AFFECTIVE SCIENCES THROUGH THE CHEMICAL SENSES

EDITED BY: Géraldine Coppin, Valentina Parma and Bettina M. Pause
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AFFECTIVE SCIENCES THROUGH THE CHEMICAL SENSES

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In people's minds, smells, flavors and affective phenomena are perceived as closely linked. But is it genuinely the case? The scientific study of this question is a rapidly expanding field, both in healthy and in clinical populations. Although still under-studied in comparison to other sensory modalities, chemical senses have proven to bring unique knowledge in the understanding of affective phenomena.

In this context, this Research Topic is aimed to offer a snapshot of the present knowledge and questions raised in this field. Topics include, but are not limited to: affects elicited by odors and/or flavors in different individuals, contexts or cultures; emotional potency of odors in guiding human behavior and cognition (e.g. attention, memory formation, decisions and choices, withdrawal and approach behavior); affects communicated by body odors; affect regulation disorders and chemosensory perception. Studies on the biological underpinnings of these effects are also included.

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Table of Contents

05 ***Editorial: Affective Sciences through the Chemical Senses***
Géraldine Coppin, Valentina Parma and Bettina M. Pause

Section 1: The prominent role of chemosensory valence

08 ***The mere exposure effect depends on an odor's initial pleasantness***
Sylvain Delplanque, Géraldine Coppin, Laurène Bloesch, Isabelle Cayeux and David Sander

15 ***The smell of death: evidence that putrescine elicits threat management mechanisms***
Arnaud Wisman and Ilan Shrira

26 ***Fancy Citrus, Feel Good: Positive Judgment of Citrus Odor, but Not the Odor Itself, Is Associated with Elevated Mood during Experienced Helplessness***
Matthias Hoenen, Katharina Müller, Bettina M. Pause and Katrin T. Lübke

33 ***Sensitivity of Physiological Emotional Measures to Odors Depends on the Product and the Pleasantness Ranges Used***
Aline M. Pichon, Géraldine Coppin, Isabelle Cayeux, Christelle Porcherot, David Sander and Sylvain Delplanque

Section 2: Affective chemosensation in the context of socio-emotional communication

45 ***Chemosensory Communication of Gender Information: Masculinity Bias in Body Odor Perception and Femininity Bias Introduced by Chemosignals During Social Perception***
Smiljana Mutic, Eileen M. Moellers, Martin Wiesmann and Jessica Freiherr

56 ***Chemosensory anxiety cues moderate the experience of social exclusion – an fMRI investigation with Cyberball***
Olga A. Wudarczyk, Nils Kohn, Rene Bergs, Raquel E. Gur, Bruce Turetsky, Frank Schneider and Ute Habel

69 ***Effect of fragrance use on discrimination of individual body odor***
Caroline Allen, Jan Havlíček and S. Craig Roberts

76 ***Body Odor Based Personality Judgments: The Effect of Fragranced Cosmetics***
Agnieszka Sorokowska, Piotr Sorokowski and Jan Havlíček

Section 3: Intersection between chemosensory and affective sciences across the development and in special populations

84 ***Positive relationship between odor identification and affective responses of negatively valenced odors***
Lenka Martinec Nováková, Dagmar Plotěná, S. Craig Roberts and Jan Havlíček

93 ***Viewing Olfactory Affective Responses Through the Sniff Prism: Effect of Perceptual Dimensions and Age on Olfactomotor Responses to Odors***
Camille Ferdenzi, Arnaud Fournel, Marc Thévenet, Géraldine Coppin and Moustafa Bensafi

103 ***The inability to self-evaluate smell performance. How the vividness of mental images outweighs awareness of olfactory performance***
Kathrin Kollndorfer, Ksenia Kowalczyk, Stefanie Nell, Jacqueline Krajnik, Christian A. Mueller and Veronika Schöpf

110 ***Unilateral Resection of the Anterior Medial Temporal Lobe Impairs Odor Identification and Valence Perception***
Stephanie A. Juran, Johan N. Lundström, Michael Geigant, Eva Kumlien, Mats Fredrikson, Fredrik Åhs and Mats J. Olsson

117 ***Odor Perception in Children with Autism Spectrum Disorder and its Relationship to Food Neophobia***
Anne-Claude Luisier, Genevieve Petitpierre, Camille Ferdenzi, Annick Clerc Bérod, Agnes Giboreau, Catherine Rouby and Moustafa Bensafi

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EDITORIAL
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Editorial: Affective Sciences through the Chemical Senses

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Keywords: olfaction, gustation, emotion, valence, affective neuroscience

The Editorial on the Research Topic

Affective Sciences through the Chemical Senses

> *Whence could it have come to me, this all-powerful joy? I was conscious that it was connected with the taste of tea and cake, but that it infinitely transcended those savors, could not, indeed, be of the same nature as theirs. Whence did it come? What did it signify? How could I seize upon and define it?*
>
> *Marcel Proust, À la Recherche du Temps Perdu (Proust, 1913–1927).*

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As the Proustian madeleine anecdote so well exemplifies, smells, and flavors can play a key role in mediating affective experiences. However, the intricacies of the human affective world, encompassing autonomic, sensory, motor, cognitive, and emotional aspects (e.g., Coppin and Sander, 2016), have to date left the link between chemosensory and affective sciences rather unexplored (e.g., Coppin et al., 2010). This Research Topic represents the efforts of two research fields to converge and explore the breath of their intersecting topics through the theoretical and experimental approaches of some of the researchers currently animating the field. The present E-book, therefore, offers a snapshot of the unique role of the chemical senses in shaping human affective experiences and vice-versa, i.e., in revealing how affective states influence chemosensation.

The prominent role of valence, the designation of positive and negative affects, in framing chemosensory experiences (e.g., Delplanque et al., 2016) made this topic the focus of several investigations included in the present Research Topic. This line of research origins from the idea that odors are powerful modulators of emotional experiences (e.g., Pause et al., 2003; Adolph and Pause, 2012). Delplanque et al. revealed that the so-called mere exposure effect depends on the initial pleasantness attributed to the odors. Indeed, the pleasantness to neutral and mildly pleasant olfactory stimuli increases following repeated exposure whereas it only marginally does that for overtly unpleasant and pleasant stimuli. These findings open an interesting speculation on the potential dangers of getting to like odors that are unpleasant and perhaps toxic, simply because they are regularly presented. The dangers of unpleasant/dangerous odors have been investigated by Wisman and Shrira, who studied participants' reactions to conscious and non-conscious exposure to putrescine, a chemical produced in the decaying tissues of dead bodies. In a set of four studies they showed, that putrescine can elicit threat management mechanisms, ranging from increased

vigilance, to avoidance, and increased implicit cognitions toward escape. The importance of the valence is again emphasized by Hoenen et al. They investigated the mood effects following exposure to different smells. Although, the exposure to the odors—including the citrus odor, commonly claimed to have uplifting effects—did not prevent the negative mood to sink in, what maintained the happiness judgments elevated was the pleasantness judgment of the odor. Additionally, Pichon et al. provided evidence on the tools to evaluate subtle differences in emotional reactions to families of odors, subserving different functions. More specifically, they demonstrated that among series of odors varying in pleasantness, physiological reactions can differentiate odors from different families (e.g., fruity, animal), but not within the same family of fine fragrances.

Another set of contributions investigated affective chemosensation in the context of socio-emotional communication. Human body odors are stimuli producing important interpersonal information, able to promote adaptive effects on cognitive–affective processes (Semin and De Groot, 2013; Parma et al., 2016; Pause, 2016). One such example refers to the communication of categorical information such as gender. The work by Mutic et al. revealed that axillary odors were perceived as masculine, irrespective of the donor's effective gender; whereas a femininity bias is introduced by chemosignals during social perception. This study emphasized the need of considering gender effects in chemosensory perception and to further research the role chemosensory communication of sex and gender information play in social perception. An example of communication of transient information is provided by Wudarczyk et al. who evaluated the neurophysiological effects of chemosignals of anxiety during a Cyberball task, a socially threatening situation. Brain activations in areas linked to social rejection were blunted while smelling the anxiety body odors, suggesting a moderation effect on the social experience of exclusion. Besides the exclusive presentation of body odors, two studies investigated the interactions between body odors and fragrances. Allen et al. investigated the complementarity between fragrances and body odors and provided an indication that the choice of different fragrances is influenced by one's body odor features. In line with this evidence, Sorokowska et al. showed that body odors are relevant cues used to gather first impression judgments of certain personality traits, which can be modulated by the use of fragranced cosmetics over the natural body odor.

The previous authors have focused on the characterization of the intersection between chemosensory and affective sciences in healthy young adults. Five contributions have additionally investigated these interactions across the development as well as in special populations. Nováková et al. showed that the knowledge regarding the odors presented is critical in the chemosensory affective experience, even at a young age. In children 8–11 years old, the pleasantness of odors was modulated by the knowledge of their identity due to prior experience, but this effect was only confined to unpleasant odors. Ferdenzi et al. examined sniffing patterns in young and older healthy adults and showed that this behavior is sensitive to subtle variations in unpleasantness, even though in the elderly with lesser extent. Therefore, sniffing may have an adaptive function to protect individuals across the lifespan from inhaling harmful substances. Aging, among other causes, is associated with a reduction of olfactory ability, which can result in hyposmia or anosmia. Kollndorfer et al. investigated such populations with respect to the difference between objective (Sniffin' Sticks) and subjective olfactory performance (odor imagery) evaluation. They found a close relationship between the vividness of mental images and self-evaluation of olfactory perception abilities, suggesting that individuals subjectively did rate their olfactory performance, even when we are not able to perceive odors. To evaluate the neural underpinnings of affective chemosensory perception, Juran et al. tested patients with unilateral resection of the anterior medial temporal lobe with several olfactory tasks. Results indicated a keen role of the temporal lobe in odor identification, and of the left anterior temporal lobe in determining the emotional saliency to odors. Last, Luisier et al. compared odor perception between children with autism spectrum disorder and typically developing children. Critically, they showed that odor hedonic reactivity relates to food neophobia in children with autism spectrum disorder, which opens fascinating avenues of research at the intersection between autism, chemical senses and food intake research.

Overall, the variety of topics, techniques and populations included in the present Research Topic can be seen as proof of the widespread effects that the chemical senses play in affective sciences and vice-versa. We thank the authors for their contributions, which have highlighted the potential boundaries of this integrated field and its richness. With this Research Topic, we hold a promise toward a deeper understanding of the interactions between chemical senses and affective sciences from a psychological and neuroscientific perspective. This investigation will bring a mutual enrichment to both chemosensory and affective sciences, which—we hope—will continue to flourish in the future.

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The mere exposure effect depends on an odor's initial pleasantness

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The mere exposure phenomenon refers to improvement of one's attitude toward an a priori neutral stimulus after its repeated exposure. The extent to which such a phenomenon influences evaluation of *a priori emotional* stimuli remains under-investigated. Here we investigated this question by presenting participants with different odors varying in *a priori* pleasantness during different sessions spaced over time. Participants were requested to report each odor's pleasantness, intensity, and familiarity. As expected, participants became more familiar with all stimuli after the repetition procedure. However, while neutral and mildly pleasant odors showed an increase in pleasantness ratings, unpleasant and very pleasant odors remained unaffected. Correlational analyses revealed an inverse U-shape between the magnitude of the mere exposure effect and the initial pleasantness of the odor. Consequently, the initial pleasantness of the stimuli appears to modulate the impact of repeated exposures on an individual's attitude. These data underline the limits of mere exposure effect and are discussed in light of the biological relevance of odors for individual survival.

Keywords: mere exposure, olfaction, pleasantness, familiarity, preference

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Introduction

More than 40 years ago, Zajonc (1968) presented his seminal work showing that "*repeated, unreinforced exposures produce an enhancement in affect toward a stimulus*" (p. 1). Since then, this *mere exposure effect* has become one of the most inspiring and studied phenomena in psychology (Bornstein, 1989; Moreland and Topolinski, 2010). In the classical paradigm used to investigate the mere exposure effect, participants are presented with a series of stimuli at different exposure frequencies within a limited time window. At a certain point, they are requested to rate their preference toward the stimuli. Experimental manipulations such as stimulus type, duration, presentation frequency, and type of ratings, as well as personality and individual variables, have been extensively studied (see Bornstein, 1989, for a review). A robust phenomenon, the mere exposure effect has been replicated in hundreds of experiments using visual, auditory (Bornstein, 1989), olfactory (e.g., Prescott et al., 2008), and recently, haptic stimuli (Jakesch and Carbon, 2012). This effect has been found even when stimuli are presented subliminally (e.g., Bornstein and D'Agostino, 1992). Hence, the mere exposure effect seems to impact any situation during which one is confronted with stimulus repetitions. It is consequently thought to constitute a key element in preference acquisition (e.g., Balogh and Porter, 1986; Schaal et al., 2000).

The vast majority of data on the mere exposure effect have been collected on meaningless *neutral* visual stimuli. In Zajonc's (1968) princeps study, for example, the subjects did not usually have "*a prior preference for the stimulus exposed*" (p. 23). The extent to which exposure could influence preferences or hedonic ratings of *a priori emotional* stimuli has rarely been investigated. This is surprising, given that encountering neutral[1] stimuli could constitute the exception, rather than the norm, in daily life. Studies examining the mere exposure effect in relation to *a priori* valenced stimuli are scarce: Although they all indicate that the initial pleasantness of a stimulus is an important variable to consider, the impact of the mere exposure effect ranges from canceling out preferences to strengthening them. For instance, Schellenberg et al. (2008) did not find any differential exposure influence on pleasantness evaluation of happy and sad musical pieces. Grush (1976) suggested that *a priori* pleasant, meaningful words became more pleasant after repeated exposures whereas *a priori* unpleasant words became more unpleasant. Evidence also suggests that exposure can improve hedonic evaluations of initially disliked harmless and caged living snakes (Litvak, 1969) and can reduce the dislike of angry faces (Young and Claypool, 2010). Using a modified prisoner's dilemma, Swap (1977) reported observing more important exposure effects (i.e., increases in interpersonal reported attraction) for rewarding partners than for punishing partners.

In the olfactory domain and with correlational approaches, several authors have described an increase in the reported pleasantness of odors with their familiarity (e.g., Engen and Ross, 1973; Lawless and Cain, 1975; Ayabe-Kanamura et al., 1998; Distel et al., 1999; Royet et al., 1999; Bensafi et al., 2002; Sulmont et al., 2002). However, Delplanque et al. (2008) showed that the correlation between pleasantness and familiarity is much more important for pleasant odors than for unpleasant ones (correlations were *not* significant for malodors). Similar results were since obtained with various set of odorants across the world (Ferdenzi et al., 2013). These results suggest that malodors are resistant to pleasantness increases that could be expected from exposure. The authors underlined the adaptive advantage of unpleasant odor processing in allowing individuals to avoid, as much as possible, the influence of exposure to the odorant (i.e., increasing familiarity) in order to maintain negative attitudes toward a potentially dangerous stimulation.

Investigating the mere exposure effect with *a priori* valenced stimuli may appear to be challenging since many studies used meaningless stimuli, e.g., geometric abstract shapes that are not valenced. In visual or auditory modalities, valenced stimuli are likely to be explicitly meaningful, as they are subjected to many regulations and high-level interpretations that could influence the mere exposure effect. In a classic review of mere exposure studies, Bornstein (1989, p. 275) highlighted "*that stimulus recognition may actually inhibit the exposure effect.*" Olfactory stimuli are putative perfect candidates in that sense, since their pleasantness is thought to be the major representation of human odorant perception (Yeshurun and Sobel, 2010) and humans do not perform well in explicit odor recognition (Issanchou et al., 2002; Stevenson, 2009).

Not only are studies investigating the mere exposure effect in relation to the *a priori* valence of stimuli scarce, but they are mainly correlational, which considerably narrows their explanatory power. They cannot demonstrate that a change in familiarity, due to exposure, *causes* a change in pleasantness. Moreover, they cannot prove that those putative changes are different along the pleasantness continuum.

In an attempt to fill this gap, the aim of the present experiment was to investigate the impact of the initial pleasantness of stimulus on the mere exposure effect by directly manipulating exposure to unpleasant, neutral, and pleasant olfactory stimulations. More precisely, we implemented a familiarization procedure for six odorants that varied in pleasantness. To avoid any confound between a mere exposure effect and habituation or desensitization effects (that are known to occur rapidly in olfaction; Cain and Johnson, 1978; Comeno-Muniz and Cain, 1995), or affective habituation (Ferdenzi et al., 2014), we did not present the odorant intensively during one session. Rather, we organized six judgment sessions separated by at least 1 day. During one session, odorants were randomly presented and participants had to rate the pleasantness, the familiarity, and the intensity of each of them. Participants' ability to recognize and label odors could not only influence their familiarity and pleasantness evaluations (Seo et al., 2008), but also the mere exposure effect itself (Bornstein, 1989). In order to assess such potential confounds linked to odors recognition, we performed a free and cued odor recognition task at the end of the familiarization procedure. In sum, if unpleasant odors are more resistant to mere exposure effect, as a previous correlational study suggests (Delplanque et al., 2008; Ferdenzi et al., 2013), we expected that changes in pleasantness ratings after repeated exposures would be less important for initially unpleasant odors than for initially neutral or pleasant ones.

Materials and Methods

Participants

Forty participants (21.72 $\pm$ 2.94 years, 10 males) took part in this experiment. They were paid 20 Swiss francs for their participation. Before starting the experiment, participants completed a consent form. They all self-reported a normal sense of smell. Participants gave written informed consent, and the study was approved by the ethical committees of the Psychology Department of the University of Geneva.

Stimuli

Six odorants provided by Firmenich, S.A. were selected [isovaleric acid (cheese), skunk (feces), leather, lilac, shampoo fragrance, and strawberry] on the basis of pleasantness ratings obtained in preceding studies (Delplanque et al., 2008; Chrea et al., 2009). Solutions (6 ml) of these odorants were injected into the absorbent core of cylindrical felt-tip pens (14 cm long, inner diameter 1.3 cm), using the same concentrations as in preceding studies

[1]In the literature on classical conditioning, a neutral stimulus is one without intrinsic motivational properties that has never been conditioned with a motivationally or emotionally relevant stimulus (see Rescorla, 1967; Balleine and Killcross, 2006; Esber and Haselgrove, 2011, for reviews).

(Delplanque et al., 2008; Chrea et al., 2009). Moreover, a small sample of Firmenich employees checked the concentrations in the pens to ensure that the odors were subjectively judged as (1) well perceived without being too strong and (2) without any notable difference in perceived intensity across all odorants. The use of this highly practical system provided by Burghart (Germany) prevents contamination by the environment. An additional pen without any odorant (blank pen) was added to the selection. Each odorant was coded by a random three-digit code and these codes were changed during the experiment to avoid recall across different sessions.

Procedure

Participants completed six judgment sessions, each separated by at least 1 day (median = 3, minimum = 1, maximum = 19). Data collection lasted 5 weeks. During each session, participants smelled the seven odor pens in random order. The interval between two odorants varied from 30 to 45 s to avoid sensory adaptation. Before testing, participants were instructed on how to smell the odorants in order to minimize the intra- and inter-participant breathing pattern variability. The instructions were as follows: when the participants saw the three-digit code on the screen, they had to (1) take the corresponding pen from the display shelf; (2) uncap the pen and breathe evenly for only one sniff with the odorant pen near the nose (about 1 cm below both nostrils); (3) cap the pen, put it back on the display shelf; and (4) use the three scales (described in detail in the next section) and wait for the signal to proceed to the next trial.

