emotion recognition between women with and

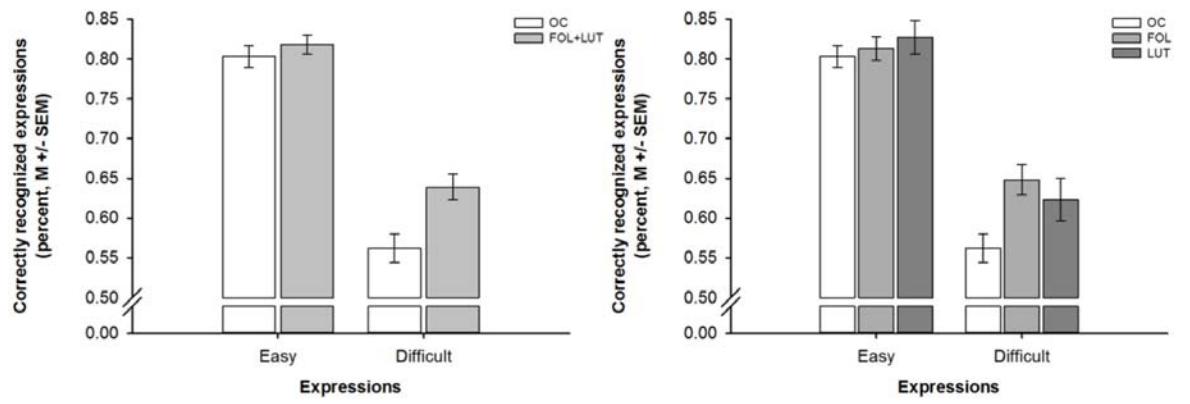

FIGURE 2 | Barplots showing difficulty-dependent differences in emotion recognition as a function of OC use and menstrual cycle phase. *Left panel*. Difficulty-dependent differences in emotion recognition between women with OC use (OC, white bars) and women without OC use who were in the follicular and luteal (FOL+LUT, light gray bars) phase. *Right panel*. Difficulty-dependent differences in emotion recognition between women with OC use (OC, white bars) and women without OC use who were in the follicular (FOL, medium gray bars) or luteal (LUT, dark gray bars) phase. Bars represent $M \pm$ SEM.

without OC use. To this end, we administered a well-established emotion recognition task to a large and homogenous sample of healthy women who were well-characterized with respect to their OC use. We run two set of analyses, a hypothesis-driven one and an exploratory one. The hypothesis-driven analyses were used to test whether women with OC would be less accurate in emotion recognition than women without OC use and the exploratory analyses were used to explore whether these differences could be explained by cyclic variations of women's progesterone and estrogen levels.

According to our hypothesis-driven analyses, women with OC use were less accurate in emotion recognition than women without OC use. Our findings are, thus, consistent with the findings of Hamstra et al. (2014, 2015) who found similar differences in emotion recognition between women with and without OC use. However, Hamstra et al. (2014, 2015) reported that these differences in emotion recognition were most pronounced during the processing of negative compared to positive expressions. We also observed that women with and without OC use tended to differ on the recognition of negative expressions (see **Table 1**), but our analyses indicated valence-unspecific rather than valence-specific differences in emotion recognition following OC use. Notably, Radke and Derntl (2016) failed to find any differences in emotion recognition between women with and without OC use, presumably because the task was not difficult enough to challenge women's emotion recognition abilities. The task employed by Radke and Derntl (2016) involved the presentation of faces showing emotional expressions of maximal intensity, whereas the task employed by Hamstra et al. (2014, 2015) involved the presentation of faces showing emotional expressions of minimal, moderate, or maximal intensity. Consequently, women's emotion recognition abilities were more challenged in the study by Hamstra et al. (2014, 2015) than in the study by Radke and Derntl (2016), thereby increasing the chance to detect even subtle differences in emotion recognition between women with and without OC use. The same may have been true for the task employed in the present study that involved the presentation of eye regions of faces expressing complex emotional expressions of varying intensities. In this respect, it is noteworthy that women with OC use showed the most pronounced impairments in emotion recognition during the processing of expressions that were difficult to recognize. It is, thus, quite likely that differences in task difficulty accounted for the inconsistent findings of previous studies (Hamstra et al., 2014, 2015; Radke and Derntl, 2016).

Our exploratory analysis revealed that women with OC use were less accurate in emotion recognition as both, women without OC use who were in the follicular phase and women without OC use who were in the luteal phase. Considering that OCs stabilize women's menstrual cycle by suppressing the rise of gonadal hormones like estrogen and progesterone (Frye, 2006) may help to understand these differences in emotion recognition. Compared to women without OC use who are in follicular or luteal phase, women with OC use show much lower estrogen and progesterone levels (Fleischman et al., 2010). Low estrogen and progesterone levels may, thus, have been responsible for the impaired emotion recognition following OC use. However, estrogen and progesterone levels are also modulated by other hormones, implying that it may be too simplistic to assume that impairments in emotion recognition were caused by estrogen and progesterone alone. Oxytocin, for example, which is also affected by OC use, may interact with estrogen and progesterone during emotion processing (Lischke et al., 2012b; Scheele et al., 2016). It may, therefore, be more appropriate to assume that OC-induced impairments in emotion recognition are caused by various hormones, including but not limited to estrogen and progesterone.

However, estrogen and progesterone probably play a major role in mediating the effects of OC use on emotion recognition (Montoya and Bos, 2017). Previous studies revealed that estrogen and progesterone levels modulate activity and connectivity changes in prefrontal and temporal brain regions that are implicated in the processing of emotional expressions (Peper et al., 2011; Toffoletto et al., 2014). Of these brain regions, the prefrontal cortex and the amygdala may be of particular relevance because the recognition of complex emotional expressions crucially depends on activity and connectivity changes in these brain regions (Baron-Cohen et al., 1999; Adolphs et al., 2002; Adams et al., 2009; Dal Monte et al., 2014). OC-induced changes in estrogen and progesterone levels are associated with changes in amygdala activity and amygdala–prefrontal connectivity in the presence (Gingnell et al., 2013; Petersen and Cahill, 2015) and absence (Lisofsky et al., 2016; Engman et al., 2017) of emotion recognition tasks. It, thus, seems plausible that differences in amygdala activity and amygdala–prefrontal connectivity accounted for differences in emotion recognition between women with and without OC use.

Overall, our findings suggest that OCs impair the recognition of complex emotional expressions that are difficult to recognize, presumably via activity and connectivity changes in prefrontal and temporal brain regions that are caused by OC-induced changes in estrogen and progesterone levels. Although these suggestions are plausible, they should be treated with caution for several reasons. First of all, our study was designed to investigate differences in emotion recognition that dependent on women's OC use rather than to investigate differences in emotion recognition that dependent on women's menstrual cycle phase. As we were primarily concerned with the recruitment of women with and without OC use, we were unable to control for an unequal distribution of women across the different cycle phases. This may have been particularly problematic with respect to women who were in the luteal phase because these women were underrepresented in the present study. Nonetheless, we tried our best to characterize the women in the different cycle phases. As women's self-reports may have been too inaccurate to determine their cycle phase, we tried to confirm their cycle phase on basis of their estrogen and progesterone levels. Similarly as in previous studies (DeBruine et al., 2005; Jones et al., 2005; Roney and Simmons, 2008), we used day-specific

reference values for an estimation of women's estrogen and progesterone levels during the respective cycle phases (Stricker et al., 2006). Following previous suggestions (Hamstra et al., 2014, 2015; Radke and Derntl, 2016), we used these hormone levels to investigate cycle-dependent differences in women's emotion recognition. Clearly, it would have been favorable to use actual instead of estimated estrogen and progesterone levels in the respective analyses. Future studies should, therefore, assess these and other hormone levels via blood or salivary samples to further investigate how different hormones levels (e.g., estrogen levels, progesterone levels, or oxytocin levels) affect emotion processing in women with and without OC use. We, thus, labeled the respective analyses "exploratory" to highlight that the respective findings have to be replicated and extended in future studies. Second, our study was designed to investigate global rather than specific effects of OC use on women's emotion recognition. Accordingly, we did not investigate whether the type (e.g., continued use, discontinued use), duration (e.g., short-term use, long-term use), or time (e.g., active use, inactive use) of OC use differentially affected the processing of emotional expressions in women. Future studies should, thus, gather more detailed information about women's OC use than those that had been assessed in the present study. This may help to reveal more specific effects of OC use on women's emotion recognition. These studies should also use emotion recognition tasks that allow a more specific assessment of the emotional expressions that are susceptible to OC effects. Whereas previous studies showed that women with OC use are impaired during the processing of negative expressions (Hamstra et al., 2014, 2015), the present study revealed that these impairments are most pronounced during the processing of expressions that are difficult to recognize. According to these findings, women with OC use may be specifically impaired during the processing of negative expressions that are difficult to recognize. Future studies that use more challenging emotion recognition tasks, like, for example, the morphed emotion recognition task (Lischke et al., 2012a), may help to identify emotion-specific impairments in women's emotion recognition. These studies may also help to determine whether these impairments occur generally during the processing of expressions that are difficult to recognize, regardless whether these expressions are complex or basic ones. Third, our study was not designed to investigate the consequences of OC-induced impairments in women's emotion recognition in interpersonal contexts. Future studies should, therefore, investigate whether these impairments alter women's ability initiate and maintain intimate relationships. Fourth, our study was designed to investigate the effects of OC use on women's emotion recognition in a quasi-experimental setting. Future studies that investigate these effects in experimental settings may be better suited to determine whether there is a causal or correlational relationship between OC use and impairments in emotion recognition.

To sum up, the findings of the present study suggest that OCs impair the recognition of complex emotional expressions that are difficult to recognize. However, these findings have to be extended and replicated in further studies before any recommendations about the current practice of OC use can be made. Considering that more and more women start using OCs shortly after onset of puberty (van Hooff et al., 1998; Krishnamoorthy et al., 2008) indicates that these types of studies are highly warranted to determine the positive and negative consequences of OC use on emotion, cognition, and behavior.

AUTHOR CONTRIBUTIONS

AL and RP designed the study, analyzed the data, and wrote the manuscript. AL, AM-M, JW, MJ, MW, and RP collected the data. AH, AM-M, JW, MJ, and MW contributed to writing, reviewing, and editing of the manuscript. All authors approved the final version of the 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/fnins.2018.01041/full#supplementary-material

REFERENCES

Adams, R. B., Rule, N. O., Franklin, R. G., Wang, E., Stevenson, M. T., Yoshikawa, S., et al. (2009). Cross-cultural reading the mind in the eyes: an fMRI investigation. *J. Cogn. Neurosci.* 22, 97–108. doi: 10.1162/jocn.2009.21187

Adolphs, R., Baron-Cohen, S., and Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. *J. Cogn. Neurosci.* 14, 1264–1274. doi: 10.1162/089892902760807258

Baron-Cohen, S., Ring, H. A., Wheelwright, S., Bullmore, E. T., Brammer, M. J., Simmons, A., et al. (1999). Social intelligence in the normal and autistic brain:

an fMRI study. *Eur. J. Neurosci.* 11, 1891–1898. doi: 10.1046/j.1460-9568.1999.00621.x

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., and Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J. Child Psychol. Psychiatry* 42, 241–251. doi: 10.1111/1469-7610.00715

Christin-Maitre, S. (2013). History of oral contraceptive drugs and their use worldwide. *Best Pract. Res. Clin. Endocrinol. Metab.* 27, 3–12. doi: 10.1016/j.beem.2012.11.004

Cohen, J. (1992). A power primer. *Psychol. Bull.* 112, 155–159. doi: 10.1037/0033-2909.112.1.155

Dal Monte, O., Schintu, S., Pardini, M., Berti, A., Wassermann, E. M., Grafman, J., et al. (2014). The left inferior frontal gyrus is crucial for reading the mind in the eyes: brain lesion evidence. *Cortex* 58, 9–17. doi: 10.1016/j.cortex.2014.05.002

Davis, M. (1983). Interpersonal Reactivity Index. A multidimensional approach to individual differences in empathy. *J. Pers. Soc. Psychol.* 44, 113–126. doi: 10.1037/0022-3514.44.1.113

Davis, M. (1994). *Empathy: A Social Psychological Approach*. Madison, WI: Brown & Benchmark's.

DeBruine, L. M., Jones, B. C., and Perrett, D. I. (2005). Women's attractiveness judgments of self-resembling faces change across the menstrual cycle. *Horm. Behav.* 47, 379–383. doi: 10.1016/j.yhbeh.2004.11.006

Derntl, B., Windischberger, C., Robinson, S., Lamplmayr, E., Kryspin-Exner, I., Gur, R. C., et al. (2008). Facial emotion recognition and amygdala activation are associated with menstrual cycle phase. *Psychoneuroendocrinology* 33, 1031–1040. doi: 10.1016/j.psyneuen.2008.04.014

Domes, G., Heinrichs, M., Michel, A., Berger, C., and Herpertz, S. C. (2007). Oxytocin improves "mind-reading" in humans. *Biol. Psychiatry* 61, 731–733. doi: 10.1016/j.biopsych.2006.07.015

Engman, J., Poromaa, I. S., Moby, L., Wikstrom, J., Fredrikson, M., and Gingnell, M. (2017). Hormonal cycle and contraceptive effects on amygdala and salience resting-state networks in women with previous affective side effects on the pill. *Neuropsychopharmacology* 43, 555–563. doi: 10.1038/npp.2017.157

Ertman, N., Andreano, J. M., and Cahill, L. (2011). Progesterone at encoding predicts subsequent emotional memory. *Learn. Mem.* 18, 759–763. doi: 10.1101/lm.023267.111

Faul, F., Erdfelder, E., Lang, A. G., and Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi: 10.3758/BF03193146

Feeser, M., Fan, Y., Weigand, A., Hahn, A., Gartner, M., Boker, H., et al. (2015). Oxytocin improves mentalizing - pronounced effects for individuals with attenuated ability to empathize. *Psychoneuroendocrinology* 53, 223–232. doi: 10.1016/j.psyneuen.2014.12.015

Fleischman, D. S., Navarrete, C. D., and Fessler, D. M. (2010). Oral contraceptives suppress ovarian hormone production. *Psychol. Sci.* 21, 750–752; authorrely 753. doi: 10.1177/0956797610368062

Franke, G. H., Jaeger, S., Glaesmer, H., Barkmann, C., Petrowski, K., and Braehler, E. (2017). Psychometric analysis of the brief symptom inventory 18 (BSI-18) in a representative German sample. *BMC Med. Res. Methodol.* 17:14. doi: 10.1186/s12874-016-0283-3

Frye, C. A. (2006). An overview of oral contraceptives: mechanism of action and clinical use. *Neurology* 66(6 Suppl. 3), S29–S36. doi: 10.1212/WNL.66.66_suppl_3.S29

Gingnell, M., Engman, J., Frick, A., Moby, L., Wikstrom, J., Fredrikson, M., et al. (2013). Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill–a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. *Psychoneuroendocrinology* 38, 1133–1144. doi: 10.1016/j.psyneuen.2012.11.006

Guastella, A. J., Einfeld, S. L., Gray, K. M., Rinehart, N. J., Tonge, B. J., Lambert, T. J., et al. (2010). Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol. Psychiatry* 67, 692–694. doi: 10.1016/j.biopsych.2009.09.020

Hamstra, D. A., de Kloet, E. R., van Hemert, A. M., de Rijk, R. H., and Van der Does, A. J. (2015). Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing. *Neuroscience* 286, 412–422. doi: 10.1016/j.neuroscience.2014.12.004

Hamstra, D. A., De Rover, M., De Rijk, R. H., and Van der Does, W. (2014). Oral contraceptives may alter the detection of emotions in facial expressions. *Eur. Neuropsychopharmacol.* 24, 1855–1859. doi: 10.1016/j.euroneuro.2014.08.015

Harkness, K., Sabbagh, M., Jacobson, J., Chowdrey, N., and Chen, T. (1999). Enhanced accuracy of mental state decoding in dysphoric college students. *Cogn. Emot.* 19, 999–1025. doi: 10.1080/02699930541000110

Hysek, C. M., Domes, G., and Liechti, M. E. (2012). MDMA enhances "mind reading" of positive emotions and impairs "mind reading" of negative emotions. *Psychopharmacology* 222, 293–302. doi: 10.1007/s00213-012-2645-9

Jones, B. C., Little, A. C., Boothroyd, L., Debruine, L. M., Feinberg, D. R., Smith, M. J., et al. (2005). Commitment to relationships and preferences for femininity and apparent health in faces are strongest on days of the menstrual cycle when progesterone level is high. *Horm. Behav.* 48, 283–290. doi: 10.1016/j.yhbeh.2005.03.010

Krishnamoorthy, N., Simpson, C. D., Townend, J., Helms, P. J., and McLay, J. S. (2008). Adolescent females and hormonal contraception: a retrospective study in primary care. *J. Adolesc. Health* 42, 97–101. doi: 10.1016/j.jadohealth.2007.06.016

Lischke, A., Berger, C., Prehn, K., Heinrichs, M., Herpertz, S. C., and Domes, G. (2012a). Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology* 37, 475–481. doi: 10.1016/j.psyneuen.2011.07.015

Lischke, A., Gamer, M., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., et al. (2012b). Oxytocin increases amygdala reactivity to threatening scenes in females. *Psychoneuroendocrinology* 37, 1431–1438. doi: 10.1016/j.psyneuen.2012.01.011

Lischke, A., Lemke, D., Neubert, J., Hamm, A. O., and Lotze, M. (2017). Inter-individual differences in heart rate variability are associated with inter-individual differences in mind-reading. *Sci. Rep.* 7:11557. doi: 10.1038/s41598-017-11290-1

Lischke, A., Pahnke, R., Koenig, J., Homuth, G., Hamm, A. O., and Wendt, J. (2018). COMT Val158Met genotype affects complex emotion recognition in healthy men and women. *Front. Neurosci.* 12:1007. doi: 10.3389/fnins.2018.01007

Lisofsky, N., Riediger, M., Gallinat, J., Lindenberger, U., and Kuhn, S. (2016). Hormonal contraceptive use is associated with neural and affective changes in healthy young women. *Neuroimage* 134, 597–606. doi: 10.1016/j.neuroimage.2016.04.042

Montoya, E. R., and Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. *Trends Cogn. Sci.* 21, 125–136. doi: 10.1016/j.tics.2016.11.005

Peper, J. S., van den Heuvel, M. P., Mandl, R. C., Hulshoff Pol, H. E., and van Honk, J. (2011). Sex steroids and connectivity in the human brain: a review of neuroimaging studies. *Psychoneuroendocrinology* 36, 1101–1113. doi: 10.1016/j.psyneuen.2011.05.004

Petersen, N., and Cahill, L. (2015). Amygdala reactivity to negative stimuli is influenced by oral contraceptive use. *Soc. Cogn. Affect. Neurosci.* 10, 1266–1272. doi: 10.1093/scan/nsv010

Radke, S., and Derntl, B. (2016). Affective responsiveness is influenced by intake of oral contraceptives. *Eur. Neuropsychopharmacol.* 26, 1014–1019. doi: 10.1016/j.euroneuro.2016.03.004

Roney, J. R., and Simmons, Z. L. (2008). Women's estradiol predicts preference for facial cues of men's testosterone. *Horm. Behav.* 53, 14–19. doi: 10.1016/j.yhbeh.2007.09.008

Scheele, D., Plota, J., Stoffel-Wagner, B., Maier, W., and Hurlemann, R. (2016). Hormonal contraceptives suppress oxytocin-induced brain reward responses to the partner's face. *Soc. Cogn. Affect. Neurosci.* 11, 767–774. doi: 10.1093/scan/nsv157

Schmidt, K. L., and Cohn, J. F. (2001). Human facial expressions as adaptations: evolutionary questions in facial expression research. *Yearb. Phys. Anthropol.* 44, 3–24. doi: 10.1002/ajpa.20001

Shaffer, J. P. (1995). Multiple hypothesis testing. *Annu. Rev. Psychol.* 46, 561–584. doi: 10.1146/annurev.ps.46.020195.003021

Stricker, R., Eberhart, R., Chevailler, M. C., Quinn, F. A., Bischof, P., and Stricker, R. (2006). Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different

phases of the menstrual cycle on the Abbott ARCHITECT analyzer. *Clin. Chem. Lab. Med.* 44, 883–887. doi: 10.1515/CCLM.2006.160

Toffoletto, S., Lanzenberger, R., Gingnell, M., Sundstrom-Poromaa, I., and Comasco, E. (2014). Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. *Psychoneuroendocrinology* 50, 28–52. doi: 10.1016/j.psyneuen.2014.07.025

van Hooff, M. H. A., Hirasing, R. A., Kaptein, M. B. M., Koppenaal, C., Voorhorst, F. J., and Schoemaker, J. (1998). The use of oral contraception by adolescents for contraception, menstrual cycle problems or acne. *Acta Obstet. Gynecol. Scand.* 77, 898–904. doi: 10.1080/j.1600-0412.1998.770905.x

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Serotonergic Contributions to Human Brain Aggression Networks

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Aggressive behavior is associated with dysfunctional frontolimbic emotion regulation circuits. Recent findings suggest serotonin as a primary transmitter for prefrontal amygdala control. However, the association between serotonin levels, amygdala regulation, and aggression is still a matter of debate. Neurobehavioral models furthermore suggest a possible mediating influence of the monoamine oxidase A gene (*MAOA*) on this brain-behavior relationship, with carriers of low expressing allele varieties being a risk group for aggression. In the present study, we investigated the influence of brain serotonin modulation and *MAOA* genotype on functional amygdala connectivity during aggressive behavior. Modulation of serotonergic neurotransmission with acute tryptophan depletion (ATD) and placebo were administered in a double-blind, cross-over design in 38 healthy male participants. Aggressive behavior was modeled in a violent video game during simultaneous assessment of brain activation with functional magnetic resonance imaging (fMRI). Trait aggression was measured with the Buss-Perry Aggression Questionnaire (BP-AQ), and *MAOA* genotypes were assessed from blood samples. Voxel-wise functional connectivity with anatomically defined amygdala was calculated from the functional data. Tryptophan depletion with ATD reduced aggression-specific amygdala connectivity with bilateral supramarginal gyrus. Moreover, ATD impact was associated with trait aggression and *MAOA* genotype in prefrontal cortex regions. In summary, serotonergic corticolimbic projections contribute to aggressive behavior. Genotype-specific vulnerability of frontolimbic projections may underlie the elevated risk in low expressing allele carriers.