Scales and Measures

In each session, participants had to complete a computer-based questionnaire. For each odorant, they were asked to judge the pleasantness, from "very unpleasant" (left side of the scale = 0) to "neutral" (middle of the scale = 300) to "very pleasant" (right side of the scale = 600); the familiarity from "not familiar at all" (left = 0) to "medium" (middle = 300) to "very familiar" (right = 600); and the subjective intensity from "not perceived" (left = 0) to "medium" (middle = 300) to "very strong" (right = 600) by placing a cursor on the continuous scale with the mouse. Participants were also informed that they could use all of the intermediate positions. At the beginning of each session, they were also asked to rate the subjective level of their hunger on a four-point scale (not at all, slightly, mildly, and strongly). At the end of the last session, they performed a free identification task during which they had to guess each odorant's name. A response was considered as correct if the participant gave the exact name of the odorant source or its synonyms (e.g., manure for feces, soap for shampoo) or the relative category (e.g., flower for lilac, cosmetic for shampoo). This was followed by a cued recognition task (similar to the Sniffin' Sticks recognition test) during which they had to find each odorant's name included in a series of three other wrong alternatives.[2]

[2]The different series of terms were (correct name in italics): Orange/Pineapple/*Strawberry*/Cassis, *Leather*/Smoke/Grass/Glue, Ham/*Cheese*/Bread/Fish, Pear/Pineapple/Prune/*Lilac*, Ammonia/Tobacco/*Feces*/Turpentine, and Chamomile/*Shampoo*/Grapefruit/Apple.

Results

Initial Ratings

At the beginning of the experiment, before any experimental exposure procedure, participants' agreement about the pleasantness of odors was high (Cronbach's alpha = 0.990; average inter-rater correlation = 0.830). The participants clearly differentiated the pleasantness of the odors [Greenhouse–Geisser corrected (G-G) repeated measures analysis of variance (ANOVA); $F(6,234) = 107.9$, $p < 0.001$, $\eta^2 = 0.73$]. Further analyses (Tukey HSD *post hoc* comparisons) revealed that all the odors were significantly different except the pair feces and cheese on the one hand and the pairs lilac/shampoo and shampoo/strawberry on the other hand (see **Figure 1A**, first session). Thus, the set of odors was composed of two unpleasant stimuli (feces and cheese), two neutral stimuli (leather and blank pen), and three pleasant stimuli (lilac, shampoo fragrance, and strawberry).

Familiarity ratings were also different across odors [$F(6,234) = 21.8$, $p < 0.001$, $\eta^2 = 0.35$], and subsequent *post hoc* analyses revealed two groups of odors. A group of similarly highly familiar odors, composed of lilac, strawberry, and shampoo, was distinguished from another group of less familiar but similar odors, composed of cheese, feces, leather, and the blank pen.

Odor intensities were also evaluated differentially [$F(6,234) = 74.86$, $p < 0.001$, $\eta^2 = 0.65$; see **Figure 1B**, first session]. The blank pen was significantly evaluated as less intense than all the other odors (*post hoc* Tukey HSD), as was the leather odor, except in comparison with lilac. Finally, strawberry was evaluated as significantly more intense than lilac.

To examine whether our odor sample was characterized by the classical positive correlation between familiarity and pleasantness, we examined the relationship between the subjective variables (Pleasantness, Familiarity, and Intensity) assessed during the first session. There was a linear and positive correlation between the pleasantness and the familiarity of the odors (Pearson $r = 0.86$, $p < 0.05$). However, the quadratic regression was also significant and the regression coefficient was more important [$r = 0.93$, $F(2,4) = 14.7$, $p < 0.05$], highlighting the weakness of the pleasantness–familiarity relationship for unpleasant odors, the correlation being reinforced as the pleasantness increased. We did not find any other significant linear or quadratic relations between the subjective measures.

Influence of Exposure on Familiarity Evaluation

To test the effectiveness of our paradigm in inducing the expected increase in evaluation of the familiarity of odors after exposure, we conducted a G-G repeated measures ANOVA with Odor (six levels) and Session (two levels) on familiarity ratings obtained in the first and sixth sessions. The main effect of Session was significant [$F(1,39) = 7.75$, $p < 0.001$, $\eta^2 = 0.24$], showing an increase in familiarity ratings between the two sessions (see **Figure 1C**). Neither the main effect of Odor nor the interaction reached significance. Thus, the procedure induced familiarization for all odors, i.e., an increase in familiarity ratings between the first and the last sessions.

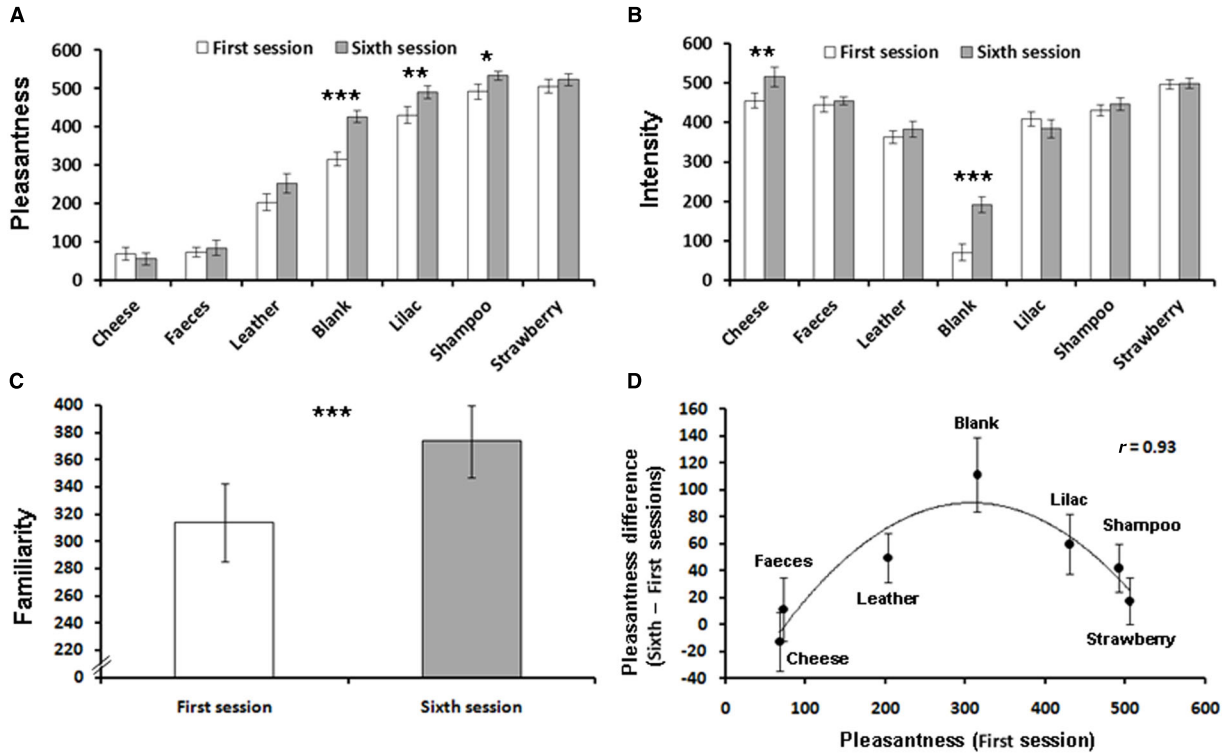

FIGURE 1 | (A) Mean pleasantness ratings for the first and sixth sessions for each odor. **(B)** Mean intensity ratings for the first and sixth sessions for each odor. **(C)** Mean odor familiarity ratings for the first and sixth sessions. **(D)** Mean pleasantness difference (sixth−first session) for each odor as a function of its initial pleasantness (first session).*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; error bars represent standard error of the mean; minimum/maximum for all scales = 0/600.

Influence of Exposure on Pleasantness Evaluation

Participants' agreement about odor pleasantness was still high after repeated exposures to odors (Cronbach's alpha = 0.993; average inter-rater correlation = 0.878). A G-G corrected repeated measures ANOVA with Odor (six levels) and Session (two levels) was performed on the pleasantness ratings obtained in the first and sixth sessions. A significant Odor × Session interaction was observed [$F(6,234) = 3.6$, $p < 0.001$, $\eta^2 = 0.08$]. ANOVAs performed for each odor revealed a marginally significant increase in pleasantness for leather [$F(1,39) = 3.22$, $p = 0.08$] and significant increases in pleasantness for the blank pen, lilac, and shampoo odors [$F_s(1,39) = 25.45, 7.2, 5.47$; $p_s < 0.001, 0.01, 0.05$; $\eta^2{}_s = 0.39, 0.15, 0.12$, respectively; see **Figure 1A**]. Thus, pleasantness representation was affected by repeated exposures, a significant increase in pleasantness with familiarization being observed only for neutral/mildly pleasant odors, but not for unpleasant or very pleasant odors.

Regression analyses were also conducted on the difference of pleasantness ratings between the sixth and the first sessions related to the pleasantness ratings of the first session. We observed a strong and significant quadratic regression [$r = 0.93$, $F(2,4) = 12.6$, $p < 0.05$; see **Figure 1D**] that remained significant when the blank pen was removed [$r = 0.93$, $F(2,3) = 10.59$, $p < 0.05$], revealing an inverse U-shape relation between pleasantness increase caused by exposure and initial pleasantness of the odor.

Influence of Exposure on Intensity Evaluation

The G-G corrected repeated measures ANOVA with Odor (six levels) and Session (two levels) performed on the intensity ratings obtained in the first and sixth sessions revealed a significant Odor × Session interaction [$F(6,234) = 6.98$, $p < 0.001$, $\eta^2 = 0.13$]. ANOVAs performed for each odor revealed significant increases in intensity for the blank pen and cheese odor [$F_s(1,39) = 19, 9.45$; $p_s < 0.001, 0.01$; $\eta^2{}_s = 0.33, 0.19$, respectively; see **Figure 1B**]. The linear correlation conducted on the difference of pleasantness and the intensity ratings between the sixth and the first sessions was not significant. This result renders the influence of intensity changes on the observed pleasantness changes due to exposure very unlikely.

Identification Scores and Hunger Level

The percentage of correct identification obtained during the free identification task was globally low (38%) but differed across odorants [Cochran Q Test, $Q(5) = 44.18$, $p < 0.001$], increasing from cheese (12.5%) to feces and lilac (27.5%), leather (32.5%), shampoo (60%), and strawberry (67.5%). Percentages obtained

in the cued recognition task were high, varying from 80 to 97% (mean = 88%) of correct responses, but were not significantly different across odorants. The mean reported hunger state was low (0.83), varying from 0.65 to 1.15, and did not significantly differ across sessions.

Discussion

In this study, we aimed to investigate the impact of the initial pleasantness of olfactory stimuli on the mere exposure effect. More precisely, odorants varying in pleasantness were presented once during six judgment sessions separated by at least 1 day to avoid any confound between a mere exposure effect and habituation or desensitization effects. This exposure procedure induced an increase in familiarity for all odors, confirming its efficiency. As expected, change in familiarity, due to exposure, caused changes in pleasantness. In particular, neutral and mildly pleasant odors were evaluated as more pleasant after the exposures than during the first session. However, these changes in pleasantness were not observed for odors that were initially unpleasant or very pleasant. The observed pattern of results is unlikely to be due to peripheral habituation since each odor was smelled only once during a particular session, and each session was separated from another by at least 1 day. In the same vein, it is unlikely that affective habituation played a role here, as intensive exposure to initially pleasant odors has been shown to reduce their pleasantness, whereas intensive exposure to initially unpleasant odors increases their pleasantness (Cain and Johnson, 1978), a pattern inconsistent with the one obtained in this study. The present data suggest that mere exposure effect is predominantly observed when initial odor evaluations are not strongly polarized on the pleasantness continuum.

As hypothesized, malodor evaluations were more resistant to the influence of repeated exposures. This result is consistent with the absence of a correlation between pleasantness and familiarity for malodors observed in correlational studies (Delplanque et al., 2008). From a functional perspective, it seems adaptive for malodor processing to allow individuals to avoid, as much as possible, the influence of exposure in order to maintain negative attitudes toward a potentially dangerous stimulation. By contrast, pleasantness evaluation of *a priori* neutral/mildly pleasant odors was affected by repeated exposures, which led to an enhancement in affect toward them. This last result constitutes the typical mere exposure effect as first described by Zajonc (1968). The gain in pleasantness due to exposures could favor approach behaviors to explore and gain information from potentially beneficial situations. The most important influence was observed for the pure neutral stimulus, i.e., the pen without odor. It is unlikely that this point has biased the whole pattern of results, since the quadratic regression conducted without this stimulus was still significant, showing that the inverse U-shape we observed was not due to this particular stimulus. This example likely better reflects that the mere exposure effect is optimally obtained for neutral stimuli.

The unexpected result of this experiment was that the most *a priori* pleasant odor's hedonic evaluation was not affected by repeated exposures. Even though this result was observed only for this most *a priori* pleasant odor (i.e., strawberry aroma), the regression analysis showed that the gain in pleasantness due to exposures weakened as pleasantness increased. This result means that less enhancement of preference occurs with exposures to an *a priori* pleasant stimulus than with an *a priori* neutral stimulus. One can wonder whether this result could be due to a rating bias, the initial pleasantness being already too high and reaching a ceiling that prevented further increases in pleasantness ratings with repeated exposures. However, the remaining space available on the scale was, on average, very close (94.8/600) to the largest pleasantness changes due to exposures (111.1/600) that was obtained for the blank pen. There was consequently potential space for increased evaluation. A more plausible explanation would be that pleasant odors are spontaneously better identified, this recognition decreasing the magnitude of the mere exposure effect as is thought to be the case with other modalities (Bornstein, 1989). A supplementary correlational analysis performed on our data revealed a significant positive linear increase in recognition success with pleasantness (Pearson $r = 0.86$, $p < 0.05$). Alternatively, when the pleasantness is initially very meaningful, there is less room for further learning and change, as the consequences of being exposed to the pleasant stimuli are well known and need no further adaptation. Thus the mechanism of increasing pleasantness to favor an approach is no longer beneficial. This interpretation could explain why there is a positive correlation between familiarity and pleasantness for *a priori* pleasant odors, as observed in correlational studies, together with the fact that pleasantness will not be further reinforced for most pleasant odors with repeated exposure, as demonstrated in our study.

The typical proposed mechanism underlying the mere exposure effect is that previous exposures to a stimulus enhance its perceptual fluency, making it more prototypical and familiar. Greater fluency then automatically generates a more positive affect that modifies pleasantness evaluation. This fluency explanation has received much experimental support in other sensory modalities (see Moreland and Topolinski, 2010, for a discussion on this topic). Sulmont et al. (2002) brought forward elements in favor of this idea in the olfactory domain by reporting that the more familiar and pleasant the odors, the simpler they are perceived by participants, whereas the number of perceived notes remained relatively independent of familiarity, suggesting that simplicity is not related to physical complexity. In this framework, our results suggest that only odors that are not *a priori* too polarized on the pleasantness continuum benefit from this fluency effect. One could speculate that this fluency gain would be inhibited for malodors, whereas fluency would reach a plateau and not be further enhanced when odors are highly pleasant.

The study of the underlying processes of the mere exposure effect has recently benefited from a new line of research based on the incorporation of the embodiment concepts in the fluency hypothesis (e.g., Moreland and Topolinski, 2010). According to this embodied fluency hypothesis, not only would the perceptual representation of a stimulus become more fluent due to repeated exposures, but so too would the stimulus-related sensorimotor

simulations (Beilock and Holt, 2007; Topolinski and Strack, 2009, 2010), since embodiment theories postulate that the stimuli representations include the sensorimotor responses associated with those stimuli (e.g., Niedenthal et al., 2005, 2009; Semin and Smith, 2008). Sniffing patterns reflecting odor pleasantness (Bensafi et al., 2003), a new line of research could investigate whether changes in the pleasantness of odors with repeated exposures are related to a specific breathing pattern (e.g., Ferdenzi et al., 2014).

In sum, this study demonstrates that mere exposure effect optimally hold for neutral and mildly pleasant olfactory stimuli and are dramatically reduced for either unpleasant or pleasant stimuli. Although this result remains to be confirmed for other sensory modalities, it suggests that mere exposure does not similarly impact all situations during which one is confronted with stimulus repetitions: Initially unbearable or exquisite events will continue to be so.

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The smell of death: evidence that putrescine elicits threat management mechanisms

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The ability to detect and respond to chemosensory threat cues in the environment plays a vital role in survival across species. However, little is known about which chemical compounds can act as olfactory threat signals in humans. We hypothesized that brief exposure to putrescine, a chemical compound produced by the breakdown of fatty acids in the decaying tissue of dead bodies, can function as a chemosensory warning signal, activating threat management responses (e.g., heightened alertness, fight-or-flight responses). This hypothesis was tested by gaging people's responses to conscious and non-conscious exposure to putrescine. In Experiment 1, putrescine increased vigilance, as measured by a reaction time task. In Experiments 2 and 3, brief exposure to putrescine (vs. ammonia and a scentless control condition) prompted participants to walk away faster from the exposure site. Experiment 3 also showed that putrescine elicited implicit cognitions related to escape and threat. Experiment 4 found that exposure to putrescine, presented here below the threshold of conscious awareness, increased hostility toward an out-group member. Together, the results are the first to indicate that humans can process putrescine as a warning signal that mobilizes protective responses to deal with relevant threats. The implications of these results are briefly discussed.

Keywords: olfaction, putrescine, threat, threat management, chemosensory cue

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Introduction

When animals die they release an unpleasant smell. A pungent component of this scent is emitted by putrescine, a volatile diamine that results from the breakdown of fatty acids in the putrefying tissue of dead bodies (Hussain et al., 2013). Interestingly, animal research shows that putrescine can function as a powerful chemosensory signal that prompts the perceiver to leave or avoid the area (Yao et al., 2009; Prounis and Shields, 2013). The aim of the present research is to show that humans respond in a similar way to putrescine, and more generally, that exposure to putrescine triggers threat management behaviors (Blanchard et al., 2001; Neuberg et al., 2011).

A growing body of research suggests that humans can identify threats via chemosignals (Chen and Haviland-Jones, 2000; Ackerl et al., 2002; Prehn et al., 2006; Mujica-Parodi et al., 2009; Zhou and Chen, 2009; de Groot et al., 2012). For instance, when people are exposed to sweat taken from donors during a fearful experience, perceivers show a heightened startle reflex (Prehn et al., 2006; Pause et al., 2009) and interpret ambiguous facial expressions as fearful (Zhou and Chen, 2009). This transmission of threat-arousing chemosignals is assumed to serve an adaptive function by orienting us to impending dangers. Indeed, the ability to detect and process chemosensory threat cues is vital for the survival of a wide range of species (Stevenson, 2010). However, thus far there is little evidence

that humans can, like other organisms, detect olfactory threat cues in the environment through means other than the chemosignals (e.g., body sweat) of conspecifics.

The decay of tissue and its resulting scent can function as a "necromone" cue that signals an animal's death to conspecifics. Alarm and avoidance behaviors (necrophobic behaviors) in response to these scents are widespread in the animal kingdom and thought to have evolved at least 420 million years ago (Yao et al., 2009). In fact, recent research shows that necrophobic behavior may have innate underpinnings through the activation of trace amine-associated receptors (TAARs), a group of specialized scent receptors in the olfactory epithelium (Hussain et al., 2013; Horowitz et al., 2014; Li and Liberles, 2015). TAARs are known to detect specific chemicals that evoke behavioral responses, without the need for prior exposure to the scents. For example, in model vertebrates, certain TAARs respond to diamines (e.g., putrescine) by producing avoidant behaviors that likely serve to defend against immediate dangers (Yoon et al., 2015). Thus, it is feasible that we have a chemosensory sensitivity to diamines like putrescine (Li and Liberles, 2015), given that their detection can aid survival (Stevenson, 2010).

A further advantage of examining putrescine as a threat stimulus is that we know what it is. Despite the impressive amount of indirect support for human chemosignals amassed in recent years, their chemical properties have yet to be identified (Wyatt, 2009). Focusing on a known compound, putrescine, enables us to directly test whether it plays a causal role in human threat responses. In a similar vein, although several studies have shown that chemosensory cues can elicit greater readiness for behavior (Bradley et al., 2001; Prehn et al., 2006), thus far there is little direct evidence that a specific chemical substance can cause overt behavioral changes in humans (Wysocki and Preti, 2004). Since exposure to putrescine elicits specific behaviors in animals (e.g., escape, avoidance), we can examine whether putrescine produces similar behaviors in humans. In sum, putrescine appears to be well-suited to test as a specific chemical compound that can act as a threat signal in humans.

Chemosensory cues can convey danger in at least two fitness-relevant domains: microbial and predator threats (Stevenson, 2010). First, olfactory information is often central to identifying the presence of pathogens. For example, pathogens can alter the scent of those who become infected, which can be detected by conspecifics (Arakawa et al., 2010; Tybur et al., 2011; Olsson et al., 2014). Similarly, the release of putrescine in decaying tissue co-occurs with the arrival of bacteria, a motivation for others to eschew physical contact with the dead body. A number of species exhibit necrophobic behaviors, and after detecting the scent emanating from dead bodies, usually respond by leaving or avoiding the area (Prounis and Shields, 2013). Second, putrescine released by decaying bodies can signal the risk of predation (Boissy et al., 1998). Since a large proportion of deaths in the wild are the result of predator attacks, putrescine would be a useful alarm cue to stay away (Misslin, 2003).

In humans, responses to specific scents can develop through learned associations between odors and personal experiences (Stevenson et al., 1998; Degel et al., 2001). For example, based on the cultural expression that when "something smells fishy" it is viewed suspiciously, exposure to fish-like odors arouses suspicion toward others and reduces cooperation, an orientation that is assumed to result from conditioned reactions to this scent (Lee and Schwarz, 2012). Likewise people may learn to associate the smell of putrescine with threats, and it is plausible that occasional exposure to putrescine, whenever it occurs, could lead to conditioned threat responses (Stevenson, 2010). However, we render it unlikely that modern humans have strong conscious associations with the scent of putrescine. Moreover, conscious scent evaluations are often inaccurate, context dependent, and colored by other sensory modalities (Sela and Sobel, 2010). In view of this, it is important to note that responses to aversive chemosensory cues do not require prior learning or conscious evaluation (Dielenberg et al., 2001; Miller and Maner, 2010; Li et al., 2007). Indeed, scents can alter our perception, cognition, behavior, and physiology (e.g., heart rate, skin conductance) even when there is no conscious scent detection (Li et al., 2007; Pause et al., 2009; Sela and Sobel, 2010; Krusemark and Li, 2012), and even after olfactory adaptation has set in (de Groot et al., 2012; Smeets and Dijksterhuis, 2014). Thus, neither prior associations with olfactory signals, nor conscious processing, are necessary conditions for people to process them as threatening (Köster et al., 2002; Williams et al., 2006; Sela and Sobel, 2010; Pause, 2012; Smeets and Dijksterhuis, 2014).

At the most basic level, threat detection increases vigilance and sharpens our reactions to events in the environment (Williams et al., 2006). For instance, detection of a predator's scent will interrupt foraging and increase behaviors (e.g., scanning the environment) that facilitate predator detection (Woody and Szechtman, 2011). Once the threat management system is engaged, it produces readiness for fight-or-flight behaviors (Cannon, 1927; Blanchard et al., 1986; Gray and McNaughton, 2003; Mobbs et al., 2009). Flight responses seek to escape the situation, whereas fight responses—whether physical or verbal aggression—are typically only used when escape is not possible. In contrast to popular belief that the dominant response to threats is to fight, flight is actually far more common (Misslin, 2003), presumably because nature selects more strongly for strategies that minimize risk. In one study, for example, when people were confronted by a threatening out-group member, they responded with aggressive readiness (fight), but only when there was little possibility of escaping; when given the option, though, participants chose to distance themselves (flight) from the other person (Cesario et al., 2010).

Overview and Hypotheses

Coming full circle, we propose that putrescine can serve as a (non-conscious) signal that initiates threat management responses. Specifically, we hypothesize that brief exposure to putrescine increases vigilance, followed by the readiness to either escape (flight), or engage in aggressive readiness (fight) when escape is not possible. Experiment 1 assessed whether putrescine (vs. ammonia and a neutral scent) increased vigilance as measured by faster responses in a

simple reaction time task. Experiments 2 and 3 assessed whether brief exposure to putrescine (vs. ammonia and neutral scent) caused participants to walk away faster from the exposure site after completing the experiment (outdoors). Experiment 3 also tested whether putrescine evoked cognitions related to escape and threat. Finally, Experiment 4 examined whether non-conscious exposure to putrescine increased aggressive readiness (e.g., defensiveness toward an out-group member). All four experiments adhered to the Declaration of Helsinki guidelines, and gained the prior approval by the University Research Ethics Committee. Written consent was obtained from all participants involved in these experiments, and all were fully debriefed.

Experiment 1: The Effect of Putrescine on Vigilance

In Experiment 1, we tested whether brief exposure to putrescine increased vigilance. To measure vigilance, we employed a task closely modeled after the shortened version of the psychomotor vigilance task (PVT; Dinges and Powell, 1985) that assessed participants' reaction times to a red dot that was presented at random intervals on a computer screen.

In addition, Experiment 1 was designed to determine whether ammonia served as an appropriate aversive control condition. Our pilot testing revealed that ammonia, unlike other aversive scents we had examined (i.e., skatole[1] and indole), was rated similarly to putrescine on repugnance, familiarity, and intensity. Moreover, previous research has used ammonia (NH_3; ammonium hydroxide) as an aversive scent prime (Rieser et al., 1976; Wise et al., 2005) and ammonia can increase trigeminal nerve activation associated with vigilance and sensory rejection, via activation of the sympathetic nervous system (Hummel and Kobal, 1992; Sekizawa and Tsubone, 1994). However, some research suggests that unpleasant ambient odors can also decrease reaction times on simple tasks like the PVT (Millot et al., 2002). In view of this, we made no specific prediction about whether ammonia, like putrescine, would enhance vigilance relative to our scentless control condition.

Method

Participants and Procedure

A sample of 60 participants (43 females; $M_{age} = 21.20$, SD = 3.20) completed the study in return for a financial incentive of 3£ (approximately $5).

Participants were randomly assigned to one of three conditions: putrescine ($C_4H_{12}N_2$; Sigma-Aldrich), ammonia (5%; NH_3; Sigma-Aldrich), or water. One hour before the start of the experiment, cotton wool pads were blotted with 2 ml of one of the three compounds, and stored separately in small (100 ml) sealable amber jars. Participants were run in our lab individually, and seated in different cubicles to avoid carryover effects of scents. The refreshment rate in each cubicle was 4–5 air changes (cycles) per hour. Furthermore, participants were booked at least 30 min apart in order to ventilate the rooms—by opening the lab room's window—between sessions. When preparing materials for the experiment, one of the researchers marked the bottom of each jar with a number code, so that the experimenters were unaware of the meaning of these codes. This basic procedure was repeated in our subsequent experiments to keep the experimenters blind to the conditions.

Participants were seated in front of a standard PC (equipped with Authorware 7.1 software) with a 17-inch screen. They were given instructions (on-screen) to open the jar, sniff the scent inside for 10 s, and close the jar. After that, they rated the scent on its intensity ("This scent is intense"; 1 = *strongly disagree* and 9 = *strongly agree*), repugnance ("This scent is repugnant"; 1 = *strongly disagree* and 9 = *strongly agree*), and familiarity ("This scent is familiar"; 1 = *strongly disagree* and 9 = *strongly agree*). Repugnance was included as evaluative rating (alongside the standard measures of intensity and familiarity) because repugnance (or disgust) is often a central component of aversive scents. Participants were then introduced to the adapted PVT, which lasted about 5 min (see Loh et al., 2004). The task instructed them to click on a red dot as quickly as possible whenever they saw the dot on the screen. Ten dots (each measuring 1 cm) were shown at different locations on the screen, and the time between appearances was randomized at variable intervals (2–45 s). As soon as participants clicked on the red dot with the mouse, a screen appeared for 5 s with the message: "prepare for next trial." Participants received two practice trials first, to get them familiar with the main task of 10 trials. Finally, after completing the PVT and filling out a standard demographic questionnaire, they were fully debriefed and thanked for their participation.

TABLE 1 | Hedonic evaluations of putrescine, ammonia, indole, "fart spray," and skatole[1] (Pilot study).

Scent primes	Putrescine	Ammonia	Indole	Skatole	Fart spray
Intensity[2]					
M	5.98_b	6.60_b	5.25_a	7.23_c	5.52_b
SD	2.50	2.46	2.15	2.08	2.07
Familiarity					
M	4.98_a	5.10_a	6.88_b	5.21_a	4.90_a
SD	2.71	2.95	2.46	2.56	2.69
Repugnance					
M	5.94_b	5.94_b	3.65_a	6.54_b	5.31_b
SD	2.65	2.55	1.78	2.94	2.63
Positivity					
M	2.63_b	2.69_b	3.81_a	2.50_b	2.67_b
SD	1.55	1.78	2.05	1.87	1.77
N	48	48	48	48	48

[1] *"How intense is this scent?", 1 Not at all and 10 Very much; "How familiar is this scent?", 1 Not at all and 10 Very much; "How repugnant is this scent?", 1 Not at all and 10 Very much; "How positive does this scent make you feel?", 1 Not at all and 10 Very much.*
[2] *Different subscripts on a hedonic dimension (within a row) indicate a significant difference of $p < 0.05$.*

[1]In line with previous research (Wheatley and Haidt, 2005), we pilot-tested a so-called "fart spray" along with skatole, indole, and ammonia, for suitability as an aversive control condition. These ratings are presented in **Table 1**. As can be seen, ammonia and fart spray were rated similarly to putrescine on all three dimensions of repugnance, familiarity, and intensity, whereas indole and skatole diverged from putrescine on at least one dimension. A disadvantage of fart spray, however, is that we could not ascertain its precise chemical compounds—its manufacturers were reluctant to disclose this information.

TABLE 2 | Scent ratings for the chemosensory primes (Experiment 1).

Chemosensory primes	Neutral	Ammonia	Putrescine
Intensity			
M	3.30	4.73	4.27
SD	1.81	1.45	1.92
Familiarity			
M	6.00	5.10	4.40
SD	0.86	2.25	1.60
Repugnance			
M	2.35	5.90	5.65
SD	1.46	1.34	1.23
N	20	20	20

Results and Discussion

Hedonic Evaluations

We began by testing our prediction, based on our pilot testing, that putrescine and ammonia would not differ from each other on repugnance, familiarity and intensity. As predicted, separate one-way between-subjects ANOVAs revealed that there was no significant difference between ammonia and putrescine on repugnance, $F(1,38) = 0.38$, $p = 0.54$, $\eta^2 = 0.01$, familiarity, $F(1,38) = 0.26$, $p = 0.26$, $\eta^2 = 0.03$, or intensity, $F(1,38) = 0.14$, $p = 0.71$, $\eta^2 = 0.004$ (see **Table 2**, for descriptive statistics). Moreover, the analyses reported below were not altered when entering all hedonic evaluations as covariates.

Reaction Times

We examined our main prediction that putrescine, relative to the neutral control condition (water), would elicit faster reaction times. In line with previous PVT research, we applied reciprocal transformation to the raw data (i.e., 1/RT). This type of transformation is standard within the PVT paradigm, as it reduces the impact of extreme scores and brings them into an acceptable range (Dinges et al., 1987; Dorrian et al., 2004). A one-way between-subjects ANOVA revealed a difference between the scent conditions, $F(2,57) = 4.32$, $p = 0.018$, $\eta^2 = 0.13$. *Post hoc* comparisons, with the raw means reported here, showed that putrescine produced faster reaction times ($M = 1.04$, SD = 0.10) than the neutral scent ($M = 1.24$, SD = 0.35; $p = 0.013$), but not compared to ammonia ($M = 1.12$, SD = 0.20; $p = 0.28$). No difference was found between the neutral and ammonia conditions ($p = 0.14$).

In sum, only putrescine caused participants to react more quickly compared to the neutral condition, supporting our hypothesis that putrescine increases vigilance. At the same time, ammonia did not increase vigilance relative to the scentless control condition. Importantly, the findings show that, consistent with our pilot study, ammonia and putrescine were evaluated similarly on repugnance, familiarity, and intensity, and were similar in the degree of vigilance they elicited. Consequently, together with previous research (Rieser et al., 1976; Wise et al., 2005), Experiment 1 indicated that ammonia would serve as an appropriate aversive control condition. Experiments 2 and 3 investigated our hypothesis that putrescine activates the motivation to escape the situation (flight).

Experiment 2: The Effect of Putrescine on Escape Behavior

Similar to Experiment 1, Experiment 2 first asked participants to rate a scent prime (putrescine vs. ammonia vs. neutral) on three dimensions: intensity, familiarity, and repugnance, then we observed whether it influenced the tendency to escape the situation. To avoid the biases associated with some operationalizations of flight in prior research (e.g., self-reported intentions, Gilbert and Gilbert, 2003), we employed an overt behavioral measure of escape (e.g., Ellsworth et al., 1972; Wisman and Koole, 2003). Specifically, we assessed whether putrescine would cause participants (who were under the impression the study was finished) to walk away more quickly over a predetermined distance of 80 m.

Method

Participants and Procedure

Forty-five participants (21 females and 24 males; $M_{age} = 27.51$, SD = 9.72) completed the study on campus. We filled three empty felt-tip pens, each with one of the three compounds (putrescine, ammonia, or water). To fill each pen, 10 ml of liquid odor was injected onto the pen's fiber rod inside the pen. The pens were then re-assembled and left to stand upside down for 24 h in order to allow the liquid to soak into the fiber rod. Just before the start of the experiment, scent blotters were marked with the scent marker pens and stored in separate sealable containers.

Participants were approached on a fixed spot on the campus and asked if they had time to participate in a brief scent test of approximately 10 min. Participants were tested individually and randomly assigned to one of three conditions (putrescine, ammonia, or water). The experimenter, blind to the conditions, presented one of the three containers to the participant, who rated the scent on intensity ("This scent is strong"; 1 = *strongly disagree* and 5 = *strongly agree*), repugnance ("This scent is repugnant"; 1 = *strongly disagree* and 5 = *strongly agree*), and familiarity ("This scent is familiar"; 1 = *strongly disagree* and 5 = *strongly agree*). After finishing and being thanked for their participation, a second experimenter—blind to the condition and hypotheses of the experiment, and out of sight of the participants—used a standard stopwatch to time how many seconds it took participants to walk away over a distance of 80 m (pre-measured before the experiment began). The recorded time constituted our dependent variable. After they reached this distance, participants were re-approached, fully debriefed and thanked again.

Results and Discussion

Hedonic Evaluations

Consistent with Experiment 1, separate one-way between-subjects ANOVAs revealed that there was no significant difference between ammonia and putrescine on repugnance, $F(1,28) = 2.30$, $p = 0.14$, $\eta^2 = 0.07$, and familiarity, $F(1,28) = 0.04$, $p = 0.75$, $\eta^2 = 0.01$. However, ammonia was rated as relatively more intense ($M = 4.73$; SD = 0.46) compared to putrescine ($M = 4.27$; SD = 0.70; $p = 0.04$; see **Table 3**). Once again, the results

TABLE 3 | Scent ratings for the chemosensory primes (Experiment 2).

Chemosensory primes	Neutral	Ammonia	Putrescine
Intensity			
M	1.53	4.73	4.27
SD	0.64	0.46	0.70
Familiarity			
M	4.75	1.60	1.67
SD	0.46	0.51	0.62
Repugnance			
M	1.73	4.47	4.80
SD	0.70	0.74	0.41
N	15	15	15

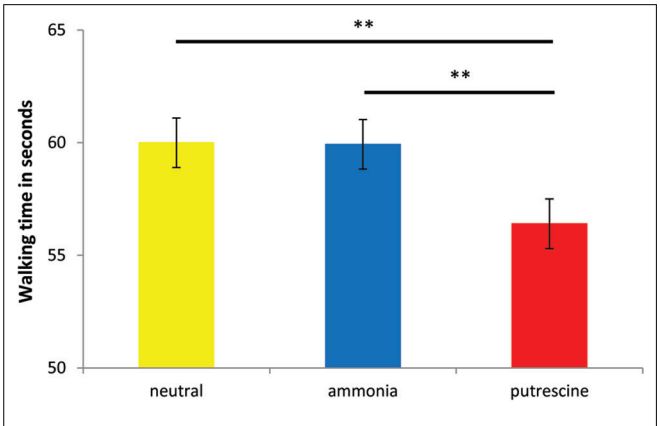

FIGURE 1 | The number of seconds it took participants to walk 80 m after exposure to the scent prime (Experiment 2). Asterisks denote that two groups differ at $^{**}p < 0.005$.

reported below were not altered when we entered the intensity (nor the other hedonic) ratings into the analyses as covariates. We also note that the results were similar whether participants rated how "intense" or "strong" the scent smelled (see Experiment 3 below).

Escape Behavior

To test our hypothesis that putrescine elicited an escape motivation, we compared our scent conditions in a one-way ANOVA, using gender as a covariate[2]. The results yielded a significant effect of the scent prime on the time it took to walk 80 m, $F(2,41) = 19.03$, $p < 0.001$, $\eta^2 = 0.48$. The only significant differences occurred between putrescine ($M = 56.40$ s; SD = 4.19) and ammonia ($M = 59.93$, SD = 5.04), and between putrescine and the neutral scent prime ($M = 60.00$, SD = 4.42; both ps < 0.005; see **Figure 1**). Thus, putrescine caused participants to walk away more quickly, supporting our assumption that putrescine evoked a stronger motivation to escape. Experiment 3 was conducted to replicate this finding, and furthermore to test whether putrescine elicited implicit cognitions related to escape and threat.

[2]Because previous research has shown that men and women tend to walk at different speeds (Chumanov et al., 2008), the results of Experiments 2 and 3 included gender as a covariate. In addition, we analyzed the results of Experiments 2 and 3 with gender as a separate factor and this did not alter the significance of the results.

Experiment 3: The Effect of Putrescine on Escape Behavior and Thoughts

The procedure for Experiment 3 was similar to Experiment 2's. First, we asked participants to evaluate the scents on the different dimensions (repugnance, familiarity, intensity). In addition, we gaged participants' implicit threat-related associations using a word stem-completion task. Specifically, this task measured the implicit accessibility of thoughts related to "escape" and "threat." We predicted that only putrescine would increase the accessibility of these cognitions. Finally, we assessed whether putrescine would cause participants to walk away more quickly over a predetermined distance of 60 m.

Method

Participants and Procedure

Sixty participants (32 females and 28 males, $M_{age} = 21.57$, SD = 1.12) completed the study on campus. Individuals were approached just outside campus on a path sloping downhill and asked if they had time to participate in a brief scent test for about 15 min.

Participants were randomly assigned to one of the three scent conditions, then they rated the scent on intensity, repugnance, and familiarity ("This scent is intense"; 1 = *strongly disagree* and 9 = *strongly agree*), repugnance ("This scent is repugnant"; 1 = *strongly disagree* and 9 = *strongly agree*), and familiarity ("This scent is familiar"; 1 = *strongly disagree* and 9 = *strongly agree*). Then, to assess cognitions relevant to the concepts of "escape" and "threat," participants completed the word-stem completion task, a widely used and well-established measurement that gaged the thought accessibility of these two concepts (Greenberg et al., 1994; Arndt et al., 1997; Lozito and Mulligan, 2010; Migo et al., 2010). Participants were asked to complete 30 word fragments, 20 of which were neutral (e.g., B_ NK could be BANK or BUNK) in terms of any particular theme, five of which could be words related to "escape" (e.g., the fragment RU_ could be completed as RUN or RUB, the latter a neutral word), and another five could be completed with a word related to "threat" (e.g., _ _ RROR could be TERROR or MIRROR). We summed the number of escape- ($M = 2.73$, SD = 1.07) and threat-related words ($M = 1.90$, SD = 0.66) that participants completed to assess the thought accessibility of these concepts. Finally, participants were again timed by a second experimenter, who was blind to the conditions and the hypotheses, for how long it took them to walk away over a distance of 60 m (due to natural constraints a slightly shorter distance was used than in Experiment 2).