Keywords: serotonin, aggression, amygdala, tryptophan depletion, PFC, supramarginal gyrus

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INTRODUCTION

Aggression is associated with dysregulation in a corticolimbic network (Davidson et al., 2000; Siever, 2008). Specifically, a deficient regulation of the amygdala via prefrontal cortex (PFC) areas has been described as a risk factor for aggressive behavior (Coccaro et al., 2011). The PFC-amygdala system supports affective control (Lederbogen et al., 2011) and regulates aggressive impulses (Bufkin and Luttrell, 2005). Evidence for this corticolimbic aggression model comes from

both structural and functional neuroimaging studies. Furthermore, reduced gray matter in PFC and inferior temporal lobe including the amygdala has been related to antisocial traits (Ermer et al., 2012; Gregory et al., 2012). In a similar vein, reduced PFC-amygdala connectivity has been described as a risk factor for trait aggression in patients with schizophrenia (Hoptman et al., 2010). In healthy individuals, enhanced amygdala activation is a neural substrate of state anger after provocation (Yu et al., 2014), whereas lateral PFC activation counteracts aggressive reactions in such situations (Achterberg et al., 2016). In summary, functioning of the PFC-amygdala regulation system seems to be central to successful emotion regulation (Banks et al., 2007), thus preventing impulsive aggression.

On the transmitter level, aggression has frequently been associated with alterations of serotonergic neural activity (Duke et al., 2013). Pharmacological serotonin challenges have been reported to influence both PFC-amygdala connectivity and aggressive feelings (Klasen et al., 2013). One established method to challenge serotonergic activity specifically is acute tryptophan depletion (ATD). In short, ATD is a neurodietary method which temporarily reduces serotonin levels via a modification of the precursor tryptophan (see Hood et al., 2005, for an overview). Lowered serotonin levels after ATD lead to increased aggression (Bjork et al., 1999; Kötting et al., 2013). On the neural level, ATD modulates functional connectivity between amygdala and lateral PFC areas (Eisner et al., 2017). Specifically, ATD challenge has been reported to reduce the processing of aggression-relevant stimuli in the amygdala-PFC system (Passamonti et al., 2012). Remarkably, the impact of ATD on aggression-related amygdala connectivity seems to depend on personality traits, with higher reward drive being associated with a larger ATD impact (Passamonti et al., 2012). The relationship, however, between ATD impact on amygdala connectivity and aggressive traits remains unknown so far.

Besides dietary and pharmacological challenges, genetic factors influencing brain serotonin levels have been associated with aggression as well. A prominent example is the Brunner syndrome, a rare genetic mutation which causes a functional knockout of the monoamine oxidase A (MAO-A) gene (*MAOA*) and is associated with higher levels of aggression and delinquency (Brunner et al., 1993). The enzyme MAO-A degrades serotonin (Shih et al., 1999), with a complete knockout leading to an excess in brain serotonin levels. Non-pathological variations in serotonin transmission caused by an upstream variable number tandem repeat (uVNTR) polymorphism of the *MAOA* gene have been associated with aggression as well (Pavlov et al., 2012). Specifically, a gene-environment interaction has been proposed as a risk factor in male carriers of low expressing gene variants (*MAOA*-L). *MAOA*-L carriers have a high vulnerability to develop aggressive behavior after the experience of childhood trauma than carriers of high expressing gene variants (*MAOA*-H, Caspi et al., 2002). An established neurobiological model proposes a reduced amygdala regulation due to a blunted serotonergic system as a neurobiological endophenotype in these risk allele carriers (Buckholtz et al., 2008). Recent findings show that trait aggression networks vary as a function of *MAOA* genotype (Klasen et al., 2018), but the role of serotonin in this brain-behavior relationship is still a matter of debate.

The present study investigated serotonergic influences on amygdala connectivity associated with aggressive behavior in 38 healthy males. Aggressive behavior was modeled via virtual reality in a violent video game. Violent video game tasks activate similar neurobiological networks compared to behavioral aggression tasks and real-life aggression (Mathiak and Weber, 2006; Weber et al., 2006). Transfer effects between playing realistic and salient violent video games and real-life aggression have been demonstrated in both experimental and longitudinal behavioral studies (Prescott et al., 2018). Thus, a violent video game task can serve as a valid aggression model and has been employed in various neuroimaging studies on aggression (e.g., Klasen et al., 2013; Zvyagintsev et al., 2016; Wolf et al., 2018). Serotonin levels were manipulated via ATD challenge in a double-bind, randomized, and placebo-controlled design. Moreover, genetic differences in serotonin effects were assessed by *MAOA* genotyping. The *MAOA* gene is located on the X chromosome; accordingly, a number of studies confirmed that genotype influences on behavior as well as on neural networks is stronger in men than in women (Kim-Cohen et al., 2006; Buckholtz et al., 2008; Buckholtz and Meyer-Lindenberg, 2008; Guo et al., 2008; Edwards et al., 2010). Following these findings, only male participants were included in the present study.

Based on previous findings (Eisner et al., 2017), we expected a reduction of aggression-specific amygdala-PFC connectivity by ATD. Moreover, we hypothesized this reduction to be associated with trait aggression, i.e., a larger ATD impact in more aggressive individuals. Finally, we sought to explore genotype influences on ATD impact. Following the assumption of enhanced neurobiological vulnerability, we expected a larger ATD effect on amygdala-PFC coupling in male carriers of low expressing *MAOA* variants (*MAOA*-L; risk allele carriers).

MATERIALS AND METHODS

Participants

Thirty eight male Caucasians (16–33 years, mean age 24.7 ± 3.6 years) participated in the study. All participants had normal or corrected to normal vision, normal hearing, no contraindications against MR investigation, no history of neurological or psychiatric illness according to the SCID screening questionnaire (Wittchen et al., 1997), and no history of psychopharmacological therapy. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). The experiment was designed according to the Code of Ethics of the Word Medical Association (Declaration of Helsinki, 2013), and the study protocol was approved by the local Ethics Committee. After detailed briefing and instruction, participants gave written informed consent.

Serotonin Challenge

The present study employed a randomized double-blind and placebo-controlled cross-over design. Each participant was

measured in an ATD and a placebo condition, taking place on separate days. Findings from a third condition (escitalopram) have previously been reported in Wolf et al. (2018). The measurements were separated by at least 1 week allowing for a sufficient washout. Condition order was randomized across participants. The ATD condition consisted of a body weight-adapted ATD drink according to the Moja-De scheme (Moja et al., 1988; Demisch et al., 2002). Placebo consisted of a tryptophan-balanced drink (PLAC) with no tryptophan depletion effect. For ATD, a tryptophan-free amino acid beverage was applied: for 10 kg body weight 0.084 g L-Isoleucine (ILE), 0.132 g L-Leucine (LEU), 0.12 g L-Lysine-HCL (LYS), 0.05 g L-Methionine (MET), 0.132 g L-Phenylalanine (PALA), 0.06 g L-Threonine (THR), and 0.096 g L-Valine (VAL). The PLAC mixture included additional 0.7 g L-Tryptophan (TRP) per 10 kg body weight and thus had no impact on 5-HT synthesis in the brain (Biskup et al., 2012). Administration order was block-randomized (block size 6). Amino acids were packed in coded containers by a person not further involved in the experimental procedure. Administration took place at the beginning of each measurement day (about 08:30 am). Functional measurements were conducted after a delay of 3 h, allowing the serotonin challenge to take effect.

Genotyping

Prior to the fMRI session, all participants underwent a 9 ml venous blood sampling. Genomic DNA was isolated from peripheral lymphocytes with a routine salting-out procedure. For the determination of the *MAOA* genotype, standard polymerase chain reaction (PCR) amplification was performed in a 25-µl volume containing 80 ng of genomic DNA, 1 unit of recombinant Taq polymerase (Invitrogen, Darmstadt/Germany), PCR buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl 2, pH 8.3), 200 mM dNTPs, and 20 pmol of each primer. *MAOA* primers

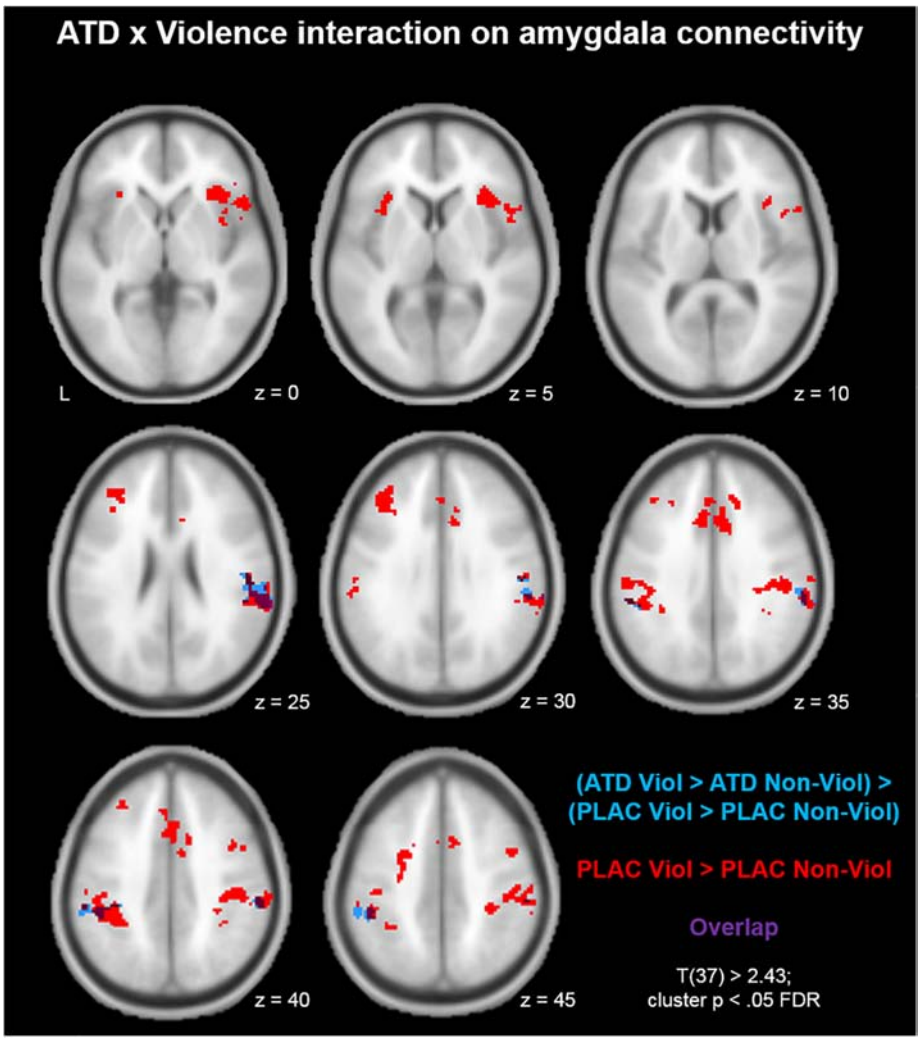

FIGURE 1 | Acute tryptophan depletion (ATD) modulation of aggression-specific amygdala connectivity. Compared to the non-violent modification, the violent game increased amygdala connectivity with bilateral supramarginal gyrus (SMG), bilateral anterior insula, dorsal ACC, left middle frontal gyrus (MFG), and somatosensory cortex (red clusters). ATD attenuated this effect in bilateral SMG (blue clusters; overlap of the contrasts shown in purple).

were obtained from Sabol et al. (1998). The PCR was run on an MJ PTC200 Temperature Cycler (Biozym, Hessisch-Oldendorf, Germany), and each of the 35 cycles consisted of a 95°C denaturation step for 45 s, a 62°C annealing step for 30 s, and, finally, a 72°C elongation step for 90 s. PCR products were run on an automated sequencing system (AB3130, Applied Biosystems,

TABLE 1 | Clusters from **Figure 1**.

Cluster	Brain region	MNI coordinates				
		x	*y*	*z*	Peak *T*	k_E
Red						
1	Supramarginal gyrus r, superior parietal lobule r, postcentral gyrus r	34	−44	62	5.28	1207
2	Supramarginal gyrus l, superior parietal lobule l, postcentral gyrus l	−56	−24	34	4.84	776
3	Inferior frontal gyrus r, anterior insula r	34	26	4	5.53	491
4	SMA r/l	12	2	72	5.89	405
5	Dorsal ACC r/l, paracingulate gyrus r/l	0	16	40	4.44	402
6	Superior frontal gyrus l, Precentral gyrus l	−24	−16	60	4.27	338
7	Middle frontal gyrus l	−38	32	32	4.94	255
8	Anterior insula l	−30	22	4	4.29	141
9	Cerebellum l	−34	−52	−34	4.67	109
10	Middle frontal gyrus l	46	2	48	4.57	99
Blue						
1	Supramarginal gyrus r	58	−32	28	−4.80	373
2	Supramarginal gyrus l	−56	−34	40	−4.84	162

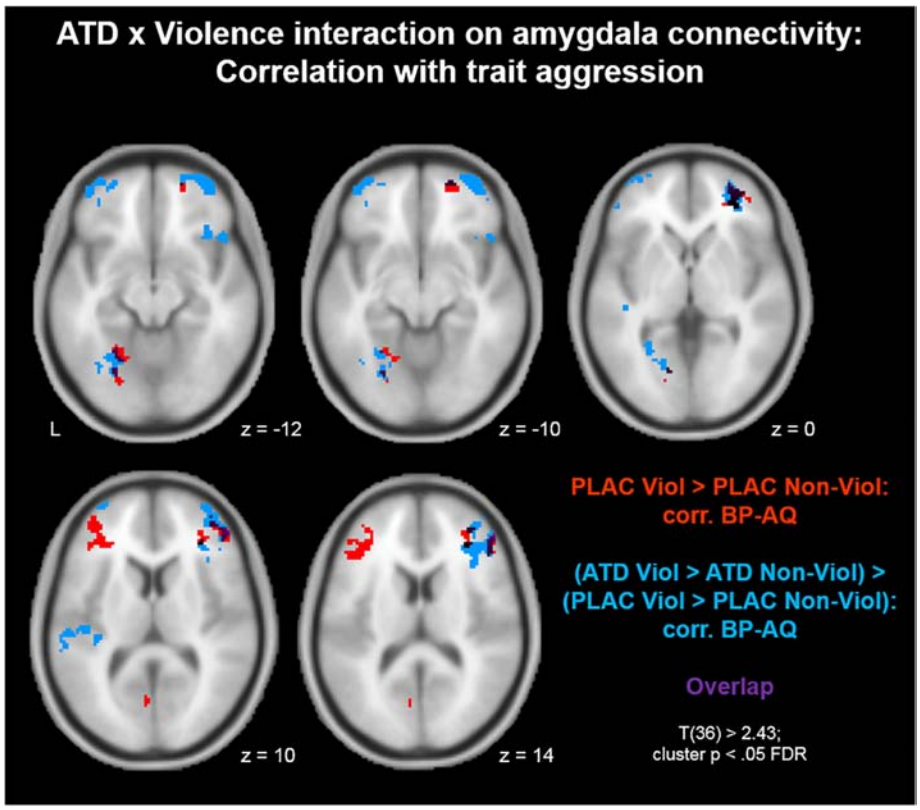

FIGURE 2 | Acute tryptophan depletion modulation of aggression-specific amygdala connectivity: Correlation with trait aggression. Positive correlations between amygdala connectivity and trait aggression were observed in bilateral inferior (IFG) and middle frontal gyri (MFG; red clusters). Similarly, ATD modulation of aggression-specific amygdala connectivity correlated with trait aggression as well. Negative correlations between contrast values and BP-AQ scores were observed in bilateral orbitofrontal cortex (OFC), IFG and MFG, left fusiform gyrus (FFG), parieto-occipital areas, and left auditory cortex (blue clusters). ATD attenuation effects on amygdala connectivity with these areas were larger in more aggressive individuals. Both contrasts overlapped in right OFC, right IFG, and left FFG (purple).

Darmstadt, Germany), and the electropherograms were analyzed with gene mapping software. According to the established classification of Sabol et al. (1998), 3.5 and 4 repeats were classified as representing a high MAO-A expression (*MAOA*-H) and 3 and 5 repeats as representing a low expression (*MAOA*-L).

Data Acquisition

During four scanning session (310 volumes each), the participants played two versions of the racing game Carmageddon: TDR 2000 (Torus Games, Bayswater, Australia, 2000) in an unrestricted manner. In its violent version, the participants were instructed to kill as many pedestrians as possible by hitting them with their car. Hitting pedestrians induced excessive blood splatter and screaming of the victims. In the non-violent version, the players' task was to hit colorful icons with their car; the absence of pedestrians in this game version did not allow for violent interactions. Hitting icons was accompanied by color explosion and by sound. Visual and auditory stimulation levels of the violent and non-violent game versions were comparable. Participants played the game with their right hand, using an MR-compatible 5-button keyboard. Visual stimulation and game sound were delivered via MR-compatible video goggles and headphones; sound levels were adjusted individually to a comfortable level. Participants played two violent and two non-violent sessions in randomized order on each measurement day. In combination with the serotonergic modulation, four conditions emerged, all of them session-wise with two sessions each [ATD Violence, ATD Non-Violence, PLAC(ebo) Violence, and PLAC Non-Violence].

Functional MRI was conducted on a 3 Tesla MR Scanner (Magnetom Trio, Siemens) with a 12-channel head coil using echo-planar imaging (EPI) sequences (TE = 28 ms, TR = 2000 ms, flip angle = 77°, voxel size = 3 × 3 mm, matrix size = 64 × 64, 34 transverse slices, 3 mm slice thickness, 0.75 mm gap). On each measurement day, 1240 functional images were obtained (4 sessions with 310 volumes). After completing the functional measurements, high-resolution T1-weighted anatomical images were performed using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TE = 2.52 ms; inversion time TI = 900 ms; TR = 1900 ms; flip angle = 9°; FOV = 256 × 256 mm^2; 1 mm isotropic voxels; 176 sagittal slices). Total scanning time (including preparations) was about 1 h.

On the first measurement day, all participants completed a validated German Version of the Buss-Perry Aggression Questionnaire (BP-AQ; Herzberg, 2003). The BP-AQ is a well-established 29-item self-report inventory for trait aggression, based on an empirically validated 4-dimensional model of aggression (Physical Aggression, Verbal Aggression, Anger, and Hostility). The BP-AQ was assessed directly after ATD or PLAC intake; thus, there was a time lag of ∼3 h between questionnaire and functional measurement. Only total BP-AQ scores were considered for the analyses.

Data Analysis

Questionnaires and demographical data were analyzed with SPSS 25 (IBM Corp., Armonk, NY, United States). Functional and anatomical image analysis was performed with the SPM toolbox *CONN* (version 17.b; Whitfield-Gabrieli and Nieto-Castanon, 2012). Preprocessing included slice time correction, 3D motion correction, Gaussian spatial smoothing (4 mm FWHM), and high-pass filtering including linear trend removal. The first five images of each session were discarded to avoid T1 saturation effects. Functional images were co-registered to anatomical data and normalized by transformation into MNI space. According

TABLE 2 | Clusters from **Figure 2**.

Cluster	Brain region	MNI coordinates				
		x	*y*	*z*	Peak *T*	k_E
Red						
1	Inferior frontal gyrus l, middle frontal gyrus, orbitofrontal cortex l	−54	34	18	4.93	375
2	Inferior frontal gyrus r, middle frontal gyrus r, orbitofrontal cortex r	20	56	−10	4.76	334
3	Superior parietal lobule l	−24	−62	52	5.40	183
4	Fusiform gyrus l, lingual gyrus l	−20	−76	−6	4.02	161
5	Cerebellum r	8	−44	−36	5.35	142
6	Lingual gyrus l, cerebellum l	−24	−56	−12	4.34	120
Blue						
1	Inferior frontal gyrus r, middle frontal gyrus r	48	34	18	−4.81	745
2	Fusiform gyrus l	−34	−76	−20	−4.98	498
3	Orbitofrontal cortex l	−42	52	−16	−4.38	267
4	Orbitofrontal cortex r	34	60	−12	−5.24	206
5	Cerebellum r	4	−82	−36	−4.38	169
6	Superior parietal lobule l	−28	−58	50	−3.92	165
7	Heschl's gyrus l, superior temporal gyrus l	−60	−30	8	−4.01	153
8	Lateral occipital cortex l	−6	−82	48	−3.50	131
9	Inferior frontal gyrus r, middle frontal gyrus r	50	18	30	−3.99	126
10	Anterior insula r	48	22	−12	−4.80	113

to the standard procedures for the removal of confounders in the CONN toolbox, 12 motion parameters (rigid body transformations and their first-order derivatives) and individual time courses from white matter and cerebrospinal fluid were extracted and regressed out of the image time series. Data analysis was restricted to a whole-brain mask in MNI space.

In a second level analysis we assessed condition-specific connectivity patterns. Four conditions entered the second level analysis: ATD Violence, ATD Non-Violence, PLAC Violence, and PLAC Non-Violence. Condition-specific connectivity was assessed over the time course of the entire session. As seed region, we employed bilateral amygdala as defined by the SPM Anatomy Toolbox (Eickhoff et al., 2005).

To assess aggression-specific amygdala connectivity in the placebo condition, we first mapped the contrast (PLAC Violence > PLAC Non-Violence). The ATD modulation of aggression-specific amygdala connectivity was then determined by the contrast (ATD Violence > ATD Non-Violence) > (PLAC Violence > PLAC Non-Violence).

Neural networks of state aggression may depend on aggressive traits as well. We therefore assessed if connectivity patterns in the above mentioned contrasts varied as a function of trait aggression. For this analysis, we correlated voxel-wise contrast values with individual BP-AQ scores, as implemented in the CONN toolbox. Finally, two samples *t*-tests were calculated to explore possible influences of *MAOA* genotypes on the above mentioned contrasts. We expected drug effects on amygdala connectivity being rather distributed and therefore allowed for a locally rather lenient threshold but a strict global correction for false discoveries. Therefore the precise locations of activation in emerging clusters need to be interpreted with caution (cf. Woo et al., 2014). All contrasts were thresholded at a voxel-wise $p < 0.01$ and corrected for multiple comparisons using false discovery rate (FDR) correction on the cluster level ($p < 0.05$).

RESULTS

ATD Modulation of Aggression-Specific Amygdala Connectivity

First, we mapped brain areas that showed aggression-specific amygdala connectivity in the placebo condition with the contrast (PLAC Violence > PLAC Non-Violence; **Figure 1**, red clusters). Clusters from this contrast are described in **Table 1** Red, including peak voxel MNI coordinates, peak t values, and cluster size. Aggression-specific amygdala connectivity encompassed bilateral supramarginal gyrus (SMG), bilateral anterior insula, dorsal ACC, left middle frontal gyrus (MFG), and somatosensory cortex (**Figure 1**, red). ATD modulation of aggression-specific amygdala connectivity was observed in bilateral SMG, as determined by the comparison (ATD Violence > ATD Non-Violence) > (PLAC Violence > PLAC Non-Violence). This contrast produced negative clusters only, i.e., ATD reduced aggression-specific amygdala connectivity in these regions (**Figure 1**, blue clusters and **Table 1**, Blue). Increases in aggression-specific amygdala connectivity after ATD were not observed. ATD modulation effects were restricted to aggression-specific areas found in the violence contrast and adjacent areas of the same anatomical structures (**Figure 1**, overlap shown in purple).

Considering potential priming effects following a violence exposure, it seems plausible that the pattern of participants starting with a violent session differed from the pattern of those participants starting with a non-violent session. To rule out such order effects, we compared the two groups on the above mentioned contrasts in additional independent samples t tests. No group differences were observed for these comparisons, neither for aggression-specific amygdala connectivity nor for its ATD modulation.

ATD Modulation of Aggression-Specific Amygdala Connectivity: Correlation With Trait Aggression

To investigate whether aggression-specific amygdala connectivity patterns varied as a function of trait aggression, we correlated voxel-wise interaction values of the contrast (PLAC Violence > PLAC Non-Violence) with individual BP-AQ scores. Positive correlations between amygdala connectivity and trait aggression were observed in bilateral inferior (IFG) and middle frontal gyri (MFG; **Figure 2**, red clusters). Clusters from this contrast are described in **Table 2** Red. Moreover, we investigated whether the ATD modulation of aggression-specific amygdala connectivity [(ATD Violence > ATD Non-Violence) > (PLAC Violence > PLAC Non-Violence)] correlated with trait aggression as well. Negative correlations between contrast values and BP-AQ scores were observed in bilateral orbitofrontal cortex (OFC), IFG and MFG, left fusiform gyrus (FFG), parieto-occipital areas, and left auditory cortex (**Figure 2**, blue and **Table 2**, Blue). Positive correlations were not observed. A substantial overlap of both contrasts was observed in right OFC, right IFG, and left FFG (**Figure 2**, purple). Amygdala connectivity with these areas was thus stronger reduced by ATD in more aggressive individuals.

Aggression in general and BP-AQ scores in specific have been shown to decline with age (e.g., Redondo et al., 2017). To rule out any potential confounding effects of participant age on aggression-specific amygdala connectivity and the ATD modulation of the latter, we performed an additional mapping of the contrasts described in **Figure 2** and controlled for age as a covariate. Results from **Figure 2** could be replicated in almost identical fashion. Thus, neither aggression networks nor their serotonergic modulation were biased by age effects.

For a better understanding of the relationship between aggression-specific amygdala connectivity and trait aggression, we furthermore mapped this contrast separately for all four dimensions of the BP-AQ (Physical Aggression, Verbal Aggression, Anger, and Hostility). The results are depicted in **Figure 3**. In summary, correlations in IFG and MFG were similar for all four dimensions, although the most prominent contributions could be assigned to the dimensions Physical Aggression (blue) and Anger (red). More dimension-specific

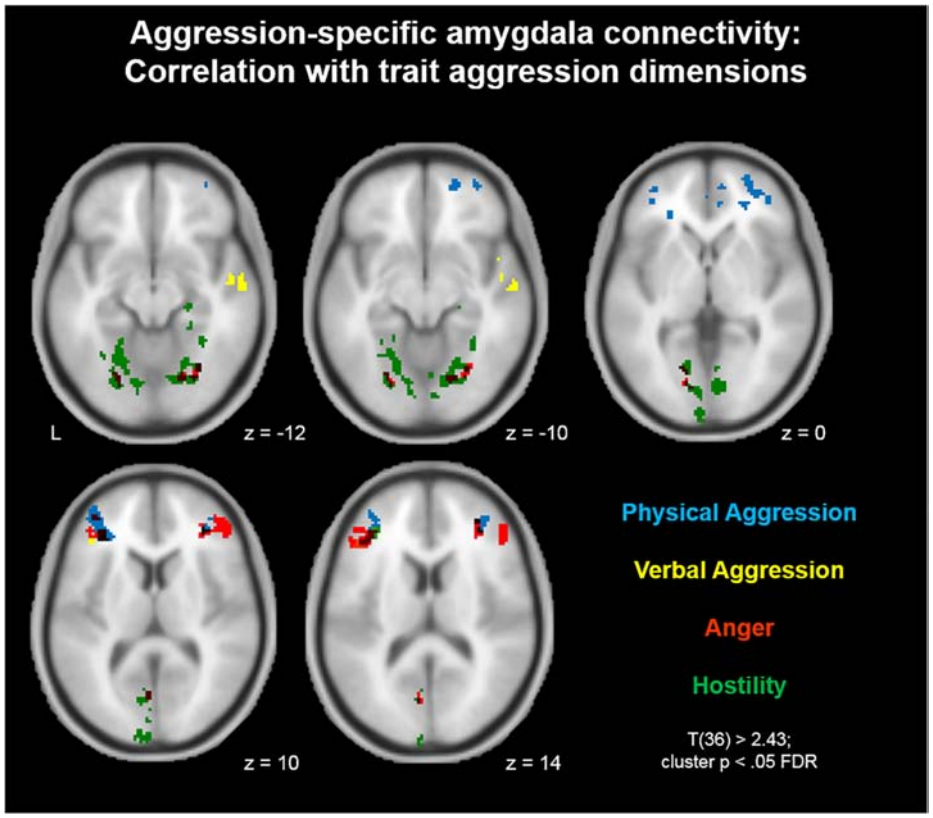

FIGURE 3 | Aggression-specific amygdala connectivity: Correlation with trait aggression dimensions. The correlation between aggression-specific amygdala connectivity and trait aggression was mapped separately for all BP-AQ dimensions. Similar correlations in IFG and MFG were observed for all four dimensions, although most prominently for Physical Aggression (blue) and Anger (red). More dimension-specific correlations were observed for visual (Anger/Hostility) and auditory (Verbal Aggression) processing streams.

correlations were observed for visual (Anger/Hostility) and auditory (Verbal Aggression) processing streams.

ATD Modulation of Aggression-Specific Amygdala Connectivity: *MAOA* Effects

Finally, we explored putative *MAOA* genotype differences regarding the ATD impact on aggression-specific amygdala connectivity. After genotyping, 11 participants (29%) were classified as *MAOA*-L carriers and 27 participants (71%) were classified as *MAOA*-H carriers, which is in line with previously reported gene frequency distributions in male Caucasian populations (Sabol et al., 1998). Allele frequencies are reported in **Table 3**.

A comparison of the two genotypes (*MAOA*-L > *MAOA*-H) on the contrast (PLAC Violence > PLAC Non-Violence) revealed a stronger amygdala connectivity with left IFG for the *MAOA*-L carriers (**Figure 4**, red clusters). Clusters from this contrast are described in **Table 4** Red. A genotype comparison on the ATD impact (ATD Violence > ATD Non-Violence) > (PLAC Violence > PLAC Non-Violence) showed a stronger attenuation effect (reduced connectivity) for the *MAOA*-L group in the left IFG (**Figure 4**, blue clusters and **Table 4**, Blue). Both contrasts yielded highly similar clusters with a substantial overlap in left IFG (**Figure 4**, purple clusters). The reversed comparison (*MAOA*-H > *MAOA*-L) yielded no significant results for both contrasts.

To explore a possible relevance of genotype differences in serotonergic modulation for aggression, we moreover correlated values from the ATD cluster (blue) with BP-AQ scores separately for both genotype groups. On average, *MAOA*-L carriers had higher BP-AQ scores than *MAOA*-H carriers [mean scores 70.64 vs. 59.52; $t(36) = 2.13$, $p < 0.05$]. We found a significant correlation between ATD modulation and trait aggression in the *MAOA*-L carriers [$r(9) = -0.71$; $p < 0.05$], with a stronger connectivity reduction via ATD (negative values) being associated with higher aggression (**Figure 4**, yellow). This effect was absent in the *MAOA*-H carriers [$r(25) = -0.13$; $p = 0.52$; **Figure 4**, green]. However, genotype differences in correlation coefficients (cf. Weaver and Wuensch, 2013) reached only trend level ($p = 0.07$) and must therefore be considered descriptive.

DISCUSSION

The present study investigated serotonergic modulation effects of aggression-specific amygdala connectivity using

ATD. We found an ATD impact on aggression-specific amygdala connectivity in bilateral SMG. Moreover, serotonergic modulation ability varied as a function of trait aggression in PFC regions, with higher aggression predicting a stronger ATD impact during virtual violence. Interaction effects in PFC were stronger in *MAOA*-L compared to *MAOA*-H carriers, emphasizing their neurobiological vulnerability as a risk group for aggressive behavior.

The topography of ATD induced clusters was largely limited to SMG areas with a aggression-specific connectivity pattern. Previous research shows that SMG connectivity is susceptible to serotonergic challenge with ATD (Helmbold et al., 2016). Functionally, the SMG supports Theory of Mind abilities (Saxe and Powell, 2006) and is a core region in the brain's empathy network (Shamay-Tsoory, 2011). As such, it is an important hub in the perception of socio-affective stimuli (Göttlich et al., 2017). Functional impairments of the SMG reduce an individual's pain empathy toward others (Coll et al., 2017). Accordingly, the SMG plays a role in aggression and violence as well. Decreased activity in a fronto-parietal network encompassing the SMG has been associated with desensitization toward violent media (Strenziok et al., 2011). Similar findings have been obtained for the amygdala. During virtual violence, amygdala activity is attenuated, indicating a suppression of the emotional response in favor of the cognitive aspects of the task (Mathiak and Weber, 2006; Weber et al., 2006). Reduced coupling of the SMG with the amygdala after ATD is also in line with findings from neuroimaging genetics, showing that the role of the SMG in aggression-related networks depends on genes influencing serotonin transmission. Specifically, a coupling of the SMG with limbic areas seems to counteract aggressive impulses (Klasen et al., 2018). Reduced connectivity between SMG and amygdala may thus be a neurobiological substrate of increased aggression after ATD (Bjork et al., 1999; Kötting et al., 2013).

TABLE 3 | *MAOA* uVNTR allele frequencies.

	Number of repeats			
	3	3.5	4	5
Number of participants	10	2	25	1

Aggression-specific amygdala networks were convergent with findings from other aggression studies and corroborate the validity of the present paradigm. Aggression-specific amygdala networks involved MFG and the nodes of the salience network in anterior insula and dorsal ACC (Seeley et al., 2007). The involvement of the salience network in aggression is well established; in specific, enhanced activity in a network of amygdala, anterior insula, and dorsal ACC is a characteristic signature of state aggression (Yu et al., 2014). Blair (2016) describes the dorsal ACC as a region of response selection, integrating action values with action outcomes, whereas the anterior insula employs this information for adjusting behavioral responses. The MFG, in turn, counteracts aggressive impulses (Achterberg et al., 2016), and enhanced connectivity of amygdala with MFG and dACC has been associated with reduced anger states after virtual aggression (Klasen et al., 2013). However, the present study did not find any significant impact of ATD on these neural systems. Instead, the findings indicate a reduced synchronization between affective and empathy networks as an

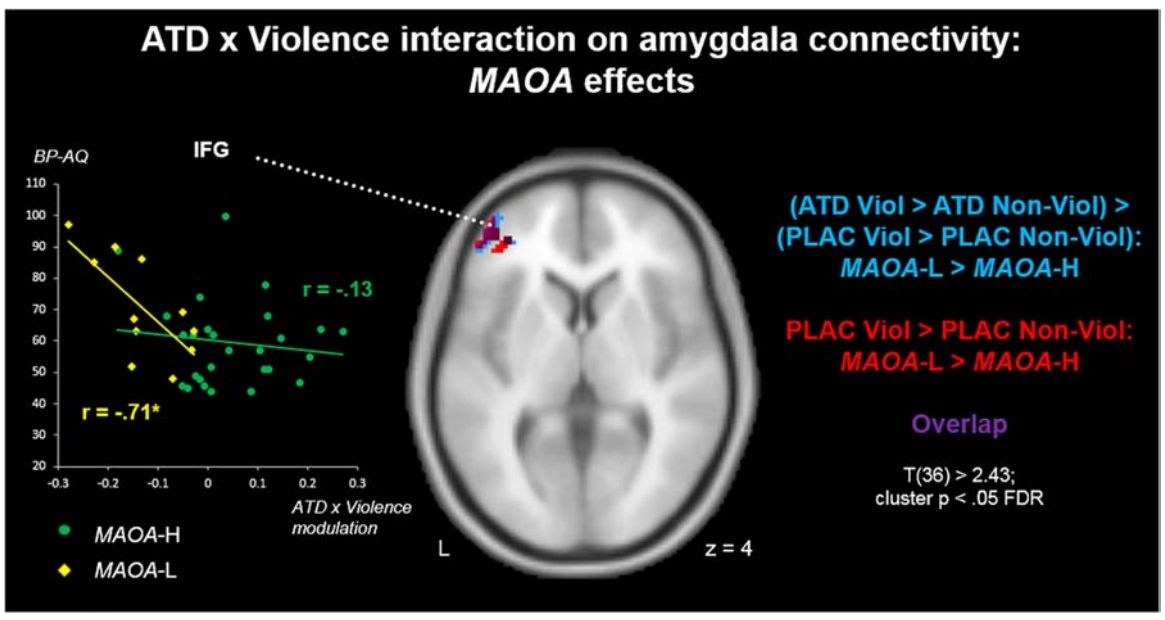

FIGURE 4 | ATD modulation of aggression-specific amygdala connectivity: *MAOA* effects. *MAOA*-L carriers had stronger aggression-specific amygdala connectivity patterns with left IFG than *MAOA*-H carriers (red). Also, this pattern was more susceptible to ATD-induced connectivity reductions in *MAOA*-L than in *MAOA*-H carriers (blue). Correlations of ATD effects with BP-AQ scores highlighted a functional significance of serotonergic IFG-amygdala projections for trait aggression in *MAOA*-L, but not *MAOA*-H carriers (left insert).