Results and Discussion

Hedonic Evaluations

Separate one-way between-subjects ANOVAs revealed no difference between the chemosensory primes on repugnance, $F(1,38) = 0.35$, $p = 0.56$, $\eta^2 = 0.01$, familiarity, $F(1,38) = 0.04$, $p = 0.85$, $\eta^2 = 0.001$, and intensity, $F(1,38) = 0.29$, $p = 0.59$, $\eta^2 = 0.008$ (see **Table 4**). Thus, participants rated ammonia and putrescine similarly to one another on each dimension. Again,

TABLE 4 | Scent ratings for the chemosensory primes (Experiment 3).

Chemosensory primes	Neutral	Ammonia	Putrescine
Intensity			
M	1.85	3.20	3.40
SD	0.99	1.32	0.99
Familiarity			
M	2.95	2.20	2.15
SD	0.83	0.89	0.75
Repugnance			
M	2.60	3.70	3.50
SD	0.60	0.98	1.15
N	20	20	20

TABLE 5 | The ratings of escape-related and threat-related cognitions for the chemosensory primes (Experiment 3).

Chemosensory primes	Neutral	Ammonia	Putrescine
Escape cognitions			
M	2.15	2.45	3.45
SD	0.99	1.05	0.69
Threat cognitions			
M	1.68	1.73	2.55
SD	0.65	0.64	0.94
N	20	20	20

the results reported below were did not differ when we entered the hedonic evaluations into the analyses as covariates.

Escape- and Threat-Related Cognitions

To test our hypothesis that putrescine elicited implicit cognitions related to escape and threat, we analyzed the escape and threat word-completion results separately. The results revealed a significant effect of scent prime on escape thought accessibility, $F(2,57) = 10.90$, $p < 0.001$, $\eta^2 = 0.28$ (see **Table 5**). Putrescine caused participants to complete word stems more frequently with escape related words ($M = 3.45$, SD $= 0.69$) than both the ammonia ($M = 2.45$, SD $= 1.05$) and the neutral scent ($M = 2.15$, SD $= 0.99$) primes (both $ps < 0.005$). Similarly, the scent primes affected the accessibility of threat-related thoughts, $F(2,57) = 8.39$, $p < 0.001$, $\eta^2 = 0.23$. Putrescine led to more threat word-stem completions ($M = 2.55$, SD $= 0.94$) than ammonia ($M = 1.73$, SD $= 0.64$) and the neutral scent ($M = 1.68$, SD $= 0.65$; both $ps < 0.005$).

Escape Behavior

Like Experiment 2, the analyses showed a significant effect of chemosensory primes on walking speed, $F(2,56) = 9.11$, $p < 0.001$, $\eta^2 = 0.24$ (see **Figure 2**). The pattern of results again showed that putrescine ($M = 33.38$, SD $= 2.99$) caused people to walk more quickly than ammonia ($M = 35.92$, SD $= 3.38$) and the neutral scent prime ($M = 37.67$, SD $= 3.13$; $p < 0.05$). Again, no difference was found between the ammonia and the neutral scent condition ($p = 0.87$).

Experiment 3 revealed that putrescine elicited implicit cognitions of escape and threat. In addition, Experiment 3 replicated the finding that putrescine increased walking speed. Thus, taken together, the results of Experiments 2 and 3 indicated

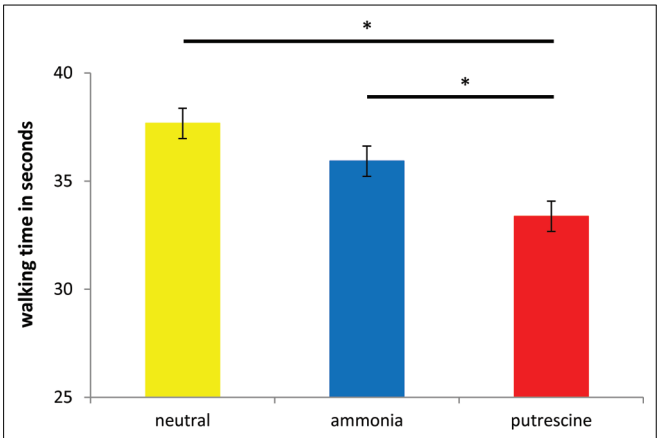

FIGURE 2 | The number of seconds it took participants to walk 60 m after exposure to the scent prime (Experiment 3). Asterisks denote that two groups differ at $^*p < 0.05$.

that putrescine motivated (automatic) escape behavior. An important feature of the settings in Experiments 2 and 3 was that participants were outdoors and in a context that facilitated the possibility that they could distance themselves from the scent.

Experiment 4: The Effects of Putrescine on Defensive Responses Toward An Out-Group

Experiment 4 sought to extend our understanding of the effects of putrescine in two important respects. First, we tested the hypothesis that *non-conscious* (unobtrusive) exposure to putrescine could elicit threat management responses. As we highlighted in the Introduction, this possibility is consistent with evidence that scent primes, even when presented at sub-threshold levels, can influence brain activation (Sobel et al., 1999), learning (Köster et al., 2002), and physiological state (Stern and McClintock, 1998). This applies similarly to aversive scent primes, which for example, have the ability to alter skin conductance (Jacquot et al., 2004), social preferences (Li et al., 2007), and cognitive performance (Epple and Herz, 1999) in ways that correspond to supraliminal exposure to aversive stimuli (Sela and Sobel, 2010). Thus, we predicted that subliminal presentation of putrescine would be capable of activating threat responses.

Second, Experiment 4 focused on the fight rather than the flight component of alarm responses. Consistent with previous research showing that implicit threat cues increase intolerance toward out-group members (Holbrook et al., 2011) and defensive responses (Blanchard et al., 2001; Wheatley and Haidt, 2005), we hypothesized that putrescine would increase defensiveness toward an out-group member, in a situation where there was no immediate opportunity to escape (Cesario et al., 2010). Like Experiment 1, we conducted this experiment in a laboratory setting. After priming the participants with one of the scents, they filled out a standard Positive And Negative Affect Scale (PANAS) that gaged their mood. Although our pilot study (see **Table 1**) and some research (e.g., Knasko, 1993) revealed that aversive scent primes do not alter mood on a conscious level, we intended

to rule out the possibility that the subliminal primes influenced participants' feelings at a conscious level. After that, they read about an out-group member—a foreign student who criticized the participants' value system—and were asked to evaluate the target. This evaluation was designed to assess how much hostility participants felt toward the target.

Method

Participants and Procedure

Sixty-nine participants (39 females and 30 males, $M_{age} = 24.00$, SD = 8.38) were run in our lab individually, in different cubicles (randomized) to avoid carryover effects of scents. Furthermore, participants were booked at least 30 min apart in order to ventilate the rooms between sessions. Upon arrival, participants were given the first of two questionnaire packets to complete. This first questionnaire consisted of demographic questions and a number of filler items. We then randomly assigned participants to their condition by marking one of the three liquid scents (putrescine, ammonia, water) to the top of each page (0.5 ml) of the second questionnaire participants were given. In the putrescine and ammonia conditions, this amounted to a very subtle scent prime that was not meant to be detected. At the conclusion of the experiment, we funnel debriefed participants to determine whether they noticed or smelled anything unusual during the study. None of them reported being aware of the scents.

The second questionnaire assessed participants' mood, and our dependent variables. First, to rule out the possibility that our results could be explained by generalized affect, participants began the second part of the questionnaire by completing the 20-item PANAS (Tellegen et al., 1988). This scale measured the extent to which each of 10 positive affect descriptors ($\alpha = 0.86$) and 10 negative affect descriptors ($\alpha = 0.85$) reflected how they felt at that moment (1 = *very slightly or not at all*, 5 = *extremely*). We computed the average positive affect ($M = 3.31$, SD = 0.68) and negative affect ($M = 1.61$, SD = 0.59) scores for everybody.

This was followed by the description and evaluation of the out-group member (Greenberg et al., 2001; Navarrete et al., 2004; Norenzayan et al., 2007). Specifically, participants read an essay supposedly written by a college student from the Middle East who was visiting the United Kingdom to study English. In this essay, the student went on to criticize Western values, predicting their eventual decline (see Norenzayan et al., 2007). Participants were then asked to evaluate the author and his message by responding to four questions on a 9-point Likert scale ["To what extent do you like the author"; "To what extent would you like to be friends with the author"; "How much would you oppose the author teaching your (future) children"; and "How much do you want the ideas of the author to be publicized"; 1 = *very much*, 9 = *not at all*]. We derived an overall out-group hostility index ($M = 5.82$, SD = 1.63) by averaging all items together ($\alpha = 0.77$), such that larger values indicated greater hostility. Finally, we measured motivation to escape the situation by timing how long it took participants to complete the second (scented) questionnaire followed by a standard demographic questionnaire (91% of the participants were native to England, 3% Greece, 4% Ireland, and 1% to the United States).

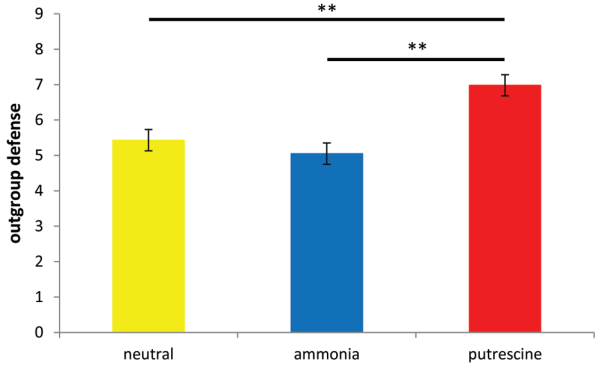

FIGURE 3 | Mean scores on the worldview defense scale for all three conditions (Experiment 4). Higher scores reflect greater hostility toward the target. Asterisks denote two groups differ at $^{**}p < 0.005$.

Results and Discussion

Ancillary Analyses

One-way ANOVAs tested whether the chemosensory primes elicited different levels of self-reported affect across the three conditions. However, the primes had no impact on positive affect $F(2,66) = 1.87$, $p > 0.16$, nor negative affect, $F(2,66) = 0.36$, $p > 0.70$. Moreover, the analyses below were no different when we used these affect measures as covariates, showing that any effect of our primes on out-group defense was not mediated by mood.

Out-Group Defense

As predicted, we found a significant effect of scent prime on defensiveness toward the author of the essay, $F(2,66) = 11.83$, $p < 0.001$, $\eta^2 = 0.26$ (see **Figure 3**). *Post hoc* analyses found that putrescine led to greater hostility ($M = 6.98$, SD = 1.42) compared to ammonia ($M = 5.05$, SD = 1.54) and the neutral conditions ($M = 5.43$, SD = 1.30; both $ps < 0.005$). There was no significant difference between the ammonia and control conditions, $p > 0.6$.

Experiment 4 supported the hypothesis that non-conscious exposure to putrescine evoked defensive responses toward an out-group member, and this effect was not due to conscious awareness of the scents, mood, or to the motivation to escape the aversive scent primes[3]. Although these results suggest that the scent primes elicited an odor percept (non-consciously), future studies may wish to control the precise intensities of the stimulus odors that are presented (e.g., using an olfactometer).

General Discussion

This research was designed to test the hypothesis that putrescine could serve as a warning signal that mobilizes protective responses to deal with threats. In four experiments, we found support for this idea: conscious and non-conscious exposure to putrescine elicited distancing and defensive reactions (e.g., fight and flight responses). Putrescine increased vigilance (Experiment 1), heightened the accessibility of escape- and threat-relevant cognitions (Experiment 3), and increased the speed participants

[3]When the amount of time participants took to complete the questionnaire was used as a covariate, the results remained significant, $F(2,65) = 13.13$, $p < 0.001$, $\eta^2 = 0.29$.

walked away from the location of the scent (Experiments 2 and 3). Experiment 4 created a situation where immediate escape was not likely and gave participants the opportunity to evaluate an out-group member. Subtle exposure to putrescine produced greater defensiveness toward the out-group member, suggesting an aggressive readiness in participants (Cesario et al., 2010). As a whole, the findings indicate that even brief exposure to putrescine mobilizes threat management responses designed to cope with environmental threats.

These are the first results to show that a specific chemical compound (putrescine) can be processed as a threat signal. Thus far, nearly all the evidence for threat chemosignals has come from those that are transmitted by body sweat (de Groot et al., 2012; Pause, 2012). Moreover, these are among the first studies that show that a specific chemical compound can cause overt behavior in humans (Wysocki and Preti, 2004). Furthermore, an advantage of isolating putrescine in threat management processes is that it may help in determining which sensory and brain pathways are involved in chemosensory threat detection and processing. For instance, research suggests that the central nucleus of the amygdala projects to the midbrain periaqueductal gray, the hypothalamus and the brainstem, which together coordinate to prepare fight-or-flight responses to threatening stimuli (Misslin, 2003). We speculate that putrescine activates a similar neurological pathway. Future research could include physiological measurements (e.g., systolic blood pressure, heart rate) to test the thesis that the observed effects of putrescine are modulated by processes originating in the sympathetic nervous system.

An important direction for future research will be to understand the precise nature of the threat produced by putrescine (e.g., microbial, predatory). Our view is that putrescine is relevant to both of these domains, though the immediate context should determine which type of threat is more primary. Recent work on TAARs has the potential to shed light on some of these mechanisms, as the activation of different receptors may function to detect specific threats, such as predators and pathogens (Li and Liberles, 2015; Pérez-Gómez et al., 2015). In addition, this research suggests that cadaverine (a compound with a similar chemical structure as putrescine; both are diamines) activates a similar pathway and produces similar escape and avoidance responses (Hussain et al., 2013; Oliveira et al., 2014) in animals. Thus, we render it likely that cadaverine evokes a similar threat response as putrescine (see Li and Liberles, 2015).

It would also be interesting to examine how putrescine detection affects sensitivity to particular types of threat and whether it produces elevated responses to certain stimuli more than others (e.g., fear- vs. disgust-based sensitivities). For instance, further research could elucidate how putrescine activates sensory acquisition (typically associated with fear experiences) and sensory rejection (associated with disgust) processes (Susskind et al., 2008), and whether exposure to putrescine augments physiological responses (e.g., heart rate, pupil dilation) that typically co-occur with adaptive responses to threats. This type of research would benefit from including individual differences in both disgust and fear sensitivity (Haidt et al., 1994; Garfinkel et al., 2014). By the same token, future work could clarify whether putrescine elicits discrete emotions (e.g., fear vs. disgust) or less specific affective states associated with negative valence and high arousal (see also Smeets and Dijksterhuis, 2014; Li and Liberles, 2015). Our findings, which showed that responses to putrescine were automatic, occurred after various lengths of delay (Experiments 1–3) and when presented at sub-threshold levels (Experiment 4), suggested that conscious evaluations are not at the heart of the observed responses to putrescine. This is consistent with our theorizing and ample work showing that chemosensory cues influence psychological and physiological operations outside of conscious awareness (for extended reviews, see Sela and Sobel, 2010; Smeets and Dijksterhuis, 2014). However, we hasten to add that more research is needed to specify the exact nature of the effects produced by the sub-threshold priming of putrescine, for instance, by varying the exposure times to putrescine, the delay after the primes, and the intensity of the putrescine stimulus.

Another important question is how specific threat management responses develop. Within non-olfactory sensory channels, for example, there may be an innate bias for humans to detect certain biologically-relevant stimuli as threatening, such as the sight of snakes and spiders (Ohman and Mineka, 2001). Although controversial in human research, some work suggests that responses to chemosensory stimuli are innate (Dielenberg et al., 2001; Misslin, 2003; Hussain et al., 2013). For instance, Soussignan et al. (1997) showed that soon after birth, butyric acid (a malodorous scent) evoked disgust reactions in neonates, a finding they claim is consistent with an innate predisposition toward ecologically-relevant scents. To test for the possibility of innate biases toward threatening chemosensory cues, it would be interesting to examine whether putrescine triggers facial expressions associated with fear or disgust in infants. In fact, research indicates that adults do not habituate so readily to the scent of putrescine emitted from rotting flesh (Roberson et al., 2008), suggesting that there might be a bias to respond warily to it.

Although the innateness of responses to chemosignals is still controversial, humans' ability to incorporate learned information into cultural practices is beyond question (Boyd and Richerson, 2005). Consequently, the magnitude of specific chemosensory threat responses could be different in cultures where people are exposed to putrescine more frequently. Likewise, reactions to putrescine may differ between cultures with different burial practices (e.g., embalming practices, the duration before burial). These factors should remind us that the context is critical to how people react to putrescine. How olfactory information modulates other sensory inputs (Zhou et al., 2012) is no doubt central to whether it will be interpreted as threatening.

One alternative theoretical perspective of our findings on the effects of putrescine is terror management theory (TMT; Greenberg et al., 1994). According to this theory, reminders of death are regulated by a "cultural anxiety buffer" that consists of beliefs and values that imbue life with meaning and the promise of immortality. Interestingly, TMT argues that a great deal of the darker side of human behavior (e.g., aggression, out-group prejudice, religious intolerance) stems from the need to maintain

and defend the integrity of this cultural anxiety buffer, due to its vital role in managing existential angst. In this view, putrescine could function as a reminder of mortality, and subsequently elicit similar defensive processes, as activated by reminders of death. We do not rule out this possibility, but render it unlikely that chemosensory threats trigger the same type of processes as those that originate from the unique human ability to reflect on the conundrum of life and death (Landau et al., 2007). Nevertheless, examining whether putrescine can be used as a subtle reminder of death, and whether it influences cultural beliefs, values, and practices, would open up fascinating directions of research.

Most research has shown that humans process threats either visually or audibly, while other animals inhabit the inaccessible world of scents. At the same time, we know that humans are guided by many of the same olfactory processes, especially when they involve fitness-relevant information. We believe that by identifying putrescine as one of these signals, a further understanding of its mechanisms can shed light on more general processes that modulate chemosensory signaling and threat management responses.

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Fancy Citrus, Feel Good: Positive Judgment of Citrus Odor, but Not the Odor Itself, Is Associated with Elevated Mood during Experienced Helplessness

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Aromatherapy claims that citrus essential oils exert mood lifting effects. Controlled studies, however, have yielded inconsistent results. Notably, studies so far did not control for odor pleasantness, although pleasantness is a critical determinant of emotional responses to odors. This study investigates mood lifting effects of d-(+)-limonene, the most prominent substance in citrus essential oils, with respect to odor quality judgments. Negative mood was induced within 78 participants using a helplessness paradigm (unsolvable social discrimination task). During this task, participants were continuously (mean duration: 19.5 min) exposed to d-(+)-limonene ($n = 25$), vanillin ($n = 26$), or diethyl phthalate ($n = 27$). Participants described their mood (Self-Assessment-Manikin, basic emotion ratings) and judged the odors' quality (intensity, pleasantness, unpleasantness, familiarity) prior to and following the helplessness induction. The participants were in a less positive mood after the helplessness induction ($p < 0.001$), irrespective of the odor condition. Still, the more pleasant the participants judged the odors, the less effective the helplessness induction was in reducing happiness ($p = 0.019$). The results show no odor specific mood lifting effect of d-(+)-limonene, but indicate a positive effect of odor pleasantness on mood. The study highlights the necessity to evaluate odor judgments in aromatherapy research.