TABLE 4 | Clusters from **Figure 4**.

Cluster	Brain region	MNI coordinates			Peak *T*	k_E
		x	*y*	*z*		
Red						
1	Inferior frontal gyrus l	−42	50	06	4.88	191
Blue						
1	Inferior frontal gyrus l	−34	46	8	−4.37	185

effect of the serotonergic challenge. Thus, the role of serotonin in aggression may be less a modulation of the emotional response, but rather an attenuation of empathy with the victim.

Moreover, our data delivered evidence that connectivity in serotonergic aggression networks varies with personality traits. Specifically, ATD effects on amygdala connectivity with IFG and OFC varied as a function of trait aggression. ATD modulation of OFC and IFG has been shown previously for response inhibition, confirming the role of prefrontal serotonergic activity for inhibitory control (Rubia et al., 2005). Accordingly, the PFC-amygdala system plays an essential role in emotion regulation (Eden et al., 2015). Impairments of this regulation system are associated with trait aggression (Raine and Yang, 2006). In a similar vein, reduced gray matter in amygdala and PFC regions including the OFC have consistently been associated with trait aggression as well (Ermer et al., 2012). Functional neuroimaging studies corroborate the relevance of this frontolimbic system for the control of aggressive impulses; specifically, reduced IFG-amygdala connectivity is frequently observed in overly aggressive individuals (see Bogerts et al., 2018, for a review). Similar findings have also been obtained for the OFC; amygdala-OFC connectivity shows a negative association with trait anger (Fulwiler et al., 2012). Although encompassing the same anatomical regions, our findings revealed an opposite pattern: trait aggression was positively associated with PFC-amygdala connectivity in the placebo condition. These findings may be explained by the neurobiology of video game violence. Aggression-enhancing effects of violent games are more pronounced in individuals with high trait aggression (Peng et al., 2008). However, aggressive actions in the game are characterized by enhanced coupling in regulatory PFC-amygdala circuits, indicating an emotion regulation in favor of the cognitive game task (Mathiak and Weber, 2006; Klasen et al., 2013). Thus, it is likely that individuals with higher trait aggression required a stronger frontolimbic emotion control for successfully performing violent game actions.

Finally, there are first indications for genotype influences on serotonergic aggression networks. In our study, carriers of the low expressing risk allele exhibited higher susceptibility of the PFC-amygdala system to ATD than high expressing allele carriers. Higher vulnerability of fronto-amygdalar regulation systems in *MAOA*-L carriers have previously been discussed as a risk factor for aggression. An established model suggests aggression-related genotype differences in amygdala control via anterior cingulate and ventromedial PFC (Buckholtz et al., 2008); however, this model has recently been challenged (Klasen et al., 2018). Our data, in turn, suggest a genotype-specific involvement of left MFG, which is in line with earlier findings on *MAOA* effects in emotion processing (Alia-Klein et al., 2009). Only in *MAOA*-L carriers, ATD impact on MFG-amygdala connectivity varied as a function of trait aggression. Thus, brain aggression networks seem to depend on *MAOA* genotypes, which is in line with recent findings (Klasen et al., 2018). In analogy to the model by Buckholtz et al. (2008), we suggest the left MFG as a *MAOA*-L-specific supplementary node for serotonergic amygdala regulation. Disruption of this regulatory system may accordingly be associated with increased risk of aggression in *MAOA*-L carriers. However, albeit a clear trend was observed, genotype differences in this analysis are descriptive.

A final interesting perspective comes from a recent investigation by Manca et al. (2018). This study revealed a role of a second *MAOA* gene polymorphism (dVNTR) on MAO-A expression. Specifically, the two VNTRs differentially affect the expression of different MAO-A isoforms. As a whole, there seem to be mechanistic interactions between the two VNTRs (Manca et al., 2018). The associations of dVNTR and dVNTR-uVNTR interactions with behavioral variables, however, have still to be established. Future studies should take these novel findings into account, which will have an impact on sample sizes as well. Given the observed frequencies of observed dVNTR-uVNTR repeat combinations (cf. Manca et al., 2018), larger samples will be required to investigate interactions between the two polymorphisms.

CONCLUSION

The present study highlights the role of serotonin in brain aggression networks. Specifically, as revealed by ATD challenge, serotonergic projections connecting limbic areas (amygdala) to empathy networks may influence the emotional assessment of aggressive actions. Additionally, amygdala connectivity with MFG and OFC is negatively correlated with trait aggression, which may constitute a supplementary emotion regulation system. Enhanced vulnerability of this system may foster aggression in *MAOA* risk allele carriers.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Code of Ethics of the Word Medical Association 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 Ethics Committee at the RWTH Aachen Faculty of Medicine.

AUTHOR CONTRIBUTIONS

KM, FZ, and RW designed the paradigm. MK, DW, and PE collected the data. MK analyzed the neuroimaging data. TE and KZ conducted the genotyping. KM and RW supported data analysis and interpretation. MK wrote and all co-authors

corrected the manuscript. All authors gave final approval of the version to be published.

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REFERENCES

Achterberg, M., van Duijvenvoorde, A. C., Bakermans-Kranenburg, M. J., and Crone, E. A. (2016). Control your anger! The neural basis of aggression regulation in response to negative social feedback. *Soc. Cogn. Affect. Neurosci.* 11, 712–720. doi: 10.1093/scan/nsv154

Alia-Klein, N., Goldstein, R. Z., Tomasi, D., Woicik, P. A., Moeller, S. J., Williams, B., et al. (2009). Neural mechanisms of anger regulation as a function of genetic risk for violence. *Emotion* 9, 385–396. doi: 10.1037/a0015904

Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J., and Phan, K. L. (2007). Amygdala-frontal connectivity during emotion regulation. *Soc. Cogn. Affect. Neurosci.* 2, 303–312. doi: 10.1093/scan/nsm029

Biskup, C. S., Sanchez, C. L., Arrant, A., Van Swearingen, A. E., Kuhn, C., and Zepf, F. D. (2012). Effects of acute tryptophan depletion on brain serotonin function and concentrations of dopamine and norepinephrine in C57BL/6J and BALB/cJ mice. *PLoS One* 7:e35916. doi: 10.1371/journal.pone.0035916

Bjork, J. M., Dougherty, D. M., Moeller, F. G., Cherek, D. R., and Swann, A. C. (1999). The effects of tryptophan depletion and loading on laboratory aggression in men: time course and a food-restricted control. *Psychopharmacology* 142, 24–30. doi: 10.1007/s002130050858

Blair, R. J. (2016). The neurobiology of impulsive aggression. *J. Child Adolesc. Psychopharmacol.* 26, 4–9. doi: 10.1089/cap.2015.0088

Bogerts, B., Schone, M., and Breitschuh, S. (2018). Brain alterations potentially associated with aggression and terrorism. *CNS Spectr.* 23, 129–140. doi: 10.1017/S1092852917000463

Brunner, H. G., Nelen, M., Breakefield, X. O., Ropers, H. H., and van Oost, B. A. (1993). Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 262, 578–580. doi: 10.1126/science.8211186

Buckholtz, J. W., Callicott, J. H., Kolachana, B., Hariri, A. R., Goldberg, T. E., Genderson, M., et al. (2008). Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. *Mol. Psychiatry* 13, 313–324. doi: 10.1038/sj.mp.4002020

Buckholtz, J. W., and Meyer-Lindenberg, A. (2008). MAOA and the neurogenetic architecture of human aggression. *Trends Neurosci.* 31, 120–129. doi: 10.1016/j.tins.2007.12.006

Bufkin, J. L., and Luttrell, V. R. (2005). Neuroimaging studies of aggressive and violent behavior: current findings and implications for criminology and criminal justice. *Trauma Violence Abuse* 6, 176–191. doi: 10.1177/1524838005275089

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., et al. (2002). Role of genotype in the cycle of violence in maltreated children. *Science* 297, 851–854. doi: 10.1126/science.1072290

Coccaro, E. F., Sripada, C. S., Yanowitch, R. N., and Phan, K. L. (2011). Corticolimbic function in impulsive aggressive behavior. *Biol. Psychiatry* 69, 1153–1159. doi: 10.1016/j.biopsych.2011.02.032

Coll, M. P., Tremblay, M. B., and Jackson, P. L. (2017). The effect of tDCS over the right temporo-parietal junction on pain empathy. *Neuropsychologia* 100, 110–119. doi: 10.1016/j.neuropsychologia.2017.04.021

Davidson, R. J., Putnam, K. M., and Larson, C. L. (2000). Dysfunction in the neural circuitry of emotion regulation–a possible prelude to violence. *Science* 289, 591–594. doi: 10.1126/science.289.5479.591

Demisch, L., Kewitz, A., and Schmeck, K. (2002). Methodology of rapid tryptophan depletion (RTD): impact of gender and body weight. *Eur. Arch. Psychiatry Clin. Neurosci.* 1:252.

Duke, A. A., Begue, L., Bell, R., and Eisenlohr-Moul, T. (2013). Revisiting the serotonin-aggression relation in humans: a meta-analysis. *Psychol. Bull.* 139, 1148–1172. doi: 10.1037/a0031544

Eden, A. S., Schreiber, J., Anwander, A., Keuper, K., Laeger, I., Zwanzger, P., et al. (2015). Emotion regulation and trait anxiety are predicted by the microstructure of fibers between amygdala and prefrontal cortex. *J. Neurosci.* 35, 6020–6027. doi: 10.1523/JNEUROSCI.3659-14.2015

Edwards, A. C., Dodge, K. A., Latendresse, S. J., Lansford, J. E., Bates, J. E., Pettit, G. S., et al. (2010). MAOA-uVNTR and early physical discipline interact to influence delinquent behavior. *J. Child Psychol. Psychiatry* 51, 679–687. doi: 10.1111/j.14697610.2009.02196.x

Eickhoff, S. B., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335. doi: 10.1016/j.neuroimage.2004.12.034

Eisner, P., Klasen, M., Wolf, D., Zerres, K., Eggermann, T., Eisert, A., et al. (2017). Cortico-limbic connectivity in MAOA-L carriers is vulnerable to acute tryptophan depletion. *Hum. Brain Mapp.* 38, 1622–1635. doi: 10.1002/hbm.23475

Ermer, E., Cope, L. M., Nyalakanti, P. K., Calhoun, V. D., and Kiehl, K. A. (2012). Aberrant paralimbic gray matter in criminal psychopathy. *J. Abnorm. Psychol.* 121, 649–658. doi: 10.1037/a0026371

Fulwiler, C. E., King, J. A., and Zhang, N. (2012). Amygdala-orbitofrontal resting-state functional connectivity is associated with trait anger. *Neuroreport* 23, 606–610. doi: 10.1097/WNR.0b013e3283551cfc

Göttlich, M., Ye, Z., Rodriguez-Fornells, A., Munte, T. F., and Kramer, U. M. (2017). Viewing socio-affective stimuli increases connectivity within an extended default mode network. *Neuroimage* 148, 8–19. doi: 10.1016/j.neuroimage.2016.12.044

Gregory, S., ffytche, D., Simmons, A., Kumari, V., Howard, M., Hodgins, S., et al. (2012). The antisocial brain: psychopathy matters. *Arch. Gen. Psychiatry* 69, 962–972. doi: 10.1001/archgenpsychiatry.2012.222

Guo, G., Ou, X.-M., Roettger, M., Shih, J. C. (2008). The VNTR 2 repeat in *MAOA* and delinquent behavior in adolescence and young adulthood: associations and *MAOA* promoter activity. *Eur. J. Hum. Genet.* 16, 626–634. doi: 10.1038/sj.ejhg.5201999

Helmbold, K., Zvyagintsev, M., Dahmen, B., Biskup, C. S., Bubenzer-Busch, S., Gaber, T. J., et al. (2016). Serotonergic modulation of resting state default mode network connectivity in healthy women. *Amino Acids* 48, 1109–1120. doi: 10.1007/s00726-015-2137-4

Herzberg, P. Y. (2003). Faktorstruktur, Gütekriterien und konstruktvalidität der deutschen Übersetzung des aggressionsfragebogens von buss und perry. *Z. Differ. Diagn. Psychol.* 24:13. doi: 10.1024/0170-1789.24.4.311

Hood, S. D., Bell, C. J., and Nutt, D. J. (2005). Acute tryptophan depletion. Part I: rationale and methodology. *Aust. N. Z. J. Psychiatry* 39, 558–564. doi: 10.1080/j.1440-1614.2005.01627.x

Hoptman, M. J., D'Angelo, D., Catalano, D., Mauro, C. J., Shehzad, Z. E., Kelly, A. M., et al. (2010). Amygdalofrontal functional disconnectivity and aggression in schizophrenia. *Schizophr. Bull.* 36, 1020–1028. doi: 10.1093/schbul/sbp012

Kim-Cohen, J., Caspi, A., Taylor, A., Williams, B., Newcombe, R., Craig, I. W., et al. (2006). MAOA, maltreatment, and gene–environment interaction predicting

children's mental health: new evidence and a meta-analysis. *Mol. Psychiatry* 11, 903–913. doi: 10.1038/sj.mp.4001851

Klasen, M., Wolf, D., Eisner, P. D., Habel, U., Repple, J., Vernaleken, I., et al. (2018). Neural networks underlying trait aggression depend on MAOA gene alleles. *Brain Struct. Funct.* 223, 873–881. doi: 10.1007/s00429-017-1528-6

Klasen, M., Zvyagintsev, M., Schwenzer, M., Mathiak, K. A., Sarkheil, P., Weber, R., et al. (2013). Quetiapine modulates functional connectivity in brain aggression networks. *Neuroimage* 75, 20–26. doi: 10.1016/j.neuroimage.2013.02.053

Kötting, W. F., Bubenzer, S., Helmbold, K., Eisert, A., Gaber, T. J., and Zepf, F. D. (2013). Effects of tryptophan depletion on reactive aggression and aggressive decision-making in young people with ADHD. *Acta Psychiatr. Scand.* 128, 114–123. doi: 10.1111/acps.12001

Lederbogen, F., Kirsch, P., Haddad, L., Streit, F., Tost, H., Schuch, P., et al. (2011). City living and urban upbringing affect neural social stress processing in humans. *Nature* 474, 498–501. doi: 10.1038/nature10190

Manca, M., Pessoa, V., Lopez, A. I., Harrison, P. T., Miyajima, F., Sharp, H., et al. (2018). The regulation of monoamine oxidase a gene expression by distinct variable number tandem repeats. *J. Mol. Neurosci.* 64, 459–470. doi: 10.1007/s12031-018-1044-z

Mathiak, K., and Weber, R. (2006). Toward brain correlates of natural behavior: fMRI during violent video games. *Hum. Brain Mapp.* 27, 948–956. doi: 10.1002/hbm.20234

Moja, E. A., Stoff, D. M., Gessa, G. L., Castoldi, D., Assereto, R., and Tofanetti, O. (1988). Decrease in plasma tryptophan after tryptophan-free amino acid mixtures in man. *Life Sci.* 42, 1551–1556. doi: 10.1016/0024-3205(88)90013-6

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Passamonti, L., Crockett, M. J., Apergis-Schoute, A. M., Clark, L., Rowe, J. B., Calder, A. J., et al. (2012). Effects of acute tryptophan depletion on prefrontal-amygdala connectivity while viewing facial signals of aggression. *Biol. Psychiatry* 71, 36–43. doi: 10.1016/j.biopsych.2011.07.033

Pavlov, K. A., Chistiakov, D. A., and Chekhonin, V. P. (2012). Genetic determinants of aggression and impulsivity in humans. *J. Appl. Genet.* 53, 61–82. doi: 10.1007/s13353-011-0069-6

Peng, W., Liu, M., and Mou, Y. (2008). Do aggressive people play violent computer games in a more aggressive way? Individual difference and idiosyncratic game-playing experience. *Cyberpsychol. Behav.* 11, 157–161. doi: 10.1089/cpb.2007.0026

Prescott, A. T., Sargent, J. D., and Hull, J. G. (2018). Metaanalysis of the relationship between violent video game play and physical aggression over time. *Proc. Natl. Acad. Sci. U.S.A.* 115, 9882–9888. doi: 10.1073/pnas.1611617114

Raine, A., and Yang, Y. (2006). Neural foundations to moral reasoning and antisocial behavior. *Soc. Cogn. Affect. Neurosci.* 1, 203–213. doi: 10.1093/scan/nsl033

Redondo, N., Pena, M. E., Grana, J. L., and Andreu, J. M. (2017). Psychometric properties of the aggression questionnaire: a replication in a sample of partner-assaultive men in psychological treatment. *Psicothema* 29, 584–589. doi: 10.7334/psicothema2016.390

Rubia, K., Lee, F., Cleare, A. J., Tunstall, N., Fu, C. H., Brammer, M., et al. (2005). Tryptophan depletion reduces right inferior prefrontal activation during response inhibition in fast, event-related fMRI. *Psychopharmacology* 179, 791–803. doi: 10.1007/s00213-004-2116-z

Sabol, S. Z., Hu, S., and Hamer, D. (1998). A functional polymorphism in the monoamine oxidase A gene promoter. *Hum. Genet.* 103, 273–279. doi: 10.1007/s004390050816

Saxe, R., and Powell, L. J. (2006). It's the thought that counts: specific brain regions for one component of theory of mind. *Psychol. Sci.* 17, 692–699. doi: 10.1111/j.1467-9280.2006.01768.x

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Shamay-Tsoory, S. G. (2011). The neural bases for empathy. *Neuroscientist* 17, 18–24. doi: 10.1177/1073858410379268

Shih, J. C., Chen, K., and Ridd, M. J. (1999). Monoamine oxidase: from genes to behavior. *Annu. Rev. Neurosci.* 22, 197–217. doi: 10.1146/annurev.neuro.22.1.197

Siever, L. J. (2008). Neurobiology of aggression and violence. *Am. J. Psychiatry* 165, 429–442. doi: 10.1176/appi.ajp.2008.07111774

Strenziok, M., Krueger, F., Deshpande, G., Lenroot, R. K., van der Meer, E., and Grafman, J. (2011). Fronto-parietal regulation of media violence exposure in adolescents: a multi-method study. *Soc. Cogn. Affect. Neurosci.* 6, 537–547. doi: 10.1093/scan/nsq079

Weaver, B., and Wuensch, K. L. (2013). SPSS and SAS programs for comparing Pearson correlations and OLS regression coefficients. *Behav. Res. Methods.*45, 880–895. doi: 10.3758/s13428-012-0289-7

Weber, R., Ritterfeld, U., and Mathiak, K. (2006). Does playing violent video games induce aggression? empirical evidenc *Media Psychol.* 8, 39–60. doi: 10.1089/cyber.2012.0230

Whitfield-Gabrieli, S., and Nieto-Castanon, A. (2012). Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* 2, 125–141. doi: 10.1089/brain.2012.0073

Wittchen, H. U., Zaudig, M., and Fydrich, T. (1997). *Strukturiertes Klinisches Interview für DSM-IV*. Göttingen: Hogrefe.