Keywords: citrus, limonene, vanillin, aromatherapy, mood, helplessness

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INTRODUCTION

The strong association of odors with emotions, both on the neurophysiological and on the experience level (e.g., Adolph and Pause, 2012), suggests that odors are effective mood regulators. Indeed, the application of aromatic compounds in order to relieve stress and pain or elevate mood is a common procedure in alternative medicine. Citrus essential oils in particular have been claimed to exert mood enhancing effects (Pimenta et al., 2012). However, studies regarding mood lifting effects of citrus odors show mixed results. In rodents the inhalation of citrus essential oils alleviates stress, and exerts anxiolytic effects (Komiya et al., 2006; Leite et al., 2008;

Lima et al., 2013). Likewise, in human's anxiolytic effects of citrus fragrances have been suggested: Patients waiting for a scheduled appointment at a dental office report reduced anxiety when orange odor is introduced as ambient fragrance (Lehrner et al., 2000, 2005). However, the anxiety reducing effect proved not to be odor specific (Lehrner et al., 2005). Others found that dentist patients' anxiety level was unaffected by any ambient odor (orange odor vs. apple; Toet et al., 2010). It has further been claimed that treatment with citrus ambient odors normalizes neuroendocrine and immune function in depressive individuals (Komori et al., 1995). Indeed, depressive individuals seem to display a specific preference for citrus fragrances (citral; Pause et al., 2001). Notably, none of the studies reporting mood enhancing effects of citrus odors examined subjective judgments of the odors' quality, although this has been identified to be a key factor determining the emotional response to odors (Herz, 2009).

Learned helplessness, a negative emotional state which is characterized by a loss of control and negative expectations regarding the future, can be used as a model for depression (Miller and Seligman, 1975). Furthermore, the state effects of helplessness resemble deviations in central odor processing of depressed individuals (Laudien et al., 2006). Learned helplessness can be induced in controlled settings using ecologically valid success–failure manipulations (Nummenmaa and Niemi, 2004; Laudien et al., 2006).

The current study investigates the mood effects of d(+)-limonene (limonene), one of the most prominent compounds in citrus essential oils (characterized as a fresh citrus orange note) within a highly controlled setting. A learned helplessness procedure was used to induce a slightly negative mood, and odor judgments as well as mood ratings were obtained prior to and following the helplessness induction.

Vanillin and diethyl phthalate served as control conditions. The introduction of a vanillin control allowed for disentangling specific odor effects from pleasantness effects, as both limonene and vanillin are generally regarded as pleasant. The diethyl phthalate control served for the discrimination of odor effects from non-specific chemosensory context effects, as diethyl phthalate was the solvent for both limonene and vanillin.

The judgment of an odor's quality as pleasant or unpleasant essentially affects the emotional response to this odor (Herz, 2009). Furthermore, beliefs about an odor (e.g., regarding an odor as unhealthy) are more important in determining the individual response to that odor than its actual biochemical properties (De Araujo et al., 2005; Laudien et al., 2008). Therefore it is expected that the experienced odor quality and not the odor itself modulates mood.

MATERIALS AND METHODS

Participants

A total of 97 volunteers participated in the experiment. All participants reported to be healthy, and free of neurological or psychiatric conditions. In order to heighten the subjective importance of the cover story (task used for employee selection in the social domain; see Cover Story), only participants working in the social domain (e.g., social worker) or studying a subject related to social sciences (e.g., psychology, educational science) were recruited (see Cover Story). Due to technical problems ($n = 9$) and disbelief in the cover story ($n = 10$) 19 participants were excluded. Of the final sample ($n = 78$), 27 participants (23 females) were included in the diethyl phthalate condition, 26 participants (22 females) were assigned to the vanillin condition, and 25 participants (21 females) were assigned to the limonene condition. Age ($M = 24$ years, $SD = 7$, range 18–59) did not differ between conditions ($p > 0.90$).

The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Mathematics and Natural Sciences of the Heinrich-Heine-University Düsseldorf. Participants gave their written informed consent and were compensated with course credit or €15. At the end of the experiment participants were debriefed and informed about the true nature of the study.

Cover Story

Participants were asked to take part in a study investigating the effects of right brain hemisphere activation on odor habituation. They were informed that they would be working on a computer-based emotional intelligence test, which leads to activation of the right brain hemisphere, while inhaling an odor. It was stated that the emotional intelligence test would usually be applied to test professional aptitude in the social domain (e.g., physiotherapy, social work, or psychotherapy). Participants were told that it was crucial to do their best at the task in order to determine whether they possessed a skill that is important for their profession. The cover story was adapted from Laudien et al. (2006).

Materials

Odors

D-(+)-limonene (97%, Sigma–Aldrich Co.; diluted 1:2 [v/v] in diethyl phthalate [99%, Merck KGaA]), Vanillin (99% Sigma–Aldrich Co.; diluted 1:10 [v/v] in diethyl phthalate [99%, Merck KGaA]), and diethyl phthalate (99%, Merck KGaA) were used as odorants. Odor concentrations of d-(+)-limonene and vanillin were chosen to be perceived as medium intense, and roughly matched for intensity (as judged by working group members).

Odors (3 ml) were dropped on cotton pads, which were placed in gas-washing bottles (100 ml volume). An air operated double diaphragm pump (Tetratec APS 50, Tetra GmbH; volumetric flow rate 14 ml/s) was used to pump ambient air through the gas-washing bottles into an oxygen mask. Air flow was controlled using computer controlled solenoid valves. Separate teflon-tubes (6 mm diameter) were used for each odor. Odors were presented continuously from the beginning of the helplessness induction until the second rating of odor quality (duration: $M = 19.5$ min, $SD = 2.5$ min).

Stimuli for the Helplessness Induction Procedure

In order to affect the participants' emotional state, an unsolvable emotional intelligence test was introduced in the cover story of the experiment (Laudien et al., 2006). A total of 175 faces (Karolinska Directed Emotional Faces System; Lundqvist et al., 1998) were presented in a facial expression assessment task.

Of these, 92% were of neutral valence (45.8% neutral, 45.8% surprise), and 8% were of negative valence (fear: 5.7%, sadness: 0.4%, anger: 1.5%, disgust: 0.8%). Stimuli were presented on a 19″ TFT monitor (Terra LCD 4319, Wortmann AG) positioned at 1 m distance using Presentation 14 (Neurobehavioural Systems Inc.).

Questionnaires

The effects of the helplessness induction were assessed on the dimensions emotional valence (−4 = negative valence, 4 = positive valence), arousal (0 = low arousal, 9 = high arousal), and dominance (0 = low dominance, 9 = high dominance) using the language-free computerized Self-Assessment Manikin (SAM; Bradley and Lang, 1994). Furthermore, participants indicated their emotional state regarding five basic emotions (anger, disgust, fear, happiness, and sadness) via computerized visual analog scales (length: 18.5 cm, range: 0–100). Odor quality was rated regarding intensity, pleasantness, unpleasantness, and familiarity using pictographic computerized nine level likert-scales, similar to the SAM.

Procedure

Participants were assigned to the three treatment groups (diethyl phthalate, limonene, vanillin) and were tested separately. At the beginning of each session, the participants indicated their baseline mood, using the SAM and emotion ratings. The participants were then asked to discriminate one deviant odor (the treatment odor) from two distractors (three alternative forced choice), which were presented in a random sequence via the oxygen mask (stimulus duration = 7 s; interstimulus interval = 7 s). Vanillin and limonene were tested against diethyl phthalate, whereas diethyl phthalate was tested against ambient air. The task was repeated five times.

Then, immediately prior to the experiment, participants rated the quality of the treatment odor regarding intensity, pleasantness, unpleasantness and familiarity. During the odor quality ratings the odor was presented continuously in order to match the odor presentation during the actual experiment.

Helplessness was induced using a facial expression classification task. A total of 264 pictures of faces were presented briefly but supraliminally (100 ms duration). Pictures were presented in random order, but no picture was repeated directly after it was shown for the first time. Participants were asked to evaluate whether these faces express either a negative or positive emotion. This was an unsolvable task due to the mostly neutral facial expressions of the stimuli presented. Decisions had to be made by mouse click within a 3-s interval. Participants were advised not to skip any pictures because all unrated faces would be counted as false. In order to induce helplessness, participants received false feedback regarding their performance over time after every 6th decision (duration: 4 s; number of feedbacks: 44, see **Figure 1**). Starting from the beginning feedback indicated "below average" and progressively worsening performance, reaching a score indicating a "quite poor performance" after the 21st trial. The feedback graphs and the meaning of the scoring were explained to the participants before testing. This procedure

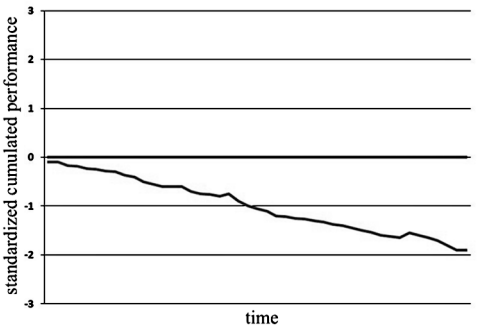

FIGURE 1 | False performance feedback. The decreasing line was introduced as the actual performance of the participant over time, the horizontal line (0) was introduced as average performance. The axes' labels were not presented to the participants.

was followed by the participants rating their mood and judging the odor's quality a second time.

Throughout the entire session, the mean ambient temperature was kept at 24°C ($SD = 1$ °C). A complete session lasted between 48 and 77 min.

Statistical Analysis

The effects of the helplessness induction procedure and the odor exposition on perceived odor quality (intensity, pleasantness, unpleasantness, familiarity) were analyzed using a 3 × 2 split-plot ANOVA with the factors odor (diethyl phthalate, vanillin, limonene) and time (prior to helplessness induction [T1], after helplessness induction [T2]). Bonferroni-corrected *t*-tests were used as *post hoc* tests ($\alpha = 0.050/3 = 0.017$).

Mood ratings (SAM ratings: emotional valence, arousal, dominance; basic emotion ratings: anger, disgust, fear, happiness, and sadness) were subjected to the same ANOVA. In order to correct for multiple tests, the significance level for the ANOVAs was bonferroni corrected to $\alpha = 0.050/8 = 0.006$.

Effects of odor hedonics on mood were assessed using a linear multivariate regression including both mean odor pleasantness and unpleasantness as predictors for difference values of emotional valence, dominance, anger, and happiness (T2 – T1). Emotional valence, dominance, anger, and happiness were chosen because these ratings proved to be affected by the helplessness induction procedure, as evident from the ANOVAs (main effect of time, see Results). Predictors were entered in the model simultaneously. In order to correct for multiple tests, the significance level for the regression models was bonferroni corrected to $\alpha = 0.050/4 = 0.013$.

RESULTS

Odor Perception

The treatment groups did not differ in their ability to detect the target odor [$\chi^2(2) = 2.13$, $p = 0.347$]. The target odor was

correctly detected at least four times (chance level < 0.05) by 69% of the participants.

Limonene ($M = 5.5$, $SD = 1.8$) was perceived as more intense than diethyl phthalate [$M = 3.4$, $SD = 1.7$; $t(50) = 4.22$, $p < 0.001$] and vanillin [$M = 4.3$, $SD = 1.1$; $t(49) = 2.88$, $p = 0.006$]. The intensity of vanillin and diethyl phthalate did not differ significantly after bonferroni-correction [$t(51) = 2.15$, $p = 0.036$; main effect odor: $F(2, 75) = 11.18$, $p < 0.001$]. During the course of the experiment strong habituation effects were evident: All odors were perceived as more intense before the helplessness induction ($M = 5.0$, $SD = 2.4$) than after the helplessness induction [$M = 3.7$, $SD = 1.9$; main effect time: $F(2, 75) = 26.12$, $p < 0.001$]. In detail, diethyl phthalate [$t(26) = 2.59$, $p = 0.016$] and limonene [$t(24) = 4.76$, $p < 0.001$] were rated as less intense after the helplessness induction, whereas ratings for vanillin did not differ between measurements [$t(25) = 1.59$, $p = 0.124$].

Odors did not differ regarding pleasantness [$F(2, 75) = 0.03$, $p = 0.972$] or unpleasantness [$F(2, 75) = 3.05$, $p = 0.053$]. All odors were rated as more unpleasant ($M = 2.2$, $SD = 1.4$) after compared to before the helplessness induction [$M = 2.7$, $SD = 2.2$; main effect time: $F(1, 75) = 5.27$, $p = 0.024$].

Vanillin ($M = 5.3$, $SD = 2.2$) and limonene ($M = 5.6$, $SD = 1.9$) were rated as more familiar than diethyl phthalate [$M = 3.3$, $SD = 2.1$; vanillin vs. diethyl phthalate: $t(51) = 3.20$, $p = 0.002$; limonene vs. diethyl phthalate: $t(50) = 4.10$, $p < 0.001$; main effect odor: $F(2, 75) = 9.02$, $p < 0.001$]. Diethyl phthalate was rated as even less familiar after the helplessness induction ($M = 2.7$, $SD = 2.3$) than before the helplessness induction [$M = 4.0$, $SD = 2.5$; $t(26) = 2.7$, $p = 0.010$], while the familiarity of limonene and vanillin did not vary over time [interaction odor × time: $F(2, 75) = 3.52$, $p = 0.035$]. For an overview of the odor quality ratings see **Table 1**.

Mood Ratings

The helplessness induction was successful. Regardless of odor condition, participants indicated they were in a more negative mood (emotional valence), more submissive (dominance), angrier (anger) and less happy (happiness) after compared to before the helplessness induction (all ps < 0.001; see **Table 2** for ANOVA results; see **Tables 3** and **4** for descriptive statistics). Odors had no effect on mood (all ps ≥ 0.067; see **Table 2**).

A model using odor pleasantness and odor unpleasantness as predictors[1] explained 12.3% (R^2) of the variance in the change of happiness over the course of the helplessness induction [$F(2, 75) = 5.28$, $p = 0.007$]. Participants reported a smaller reduction of happiness the more pleasant [$\beta = -0.268$, $t(75) = 2.48$, $p = 0.019$] and, by trend, the less unpleasant they rated the odor [$\beta = 0.191$, $t(75) = 1.74$, $p = 0.087$]. A similar effect was found for emotional valence: Participants reported a more negative valence after the helplessness induction the more unpleasant the odor was rated [$\beta = 0.250$, $t(75) = 2.23$, $p = 0.028$]. However, after bonferroni-correction the overall model predicting emotional valence is not considered significant [$F(2, 75) = 3.60$, $p = 0.032$].

The odors' pleasantness and unpleasantness cannot predict the change in dominance or anger ratings over the course of the helplessness induction, after bonferroni-correction is applied (see **Table 5**).

ANCOVAs including the factors of the original ANOVAs (odor and time) and odor pleasantness as well as odor unpleasantness as covariates support the previous ANOVAs' results: Mood ratings still are unaffected by odor (all $ps > 0.4$, except for sadness ratings, odor × time: $p = 0.074$).

Also the results of the regression analysis are replicated: Participants show a smaller happiness reduction the more pleasant [time × pleasantness: $F(1, 73) = 5.42$, $p = 0.023$] and the less unpleasant they rated the odor [time × unpleasantness: $F(1, 73) = 3.97$, $p = 0.050$]. Further, participants reported a more negative valence after the helplessness induction the more unpleasant the odor was rated [time × pleasantness: $F(1, 73) = 5.05$, $p = 0.028$].

DISCUSSION

The current study aimed at investigating whether the odor of limonene would be especially potent in preventing the induction of negative mood by a learned helplessness procedure. However, the present results indicate that limonene, like the control odors (vanillin, diethyl phthalate), was ineffective at preventing negative mood, even though the current design achieved a statistical power of 0.97 (medium effect sizes

[1]Note that ratings for pleasantness and unpleasantness can both be used as predictors in the multivariate regression, since they are not correlated ($r = -0.174$, $p = 0.129$).

TABLE 1 | Descriptive values of odor quality ratings.

	Diethyl phthalate				Vanillin				Limonene			
	T_1		T_2		T_1		T_2		T_1		T_2	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Intensity	4.0	2.4	2.8	1.7	4.6	1.7	3.9	1.6	6.5	2.2	4.4	2.1
Pleasantness	4.7	2.7	4.6	2.4	5.0	2.1	4.5	2.1	5.1	2.1	4.4	2.4
Unpleasantness	1.9	1.2	2.6	1.7	1.9	1.5	2.4	2.1	3.0	1.8	3.3	2.5
Familiarity	4.0	2.5	2.7	2.3	5.2	2.9	5.3	2.2	5.6	1.9	5.6	2.1

T_1, before helplessness induction, T_2, after helplessness induction, M, mean, SD, standard deviation.

TABLE 2 | Effects of odor and helplessness induction (time) on mood.

	Odor			Time			Odor × Time		
	F(2, 75)	*P*	η_p^2	*F*(1, 75)	*p*	η_p^2	*F*(2, 75)	*p*	η_p^2
Valence	0.77	0.466	0.020	55.94	<0.001	0.427	0.01	0.987	<0.001
Arousal	0.79	0.458	0.021	7.34	0.008	0.089	1.06	0.508	0.018
Dominance	0.86	0.426	0.022	18.31	<0.001	0.196	0.42	0.662	0.011
Anger	0.04	0.953	0.002	49.50	<0.001	0.398	0.08	0.921	0.001
Fear	0.06	0.934	0.002	0.58	0.450	0.088	2.64	0.078	0.066
Disgust	2.17	0.121	0.055	0.40	0.533	0.005	0.77	0.467	0.020
Happiness	0.67	0.515	0.018	77.96	<0.001	0.510	0.25	0.779	0.007
Sadness	0.36	0.940	0.010	4.06	0.047	0.051	2.81	0.067	0.070

Bonferroni adjusted significance level: α = 0.006.

TABLE 3 | Descriptive values of mood ratings (SAM).

	Diethyl phthalate				Vanillin				Limonene			
	T_1		T_2		T_1		T_2		T_1		T_2	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Valence	1.9	1.3	0.4	2.1	1.5	1.3	0.0	1.4	1.9	1.4	0.4	1.7
Arousal	4.5	1.9	5.1	2.1	4.2	1.3	4.4	1.5	4.2	1.2	5.0	1.6
Dominance	5.6	1.7	5.1	1.6	5.7	1.5	5.2	1.5	6.2	1.3	5.5	1.3

T_1, before helplessness induction, T_2, after helplessness induction, M, mean, SD, standard deviation.

TABLE 4 | Descriptive values of mood ratings (basic emotions).

	Diethyl phthalate				Vanillin				Limonene			
	T_1		T_2		T_1		T_2		T_1		T_2	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Anger	7.6	13.5	28.4	25.1	7.7	10.9	25.7	26.8	8.0	13.3	27.6	25.2
Fear	7.1	12.5	10.3	16.6	11.2	22.0	7.2	15.8	7.8	10.6	12.4	16.1
Disgust	7.1	11.1	5.8	10.2	7.1	13.1	7.8	14.9	11.2	14.9	15.2	22.2
Happiness	49.3	19.5	35.8	23.4	53.9	22.7	39.0	18.7	54.6	14.8	42.4	18.8
Sadness	6.3	11.5	13.2	17.6	14.2	23.9	11.7	20.0	5.9	11.4	13.0	21.2

T_1, before helplessness induction, T_2, after helplessness induction, M, mean, SD, standard deviation.

TABLE 5 | Parameters for regression model with odor pleasantness and unpleasantness as predictors.