Wolf, D., Klasen, M., Eisner, P., Zepf, F. D., Zvyagintsev, M., Palomero-Gallagher, N., et al. (2018). Central serotonin modulates neural responses to virtual violent actions in emotion regulation networks. *Brain Struct. Funct.* 223, 3327–3345. doi: 10.1007/s00429-018-1693-2

Woo, C. W., Krishnan, A., and Wager, T. D. (2014). Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations. *Neuroimage* 91, 412–419. doi: 10.1016/j.neuroimage.2013.12.058

Yu, R., Mobbs, D., Seymour, B., Rowe, J. B., and Calder, A. J. (2014). The neural signature of escalating frustration in humans. *Cortex* 54, 165–178. doi: 10.1016/j.cortex.2014.02.013

Zvyagintsev, M., Klasen, M., Weber, R., Sarkheil, P., Esposito, F., Mathiak, K. A., et al. (2016). Violence-related content in video game may lead to functional connectivity changes in brain networks as revealed by fMRI-ICA in young men. *Neuroscience* 320, 247–258. doi: 10.1016/j.neuroscience.2016.01.056

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OPINION
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Can Machine Learning Approaches Lead Toward Personalized Cognitive Training?

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Keywords: cognitive training, cognitive remediation, machine learning, treatment adaptation, treatment selection

INTRODUCTION

Cognitive training efficacy is controversial. Although many recent studies indicate that cognitive training shows merit, others fail to demonstrate its efficacy. These inconsistent findings may at least partly result from differences in individuals' ability to benefit from cognitive training in general, and from specific training types in particular. Consistent with the move toward personalized medicine, we propose using machine learning approaches to help optimize cognitive training gains.

COGNITIVE TRAINING: STATE-OF-THE-ART FINDINGS AND DEBATES

Cognitive training targets neurobiological mechanisms underlying emotional and cognitive functions. Indeed, Siegle et al. (2007) suggested that cognitive training can significantly improve mood, daily functioning, and cognitive domains. In recent years, various types of cognitive training have been researched. Frequently researched training types include *cognitive bias modification* (CBM) aims to modify cognitive processes such as interpretations and attention, making these more adaptive and accommodating to real-life demands (Hallion and Ruscio, 2011); *inhibitory training* seeks to improve inhibitory control and other executive processes, thus helping regulate behavior and emotion (Cohen et al., 2016; Koster et al., 2017); *working memory training* targets attentional resources, seeking to increase cognitive abilities by improving working memory capacities (Melby-Lervåg and Hulme, 2013). All these types demonstrated major potential in improving psychopathological symptoms or enhancing cognitive functions (Jaeggi et al., 2008; Hakamata et al., 2010).

Despite the accumulating body of evidence suggesting that cognitive training is a promising research path with major clinical potential, questions remain regarding its efficacy, and generalizability. Recent meta-analyses further corroborate this (for a discussion, see Mogg et al., 2017; Okon-Singer, 2018). For example, several research groups tested CBM studies using meta-analyses. Hakamata et al. (2010) analyzed twelve studies (comprising 467 participants from an anxious population), reporting positive moderate effects of training on anxiety symptom improvement. Yet two other meta-analyses focusing on both anxiety and depression (49 and 45 studies, respectively) demonstrated small effect sizes and warned of possible publication bias (Hallion and Ruscio, 2011; Cristea et al., 2015). These inconsistent results raise important questions about training efficacy. Several factors have been suggested as potential sources of this variability in effect size, including differences in inclusion criteria and quality of the studies included (Cristea et al., 2015).

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As in the CBM literature, meta-analyses of working memory training also yielded divergent results. Au et al. (2015) analyzed twenty working memory training studies comprising samples of healthy adults and reported small positive effects of training on fluid intelligence. The authors suggested that the small effect size underestimates the actual training benefits and may result from methodological shortcomings and sample characteristics, stating that "it is becoming very clear to us that training on working memory with the goal of trying to increase fluid intelligence holds much promise" (p. 375). Yet two other meta-analyses of working memory (87 and 47 studies, respectively) described specific improvements only in the trained domain (i.e., near transfer benefits) and few generalization effects in other cognitive domains (Schwaighofer et al., 2015; Melby-Lervåg et al., 2016). As with CBM, these investigations did not include exactly the same set of studies, making it difficult to infer the reason for the discrepancies. Nevertheless, potential factors contributing to variability in intervention efficacy include differences in methodology and inclusion criteria (Melby-Lervåg et al., 2016).

Some scholars suggested that the inconsistent results seen across types of training may be result from the high variability in training features, such as dose, design type, training type, and type of control groups (Karbach and Verhaeghen, 2014). For example, some studies suggest that only active control groups should be used and that using untreated controls is futile (Melby-Lervåg et al., 2016), while others discovered no significant difference between active and passive control groups (Schwaighofer et al., 2015; Weicker et al., 2016). Researchers have also suggested that the type of activity assigned to the active control group (e.g., adaptive or non-adaptive) may influence effect sizes (Weicker et al., 2016). Adaptive control activity may lead to underestimation of training benefits, while non-adaptive control activity may yield overestimation (von Bastian and Oberauer, 2014).

Training duration has also been raised as a potential source of variability. Weicker et al. (2016) suggested that the number of training sessions (but not overall training hours) is positively related to training efficacy in a brain injured sample. While only studies with more than 20 sessions demonstrated a long-lasting effect. In a highly influential working memory paper, Jaeggi et al. (2008) compared different numbers of training sessions (8–19). Outcomes demonstrated a dose-dependency effect: the more training sessions participants completed, the greater the "far transfer" improvements. In contrast, in a 2014 meta-analytical review Karbach and Verhaeghen reported no dose–dependency, as overall training time did not predict training effects. This is somewhat consistent with the findings of Lampit et al. (2014) meta-analysis, which indicated that only three or fewer training sessions per week were beneficial in training healthy older adults in different types of cognitive tasks. Furthermore, even time gaps between training sessions when the overall number of sessions is fixed may be influential. A study that specifically tested the optimal intensity level of working memory training revealed that distributed training (16 sessions in 8 weeks) was more beneficial than high intensity training (16 sessions in 4 weeks) (Penner et al., 2012). In sum, literature reviews maintain that this large variability in training hampers attempts to evaluate the findings (Koster et al., 2017; Mogg et al., 2017).

So far, the majority of studies in the field of cognitive training have been concerned mainly with establishing the average effectiveness of various training methods, with studies based on combined samples comprising individuals who profited from training and those who did not. Therefore, the samples' heterogeneity might be too high to evaluate efficacy for the "average individual" in each sample. We contend that focusing on the average individual contributes to the inconsistent findings, as is also the case with other interventions aimed at improving mental health (Zilcha-Mano, 2018). We argue that the inconsistent findings and large heterogeneity in studies evaluating cognitive training efficacy do not constitute interfering noise but rather provide important information that can guide us in *training selection*. In addition to selecting the optimal training for each individual, achieving maximum efficacy also requires adapting the selected training to each individual's characteristics and needs (Zilcha-Mano, 2018). In line with this notion, training games studies (i.e., online training platforms displayed in a game-like format) showcased different methods which personalized cognitive training by (a) *selecting* the type of training according to a baseline cognitive strengths and weaknesses evaluation or the intent of the trainee, and (b) *adapting* the ongoing training according to the individual's performance (Shatil et al., 2010; Peretz et al., 2011; Hardy et al., 2015). Until now, however, training personalization was made by pre-exist defined criteria and rationale (i.e., individual's weaknesses and strengths, individual's personal preference). Additional method for personalization, that is becoming increasingly popular in recent years, is data-driven personalization implemented by machine learning algorithms (Cohen and DeRubeis, 2018).

The observed variation in efficacy found in cognitive training studies may serve as a rich source of information to facilitate both intervention selection and intervention adaptation—the two central approaches in personalized medicine (Cohen and DeRubeis, 2018). Intervention selection seeks to optimize intervention efficacy by identifying the most promising type of intervention for a given individual based on as many pre-training characteristics as possible (e.g., age, personality traits, cognitive abilities). Machine learning approaches are especially suitable for such identification because they enable us to choose the most critical items for guiding treatment selection without relying on specific theory or rationale. In searching for a single patient characteristic that guides training selection, most approaches treat all other variables as noise. It is more intuitive, however, to hypothesize that no single factor is as important in identifying the optimal training for an individual as a set of interrelated factors. Traditional approaches to subgroup analysis, which tests each factor as a separate hypothesis, can lead to erroneous conclusions due to multiple comparisons (inflated type I errors), model misspecification, and multicollinearity. Findings may also be affected by publication bias because statistically significant moderators have a better chance of being reported in the literature. Machine learning

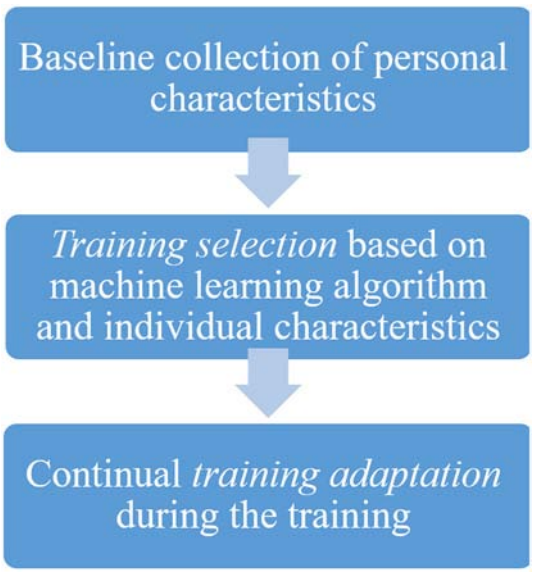

FIGURE 1 | Flow diagram of personalized cognitive training process.

approaches make it feasible to identify the best set of patient characteristics to guide intervention and training selection (Cohen and DeRubeis, 2018; Zilcha-Mano et al., 2018). With that said, given the flexibility of methods like decision tree analyses, there is a risk of overfitting that reduces validity for inference out of sample, such that the model will fit specifically the sample on which it was built and may be therefore unlikely to be generalizable in an independent application (Ioannidis, 2005; Open Science Collaboration, 2015; Cohen and DeRubeis, 2018). Thus, it is important to test out-of-sample prediction, either on a different sample or a sub-sample of the original sample on which the model was not built (e.g., cross-validation).

An example of treatment selection from the field of antidepressant medication (ADM) demonstrates the utility of this approach. Current ADM treatments are ineffective for up to half the patients, despite much variability in patient response to treatments (Cipriani et al., 2018). Researchers are beginning to realize the benefits of implementing machine learning approaches in selecting the most effective treatment for each individual. Using the gradient boosting machine (GBM) approach, Chekroud et al. (2016) identified 25 variables as most important in predicting treatment efficacy and were able to improve treatment efficacy in 64% of responders to medication—a 14% increase.

Whereas, *training selection* affects pre-treatment decision-making, *training adaptation* focuses on continuously adapting the training to the individual (see **Figure 1**). A patient's baseline characteristics (e.g., age, personality traits, cognitive abilities) and individual training performance trajectory can be used to tailor the training parameters (training type, time gaps between sessions, number of sessions, overall training hours) to achieve optimal performance. Collecting information from a sample of patients with similar baseline characteristics that underwent the same intervention yields an expected trajectory. Deviations from this expected trajectory act as warning signs and can help adapt the training parameters to the individual's needs (Rubel and Lutz, 2017).

An example of treatment adaptation comes from the field of psychotherapy research, where a common treatment adaptation method involves providing therapists with feedback on their patients' progress. This method was developed to address the problem that many therapists are not sufficiently aware of their patients' progress. While many believe they are able to identify when their patients are progressing as expected and when not, in practice this may not be true (Hannan et al., 2005). Many studies have demonstrated the utility of giving therapists feedback regarding their patients' progress (Lambert et al., 2001; Probst et al., 2014). Shimokawa et al. (2010) found that although some patients continue improving and benefitting from therapy (on-track patients—OT), others seem to deviate from this positive trajectory (not-on-track patients—NOT). These studies provided clinicians feedback on their patients' state so they could better adapt their therapy to the patients' needs. This in turn had a positive effect on treatment outcomes in general, especially outcomes for NOT patients, to the point of preventing treatment failure. These treatment adaptation methods have recently evolved to include implementations of the nearest neighbor machine learning approach originating in avalanche research (Brabec and Meister, 2001), as well as other similar approaches to better predict an individual's optimal trajectory and identify deviations from it (Rubel et al., in press).

Machine learning approaches may thus be beneficial in the efforts of progressing toward personalized cognitive training. The inconsistencies between studies in terms of the efficacy of CBM, inhibitory training, and working memory training can serve as a rich and varied source to guide the selection and adaptation of effective personalized cognitive training. In this way, general open questions such as optimal training duration and time gaps between sessions will be replaced with specific questions about the training parameters most effective for each individual.

AUTHOR CONTRIBUTIONS

RS managed the planning process of the manuscript, performed all administrative tasks required for submission and drafted the manuscript. HO-S and SZ-M took part in planning, supervision, brainstorming, and writing the manuscript. ST took part in brainstorming and writing the manuscript.

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REFERENCES

Au, J., Sheehan, E., Tsai, N., Duncan, G. J., Buschkuehl, M., and Jaeggi, S. M. (2015). Improving fluid intelligence with training on working memory: a meta-analysis. *Psychon. Bull. Rev.* 22, 366–377. doi: 10.3758/s13423-014-0699-x

Brabec, B., and Meister, R. (2001). A nearest-neighbor model for regional avalanche forecasting. *Ann. Glaciol.* 32, 130–134. doi: 10.3189/172756401781819247

Chekroud, A. M., Zotti, R. J., Shehzad, Z., Gueorguieva, R., Johnson, M. K., Trivedi, M. H., et al. (2016). Cross-trial prediction of treatment outcome in depression: a machine learning approach. *Lancet Psychiatr.* 3, 243–250. doi: 10.1016/S2215-0366(15)00471-X

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., et al. (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet* 391, 1357–1366. doi: 10.1016/S0140-6736(17)32802-7

Cohen, N., Margulies, D. S., Ashkenazi, S., Schäfer, A., Taubert, M., Henik, A., et al. (2016). Using executive control training to suppress amygdala reactivity to aversive information. *Neuroimage* 125, 1022–1031. doi: 10.1016/j.neuroimage.2015.10.069

Cohen, Z. D., and DeRubeis, R. J. (2018). Treatment selection in depression. *Ann. Rev. Clin. Psychol.* 14, 209–236. doi: 10.1146/annurev-clinpsy-050817-084746

Cristea, I. A., Kok, R. N., and Cuijpers, P. (2015). Efficacy of cognitive bias modification interventions in anxiety and depression: meta-analysis. *Br. J. Psychiatr.* 206, 7–16. doi: 10.1192/bjp.bp.114.146761

Hakamata, Y., Lissek, S., Bar-Haim, Y., Britton, J. C., Fox, N. A., Leibenluft, E., et al. (2010). Attention bias modification treatment: a meta-analysis toward the establishment of novel treatment for anxiety. *Biol. Psychiatr.* 68, 982–990. doi: 10.1016%2Fj.biopsych.2010.07.021

Hallion, L. S., and Ruscio, A. M. (2011). A meta-analysis of the effect of cognitive bias modification on anxiety and depression. *Psychol. Bull.* 137, 940–58. doi: 10.1037/a0024355

Hannan, C., Lambert, M., Harmon, C., Nielsen, S., Smart, D., Shimokawa, K., et al. (2005). A lab test and algorithms for identifying clients at risk for treatment failure. *J. Clin. Psychol.* 61, 155–163. doi: 10.1002/jclp.20108

Hardy, J. L., Nelson, R. A., Thomason, M. E., Sternberg, D. A., Katovich, K., Farzin, F., et al. (2015). Enhancing cognitive abilities with comprehensive training: a large, online, randomized, active-controlled trial. *PLoS ONE* 10:e0134467. doi: 10.1371/journal.pone.0134467

Ioannidis, J. P. A. (2005). Why most published research findings are false. *PLoS Med.* 2:e124. doi: 10.1371/journal.pmed.0020124

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Karbach, J., and Verhaeghen, P. (2014). Making working memory work: a meta-analysis of executive-control and working memory training in older adults. *Psychol. Sci.* 25, 2027–2037. doi: 10.1177%2F0956797614548725

Koster, E. H., Hoorelbeke, K., Onraedt, T., Owens, M., and Derakshan, N. (2017). Cognitive control interventions for depression: a systematic review of findings from training studies. *Clin. Psychol. Rev.* 53, 79–92. doi: 10.1016/j.cpr.2017.02.002

Lambert, M. J., Whipple, J. L., Smart, D. W., Vermeersch, D. A., Nielsen, S. L., and Hawkins, E. J. (2001). The effects of providing therapists with feedback on patient progress during psychotherapy: are outcomes enhanced? *Psychother. Res.* 11, 49–68. doi: 10.1080/713663852

Lampit, A., Hallock, H., and Valenzuela, M. (2014). Computerized cognitive training in cognitively healthy older adults: a systematic review and meta-analysis of effect modifiers. *PLoS Med.* 11:e1001756. doi: 10.1371/journal.pmed.1001756

Melby-Lervåg, M., and Hulme, C. (2013). Is working memory training effective? A meta-analytic review. *Dev. Psychol.* 49, 270–91. doi: 10.1037/a0028228

Melby-Lervåg, M., Redick, T. S., and Hulme, C. (2016). Working memory training does not improve performance on measures of intelligence or other measures of "far transfer" evidence from a meta-analytic review. *Perspect. Psychol. Sci.* 11, 512–534. doi: 10.1177/1745691616635612

Mogg, K., Waters, A. M., and Bradley, B. P. (2017). Attention bias modification (ABM): review of effects of multisession ABM training on anxiety and threat-related attention in high-anxious individuals. *Clin. Psychol. Sci.* 5, 698–717. doi: 10.1177%2F2167702617696359

Okon-Singer, H. (2018). The role of attention bias to threat in anxiety: mechanisms, modulators and open questions. *Curr. Opin. Behav. Sci.* 19, 26–30. doi: 10.1016/j.cobeha.2017.09.008

Open Science Collaboration (2015). Estimating the reproducibility of psychological science. *Science* 349:aac4716. doi: 10.1126/science.aac4716

Penner, I. K., Vogt, A., Stöcklin, M., Gschwind, L., Opwis, K., and Calabrese, P. (2012). Computerised working memory training in healthy adults: a comparison of two different training schedules. *Neuropsychol. Rehabil.* 22, 716–733. doi: 10.1080/09602011.2012.686883

Peretz, C., Korczyn, A. D., Shatil, E., Aharonson, V., Birnboim, S., and Giladi, N. (2011). Computer-based, personalized cognitive training versus classical computer games: a randomized double-blind prospective trial of cognitive stimulation. *Neuroepidemiology* 36, 91–99. doi: 10.1159/000323950

Probst, T., Lambert, M. J., Dahlbender, R. W., Loew, T. H., and Tritt, K. (2014). Providing patient progress feedback and clinical support tools to therapists: Is the therapeutic process of patients on-track to recovery enhanced in psychosomatic in-patient therapy under the conditions of routine practice? *J. Psychosom. Res.* 76, 477–484. doi: 10.1016/j.jpsychores.2014.03.010

Rubel, J.A. Giesemann, J., Zilcha-Mano, S., and Lutz, W. (in press). Predicting process-outcome associations in psychotherapy based on the nearest neighbor approach: the case of the working alliance. *Psychotherapy Research*.