	Overall model			Odor pleasantness			Odor unpleasantness		
	R^2	*F*(2, 75)	*p*	β	*t*(75)	*p*	β	*t*(75)	*p*
Valence	0.088	3.60	0.032	−0.121	1.08	0.285	0.250	2.23	0.028
Dominance	0.010	0.37	0.691	−0.050	0.43	0.667	0.077	0.66	0.511
Anger	0.043	1.67	0.196	0.022	0.194	0.846	−0.201	1.75	0.084
Happiness	0.123	5.28	0.007	−0.264	2.40	0.019	0.191	1.74	0.087

Bonferroni adjusted significance level for the overall model: α = 0.013.

assumed [$f = 0.25$, Cohen, 1988]). Moreover, the observed null effect is independent of the application of a bonferroni-correction. Thus, the current results are in line with Toet et al. (2010), who also could not show a mood lifting effect of orange odor, and seem to contradict those studies showing positive effects of orange odor on mood (Lehrner et al., 2000, 2005).

On the other hand, the effectiveness of the helplessness induction varied between individuals in accordance with their ratings of the odors' pleasantness. In detail, the more pleasant

the odors were rated, the less successful (in terms of a smaller decrease in happiness) the helplessness induction was. Moreover, it is possible to assume that these differences in perceived odor pleasantness actually caused the mood stabilizing effect (instead of happiness affecting odor pleasantness): Odor pleasantness was rated the same prior and after the helplessness induction. Therefore, the respective pleasantness judgment can be considered as having been evident before any changes in mood occurred.

Taken together, this pattern indicates that mood lifting effects of limonene and vanillin can primarily be attributed to their pleasantness and not to their specific aromatic profile or chemical structure. These results are in line with studies showing effects of pleasant odors on the autonomic nervous system congruent with positive mood (e.g., Alaoui-Ismaïli et al., 1997; Heuberger, 2001). Thus, odors might indeed work as mood enhancers, as long as they are perceived as pleasant. As learned helplessness, which was utilized within the current study to induce negative mood, is regarded as an etiologic model for depression, the current work especially underlines the close connectivity between odors and emotions in the context of depression (Pause et al., 2003; Schablitzky and Pause, 2014). Our results further suggest that being exposed to pleasant odors might attenuate the experience of negative mood in a situation typically involved in the development of depressive symptomatology. Pleasant odors might therefore be an additional support in the treatment of depressive symptoms.

It could be speculated that specific mood enhancing effects of limonene might have been prevented by its potentially irritating properties (Larsen et al., 2000). However, a reduction in perceived intensity over the course of the experiment suggests that the participants showed perceptual habituation. Habituation indicates that the olfactory properties of limonene dominated, as trigeminal stimulation should rather have led to sensitization (Hummel and Kobal, 1999; Hummel, 2000).

It could be argued that the generalizability of the current results might be somewhat limited due to an overrepresentation of females within the sample. However, according to previous studies, gender does not modulate the effects of pleasant and unpleasant odors on mood (Marchand and Arsenault, 2002), rendering a similar gender bias within the current results unlikely. Further, as women were equally distributed among the odor groups, possible odor effects could not have been confounded by gender.

So far, research examining the potential of odors – and citrus odors in particular – to prevent negative mood has yielded inconclusive results. The current data suggest that such conflicting results might be related to odor pleasantness judgments varying between individuals and from study to study, rendering the respective odors either effective or ineffective mood enhancers. Therefore, the current study is in line with studies showing that judgments about an odor are more important in determining the response to it than its biochemical properties (De Araujo et al., 2005; Laudien et al., 2008) and Herz's (2009) conclusion, that the effects of aromatherapy in humans may primarily be attributed to psychological effects.

CONCLUSION

The current study indicates that odor pleasantness and not limonene itself has a mood enhancing effect. Odor effects in humans are provoked by the individual perception of a particular odor, and not by the intrinsic properties of the odor. Thus, the study highlights the necessity to evaluate the odor judgments of the participants in aromatherapy research.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Sensitivity of Physiological Emotional Measures to Odors Depends on the Product and the Pleasantness Ranges Used

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Emotions are characterized by synchronized changes in several components of an organism. Among them, physiological variations provide energy support for the expression of approach/avoid action tendencies induced by relevant stimuli, while self-reported subjective pleasantness feelings integrate all other emotional components and are plastic. Consequently, emotional responses evoked by odors should be highly differentiated when they are linked to different functions of olfaction (e.g., avoiding environmental hazards). As this differentiation has been observed for contrasted odors (very pleasant or unpleasant), we questioned whether subjective and physiological emotional response indicators could still disentangle subtle affective variations when no clear functional distinction is made (mildly pleasant or unpleasant fragrances). Here, we compared the sensitivity of behavioral and physiological [respiration, skin conductance, facial electromyography (EMG), and heart rate] indicators in differentiating odor-elicited emotions in two situations: when a wide range of odor families was presented (e.g., fruity, animal), covering different functional meanings; or in response to a restricted range of products in one particular family (fragrances). Results show clear differences in physiological indicators to odors that display a wide range of reported pleasantness, but these differences almost entirely vanish when fragrances are used even though their subjective pleasantness still differed. Taken together, these results provide valuable information concerning the ability of classic verbal and psychophysiological measures to investigate subtle differences in emotional reactions to a restricted range of similar olfactory stimuli.

Keywords: odor perception, emotion, psychophysiology, pleasantness, subjective sensitivity, physiological sensitivity, fragrance

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INTRODUCTION

Olfaction stands out in the sensory landscape for its peculiar and intimate connection with the world of emotions, which may stem from the distinctive anatomical overlap between olfactory- and emotion-related neural structures (Carmichael et al., 1994; Smeets and Dalton, 2002; Anderson et al., 2003; Grabenhorst et al., 2007; Zelano et al., 2007). The majority of consciously perceived

odors tend to be salient, compared with stimuli from other modalities, because of the prominent presence of their hedonic dimension (Mohanty and Gottfried, 2013). Odors surround us in everyday life and affect our behavior (Bensafi et al., 2002a; Li et al., 2007), our mood, and our well-being (Alaoui-Ismaïli et al., 1997; Rétiveau et al., 2004; Warrenburg, 2005. This is attested by the importance of perfumery since the earliest civilization (Le Guérer, 1994), the significantly impoverished quality of life observed in individuals suffering from olfactory impairment (Hummel and Nordin, 2005; Landis et al., 2009; Croy et al., 2012; Keller and Malaspina, 2013), and the influence that odors exert on various behavioral and cognitive processes such as memory or preference acquisition (Leppanen and Hietanen, 2003; Herz et al., 2004a).

Emotions are characterized by synchronized changes in several components of the organism: subjective, physiological, expressive, cognitive, and motivational (Scherer, 1982, 2001). Experimental research using olfactory stimulations has demonstrated changes in these components as a function of odor pleasantness. At the subjective level, self-reports (e.g., on liking scales) are used extensively to characterize individual preferences (Degel et al., 2001; Savic et al., 2002; Howard et al., 2009; Pause et al., 2009; Adolph et al., 2010; Li et al., 2010; Gelstein et al., 2011; Coppin et al., 2012). Self-reported measures of preference are deeply influenced by contextual factors and individual states, as the subjective response to smell is notoriously flexible (see Coppin and Sander, 2011 for a review). Hedonic responses to olfactory stimulations can be modulated by processes such as mere exposure (Delplanque et al., 2008, 2015), decision making (Coppin et al., 2010), associative learning (Herz et al., 2004b), or verbal context (Herz, 2003; Bensafi et al., 2007). According to appraisal theories, including the component process model (Scherer, 1982, 2001), the physiological component of the emotional response is a support for adapted responses and energy that provides for the expression of these action tendencies. Extensive experimental evidence shows that olfactory stimulations induce differential responses at the physiological level according to their pleasantness, readily affecting heart rate, which has been shown to decrease as a function of odor hedonicity (Alaoui-Ismaïli et al., 1997; Bensafi et al., 2002b; Delplanque et al., 2009), while other indicators such as skin conductance and pupillary light reflex are also sensitive to arousal (Bensafi et al., 2002b; Bradley et al., 2008; Sequeira et al., 2009). Finally, the expressive component of the emotional response is subtended by the motor system and is responsible for communication of reaction and behavioral intention. Odor pleasantness also affects facial expression, inducing differences in EMG activity. Facial muscles responsible for frowning (corrugator) and for smiling (zygomaticus) respond differentially to pleasant and unpleasant odors (e.g., Bensafi et al., 2002c; Soussignan et al., 2005; Armstrong et al., 2007; Delplanque et al., 2009).

Most previous experiments have used varied olfactory stimuli, spanning a wide valence spectrum (i.e., very unpleasant to very pleasant; see Mohanty and Gottfried, 2013 for a review), which increases the likelihood of observing clear-cut differences in all components of the emotional response. A comparison between physiological and self-reported responses to olfactory stimulations (Alaoui-Ismaïli et al., 1997) has revealed that the correlation between these two indicators is good, as long as the stimulations are well contrasted in terms of subjectively reported valence and are of different types (e.g., food, cosmetics, animal). Certain types of odors, such as essential oils or fine perfumes, can be considered as belonging to one particular odor family—fragrances—in which marked differences in self-reported pleasantness can nonetheless be observed (Rétiveau et al., 2004).

Subjective reports appear to provide subtle valence differences that are found even when the odors belong to the same family. Subjective feelings integrate all other emotional components and are plastic (Scherer, 1982, 2001). By contrast, the physiological component supports adapted responses and energy, providing for the expression of more hard-wired action tendencies. This component is less likely than subjective feelings to be able to differentiate subtle differences in valence for odors of the same family. Here, we illustrate this point by presenting the results of two studies that assess subjective, physiological, and expressive components of emotion in response to olfactory stimuli. We compared two conditions: (1) *Odors*: when olfactory stimulations were strongly differentiated and belonged to different odor families (food, floral, animal, perfumes, etc.), and (2) *Fragrances*: when olfactory stimulations belonged to a particular family, i.e., fine perfumes. The objectives of this study were (1) to replicate the classic distinction observed in emotional components (subjective, physiological, and expressive) in response to well-differentiated olfactory stimulations (i.e., pleasant and unpleasant odors); and (2) to evaluate whether these components remain sensitive enough to differentiate between the emotional reactions associated with family related olfactory stimulations (i.e., fragrances). If indeed the subjective component is more malleable than the physiological component, then subjective differences should arise regardless of width of the pleasantness spectrum examined, whereas physiological differences would appear only in the case of larger differences.

Because olfactory preferences are highly individual, we did not contrast the different dependent variables (i.e., subjective, physiological, and expressive) by olfactory stimuli, but performed individual selections, grouping each individual's most pleasant and most unpleasant olfactory stimuli.

MATERIALS AND METHODS

We analyzed non-published data acquired previously by Delplanque et al. (2009). In this study, participants were presented with a set of varied "sample" and "target" odors and given no information about them. Sample odors were presented first as an encoding condition, whereas target odors were presented second, as a retrieval condition. Only target odors were previously analyzed to be included in Delplanque et al. (2009). Here, we analyzed responses to the sample odors. Emotional responses to these odors were compared with those obtained in an independent sample of participants presented with a set of fragrances. Given the strong inter-individual variability of

olfactory preferences (Herz and Von Clef, 2001; de Araujo et al., 2005; Keller et al., 2007), we conducted our analyses on the basis of individual judgments as opposed to averaging the subjective ratings for a given odor.

Participants

Two different groups of nonsmoking participants (Group 1 and Group 2), all University of Geneva students, were recruited through ads posted in a university building. Group 1 consisted of 18 participants (9 females, right handed; mean age = 27.1 ± 6.2 years) and was provided with pleasant and unpleasant odors (Delplanque et al., 2009). Group 2 consisted of 21 participants (all females; mean age = 22.7 ± 3.3 years) and was provided with fragrances. Participants were individually tested and paid 50 Swiss Francs (approximately $50) for their participation. On testing days, participants were asked not to wear any perfume. They all self-reported a normal sense of smell and were free from respiratory infections when they participated. None of the participants reported any mental illnesses that could have affected their emotional responses to stimuli. Written consent was obtained from all participants before starting the experiment in accordance with the Declaration of Helsinki, and the study was approved by the ethical committees of the Geneva University Hospital and of the Psychology Department of the University of Geneva. In Group 1, two participants were excluded because of acquisition artifacts in facial muscle activity (both the corrugator and the zygomaticus muscles), leaving 19 participants for analysis. In Group 2, participants were excluded because of acquisition artifacts in activities of the corrugator (one participant) and zygomaticus muscles (two participants), leaving 16 and 17 participants for analysis on these two variables, respectively.

Stimuli

All olfactory stimuli ("*Odors*" and "*Fragrances*") were injected into the tampon of cylindric felt-tip pens (14 cm long, inner diameter 1.3 cm). The use of these highly practical devices (provided by Burghart, Germany) avoids any contamination of the environment.

Odors

Thirty-two *a priori* pleasant and unpleasant odorants (**Table 1**) were selected on the basis of a previous study conducted on 66 participants, who evaluated 51 odorants according to subjective intensity, pleasantness, and familiarity (see Delplanque et al., 2008, 2009). The aim of this large selection was to obtain an array of odorants with a wide pleasantness spectrum. For practical reasons, we labeled this first choice of odorants as "*Odors*".

Fragrances

Nine additional fine perfumes (**Table 2**) were selected on the basis of a preliminary study performed on 60 undergraduate

TABLE 1 | Odors.

Unpleasant odors	Concentration (% in DIPG)	Odor family	CAS	Pleasant odors	Odor family	Concentration (% in DIPG)	CAS
Aladinate*	20	Floral	341017-24-1	Amyl acetate*	Fruity	20	628-63-7
Beer	20	Savory food		Basil	Green	5	
Body odor (synthetic)	Pure	Animal		Bornyl acetate*	Camphor	20	125-12-2
Carbinol*	5	Earthy	700-06-1	Cake	Sweet food	20	
Caproic acid*	20	Animal	142-52-1	Cassis bud	Fruity	20	
Diacetyl*	50	Buttery	431-03-8	Classic body lotion fragrance	Detergent	5	
Durian	20	Fruity		Classic detergent fragrance	Detergent	1	
Dynascone*	20	Amber, Musky	0056973-85-4	Classic shampoo fragrance	Detergent	10	
Framboisone*	50	Fruity		Classic soap fragrance	Detergent	10	
Ghee	5	Savory food		Fig	Fruity	10	
Isobutyl quinoline*	20	Animal	93-19-6	Geraniol*	Floral	20	106-24-1
Isobutyric acid*	10	Pungent, Animal	79-31-2	Green tea	Floral green	10	
Isovaleric acid*	1	Pungent, Animal	503-74-2	Honey	Sweet food	10	
Landes wood	5	Woody		Lavender	Floral	10	
Leather	5	Animal		Lilac	Floral	10	
Melonal*	50	Fruity	106-72-9	Lime	Citrus	20	
Octamylamine*	5	Fishy-oily	502-59-0	Linalol*	Floral	10	78-70-6
Octanol*	5	Oily	11-87-5	Magnolia grandifolia	Floral	20	
Paracresol*	1	Animalic	106-44-5	Methyl-salicylate*	Aromatic	10	119-36-8
Rancid butter	20	Savory food		Neroli	Floral	5	
Sclarymol*	1	Sulfury		Peach	Fruity	10	
Skunk	10	Animal		Pineapple	Fruity	10	
Sulfox	0.05	Sulfury		Tiare	Floral	Pure	
Yogurt	10	Sweet food		Tutti frutti	Fruity	10	

**Single odorant molecule. CAS molecule numbers are provided where available.*

TABLE 2 | Fragrances.

Fragrance	Brand	Notes
Angel	Thierry Mugler	Oriental – Vanilla
Chanel n°5	Chanel	Floral – Aldehyde
Ck One	Calvin Klein	Citrus – Aromatic
Flower	Kenzo	Cedarwood – Amber – Musks
J'adore	Dior	Floral – Fruity
Light blue	Dolce & Gabbana	Floral – Fruity
Romance	Ralph Lauren	Floral – Fruity
Samsara	Guerlain	Oriental – Woody
Trésor	Lancôme	Floral – Rose Violet

students (60 females; mean age = 20.27 ± 3.1 years). The primary interest of that study was to assess the influence of contextual information on fragrance evaluation. We chose fragrances that were well-known in the French and Swiss markets. In addition, the fragrances were well characterized to ensure good perceptual variability (see **Table 2**). For practical reasons, we labeled this second choice of odorants as "*Fragrances*".

Experimental Procedures

Participants were told that they would be provided with olfactory stimuli to evaluate. During one session, they smelled the 32 odor-containing (Group 1, *Odors*) or the nine fragrance-containing (Group 2, *Fragrances*) pens in random order in successive trials. For each trial, an experimenter seated near the participant in a well-ventilated room then placed an odor pen about 1 cm below the participant's nostrils for 2 s. Before testing, participants were instructed via computer to smell the odorants according to a particular procedure to minimize variability in intra- and inter-participant breathing patterns (Jung et al., 2006; Delplanque et al., 2009). The participants first had to breathe out deeply through the mouth, wait for the request to inhale (a word presented on a screen in front of the participant), breathe in evenly with the felt-tip pen containing the odorant under the two nostrils, and then rest and relax for 15 s.

The presentation of the olfactory stimulus to the participant was followed by the completion of subjective ratings assessing intensity, hedonicity, and familiarity. The interval between two stimuli was 15 s to avoid sensory adaptation.

Subjective Ratings

Participants rated the hedonicity, intensity, and familiarity of the olfactory stimuli that they were presented with on continuous 10 cm scales from *very unpleasant* (left of the scale = 0 cm) to *neutral* (middle of the scale, 5 cm) to *very pleasant* (right of the scale, 10 cm); from *not perceived* (or low intensity, left) to *medium* (middle) to *strong* (or high intensity, right); and from *not familiar at all* (left) to *very familiar* (right), respectively, (see Delplanque et al., 2009 for details).

Apparatus and Physiological Recordings

Physiological signals were assessed with the TEL 100 Remote Monitoring System (Group 1) and the MP150 (Group 2) system of Biopac (Santa Barbara, CA, USA) with separate settings for the electrocardiogram, electrodermal activity, and respiratory activities. Signals were transferred from the experimental room to the MP100 Acquisition Unit (16 bit A/D conversion) in an adjacent room and stored on computer hard disk (sampling rate 500 Hz). Respiratory activity was assessed by placing two respiration belts on the participant that measured abdominal and thoracic expansion and contraction. Electrodermal activity was recorded (high-pass filter: 0.025 Hz) by the constant-voltage method (0.5 V). Beckman Ag–AgCl electrodes (8 mm diameter active area) filled with a skin conductance paste (Biopac) were attached to the palmar side of the middle phalanges of the second and third fingers of the participants' non-dominant hand. Heart rate was assessed by fixing Biopac pregelled disposable electrodes under the participants' left and right wrists. A third electrode was placed on the left ankle. The signal was amplified by 1,000 and low-pass filtered (30 Hz). Electrocardiographic R waves were detected offline, and intervals between heartbeats were converted into heart rate, expressed in beats per minute (BPM). Surface electromyography (EMG) was collected, digitized, and stored (bandwidth 0.1 to 417 Hz, sample rate: 2,048 Hz) with a BIOSEMI Active-Two amplifier system (BioSemi Biomedical Instrumentation, Amsterdam, the Netherlands). Six active electrodes were placed over the right frontalis, corrugator, and zygomaticus regions of the face, corresponding to three distinct bipolar montages of interest (Fridlung and Cacioppo, 1986). Two additional electrodes placed above the inion (the common mode sense active electrode and the driven right leg passive electrode) were used as recording references and ground electrodes[1]. Conventional bipolar montages were then calculated from electrode pairs for each muscle by subtracting the activity of one electrode placed over the muscle from the activity of the other nearby electrode in Brain Vision Analyzer software (Brain Products, Gilching, Germany). Signals were then filtered with a 20 to 400 Hz band-pass digital filter, rectified, and low-passed filtered below 40 Hz.