Rubel, J. A., and Lutz, W. (2017). "How, when, and why do people change through psychological interventions?—Patient-focused psychotherapy research," in *Routine Outcome Monitoring in Couple and Family Therapy*,eds T. Tilden and B. Wampold (Cham: Springer), 227–243. doi: 10.1007/978-3-319-50675-3_13

Schwaighofer, M., Fischer, F., and Bühner, M. (2015). Does working memory training transfer? A meta-analysis including training conditions as moderators. *Educ. Psychol.* 50, 138–166. doi: 10.1080/00461520.2015.1036274

Shatil, E., Metzer, A., Horvitz, O., and Miller, A. (2010). Home-based personalized cognitive training in MS patients: a study of adherence and cognitive performance. *NeuroRehabilitation* 26, 143–153. doi: 10.3233/NRE-2010-0546

Shimokawa, K., Lambert, M. J., and Smart, D. W. (2010). Enhancing treatment outcome of patients at risk of treatment failure: meta-analytic and mega-analytic review of a psychotherapy quality assurance system. *J. Consult. Clin. Psychol.* 78, 298–311. doi: 10.1037/a0019247

Siegle, G. J., Ghinassi, F., and Thase, M. E. (2007). Neurobehavioral therapies in the 21st century: summary of an emerging field and an extended example of cognitive control training for depression. *Cognit. Ther. Res.* 31, 235–262. doi: 10.1007/s10608-006-9118-6

von Bastian, C. C., and Oberauer, K. (2014). Effects and mechanisms of working memory training: a review. *Psychol. Res.* 78, 803–820. doi: 10.1007/s00426-013-0524-6

Weicker, J., Villringer, A., and Thöne-Otto, A. (2016). Can impaired working memory functioning be improved by training? A meta-analysis with a special focus on brain injured patients. *Neuropsychology* 30, 190–212. doi: 10.1037/neu0000227

Zilcha-Mano, S. (2018). Major developments in methods addressing for whom psychotherapy may work for and why. *Psychother. Res.* 1–16. doi: 10.1080/10503307.2018.1429691

Zilcha-Mano, S., Roose, S. P., Brown, P. J., and Rutherford, B. R. (2018). A machine learning approach to identifying placebo responders in late-life depression trials. *Am. J. Geriatr. Psychiatr.* 26, 669–677. doi: 10.1016/j.jagp.2018.01.001

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Oxytocin Modulates the Cognitive Appraisal of the Own and Others Close Intimate Relationships

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Close and intimate relationships are important promoters of health. Oxytocin and its association with social cognition have been investigated in a large number of studies, especially highlighting the neuropeptide's involvement in attachment behavior and intimate relationships. However, mixed findings on exogenous oxytocin application have led to the focus on moderators and mediators, suggesting that the effects are depended on specific factors – namely context and salience. The objective of the current study was to assess the effect of intranasal oxytocin on social appraisal of own and others' close intimate relationship characteristics. Different characteristics of relationships, including trust or closeness, between romantic couples (unknown and own) were assessed using the Couple Appraisal Task. In a randomized controlled double-blind cross-over within subject design, N = 71 healthy men and women were investigated after receiving first intranasal oxytocin and 2 weeks later placebo, or vice versa. We found an oxytocin-induced increase in the positive appraisal of one's own overall relationship characteristics but not in the evaluation of the relationship of others. The present study – one of the first of its kind administrating oxytocin in a repeated measures cross-over design – adds further evidence to the mediating role of oxytocin in social cognition, specifically with regard to romantic relationship characteristics.

Keywords: oxytocin, couple relationships, relationship appraisal, social cognition, repeated-measures-cross-over-design

INTRODUCTION

Healthy and supportive intimate relationships promote well-being, buffer against the development of mental and physical illnesses, and improve recovery in burdened individuals (Holt-Lunstad et al., 2010; Ditzen and Heinrichs, 2014). In contrast, low-quality relationships can induce dysfunctional processes and contribute to the development of disease, if an individual is vulnerable (Fincham and Beach, 2010; Robles, 2014; Holt-Lunstad et al., 2015; Kiecolt-Glaser and Wilson, 2017).

Close positive relationships with a significant other are described by a variety of important relationship characteristics such as social support (Holt-Lunstad et al., 2008), attachment and affection (Noftle and Shaver, 2006; Hadden et al., 2014), intimacy (Aron et al., 2000;

Rubin and Campbell, 2012; Ferreira et al., 2014), dependency (DeHart et al., 2004; Valor-Segura et al., 2014), commitment (Stanley et al., 2010), cohesion or security (Baumeister and Leary, 1995; Olson, 2011; Kazmierczak and Blazek, 2015), as well as trust or trustworthiness (see for overall quality of relationship characteristics Schneider et al., 2011; Aguilar-Raab et al., 2015, 2018). This is also evident in theoretical models describing high functioning relationships, such as Gottman's Sound Relationship House Theory (Gottman and Gottman, 2017) or Sternberg's Triangular Theory of Love (Sternberg, 1986; Lemieux and Hale, 2000), the latter defining emotional, motivational, and cognitive components.

Despite the evidence of their impact on health, relatively little is known about the neurochemical mechanisms and their interconnected and complex functioning, that underpin relationship characteristics shaping social interactions with significant others.

On a neuroendocrine level, the neuropeptide and hormone oxytocin (OT) is considered to be involved in emotional and cognitive processes within intimate relationships (Hurlemann and Scheele, 2016; Feldman, 2017). On the one hand, OT is assumed to be released during partner contract (Grewen et al., 2005). On the other hand, when given exogenously, it has been shown to dampen stress-sensitive activation of the hypothalamic pituitary adrenal (HPA) axis and to enhance positive behavior during couple conflict (Ditzen et al., 2009, 2012). Furthermore, it seems to stimulate the central reward system when viewing pictures or feeling touch of the partner (Scheele et al., 2013). One functional aspect of these effects might be seen in the protection of the own established relationship via increasing the distance to potential other partners such as an attractive unacquainted person (Scheele et al., 2012). In addition, it strengthens sexual experiences with the partner (Behnia et al., 2014). Another function can be to increase the beneficial effects of partner support and touch, e.g., when experiencing acute pain (Kreuder et al., 2018). Health-promoting effects of partner contact in combination with OT are assumed to be a result of a learned association of safety experiences during the individual relationship history (Eckstein et al., 2018b).

Experimental manipulations of intranasally administered OT in association with social cognition have increased tremendously in the last two decades. These studies suggest that OT cannot be regarded as a "love-hormone" per se (Guzman et al., 2013) but instead much depends on the context (with e.g., strong social in- and out-group effects; De Dreu et al., 2010, 2011). Most importantly, the individual significance and direction of OT effects seems to depend on personal experience (Heim et al., 2008; Bertsch et al., 2013).

Intranasally administered OT has been shown to reduce the neuroendocrine response to stressful social interactions (Heinrichs et al., 2003; Ditzen et al., 2009; Zietlow et al., 2018). The administration of intranasal OT has also revealed mediation effects in the framework of social cognition and perception, such as a better ability to infer the affective state of others based on a more accurate appraisal of social cues of the eye region (Domes et al., 2007). Some findings support evidence for OT to promote qualities like generosity, cooperation, and trust (Zak et al., 2004; Kosfeld et al., 2005; Theodoridou et al., 2009). Furthermore, Fischer-Shofty et al. (2013) for example could show that OT had an overall effect on improving accurate perception of social interactions based on an Interpersonal Perception Task. On a central nervous system level, the key regions of OT effects comprise among others - the striatum, amygdala, cingulate, and insula (Winston et al., 2002; Watabe et al., 2011; Xu et al., 2012; Bethlehem et al., 2013; Scheele et al., 2013).

However, so far, findings on OT and social cognition yielded mixed and inconclusive results. For example, for effects of OT on trust, a recent review and meta-analysis summarized the available data quite critically especially in terms of variations in applied methods assessing OT, for example in peripheral bodily fluids (Nave et al., 2015): Therefore, they call for further research critically investigating the role of OT for trust-related processes, and for replicating findings and publishing controversial or null-findings.

Besides that, OT exhibits effects especially toward difficult or ambiguous items (Domes et al., 2007), and leads to higher concordant ratings for self- and other judgments (Colonnello et al., 2013). In addition, the novelty or hormonal state at prior exposure to the stimuli can play a significant role (Wallen and Rupp, 2010; Tops et al., 2013; Eckstein et al., 2018a). Shamay-Tsoory and Abu-Akel (2016) suggested that one possible mechanism to explain these findings is an influence of OT on salience and attention orientation toward social stimuli with dependence on the personal baseline characteristics such as gender, relationship status, or individual experiences. If social bonding, trust, and attachment are highly salient in individuals based on the involvement in a romantic relationship, OT can probably rather strengthen this salience of these important relationship characteristics (Scheele et al., 2012), and thereby enhance pre-existing tendencies. One underlying mechanism could be *a priori* individual differences in receptor density or sensitivity, which have been suggested in animal studies investigating local receptor distribution and sensitivity in the central nervous system (Insel and Shapiro, 1992; Walum and Young, 2018).

The potential influence of intranasally administered OT on how people perceive and evaluate important aspects of the relationship quality of others' as well as their own close relationships, has not been fully understood so far. These processes likely form the basis of the hypothesized relevance of OT for the health-promoting effect of social relationships.

The present research aimed to investigate the influential role of OT on cognitive appraisals of romantic relationships and important relationship-defining characteristics. Using a previously published standard set of pictures and criteria (Bilderbeck et al., 2011), the question was on how OT would influence the study participants' appraisal of their own and others' relationships – taking into account the physical proximity (with vs. without physical contact) of the unknown couples shown on a set of photographs as potential mediator of perceived bonding (Bilderbeck et al., 2011).

To control for potential person-related factors, we applied a controlled double-blind cross-over within-subject design allowing to test order-effects (Kim et al., 2015; Eckstein et al.,

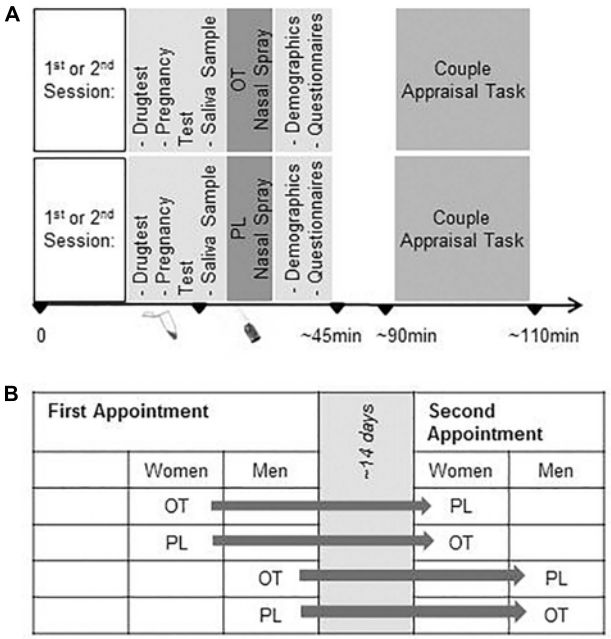

FIGURE 1 | (A) Experimental Procedure, and **(B)** Randomized Cross-Over Design. OT, oxytocin; PL, placebo.

2018a). Both, healthy women and men received first intranasal OT and 2 weeks later Placebo (PL), or vice versa. We hypothesized that OT – compared to PL – would lead to higher positive ratings of both other and own relationship characteristics in couples, such as intimacy or trust.

MATERIALS AND METHODS

Participants

The study was conducted at University of Zurich, Switzerland. In a repeated-measures design, initially $N = 84$ heterosexual men and women were randomly and double blind assigned to receiving intranasal OT in the first session and PL in the second session or vice versa (see **Figures 1A,B**). Prior to the experimental sessions, a telephone screening was conducted to exclude participants with the following criteria: Chronic physical or mental illness, regular smoking, alcohol consumption or drug abuse, medication intake, including intake of hormonal contraceptives, and BMI above 25 or below 18. Additional exclusion criteria for women were irregular menstrual cycle (<23/>35 days), current pregnancy, and breastfeeding. All women were naturally cycling and scheduled balanced for cycle phase (50% were invited for the first session during the mid-luteal cycle phase and 50% during follicular phase according to repeated self-report and repeated monitoring).

Participants were recruited via university advertisements and public media and received either financial incentive (100 CHF) or study credits. All participants provided written informed consent before beginning with the experimental sessions. The study was approved by the local and cantonal ethics committee of the Canton of Zurich (2009/0063/5) as well as by Swissmedic, conducted in compliance with the Declaration of Helsinki and monitored from the Clinical Trials Unit (CTU) of the University of Zurich.

A total of $N = 71$ participants with $N = 38$ female and $N = 33$ male adult participants with a mean age of $M = 26.37$ ($SD = 5.36$; age range 21–45 years) were included in the final data analysis. For reasons of technical difficulties, missing data and dropout, $N = 13$ participants were previously excluded. Of the final sample, $N = 33$ (46.5%) received OT and $N = 38$ (53.5%) received PL first. $N = 40$ (56.3%) participants indicated to be single, whereas $N = 31$ (43.7%) specified to live in a romantic relationship – the latter sub-sample was used for the analysis of the Couples Appraisal Task (CAT) regarding the participants' own relationship. This sub-sample stated to be highly satisfied with their partnership, as suggested from their scores in the Relationship Assessment Scale (seven item RAS, German version; mean score range 2.86–5.00; 4th and 7th item were recoded; higher ratings indicate higher relationship satisfaction) with $M = 4.21$ (SD = 0.11). **Table 1** shows all other sample characteristics – from age for all subgroups, nationality up to annual income and others.

Procedure and Tasks

The study was conducted in two identically structured experimental sessions on separate days, scheduled approx. 14 days apart ($M = 16.47$ days between assessments, SD = 10.80), between 3 – 9 pm. Prior to the investigation, participants received information about the experimental sessions along with instructions to abstain from smoking, caffeine, medication, and alcohol as well as excessive sports on the days of the investigation. At the beginning of each appointment, detailed information about the procedures and confidentiality was given and written consent was obtained. A multi drug screening (M-3-1-DT, Diagnostik Nord, Schwerin, Germany), and for women additionally, a pregnancy test was done (Evial, Inopharm, Muri near Bern, Switzerland). Participants gave a saliva sample for the assessment of gonadal hormones in order to verify the women's menstrual cycle phase.

Subsequently, the participants self-administered either 24 IU of intranasally OT (three puffs per nostril; Syntocinon Spray, Novartis, Basel, Switzerland) or PL (containing identical ingredients except for the peptide; cantonal pharmacy of Zurich) under the supervision of the study coordinator.

The trials started 45 min after OT application and took about 20 min.

Couple Appraisal Task

We used the German version of the "Couple Appraisal Task," (CAT Bilderbeck et al., 2011), to assess the evaluation of different couple specific characteristics. The CAT contains presentations of 24 pictures of heterosexual couples at different ages. Pictures were taken when couples stood outside in a neutral setting. All couples look directly into the camera and men and women show a neutral facial expression. In 12 photographs, the couples touch each other with a romantic gesture, such as holding hands or putting their arms around their shoulders (see **Figure 2A**),

TABLE 1 | Sample characteristics.

		Both groups			Group 1			Group 2		
		all	female	male	all	female	male	all	female	male
	n	71	38	33	33	19	14	38	19	19
Age	*M*	26.37	25.47	27.39	26.39	26.53	26.21	26.34	24.42	28.26
	SD	5.36	4.37	6.23	0.50	5.25	5.73	5.42	3.07	6.58
	range	21;42	21;38	21;42	21;40	21;38	21;40	21;42	21;30	21;42
Nationality	CH	78.9	71.1	87.9	78.8	78.9	78.6	78.9	63.2	94.7
	D	5.6	7.9	3.0	3.0	5.3	0	7.9	10.5	5.3
	other	15.5	21.1	9.1	18.2	15.8	21.4	13.2	26.3	0
Relationship	no	56.3	52.6	60.6	57.6	52.6	64.3	55.3	52.6	57.9
	yes	43.7	47.4	39.4	42.4	47.4	35.7	44.7	47.4	42.1
Sex. Orient.	Heterosex.	97.2	94.7	100	93.9	89.5	100	100	100	100
	Bisex.	2.8	5.3	0	6.1	10.5	0	0	0	0
Edu. Level	Primary	1.4	0	3.0	3.0	0	7.1	0	0	0
	Middle	1.4	2.6	0	3.0	5.3	0	0	0	0
	Apprenticeship	5.6	2.6	9.1	0	0	0	10.5	5.3	15.8
	Vocational	4.2	5.3	3.0	6.1	5.3	7.1	2.6	5.3	0
	Baccalaureate	49.3	50.0	48.5	48.5	42.1	57.1	50.0	57.9	42.1
	Uni. Degr.	36.6	36.8	36.4	36.4	42.1	28.6	36.8	31.6	42.1
	other	1.4	2.6	0	3.0	5.3	0	0	0	0
Job	no	40.8	34.2	48.5	45.5	36.8	57.1	36.8	31.6	42.1
	yes	59.2	65.8	51.5	54.5	63.2	42.9	63.2	68.4	57.9
Income	No income	4.2	0	9.1	0	0	0	7.9	0	15.8
	Student	63.4	65.8	60.6	72.7	68.4	78.6	55.3	63.2	47.4
	≤50.000	25.4	26.3	24.2	18.2	15.8	21.4	31.6	36.8	26.3
	≤100.000	7.0	7.9	6.1	9.1	15.8	0	5.3	0	10.5

n, Sample size; Group 1, received OT first, PL at the second measurement point; Group 2, received PL first, OT at the second measurement point; Nationalities: CH, Switzerland, D, Germany; Sex. Orient., sexual orientation; Heterosex., heterosexual; Bisex., bisexual; Edu.Level, highest educational level; Primary, primary school; Middle, middle school; Apprenticeship, apprenticeship certificate; Vocational, vocational school-leaving certificate; Baccalaureate, baccalaureate diploma/high school graduation; Uni. Degr., university degree; Income in Swiss francs; all other characteristic values, data in percent.

whereas in the other 12 pictures couples are standing slightly apart (see **Figure 2B**). The same set of pictures was used for both assessment time points.

Participants were asked to focus on each picture without time limit and to assess the following questions on a seven-point scale from 1, "not at all" to 7, "very": (1) "How strongly do the two partners support each other?" (supportive); (2) "How strong is the intimacy between the two partners?" (intimate); (3) "How independent are the two partners from each other?" (independent); (4) "What is the commitment between the partners?" (committed); (5) "How romantic is the relationship between the two of them?" (romantic); (6) "How trustful is the relationship between the two of them?" (trusting); (7) "How certain is the relationship between the two of them?" (certainty of relationship); (8) "How well do the partners fit together?" (fitting); (9) "How well do they handle possible conflicts?" (conflict resolution); and (10) "How good is the physical relationship between the two of them?" (good physical relationship).

To investigate couple appraisal with regard to the own romantic relationship, participants, who were currently and exclusively dating, were asked to send a picture of themselves together with their partner prior to the first assessment. The same criteria as described above were required for photograph acquisition.

First the pictures of the unknown couples where presented, and if the participant was living in a romantic relationship, the picture of him-/herself together with the partner followed as one additional picture.

The ten CAT ratings of the 24 pictures of unknown couples showed good reliability (Cronbach's Alpha = 0.96, based on $N = 71$), whereas the ten CAT ratings of the participants' own couple pictures (based on the sub-sample of $N = 31$) yielded lower reliability (Cronbach's Alpha = 0.76).

Statistical Analysis

For the CAT task we calculated mean values of the ten CAT ratings of the first set of 12 pictures showing couples with body contact, and of the second set of 12 pictures showing couples without body contact. Finally we calculated mean values of all couple pictures, and for the analysis of those participants currently in a relationship (sub-group analysis) we calculated mean values of the ten CAT ratings regarding their own couple picture.

For all statistical analyses we used IBM SPSS Version 22. Repeated measures ANOVAs were used to calculate the effects of

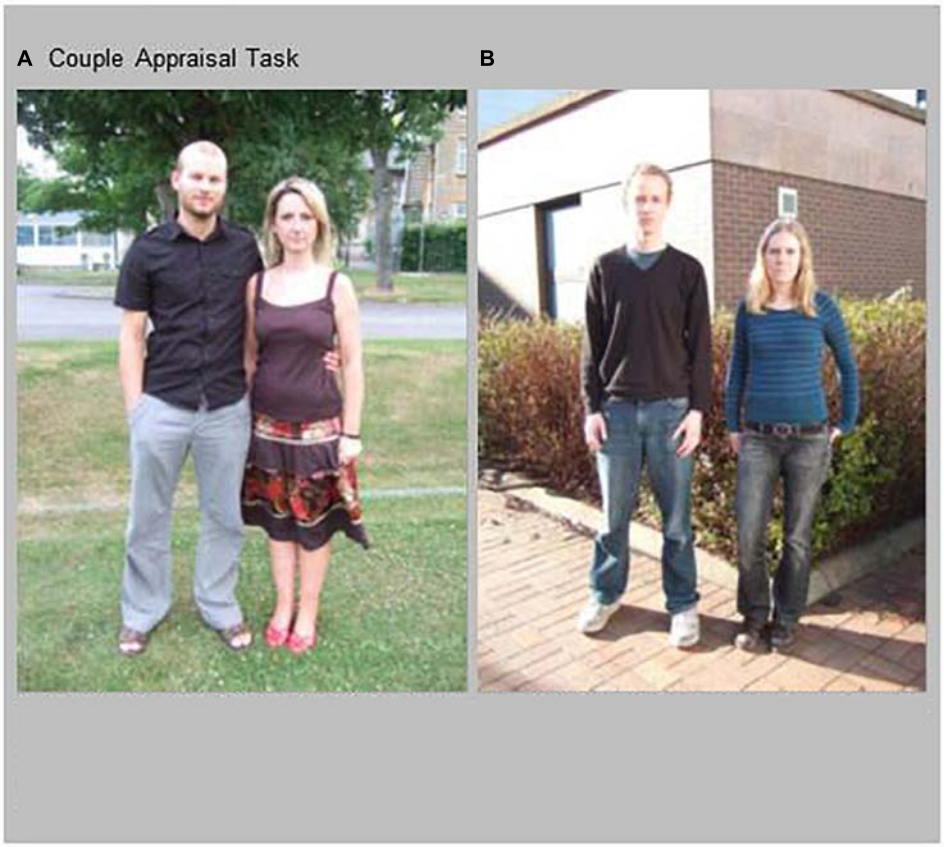

FIGURE 2 | CAT Examples. Couples **(A)** with body contact, **(B)** without body contact. Written informed consent was obtained from these individuals for the publication of the two pictures.

OT vs. PL (treatment factor) on the different ratings taking into account the two measurement time-points.

In line with Bilderbeck et al., 2011, we aimed to investigate differential effects of pictures with body contact and without body contact. We conducted *t*-tests or 2 × 2 repeated measures ANOVA (treatment 2 levels: OT/PL; 2 levels with pictures with body contact/without body contact). Then we exploratively ran (2 × 10) ANOVAs one for each CAT rating. These were uncorrected for multiple testing. Finally, we analyzed the subset of pictures of the own relationship. In all analyses, we added between-subject factors for sex (male vs. female) and order of assessment (OT vs. PL in first session).

RESULTS

The groups (receiving OT or PL first) did not differ in any of the demographic variables, which indicates successful randomization within the group assignments: gender: $\chi 2$ (1) = 0.41, p = 0.52; age: $\chi 2$ (20) = 16.12, p = 0.71; education: $\chi 2$ (6) = 7.43, p = 0.28; employment: $\chi 2$ (1) = 0.54, p = 0.46; and relationship status: $\chi 2$ (1) = 0.04, p = 0.85. Additionally, the groups of female and male participants did not differ with regard to age: $\chi 2$ (20) = 15.70, p = 0.74.

The ratings of the images of the unknown couples with and without body contact showed significant differences in a *t*-test – revealing higher ratings of the positive partnership characteristics in the couple pictures with body contact: $t(70) = 9.12$. $p = 0.000$.

CAT mean scores and standard deviations are depicted in **Table 2**, differentiated by OT vs. PL, by gender and by order.

Appraisal of Other Couples' Relationship Characteristics in Pictures With Versus Without Body Contact

Results of the repeated measures (2 × 2) ANOVA (treatment OT/PL, pictures with/without body contact) with the mean scores of the ten CAT ratings of the 12 couple pictures as dependent variable showed a significant main effect of body contact with higher CAT-ratings of body contact pictures: $F(1,67) = 95.48$, $p = 0.000$, $\eta^2 = 0.588$ (this and all following η^2 are *partial* η^2), no interaction effect of body contact and sex $F(1,67) = 1.37$, $p = 0.247$, $\eta^2 = 0.020$, or body contact and order $F(1,67) = 0.06$, $p = 0.814$, $\eta^2 = 0.001$.

No significant results were found for OT treatment $F(1,67) = 1.94$, $p = 0.169$, $\eta^2 = 0.028$, no interaction effect of treatment and sex $F(1,67) = 0.40$, $p = 0.529$, $\eta^2 = 0.006$, and

TABLE 2 | Couple Appraisal Task (CAT) Ratings*.

			Unknown couples		Own relationship	
category	sex	order	*n*	*M(SD)*	*n*	*M(SD)*
Oxytocin	male	Placebo → Oxytocin	19	4.60(0.49)	8	5.73(0.72)
		Oxytocin → Placebo	14	4.42(0.59)	5	6.05(0.52)
		total	33	4.52(0.53)	13	5.86(0.65)
	female	Placebo → Oxytocin	19	4.65(0.64)	9	5.90(0.79)
		Oxytocin → Placebo	19	4.65(0.59)	9	6.10(0.48)
		total	38	4.65(0.61)	18	5.94(0.64)
	total	Placebo → Oxytocin	38	4.62(0.56)	17	5.34(0.74)
		Oxytocin → Placebo	33	4.55(0.59)	14	9.09(0.48)
		total	71	4.59(0.57)	31	5.95(0.64)
Placebo	male	Placebo → Oxytocin	19	4.55(0.42)	8	5.34(0.74)
		Oxytocin → Placebo	14	4.28(0.58)	5	6.08(0.77)
		total	33	4.44(0.51)	13	5.63(0.81)
	female	Placebo → Oxytocin	19	4.68(0.63)	9	5.70(0.77)
		Oxytocin → Placebo	19	4.55(0.38)	9	5.89(0.56)
		total	38	4.62(0.52)	18	5.79(0.66)
	total	Placebo → Oxytocin	38	4.61(0.53)	17	5.53(0.76)
		Oxytocin → Placebo	33	4.44(0.49)	14	5.96(0.62)
		total	71	4.53(0.52)	31	5.72(0.72)

*n, Sample size, M, Mean, SD, Standard deviation. Order: Placebo → Oxytocin, received Placebo first, Oxytocin at the second measurement point; Oxytocin → Placebo, received Oxytocin first, Placebo at the second measurement point. * overall CAT mean values, all pictures (with and without body contact).*

no interaction effect of treatment and order $F(1,67) = 1.30$, $p = 0.259$, $\eta^2 = 0.019$.

Appraisal of Other Couples' Relationship Characteristics

Results of the repeated measures ANOVA (treatment OT/PL by time and sex) with the mean scores of the ten CAT ratings of all couple pictures (with and without body contact) as dependent variable revealed no overall main effect of OT treatment $F(1,67) = 1.94$, $p = 0.169$, $\eta^2 = 0.028$, no interaction effect of treatment and sex $F(1,67) = 0.40$, $p = 0.529$, $\eta^2 = 0.006$, and no interaction effect of treatment and order $F(1,67) = 1.298$, $p = 0.259$, $\eta^2 = 0.019$.

Analog analyses of the single CAT score levels, for example for the trust ratings, showed no significant effect of OT treatment $F(1,67) = 1.742$, $p = 0.191$, $\eta^2 = 0.025$, no interaction effect of treatment and sex $F(1,67) = 2.546$, $p = 0.115$, $\eta^2 = 0.037$ and no interaction effect treatment and order $F(1,67) = 0.036$, $p = 0.850$, $\eta^2 = 0.001$ (see **Appendix A** for the non-significant findings of all CAT scores). A significant interaction effect of treatment and order was found regarding the couple characteristic "romantic" with $F(1,67) = 4.436$, $p = 0.039$, $\eta^2 = 0.062$. When participants first received PL, OT led to higher ratings of the couples to be more romantic.

Appraisal of the Own Relationship's Characteristics

The analysis (repeated measures ANOVA with treatment OT/PL by time and sex) within the sub-sample of participants currently living in a romantic relationship ($N = 31$) of the mean scores of the ten CAT ratings regarding their own relationship (evaluation of their own couple picture) exhibited a main treatment effect $F(1,27) = 4.229$, $p = 0.05$, $\eta^2 = 0.135$, suggesting higher positive couple appraisals toward the participants' own relationship under OT, see **Figure 3**. No interaction effect of treatment and sex $F(1,27) = 0.021$, $p = 0.886$, $\eta^2 = 0.001$, and no interaction effect of treatment and order $F(1,27) = 1.14$, $p = 0.295$, $\eta^2 = 0.041$ were found. Analysis of the specific and single CAT scores showed a significant interaction effect of treatment and order on the appraisal of one's own relationship in "conflict resolution capacities" with $F(1,27) = 5.952$, $p = 0.02$, $\eta^2 = 0.181$. When participants received PL first, OT resulted in higher ratings of conflict resolution capacities. For all other individual CAT ratings, analysis revealed no significant results, including the trust ratings: no trust main effect of OT treatment $F(1,27) = 0.16$, $p = 0.69$, $\eta^2 = 0.006$, no interaction effect of treatment and sex $F(1,27) = 0.759$, $p = 0.391$, $\eta^2 = 0.027$, and no interaction effect of treatment and order $F(1,27) = 0.028$, $p = 0.869$, $\eta^2 = 0.001$ (see **Appendix A** for the non-significant findings of all other CAT characteristics of one's own relationship).

Interaction Effects of OT/PL of Other vs. Own Relationship's Characteristics (Based on the Sub-Sample of *n* = 31)

The repeated measures ANOVA analyses resulted in a significant within-subject effect with $F(2,26) = 4.31$, $p = 0.024$, $\eta^2 = 0.249$, indicating higher CAT ratings under OT vs. PL for the own couple vs. the unknown couples pictures, see **Figure 3**.

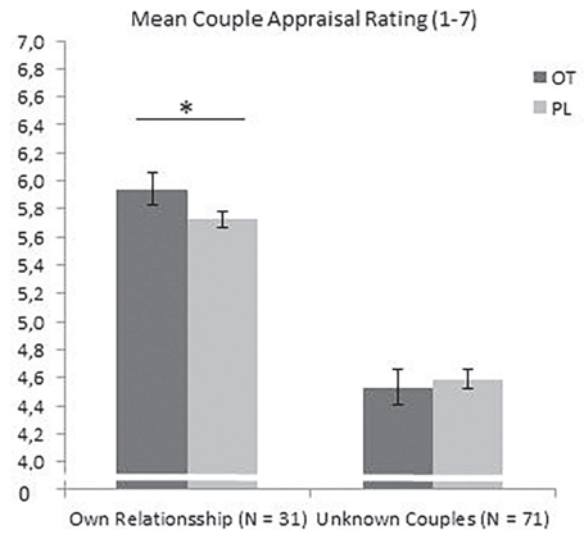

FIGURE 3 | OT increased the CAT ratings toward the own relationship in the subsample of pair-bonded individuals, but not in the whole sample toward the unknown couples. *p <0.05.

DISCUSSION

The present study investigated the effects of intranasally administered OT vs. PL in a cross-over repeated measures design during a task testing for relevant and specific relationship characteristics, namely support, intimacy, independence, commitment, degree of being romantic, trust, security, fitting together, conflict resolution, and good physical relationship. Applying the Couple Appraisal Task (Bilderbeck et al., 2011) men's and women's evaluation of these characteristics in pictures depicting unknown couples (with and without body contact) and the participants themselves with their own partner were investigated.

While OT did not affect positive appraisals toward couple pictures unknown to the study participants – regardless of whether the couples were depicted with or without physical contact, OT significantly increased positive appraisals of these same characteristics regarding the participants' own relationship. This effect was moderated neither by sex nor order of substance application.

These findings are in line with theoretical models and empirical data on OT's involvement in close attachment bonds and romantic relationships, especially inducing social affiliative behavior (Taylor, 2006; Feldman, 2012, 2017). Above this, this data replicates the results from Scheele et al. (2013), where OT selectively increased positive ratings and reward-related brain activity toward the own partner vs. an unfamiliar woman. Here our data suggests that it is the overall appraisal rather than the evaluation of individual relationship characteristics, which is influenced by OT.

One underlying mechanism of these effects seems to be in the stimulation of the central nervous reward system and related dopamine activation (Walum and Young, 2018), an effect supported with data on region-specific activation in the nucleus accumbens and anterior cingulate cortex following OT administration (Scheele et al., 2013, 2015; Kreuder et al., 2017). Therefore, OT might act by increasing the rewarding aspects of the own relationship, specifically.

At the same time, the non-significant findings on the overall estimation of relationship characteristics of unknown couples shown as photographs might point to the fact that OT effects depend on individual experiences and on affiliative motivation with regard to these social stimuli. If confronted with social characteristics not linked to oneself and thereby lacking strong emotional relevance and personal involvement, OT might not necessarily influence cognition.

Above this, rather than turning social perception and interpretations toward an overall more positive view, OT was suggested to increase the stimulus materials' salience and relevance (Shamay-Tsoory et al., 2009; Harari-Dahan and Bernstein, 2014) through influence on the amygdala and striatum, as well as the medial prefrontal cortex. In addition, it has also been suggested, that OT especially acts on salient stimuli. Our own data of another study suggests that OT increases the strong salience of negative social feedback (Eckstein et al., 2014). Another study suggested that OT increased the self-perception of positive personality traits (Cardoso et al., 2012). With regard to the present study sample, this might be of particular importance, because overall, relationship satisfaction in the current study sample was quite high (see methods section), therefore pictures of the own relationship were highly salient and very likely positively attributed. Increasing the salience of own relationship characteristics in these genuinely happy and healthy study participants might explain the specific OT effects on the participants' own relationship characteristics. In contrast, couple appraisals regarding other – unknown – less salient couples might not be influenced by OT over this mechanism.

It might be argued, that endogenous OT mechanisms differ between singles without a partner and individuals in a couple relationship. In a relationship, there is probably more frequent endogenous stimulation of OT by social touch or sexual intimacy (de Jong et al., 2015), therefore bonded individuals may have different receptor sensitivity or density than singles, similar to what has been proposed in studies with voles (Insel and Shapiro, 1992). To date such differences cannot be tested in the living human brain.

Limitations

The present study has some shortcomings. The CAT is an established measure (Bilderbeck et al., 2011), but the variance of perceived relationship characteristics in others vs. for the own relationship is yet to be investigated. Our data speak for a ceiling effect in favor of positive appraisals of the participant's own positive relationship characteristics. However, the differential effect of OT on others and the own relationship characteristics might be due to the different picture samples analyzed. Using the CAT in real-time couple interaction tasks (Ditzen et al., 2007; Jarnecke et al., 2018) or study designs with ecological momentary assessments in daily life might increase the validity

and generalizability of findings (c.f., Timmons et al., 2017; Doerr et al., 2018).

OT-induced increases in the CAT for the overall appraisal of one's own relationship characteristics were not specific for gender. This missing effect might be due to the small sample size and poor statistical power. In contrast, for example, to our own previous results (Eckstein et al., 2018a), here the OT effect was dependent on order of treatment application only for the single aspect of "conflict resolution." The missing order or carryover effect might be explained with the specific stimulus material used in this study: pictures of the own couple in comparison to pictures showing unknown couples. While in previous datasets, the stimulus material was new – and with the first presentation of these new and unacquainted stimuli there was a particularly strong OT-effect found, the own partner and photographs of the own relationship were well-known to the participants. Therefore, whether the single order-effect indicates that OT – compared to PL – had a specific impact on the evaluation of conflict resolution at the second time point, or if this is merely a spurious result due to Alpha error accumulation in multiple testing, cannot be judged with certainty and requires further exploration in the future.

Furthermore, we assessed naturally cycling women only, which does not allow for conclusions on women using hormonal contraception (Scheele et al., 2015). Gonadal hormones and opioids have been related to OT functioning (Champagne et al., 2001; Choleris et al., 2003), therefore it might be useful to systematically design future studies assessing those in order to get a full picture of the underlying complex mechanisms. Another relevant moderator, the menstrual cycle phase could not be analyzed due to small sub-group numbers and inconsistencies in self-report and endocrine markers. Still, we controlled for the cycle phase, since a recent meta-analysis shows that the endogenous oxytocin concentration in women increases or decreases depending on the respective cycle phase (Engel et al., 2019).

Moreover, it needs to be addressed that we did not measure endogenous OT levels or other neuroendocrine factors such as vasopressin, which are also important in the context of social cognition. In addition, there is the difficulty that so far hardly anything is known about daily variability of OT.

Additional factors such as genetic or epigenetic variables have also been shown to play an important role in the context of social bonds and social behavior (Jacob et al., 2007; Krueger et al., 2012; Massey et al., 2015; Feldman et al., 2016). Larger and representative biomarker studies can inform on these effects (see for example Walum et al., 2012), however, usually, these studies do not have repeated-measures behavioral data.

Moreover, due to the assumed publication bias in OT literature (Lane et al., 2016), it continues to be unknown what other tasks or effects of OT have already been tested but have not been published. Thus, the publication of null findings and unexpected results should be encouraged.

Altogether our results are in line with previous data on the modulating role of OT on couple behavior and bonding (Hurlemann and Scheele, 2016; Feldman, 2017), but also suggest that OT might not serve as a "love hormone" or rose-colored spectacles regarding romantic relationships overall. Rather, in this present sample of individuals in a genuinely happy romantic relationship, OT might have increased perceived salience and, thereby, positive appraisals of one's own relationship. It stands to find out, whether indeed OT might serve as a possible pharmacological intervention in order to improve unhappy couple relationships. Thus, the present study adds further evidence to the mediating role of OT in social cognition and specifically estimating one's own relationship characteristics. Future research should systematically investigate and replicate findings on neurobiological person-related factors and specific skill requirements in different tasks, relationship types and levels of relationship quality.

ETHICS STATEMENT

All participants provided written informed consent before beginning with the experimental sessions. The study was approved by the local and cantonal ethics committee of the Canton of Zurich (2009/0063/5) as well as by the Swissmedic, conducted in compliance with the Declaration of Helsinki and monitored from the Clinical Trials Unit (CTU) of the University of Zurich.

AUTHOR CONTRIBUTIONS

BD, SG, MP, IG, MH, and UE designed the experiments. MP and SG conducted the experiments. AB conceptualized and tested the CAT and provided the CAT-stimuli. CA-R and ME analyzed the data. CA-R, ME, and BD wrote the 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/fnins.2019.00714/full#supplementary-material

REFERENCES

Aguilar-Raab, C., Grevenstein, D., Gotthardt, L., Jarczok, M. N., Hunger, C., Ditzen, B., et al. (2018). Changing me, changing us: relationship quality and collective efficacy as major outcomes in systemic couple therapy. *Fam. Process* 57, 342–358. doi: 10.1111/famp.12302

Aguilar-Raab, C., Grevenstein, D., and Schweitzer, J. (2015). Measuring social relationships in different social systems: the construction and validation of the evaluation of social systems (EVOS) scale. *PLoS One* 10:e0133442. doi: 10.1371/journal.pone.0133442

Aron, A., Norman, C. C., Aron, E. N., McKenna, C., and Heyman, R. E. (2000). Couples' shared participation in novel and arousing activities and experienced relationship quality. *J. Pers. Soc. Psychol.* 78, 273–284. doi: 10.1037/0022-3514.78.2.273

Baumeister, R. F., and Leary, M. R. (1995). The need to belong: desire for interpersonal attachments as a fundamental human motivation. *Psychol. Bull.* 117, 497–529.