Physiological Data Analyses

Respiration Parameters

The voltage amplitude of the inhalation phase during the olfactory stimulus presentation was reported and constitutes the main respiratory control.

Electrodermal Activity

Specific skin conductance responses (SCRs) to odors were measured in microSiemens and analyzed offline. They were scored as changes in conductance starting in the -s to 4-s interval after the beginning of inhalation (Dawson et al., 1990). SCRs were square root transformed to normalize the data (Edelberg, 1972).

Facial Muscle Activity

Electromyography amplitude during the 1 s before olfactory stimulus presentation served as the baseline. To allow us to examine the temporal profiles of facial EMG for 5 s after inhalation of different olfactory stimuli, we expressed mean

[1]http://www.biosemi.com/faq/cms&drl.htm

EMG amplitudes during subsequent 1 s time intervals as a percentage of the mean amplitude of the baseline. Percentage scores were introduced to standardize the widely differing absolute EMG amplitudes of individual participants and thus enable comparison between individuals and groups (e.g., de Wied et al., 2006).

Heart Rate

The biphasic heart response consists of cardiac acceleration peaking at about 3 s followed by a decrease in heart rate, with a minimum reached at about 6 s after the onset of inspiration (see Delplanque et al., 2009). We analyzed the maximum negative variation in the 5 to 8 s window following stimulus presentation (heart rate deceleration) to investigate whether this phase was sensitive to stimulus pleasantness. The heart rate time course during the 10 s before olfactory stimulus presentation served as the baseline. We averaged the heart rate values within successive 200 ms periods, leading to 15 heart rate scores during the 3 s interval. We then expressed these 15 heart scores as a percentage of the BPM of the baseline. Percentage scores were introduced to standardize the differing absolute BPM variations of individual participants and thus enable comparison between individuals and groups.

Statistical Analyses

In order to obtain our intra-subject measures, two types of odors and fragrances were distinguished on the basis of each participant's own ratings: *pleasant* (two highest hedonicity scores) and *unpleasant* (two lowest hedonicity scores). We also performed correlations between the mean pleasantness rating of each odorant stimulus corresponding to a given hedonic order (1: least liked odorant to 32: most liked odorant) across individuals and the strength of its corresponding physiological response (heart rate or electrodermal response).

We computed a mixed model analysis of variance (ANOVA), with pleasantness (2: *pleasant, unpleasant*) as the within-subject repeated factor and group (2: *Odor, Fragrance*) as the between-subject factor to analyze subjective ratings, heart rate, electrodermal response, and respiratory parameters.

In the case of facial muscle activity, a Time factor (five: *0–1,1–2,2–3,3–4,4–5 s*) was added to account for the temporal evolution of the signal, decomposed in five 1 s time intervals. We tested the significance of paired comparisons between experimental conditions, using Tukey *post hoc* comparisons (PHCs). All tests were performed by using STATISTICA 12[2].

RESULTS

Subjective Ratings

The analysis performed on hedonicity, familiarity, and intensity ratings revealed a main effect of pleasantness on these three indicators [$F(1,35) = 839.03$, $p < 0.001$, $\eta^2_s = 0.96$; $F(1,35) = 77.98$ $p < 0.001$, $\eta^2 = 0.69$; and $F(1,35) = 7.28$, $p = 0.011$, $\eta^2 = 0.17$, respectively]. Pleasant stimuli (*odors* and *fragrances*) were systematically evaluated as being more pleasant (average: 8.44) than unpleasant stimuli (1.81), confirming that a clear hedonic distinction was made (**Figure 1A**) based on each participant's own evaluation. Pleasant stimuli were also perceived as being more familiar (7.43) than unpleasant stimuli (4.22; **Figure 1B**). The group × pleasantness interaction was significant for both hedonic and familiarity ratings [$F(1,35) = 55.92$, $p < 0.001$, $\eta^2 = 0.61$; $F(1,35) = 12.12$, $p = 0.001$, $\eta^2 = 0.26$], revealing a more pronounced hedonic distinction according to pleasantness for *Odors* (PHC $p < 0.001$ for hedonicity and familiarity) compared with *Fragrances* (PHCs $p < 0.003$ for hedonicity and familiarity), since unpleasant *Odors* were rated lower than unpleasant *Fragrances* (**Figure 1A**; PHC, $p < 0.001$). This interaction was not significant for intensity ratings [$F(1,35) = 0.14$, $p = 0.709$, n.s., $\eta^2 = 0.004$; **Figure 1C**],

[2]http://www.statsoft.com

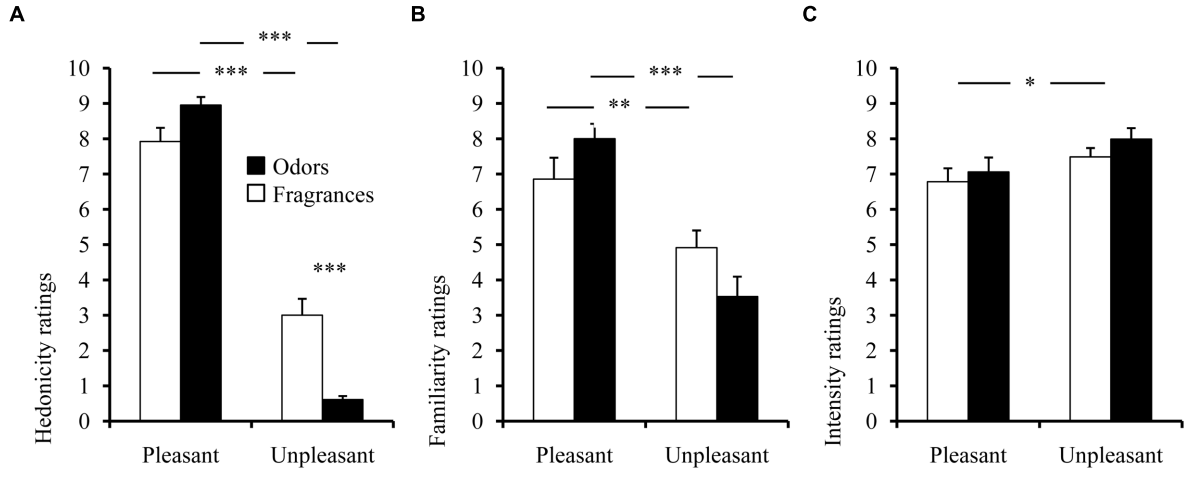

FIGURE 1 | Mean subjective ratings of (A) hedonicity, (B) familiarity, and (C) intensity of *Odors* and *Fragrances* according to pleasantness. Vertical bars denote standard errors to the mean. Significance levels for pleasantness effect: n.s.: not significant, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

indicating that unpleasant olfactory stimuli were more intense (7.47) than pleasant stimuli (6.92), regardless of the pleasantness spectrum (*Odors* or *Fragrances*).

Peripheral Physiology

Group × pleasantness interactions were also observed for both peripheral physiological measures, i.e., electrodermal activity and heart rate [$F(1,35) = 5.75$, $p = 0.022$, $\eta^2 = 0.14$; $F(1,35) = 7.33$, $p = 0.010$, $\eta^2 = 0.17$, respectively]. Unpleasant *Odors* elicited stronger SCRs than did pleasant *Odors* (PHC, $p = 0.033$) and unpleasant *Fragrances* (PHC, $p < 0.001$; **Figure 2A**).

Unpleasant *Odors* also specifically induced a weaker heart deceleration than did pleasant *Odors* (PHC, $p = 0.007$), unlike *Fragrances* in which this effect was not significant (**Figure 2C**).

Since both interactions were significant, we performed separate regression analyses between mean hedonicity ratings and SCRs or heart rate, for *Odors* and *Fragrances*, respectively. A significant U-shaped quadratic correlation was found for *Odors* on the SCRs only ($r^2 = 0.26$, $p = 0.013$), with higher SCRs in response to *Odors* on the extremes of the valence spectrum (very unpleasant or very pleasant), but lower responses to (neutral) *Odors* in the middle of the spectrum (**Figure 2B**). These results were confirmed by a supplementary statistical analysis conducted on electrodermal responses to *Odors*. We conducted a repeated measures ANOVA with pleasantness as **three-level** within-subject repeated factor, in which we took into account a third type of neutral *Odors* (two hedonicity scores located around the median score), in addition to pleasant and unpleasant ones. This analysis revealed a main pleasantness effect [$F(2,34) = 8.31$, $p = 0.001$, $\eta^2 = 0.33$]. A subsequent *post hoc* planned quadratic comparison was performed, with weights of 1, –2, and 1 assigned to pleasant, neutral and unpleasant *Odors*, respectively. This planned comparison was significant [$F(1,17) = 13.47$, $p = 0.002$], confirming that lower SCRs were elicited in response to neutral *Odors* compared to pleasant and unpleasant ones.

In addition, *Odor*-induced heart rate variations correlated negatively with hedonic scores ($r^2 = 0.43$, $p < 0.001$; **Figure 2D**). However, no significant correlations with *Fragrance* hedonicity ratings were found for either fragrance-induced SCRs or heart rate variations.

Finally, we examined the effects of stimulus pleasantness on respiratory control measures to rule out any confounds that could cause differences at the physiological level. No significant effects of stimulus pleasantness were found on any of the respiratory control measures [$F(1,35) = 2.96$, $p = 0.094$, n.s., $\eta^2 = 0.03$, and $F(1,35) = 0.27$, $p = 0.600$, n.s., $\eta^2 = 0.01$, for abdominal and thoracic respirations, respectively; **Figures 2E,F**], although the general thoracic respiratory amplitude was higher in the *Fragrance* group [$F(1,35) = 7.52$, $p = 0.001$, $\eta^2 = 0.18$].

Facial Muscle Activity

In general, *Odors* elicited a much stronger expressive activity than did *Fragrances* [main group effects: $F(1,33) = 4.74$, $p = 0.037$, $\eta^2 = 0.3$, and $F(1,33) = 8.75$, $p = 0.006$, $\eta^2 = 0.21$, for corrugator and zygomaticus, respectively]. We found a significant triple Time × Pleasantness × Group interaction for corrugator activity [$F(4,132) = 2.45$, $p = 0.050$, $\eta^2 = 0.07$]. In order to examine these effects in more detail, we performed two separate secondary ANOVAs on corrugator activity, where Time (5: corresponding to 5 s × 1 s windows) was introduced as a multiple dependent variable and pleasantness (2) as a within-subject factor for *Odors* and *Fragrances* separately, since muscular activity shows a sequential evolution (see Delplanque et al., 2009).

These analyses revealed a Time × Pleasantness interaction in *Odor*-induced corrugator activity [$F(4,64) = 2.67$, $p = 0.040$, $\eta^2 = 0.14$], with an increase in the percentage of muscular activity in response to unpleasant *Odors* as compared with pleasant *Odors* in all time windows except the first one (PHC $ps \leq 0.004$; **Figure 3A**). For better visualization of the effect, the continuous evolution of corrugator activity was plotted both as a function of time and of hedonicity scores. The resulting 3D plot showed a combined slope increasing across time toward lower hedonic values of *Odors* (**Figure 3B**). *Fragrance*-induced corrugator activity increased both as a function of unpleasantness [$F(1,17) = 5.19$, $p = 0.036$, $\eta^2 = 0.23$] and of time [$F(4,68) = 9.83$, $p < 0.001$, $\eta^2 = 0.36$; **Figure 3C**], although this increase was relatively small compared with that induced by *Odors* (**Figure 3D**).

The zygomaticus also showed increased activity in response to both pleasant olfactory stimuli [main pleasantness effect: $F(1,33) = 6.50$, $p = 0.016$, $\eta^2 = 0.16$; **Figure 4A**], although the increase in activity over time was more important for *Odors* than for *Fragrances* [Time × Group interaction: $F(4,132) = 3.94$, $p = 0.005$, $\eta^2 = 0.11$; **Figure 4B**].

DISCUSSION

In this experiment, we assessed whether subjective, physiological, and expressive indicators differentiate between different ranges of odor and fragrance pleasantness. Our results showed strong distinctions of pleasant and unpleasant *Odors* on the basis of subjective, physiological, and expressive data, in agreement with previous studies (Alaoui-Ismaïli et al., 1997; Bensafi et al., 2002b; Armstrong et al., 2007; Bradley et al., 2008; Delplanque et al., 2009; Sequeira et al., 2009). On the other hand, *Fragrances*, belonging to a more restricted pleasantness spectrum, were mostly differentiated on the basis of their subjective ratings, rather than physiological and expressive indicators.

More specifically, subjective ratings were sensitive to pleasantness, with unpleasant olfactory stimuli perceived as being less familiar and more intense, in line with previous findings (Doty, 1975; Ayabe-Kanamura et al., 1998; Royet et al., 1999; Delplanque et al., 2008), although this distinction between pleasant and unpleasant olfactory stimuli was stronger for *Odors* than for *Fragrances*. At the physiology level, heart rate differentiated between levels of *Odor* pleasantness linearly: the more pleasant the *Odor*, the stronger the decrease, which is in line with previous findings (Soussignan et al., 2005; Delplanque et al., 2009). Electrodermal responses were sensitive to either very pleasant or very unpleasant stimuli. The supplementary analyses performed with an additional category of neutral *Odors* revealed

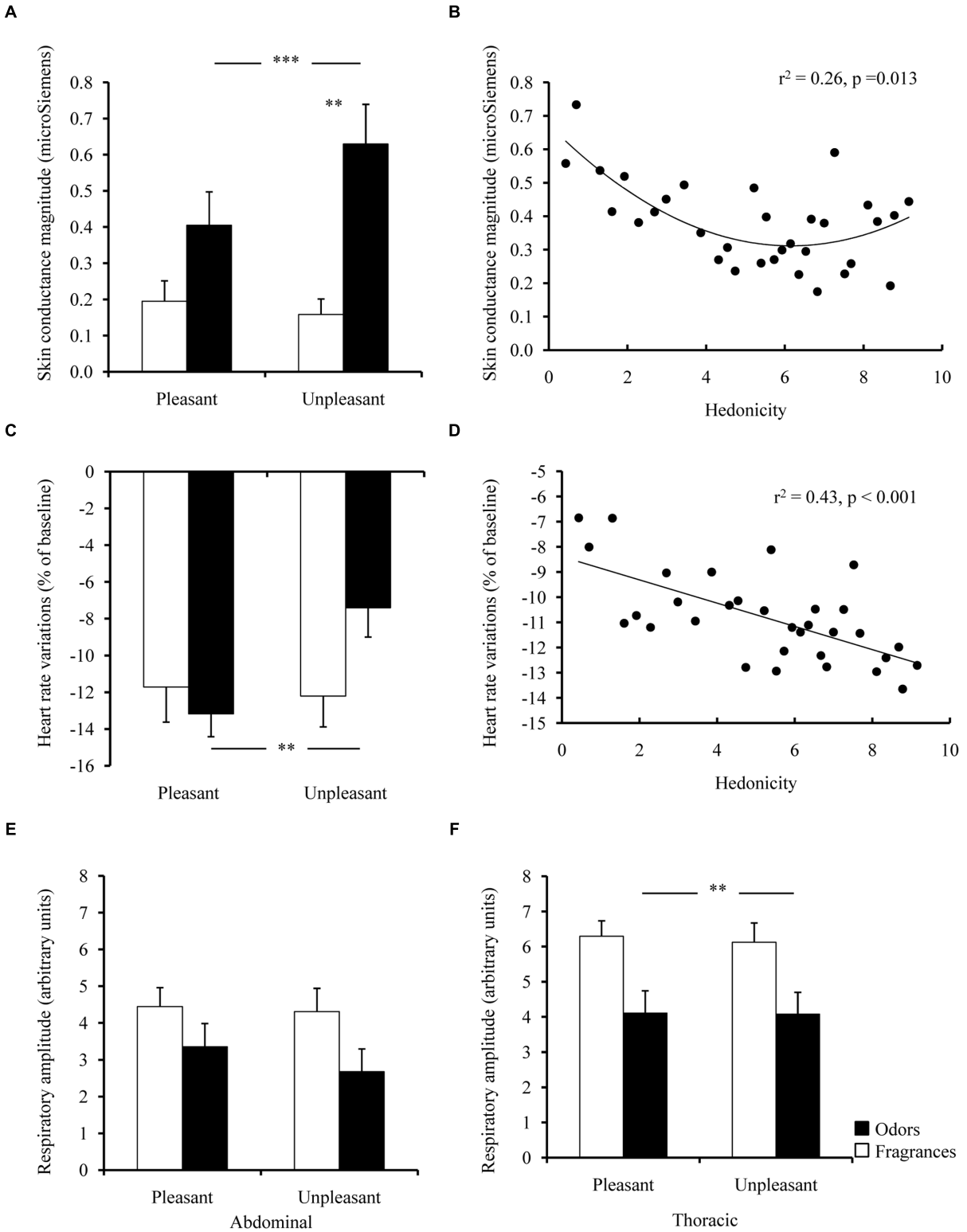

FIGURE 2 | Peripheral physiology. (A) Skin conductance, **(C)** heart rate variation (BPM; 5–8 s after stimulus presentation), and **(E)** abdominal and **(F)** thoracic respiratory amplitudes for the intra-individually determined pleasant and unpleasant *Odors* and *Fragrances*. Significant correlations between mean ratings for all odors corresponding to a given hedonic order across individuals **(B)** skin conductance responses and **(D)** heart rate variations. In the graph abscissa, odors pleasantness ratings are arranged from those of least liked odors (corresponding to hedonic order 1), to those of most liked odors (corresponding to hedonic order 32). Vertical bars denote standard errors to the mean. Significance levels for pleasantness effect: n.s.: not significant, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

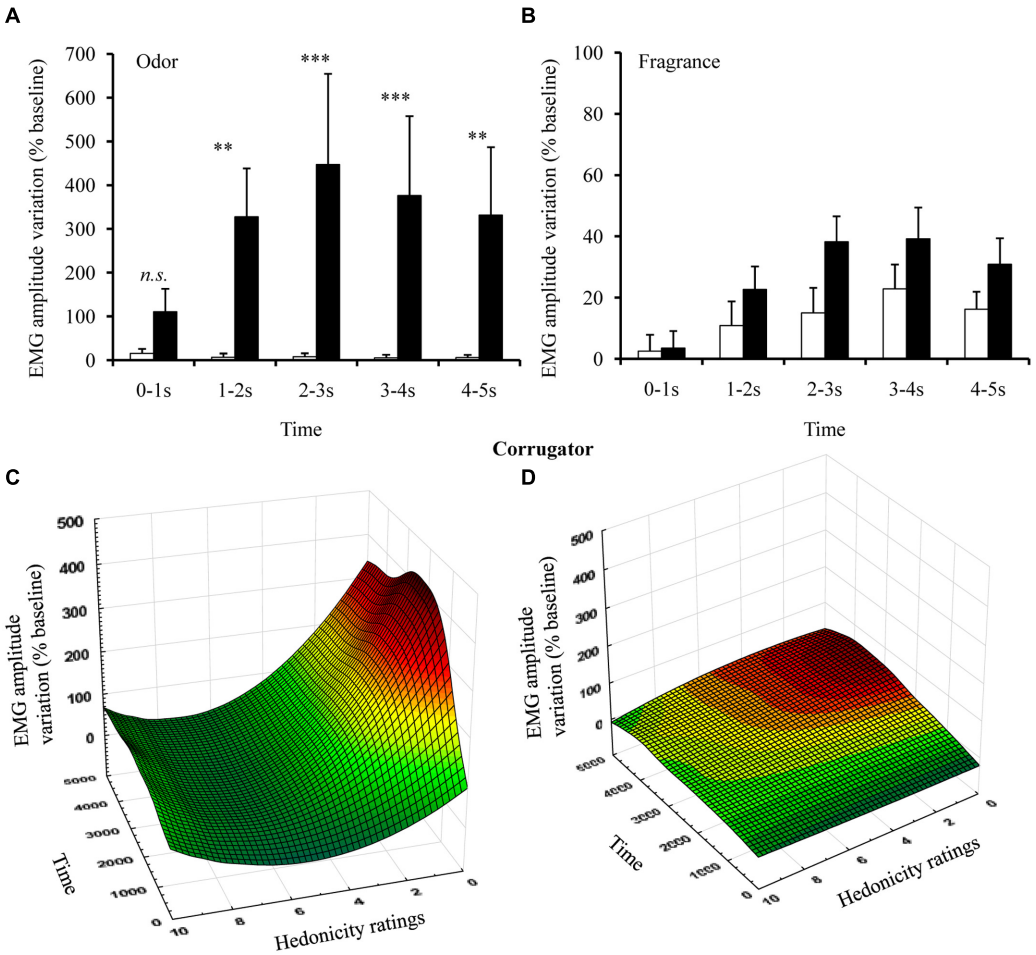

FIGURE 3 | Corrugator muscle activity. (A) *Fragrance*- and **(C)** *Odor*-related corrugator activities (EMG; % of baseline) for the intra-individually determined pleasant and unpleasant odors. **(B)** *Fragrance*- and **(D)** *Odor*-related corrugator activity changes (EMG; % of baseline) as a function of time (ms) and hedonicity for all stimuli. Vertical bars denote standard errors to the mean. Significance levels for pleasantness effect: n.s.: not significant, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