Behnia, B., Heinrichs, M., Bergmann, W., Jung, S., Germann, J., Schedlowski, M., et al. (2014). Differential effects of intranasal oxytocin on sexual experiences and partner interactions in couples. *Horm. Behav.* 65, 308–318. doi: 10.1016/j.yhbeh.2014.01.009

Bertsch, K., Schmidinger, I., Neumann, I. D., and Herpertz, S. C. (2013). Reduced plasma oxytocin levels in female patients with borderline personality disorder. *Horm. Behav.* 63, 424–429. doi: 10.1016/j.yhbeh.2012.11.013

Bethlehem, R. A., van Honk, J., Auyeung, B., and Baron-Cohen, S. (2013). Oxytocin, brain physiology, and functional connectivity: a review of intranasal oxytocin fMRI studies. *Psychoneuroendocrinology* 38, 962–974. doi: 10.1016/j.psyneuen.2012.10.011

Bilderbeck, A. C., McCabe, C., Wakeley, J., McGlone, F., Harris, T., Cowen, P. J., et al. (2011). Serotonergic activity influences the cognitive appraisal of close intimate relationships in healthy adults. *Biol. Psychiatry* 69, 720–725. doi: 10.1016/j.biopsych.2010.12.038

Cardoso, C., Ellenbogen, M. A., and Linnen, A.-M. (2012). Acute intranasal oxytocin improves positive self-perceptions of personality. *Psychopharmacology* 220, 741–749. doi: 10.1007/s00213-011-2527-6

Champagne, F., Diorio, J., Sharma, S., and Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12736–12741. doi: 10.1073/pnas.221224598

Choleris, E., Gustafsson, J. -Å., Korach, K. S., Muglia, L. J., Pfaff, D. W., and Ogawa, S. (2003). An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-α and-β knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6192–6197. doi: 10.1073/pnas.0631699100

Colonnello, V., Chen, F. S., Panksepp, J., and Heinrichs, M. (2013). Oxytocin sharpens self-other perceptual boundary. *Psychoneuroendocrinology* 38, 2996–3002. doi: 10.1016/j.psyneuen.2013.08.010

De Dreu, C. K., Greer, L. L., Van Kleef, G. A., Shalvi, S., and Handgraaf, M. J. (2011). Oxytocin promotes human ethnocentrism. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1262–1266. doi: 10.1073/pnas.1015316108

De Dreu, C. K. W., Greer, L. L., Handgraaf, M. J. J., Shalvi, S., Van Kleef, G. A., Baas, M., et al. (2010). The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science* 328, 1408–1411. doi: 10.1126/science.1189047

de Jong, T. R., Menon, R., Bludau, A., Grund, T., Biermeier, V., Klampfl, S. M., et al. (2015). Salivary oxytocin concentrations in response to running, sexual self-stimulation, breastfeeding and the TSST: the regensburg oxytocin challenge (ROC) study. *Psychoneuroendocrinology* 62, 381–388. doi: 10.1016/j.psyneuen.2015.08.027

DeHart, T., Pelham, B., and Murray, S. (2004). Implicit dependency regulation: self-esteem, relationship closeness, and implicit evaluations of close others. *Soc. Cogn.* 22, 126–146. doi: 10.1521/soco.22.1.126.30986

Ditzen, B., and Heinrichs, M. (2014). Psychobiology of social support: the social dimension of stress buffering. *Restor Neurol. Neurosci.* 32, 149–162. doi: 10.3233/rnn-139008

Ditzen, B., Nater, U. M., Schaer, M., La Marca, R., Bodenmann, G., Ehlert, U., et al. (2012). Sex-specific effects of intranasal oxytocin on autonomic nervous system and emotional responses to couple conflict. *Soc. Cogn. Affect. Neurosci.* 8, 897–902. doi: 10.1093/scan/nss083

Ditzen, B., Neumann, I. D., Bodenmann, G., von Dawans, B., Turner, R. A., Ehlert, U., et al. (2007). Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women. *Psychoneuroendocrinology* 32, 565–574. doi: 10.1016/j.psyneuen.2007.03.011

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., and Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol. Psychiatry* 65, 728–731. doi: 10.1016/j.biopsych.2008.10.011

Doerr, J. M., Nater, U. M., Ehlert, U., and Ditzen, B. (2018). Co-variation of fatigue and psychobiological stress in couples' everyday life. *Psychoneuroendocrinology* 92, 135–141. doi: 10.1016/j.psyneuen.2018.01.016

Domes, G., Heinrichs, M., Michel, A., Berger, C., and Herpertz, S. C. (2007). Oxytocin improves "mind-reading" in humans. *Biol. Psychiatry* 61, 731–733. doi: 10.1016/j.biopsych.2006.07.015

Eckstein, M., Bamert, V., Stephens, S., Wallen, K., Young, L., Ehlert, U., et al. (2018a). w. *Soc. Neurosci.* doi: 10.1016/S0168-1591(02)00254-X [Epub ahead of print].

Eckstein, M., de Minas, A. C. A., Scheele, D., Kreuder, A.-K., Hurlemann, R., Grinevich, V., et al. (2018b). Oxytocin for learning calm and safety. *Int. J. Psychophysiol.* 136, 5–14. doi: 10.1016/j.ijpsycho.2018.06.004

Eckstein, M., Scheele, D., Weber, K., Stoffel-Wagner, B., Maier, W., and Hurlemann, R. (2014). Oxytocin facilitates the sensation of social stress. *Hum. Brain Mapp.* 35, 4741–4750. doi: 10.1002/hbm.22508

Engel, S., Klusmann, H., Ditzen, B., Knaevelsrud, C., and Schumacher, S. (2019). Menstrual cycle-related fluctuations in oxytocin concentrations: a systematic review and meta-analysis. *Front. Neuroendocrin.* 52, 144–155. doi: 10.1016/j.yfrne.2018.11.002

Feldman, R. (2012). Oxytocin and social affiliation in humans. *Horm. Behav.* 61, 380–391. doi: 10.1016/j.yhbeh.2012.01.008

Feldman, R. (2017). The neurobiology of human attachments. *Trends Cogn. Sci.* 21, 80–99. doi: 10.1016/j.tics.2016.11.007

Feldman, R., Monakhov, M., Pratt, M., and Ebstein, R. P. (2016). Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. *Biol. Psychiatry* 79, 174–184. doi: 10.1016/j.biopsych.2015.08.008

Ferreira, L. C., Narciso, I., Novo, R. F., and Pereira, C. R. (2014). Predicting couple satisfaction: the role of differentiation of self, sexual desire and intimacy in heterosexual individuals. *Sex Relat. Ther.* 29, 390–404. doi: 10.1080/14681994.2014.957498

Fincham, F. D., and Beach, S. R. H. (2010). Marriage in the new millennium: a decade in review. *J. Marriage Fam.* 72, 630–649. doi: 10.1111/j.1741-3737.2010.00722.x

Fischer-Shofty, M., Levkovitz, Y., and Shamay-Tsoory, S. G. (2013). Oxytocin facilitates accurate perception of competition in men and kinship in women. *Soc. Cogn. Affect. Neurosci.* 8, 313–317. doi: 10.1093/scan/nsr100

Gottman, J., and Gottman, J. (2017). The natural principles of love. *J. Fam. Theory Rev.* 9, 7–26. doi: 10.1111/jftr.12182

Grewen, K. M., Girdler, S. S., Amico, J., and Light, K. C. (2005). Effects of partner support on resting oxytocin, cortisol, norepinephrine, and blood pressure before and after warm partner contact. *Psychosom. Med.* 67, 531–538. doi: 10.1097/01.psy.0000170341.88395.47

Guzman, Y. F., Tronson, N. C., Jovasevic, V., Sato, K., Guedea, A. L., Mizukami, H., et al. (2013). Fear-enhancing effects of septal oxytocin receptors. *Nat. Neurosci.* 16, 1185–1187. doi: 10.1038/nn.3465

Hadden, B. W., Smith, C. V., and Webster, G. D. (2014). Relationship duration moderates associations between attachment and relationship quality: meta-analytic support for the temporal adult romantic attachment model. *Pers. Soc. Psychol. Rev.* 18, 42–58. doi: 10.1177/1088868313501885

Harari-Dahan, O., and Bernstein, A. (2014). A general approach-avoidance hypothesis of oxytocin: accounting for social and non-social effects of oxytocin. *Neurosci. Biobehav. Rev.* 47, 506–519. doi: 10.1016/j.neubiorev.2014.10.007

Heim, C., Young, L. J., Newport, D. J., Mletzko, T., Miller, A. H., and Nemeroff, C. B. (2008). Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Mol. Psychiatry* 14, 954–958. doi: 10.1038/mp.2008.112

Heinrichs, M., Baumgartner, T., Kirschbaum, C., and Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol. Psychiatry* 54, 1389–1398. doi: 10.1016/s0006-3223(03)00465-7

Holt-Lunstad, J., Birmingham, W., and Jones, B. Q. (2008). Is there something unique about marriage? The relative impact of marital status, relationship quality, and network social support on ambulatory blood pressure and mental health. *Ann. Behav. Med.* 35, 239–244. doi: 10.1007/s12160-008-9018-y

Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., and Stephenson, D. (2015). Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspect. Psychol. Sci.* 10, 227–237. doi: 10.1177/1745691614568352

Holt-Lunstad, J., Smith, T. B., and Layton, J. B. (2010). Social relationships and mortality risk: a meta-analytic review. *PLoS Med.* 7:e1000316. doi: 10.1371/journal.pmed.1000316

Hurlemann, R., and Scheele, D. (2016). Dissecting the role of oxytocin in the formation and loss of social relationships. *Biol. Psychiatry* 79, 185–193. doi: 10.1016/j.biopsych.2015.05.013

Insel, T. R., and Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci. U.S.A.* 89, 5981–5985. doi: 10.1073/pnas.89.13.5981

Jacob, S., Brune, C. W., Carter, C., Leventhal, B. L., Lord, C., and Cook, E. H. Jr. (2007). Association of the oxytocin receptor gene (OXTR) in caucasian children and adolescents with autism. *Neurosci. Lett.* 417, 6–9. doi: 10.1016/j.neulet.2007.02.001

Jarnecke, A. M., Barden, E., Back, S. E., Brady, K. T., and Flanagan, J. C. (2018). Intimate partner violence moderates the association between oxytocin and reactivity to dyadic conflict among couples. *Psychiatry Res.* 270, 404–411. doi: 10.1016/j.psychres.2018.10.003

Kazmierczak, M., and Blazek, M. (2015). Attachment styles as predictors of the perception of couples' cohesion. *Soc. Behav. Pers.* 43, 1055–1056. doi: 10.2224/sbp.2015.43.6.1055

Kiecolt-Glaser, J. K., and Wilson, S. J. (2017). Lovesick: how couples' relationships influence health. *Annu. Rev. Clin. Psychol.* 13, 421–443. doi: 10.1146/annurev-clinpsy-032816-045111

Kim, Y.-R., Eom, J.-S., Yang, J.-W., Kang, J., and Treasure, J. (2015). The impact of oxytocin on food intake and emotion recognition in patients with eating disorders: a double blind single dose within-subject cross-over design. *PLoS One* 10:e0137514. doi: 10.1371/journal.pone.0137514

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., and Fehr, E. (2005). Oxytocin increases trust in humans. *Nature* 435, 673–676. doi: 10.1038/nature03701

Kreuder, A. K., Scheele, D., Wassermann, L., Wollseifer, M., Stoffel-Wagner, B., Lee, M. R., et al. (2017). How the brain codes intimacy: the neurobiological substrates of romantic touch. *Hum. Brain Mapp.* 38, 4525–4534. doi: 10.1002/hbm.23679

Kreuder, A. K., Wassermann, L., Wollseifer, M., Ditzen, B., Eckstein, M., Stoffel-Wagner, B., et al. (2018). Oxytocin enhances the pain-relieving effects of social support in romantic couples. *Hum. Brain Mapp.* 40, 242–251. doi: 10.1002/hbm.24368

Krueger, F., Parasuraman, R., Iyengar, V., Thornburg, M., Weel, J., Lin, M., et al. (2012). Oxytocin receptor genetic variation promotes human trust behavior. *Front. Hum. Neurosci.* 6:4. doi: 10.3389/fnhum.2012.00004

Lane, A., Luminet, O., Nave, G., and Mikolajczak, M. (2016). Is there a publication bias in behavioural intranasal oxytocin research on humans? Opening the file drawer of one laboratory. *J. Neuroendocrinol.* 28. doi: 10.1111/jne.12384

Lemieux, R., and Hale, J. L. (2000). Intimacy, passion, and commitment among married individuals: further testing of the triangular theory of love. *Psychol. Rep.* 87, 941–948. doi: 10.2466/pr0.2000.87.3.941

Massey, S. H., Estabrook, R., O'Brien, T. C., Pine, D. S., Burns, J. L., Jacob, S., et al. (2015). Preliminary evidence for the interaction of the oxytocin receptor gene (oxtr) and face processing in differentiating prenatal smoking patterns. *Neurosci. Lett.* 584, 259–264. doi: 10.1016/j.neulet.2014.10.049

Nave, G., Camerer, C., and McCullough, M. (2015). Does oxytocin increase trust in humans? A critical review of research. *Perspect. Psychol. Sci.* 10, 772–789. doi: 10.1177/1745691615600138

Noftle, E. E., and Shaver, P. R. (2006). Attachment dimensions and the big five personality traits: associations and comparative ability to predict relationship quality. *J. Res. Pers.* 40, 179–208. doi: 10.1016/j.jrp.2004.11.003

Olson, D. H. (2011). FACES IV and the circumplex model: validation study. *J. Marital Fam. Ther.* 37, 64–80. doi: 10.1111/j.1752-0606.2009.00175.x

Robles, T. F. (2014). Marital quality and health: implications for marriage in the 21st Century. *Curr. Dir. Psychol. Sci.* 23, 427–432. doi: 10.1177/0963721414549043

Rubin, H., and Campbell, L. (2012). Day-to-day changes in intimacy predict heightened relationship passion, sexual occurrence, and sexual satisfaction: a dyadic diary analysis. *Soc. Psychol. Personal. Sci.* 3, 224–231. doi: 10.1177/1948550611416520

Scheele, D., Plota, J., Stoffel-Wagner, B., Maier, W., and Hurlemann, R. (2015). Hormonal contraceptives suppress oxytocin-induced brain reward responses to the partner's face. *Soc. Cogn. Affect. Neurosci.* 11, 767–774. doi: 10.1093/scan/nsv157

Scheele, D., Striepens, N., Güntürkün, O., Deutschländer, S., Maier, W., Kendrick, K. M., et al. (2012). Oxytocin modulates social distance between males and females. *J. Neurosci.* 32, 16074–16079. doi: 10.1523/JNEUROSCI.2755-12.2012

Scheele, D., Wille, A., Kendrick, K. M., Stoffel-Wagner, B., Becker, B., Güntürkün, O., et al. (2013). Oxytocin enhances brain reward system responses in men viewing the face of their female partner. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20308–20313. doi: 10.1073/pnas.1314190110

Schneider, I. K., Konijn, E. A., Righetti, F., and Rusbult, C. E. (2011). A healthy dose of trust: the relationship between interpersonal trust and health. *Pers. Relatsh.* 18, 668–676. doi: 10.1111/j.1475-6811.2010.01338.x

Shamay-Tsoory, S. G., and Abu-Akel, A. (2016). The social salience hypothesis of oxytocin. *Biol. Psychiatry* 79, 194–202. doi: 10.1016/j.biopsych.2015.07.020

Shamay-Tsoory, S. G., Fischer, M., Dvash, J., Harari, H., Perach-Bloom, N., and Levkovitz, Y. (2009). Intranasal administration of oxytocin increases envy and schadenfreude (gloating). *Biol. Psychiatry* 66, 864–870. doi: 10.1016/j.biopsych.2009.06.009

Stanley, S. M., Rhoades, G. K., and Whitton, S. W. (2010). Commitment: functions, formation, and the securing of romantic attachment. *J. Fam. Theory Rev.* 2, 243–257. doi: 10.1111/j.1756-2589.2010.00060.x

Sternberg, R. J. (1986). A triangular theory of love. *Psychol. Rev.* 93, 119–135. doi: 10.1037/0033-295X.93.2.119

Taylor, S. E. (2006). Tend and befriend: biobehavioral bases of affiliation under stress. *Curr. Dir. Psychol. Sci.* 15, 273–277. doi: 10.1111/j.1467-8721.2006.00451.x

Theodoridou, A., Rowe, A. C., Penton-Voak, I. S., and Rogers, P. J. (2009). Oxytocin and social perception: oxytocin increases perceived facial trustworthiness and attractiveness. *Horm. Behav.* 56, 128–132. doi: 10.1016/j.yhbeh.2009.03.019

Timmons, A. C., Baucom, B. R., Han, S. C., Perrone, L., Chaspari, T., Narayanan, S. S., et al. (2017). New frontiers in ambulatory assessment: big data methods for capturing couples' emotions, vocalizations, and physiology in daily life. *Soc. Psychol. Personal. Sci.* 8, 552–563. doi: 10.1177/1948550617709115

Tops, M., Huffmeijer, R., Linting, M., Grewen, K., Light, K., Koole, S., et al. (2013). The role of oxytocin in familiarization-habituation responses to social novelty. *Front. Psychol.* 4:761. doi: 10.3389/fpsyg.2013.00761

Valor-Segura, I., Expósito, F., Moya, M., and Kluwer, E. (2014). Don't leave me: the effect of dependency and emotions in relationship conflict. *J. Appl. Soc. Psychol.* 44, 579–587. doi: 10.1111/jasp.12250

Wallen, K., and Rupp, H. A. (2010). Women's interest in visual sexual stimuli varies with menstrual cycle phase at first exposure and predicts later interest. *Horm. Behav.* 57, 263–268. doi: 10.1016/j.yhbeh.2009.12.005

Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., et al. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biol. Psychiatry* 71, 419–426. doi: 10.1016/j.biopsych.2011.09.002

Walum, H., and Young, L. J. (2018). The neural mechanisms and circuitry of the pair bond. *Nat. Rev. Neurosci.* 19, 643–654. doi: 10.1038/s41583-018-0072-76

Watabe, M., Ban, H., and Yamamoto, H. (2011). Judgments about others' trustworthiness: an fMRI study. *Lett. Evol. Behav. Sci.* 2, 28–32. doi: 10.1016/j.psyneuen.2019.04.014

Winston, J. S., Strange, B. A., O'Doherty, J., and Dolan, R. J. (2002). Automatic and intentional brain responses during evaluation of trustworthiness of faces. *Nat Neurosci.* 5, 277–283. doi: 10.1038/nn816

Xu, X., Brown, L., Aron, A., Cao, G., Feng, T., Acevedo, B., et al. (2012). Regional brain activity during early-stage intense romantic love predicted relationship outcomes after 40 months: an fMRI assessment. *Neurosci. Lett.* 526, 33–38. doi: 10.1016/j.neulet.2012.08.004

Zak, P. J., Kurzban, R., and Matzner, W. T. (2004). The neurobiology of trust. *Ann. N. Y. Acad. Sci.* 1032, 224–227. doi: 10.1196/annals.1314.025

Zietlow, A.-L., Eckstein, M., Nonnenmacher, N., Reck, C., Schaer, M., Bodenmann, G., et al. (2018). Dyadic coping and its underlying neuroendocrine mechanisms–implications for stress regulation. *Front. Psychol.* 9:2600. doi: 10.3389/fpsyg.2018.02600

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