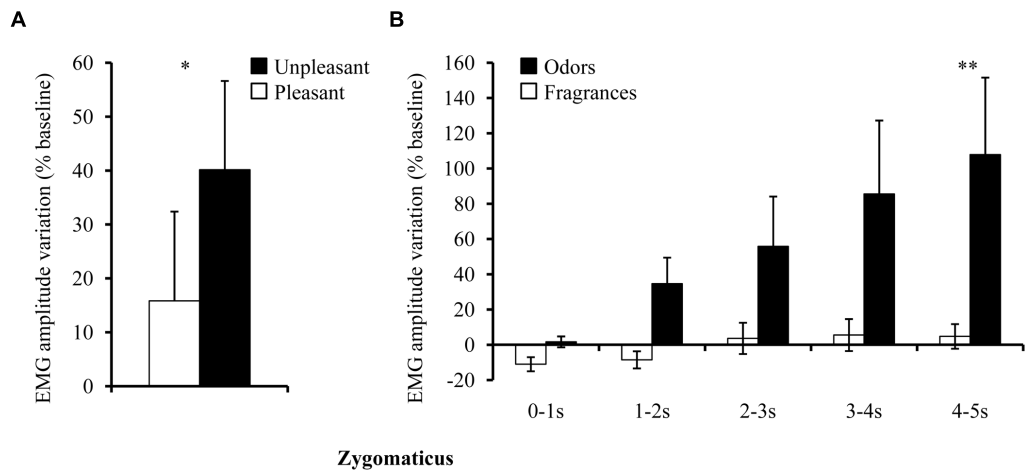

FIGURE 4 | Zygomaticus muscle activity (EMG; % of baseline) for *Fragrance* and *Odors*. (A) Main pleasantness effect. **(B)** Main group effect. Vertical bars denote standard errors to the mean. Significance levels for pleasantness effect: n.s.: not significant, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

weaker responses to neutral stimuli, suggesting sensitivity to arousing stimulations, in keeping with the U-shaped relation between odor hedonicity and arousal (Doty, 1975; Bensafi et al., 2002b,c; Winston et al., 2005).

No statistically significant modulation of these two physiological indicators (heart rate, SCR) was observed for *Fragrances*, however, suggesting that the sensitivity of physiological indicators to related odors with a narrow range of pleasantness, such as fragrances, is limited, even although subjective hedonic differentiations were clearly reported by the participants. The fact that no effect of pleasantness was observed in any of the respiratory control measures indicates that it is unlikely that the differences observed at the physiological level could be caused by differential patterns of respiration as a function of odor pleasantness. Finally, pleasantness was also differentiated at the expressive level through corrugator activation and, to a lesser extent, through zygomaticus activation, echoing prior results (Bensafi et al., 2002c; Soussignan et al., 2005; Armstrong et al., 2007; Delplanque et al., 2009). The expressive component responded to both *Odors* and *Fragrances*, although *Fragrance*-related activity was much weaker.

This experiment provided information concerning the ability of classic psychophysiological measures to investigate subtle differences in emotional reaction to olfactory stimuli, as it sheds light on the relation between physiological indicators and subjective ratings when characterizing odors with a wide range of pleasantness versus fragrances with a narrow range of pleasantness. Whereas there were clear differences in physiological reactions to odors that were very different in terms of pleasantness, those differences almost entirely vanished when a particular family of products (i.e., only fragrances) with a restricted range of pleasantness was tested. This does not mean that finding subtle physiological differences in response to a restricted range of products is not possible. Rather, it seems that with classic and easy-to-set-up measures, such subtle differences are unlikely to be observed.

Apart from the technical and methodological constraints, there are clear theoretical reasons to expect such a pattern of results. According to appraisal theories of emotion, e.g., the component process model (Scherer, 1982, 2001), the subjective feeling and the physiological response associated with a specific stimulus (e.g., a given odorant) are separate components whose synchronized modification entails an emotional percept. Although related, subjective feeling and physiological response reflect different components of the emotional response. A modification of the subjective feeling component—which is considered to reflect changes in the other components—will not necessarily entail a difference in the physiological or EMG data, the latter reflecting the expressive component. Our results emphasize the importance of measuring several components of an emotional episode.

On the other hand, the physiological responses observed during an emotional episode should be adapted to the demands of the physical and social environment in order to prepare the individual for action (Frijda, 1987; Sander et al., 2005). Similar to emotional cues triggering adaptive behaviors in reaction to environmental events, olfactory stimuli modulate motivational states in a powerful fashion through their relevance, for example, when malodors induce avoidance reactions through the elicitation of profound aversion or disgust (Royet et al., 2001; Gottfried et al., 2002; Anderson et al., 2003). Olfactory stimuli are thus prone to inducing behavioral adaptations to changes in the environment (Pause et al., 2003), resulting in approach or avoid action tendencies (Frijda, 1987). Olfactory stimuli can even be involved in more complex functions, classified as adaptive behaviors for survival: ingestion, hazard avoidance, social communication, and emotional contagion (see Stevenson, 2010 for a review).

Characterizing consumer preferences by objective physiological and/or EMG measures is a goal that many industries would currently like to attain. These measured responses should be able to differentiate among odors that evoke representations linked to different functions of olfaction (Stevenson, 2010), scattered along a wide pleasantness spectrum. In contrast, it is unlikely that the physiological system would respond differentially when the range of pleasantness is narrow, as is the case with fragrances.

Such subtle differences are well characterized by subjective appreciations, as previous evidence suggested that odor-elicited feelings are complex and varied (Chrea et al., 2009). Aside from the utilitarian functions they embody, odor-borne feelings may also be related to more elaborate forms of hedonic appreciation, such as complex esthetic feelings experienced with music (Zentner et al., 2008; Trost et al., 2012). Odor-borne feelings can be accurately described by specific semantic scales (Chrea et al., 2009; Ferdenzi et al., 2011, 2013; Delplanque et al., 2012), which are a reliable tool for the discrimination of products with similar liking scores such as fragrances or flavored products (Porcherot et al., 2010). In the domain of fragrances, differences in ratings of liking have been found when the same fragrances are rated with or without brand labels (Moskowitz, 1979), an effect commonly observed in food perception (Spinelli et al., 2015). Therefore, an important dimension to consider when it comes to fragrances—in particular, fine perfumes—is luxury because of its ecological occurrence in brand information. A luxurious qualification confers additional value and satisfaction to a given product, as well as supplementary information about its source, yet it may not reflect urgent necessities (Kapferer, 1997; Megehee and Spake, 2012) or differential survival-related functions (e.g., they would all be related to well-being; see Stevenson, 2010). It would thus be interesting to investigate the extent to which self-reported and psychophysiological measures could be influenced by information regarding the luxurious character of a fine perfume. This could be done by presenting the same group of participants with fragrances, with and without the corresponding labels, truthful or not, on different days. Aside from liking, the rewarding sensation experienced during any agreeable sensory stimulation also includes a "wanting" component, which translates into motivation to invest effort in order to obtain such a reward (Berridge and Robinson, 1998; Pool et al., 2015). By measuring, for example, the willingness to pay for a specific product, the wanting component would allow a more complete picture of fragrance-based elicited reward and would

perhaps enhance the discriminative power of subjective measures for similarly pleasant products.

CONCLUSION

In summary, this study shows that emotions elicited by odors that display a wide range of reported pleasantness can be distinguished by both subjective feeling and physiological indicators. These physiological differences almost entirely vanish when odorants belong to a much more restricted pleasantness range, even though the subjective feelings still differ. This work contributes to the literature on emotions by emphasizing the multi-componential nature of emotion and the importance of considering several components when studying olfactory-induced emotions. Finally, our results address the current trend found in many industries to characterize consumer behavior by using physiological measures. Although differences can be expected in response to heterogeneous products in terms of pleasantness, physiological measures appear to show limited sensitivity in distinguishing among similarly pleasant products.

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Chemosensory Communication of Gender Information: Masculinity Bias in Body Odor Perception and Femininity Bias Introduced by Chemosignals During Social Perception

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Human body odor is a source of important social information. In this study, we explore whether the sex of an individual can be established based on smelling axillary odor and whether exposure to male and female odors biases chemosensory and social perception. In a double-blind, pseudo-randomized application, 31 healthy normosmic heterosexual male and female raters were exposed to male and female chemosignals (odor samples of 27 heterosexual donors collected during a cardio workout) and a no odor sample. Recipients rated chemosensory samples on a masculinity-femininity scale and provided intensity, familiarity and pleasantness ratings. Additionally, the modulation of social perception (gender-neutral faces and personality attributes) and affective introspection (mood) by male and female chemosignals was assessed. Male and female axillary odors were rated as rather masculine, regardless of the sex of the donor. As opposed to the masculinity bias in the odor perception, a femininity bias modulating social perception appeared. A facilitated femininity detection in gender-neutral faces and personality attributes in male and female chemosignals appeared. No chemosensory effect on mood of the rater was observed. The results are discussed with regards to the use of male and female chemosignals in affective and social communication.

Keywords: sex, sexual dimorphism, sex recognition, mood, body odor, olfaction

INTRODUCTION

Humans, although seen as the most highly scented apes (Stoddart, 1990), have been less extensively studied compared to non-primate mammals when it comes to chemosensory communication. Nonetheless, human chemosignalling research has revealed that stable and temporal features of a sender are communicated through the chemical senses (Lübke and Pause, 2015). Especially, when male and female communication via axillary odor is studied, features of a sender affect various levels in a receiver, e.g., social behavior (Frumin et al., 2015), emotional perception (Zhou and Chen, 2009; Albrecht et al., 2011), memory function (Alho et al., 2015),

social evaluation (Mitro et al., 2012), attractiveness, and mating preferences (Wedekind and Füri, 1997; Thornhill et al., 2003; Havlicek et al., 2005).

Research on gender-related differences in chemosensory communication reveals the impact of chemosignals on sexual attraction and mate choice (Doty and Cameron, 2009). Chemosensory mate perception is largely affected by female relationship status and menstrual cycle phase (Havlicek et al., 2005; Rantala et al., 2006) as well as intake of hormonal contraception (Roberts et al., 2008). In a study on the chemosensory effect on sexual attraction and mate choice in male raters, it was found that male raters can distinguish between ovulating and non-fertile female body odor (Kuukasjärvi et al., 2004) and that they display higher testosterone levels during exposure to an ovulating female's body odor (Miller and Maner, 2009). Female odor raters explicitly value male body odor pleasantness (Herz and Cahill, 1997; Herz and Inzlicht, 2002) and are able to assess male attractiveness and fluctuating asymmetry, a marker of developmental stability (Thornhill and Gangestad, 1999).

More importantly, features of the receiver such as biological sex and sexual orientation (Sergeant et al., 2007; Lübke et al., 2012), hormonal status (Roberts et al., 2008) or chemosensory sensitivity to chemosignals (Pause et al., 1999) influence the chemosensory communication process. Nevertheless, body odor sampling studies with both male and female donors are still rare. Mere sex discrimination ability based on female and male axillary odor has been examined in previous research (Russell, 1976; Hold and Schleidt, 1977; Schleidt, 1980; Doty, 1981) stating that humans are able to marginally discriminate between male and female axillary odor. Male body odor is perceived as more musky (Russell, 1976), more intense and less pleasant than female body odor (Hold and Schleidt, 1977; Doty et al., 1978; Schleidt et al., 1981; Mitro et al., 2012). It has further been established that higher chemosensory discrimination of body odors is more frequent for female raters and that the body odor of the opposite sex is expected to smell more pleasant (Hold and Schleidt, 1977; Sergeant, 2010; Mitro et al., 2012).

Ample evidence is pointing to sex-specific differences in male and female body odor. Chemical analyses of volatile compounds in axillary sweat provide information about distinct chromatographic profiles of male and female samples (Penn et al., 2007), and even non-volatile odor precursors of axillary sweat (fatty acids and thiols) were shown to vary concentration-wise in a sex-specific manner (Troccaz et al., 2009). These findings support the idea that sex-related body odor differences do not only exist but can be also communicated among individuals.

Besides natural axillary odor, chemical compounds that are most commonly supposed to have a communicative function are applied to explore chemosensory communication of sex information (e.g., Gustavson et al., 1987; Jacob and McClintock, 2000; Savic et al., 2001; Wysocki and Preti, 2004; Grammer et al., 2005; Lundström et al., 2006; Olsson et al., 2006; Wyart et al., 2007; Zhou et al., 2014) and were shown to affect masculinity and femininity ratings of schematic body movements (Zhou et al., 2014).

Concluding from chemosensory research on sex and gender communication, the conveyed chemosensory information seems to be modulated by the sex of the sender (donor) and the receiver (rater). Taking both factors into account is crucial for an accurate investigation of the still poorly understood chemosensory effect of male and female body odor on higher cognition, emotion, and behavior in a receiver. As it was shown that sex and age of a donor induce rapid mood changes in receivers (e.g., Chen and Haviland-Jones, 1999), affective and social communication via the chemical senses can only be accurately examined in case modulating effects of communicated gender information from the sender to the receiver are known. A study applying the putative chemosignal androstadienone to male and female participants (Hummer and McClintock, 2009) revealed that emotional information processing was altered during its exposure compared to a control odor (clove). While subliminal face processing and perception of emotional words was affected by androstadienone, emotional introspection (mood) was not affected. This finding relates to the discussion by Grammer et al. (2005) of whether chemosignals rather influence socially oriented perception of conspecifics (e.g., evaluation of others, sexual attractant) or self-perception (e.g., as mood enhancer or modulator) in human chemosignalling in general as well as during chemosensory gender communication. This question had not been considered before.

Therefore, we aim to systematically examine the chemosensory information emitted from male and female donors to male and female raters in odor perception, social perception and emotional introspection. We hypothesize that, in a chemosensory rating task, male and female chemosensory samples produce distinguishable intensity and pleasantness ratings. In a masculinity-femininity rating task, we expect male and female chemosensory samples to be correctly assigned by a collective of male and female raters. Furthermore, and beyond mere communication of sex information, we explore whether body odors modulate social or self-perception. As our chemosensory samples convey social information, we expect the perception of social stimuli (in a personality rating task) and conspecifics (in face and word rating tasks) to be modulated rather than introspection (mood rating). More precisely, regarding the rating gender-neutral personality attributes and faces, we expect female chemosensory samples to be associated with a femininity bias and male chemosensory samples to be associated with a masculinity bias.

MATERIALS AND METHODS

Participants

The present study was carried out in accordance with the recommendations of the Ethics committee of the medical faculty of RWTH Aachen University and in accordance with the Declaration of Helsinki with written informed consent provided from all participants. In total, 32 healthy participants (raters) took part in the experiment. Participants were healthy, heterosexual (Martins et al., 2005) non-smokers with no current medication or drug intake (Doty and Bromley, 2004). All eligible participants rated their current sexual behavior (past 12 months)

as exclusively heterosexual on the 7-point Kinsey Scale (Kinsey et al., 1948), ranging from 0 (exclusively heterosexual with no homosexual behavior) to 6 (exclusively homosexual with no heterosexual behavior). To ensure that no exogenous odors contaminate the body odors, dietary and hygienic instructions for 2 days prior to the experiment included abstinence from alcohol, caffeine, garlic, onions, spices and the use of deodorants, body fragrances and lotions (Albrecht et al., 2011). Participants showered with scent-free body wash and shampoo, did not shave the armpits and refrained from visiting public pools and saunas. Female participants were scheduled to always participate in the same phase of their menstrual cycle. All females stated to be non-pregnant, did not take hormonal contraception, had experienced a regular menstrual cycle during the six months preceding their participation and were always tested in the same cycle phases each (follicular phase: $n = 4$, periovulatory phase: $n = 5$ and luteal phase: $n = 7$). Phases were defined as a count of post-menstrual onset days based on self-report (Lundström et al., 2006) e.g., for a menstrual cycle length of 28 days, we defined follicular phase from day 1 to 11, periovulatory phase from day 12 to 16 and luteal phase from day 17 to 28. One participant was excluded due to a lack of task compliance. The final sample consisted of $n = 31$ participants (age range = 19–47 years), including 15 males ($M = 27.80$ years, $SD = 8.83$ years) and 16 females ($M = 29.56$ years, $SD = 9.33$ years). The two groups did not differ in age, $t(29) = 0.539$, $p = 0.594$. The odor identification test MONEX-40 (Freiherr et al., 2012) classified all participants as normosmic ($M = 32.20$, $SD = 2.77$; range = 26–38).

Donation Procedure and Chemosensory Samples

In total, 29 healthy participants (donors) took part in the body odor donation. Female participants did not take hormonal contraception, experienced regular menstrual cycles and stated to be non-pregnant. Donors underwent the same dietary and hygienic instructions as the raters. Two participants were excluded due to acute medication intake prior to the experiment and blood circulation problems. The final sample consisted of $n = 27$ participants (range = 20–49 years), including 14 males ($M = 25.93$ years; $SD = 8.87$ years) and 13 females ($M = 26.31$ years; $SD = 7.54$ years). The two groups did not differ in age, $t(25) = 0.119$, $p = 0.906$.

Upon arrival, participants were informed about the purpose of the study and screened for dietary and hygienic compliance. After cleaning their armpits with scent-free wipes, cotton pads were attached under both armpits. Participants wore a long-sleeved cotton shirt washed with scent-free detergent. In order to increase sweat production, all participants wore a synthetic raincoat. They exercised in a training room with room temperature on an ergometer for 20 min with 100 W/h and 60–80 cycles per minute. After a short break, the pads were replaced and the donation procedure was repeated. Before and after each donation, pulse and blood pressure were assessed (Omron IntelliTM sense, R7 HEM-632-E2; Omron Healthcare Co., Ltd. Kyoto, Japan). To ascertain a correct value, three measurements were performed (immediately after one another) and their mean was used as a final value. This resulted in three measurements in total: before the first 20 min donation, during the break between the first and the second 20 min donation and after the second 20 min donation. Upon successful completion of the donation, the donors were paid 20 Euros.

Immediately after donation, the chemosensory pads underwent an olfactory examination by the experimenter. Pads were not included in case the body odor was not free from perceivable exogenous odors (such as perfume, smoke or spices) or unusual odor intensity was detected ($n = 2$). To minimize odor contamination, pad handling was performed after disinfection of utensils and hands with isopropanol (70%). Each pad was cut in sixteen parts (quadrants of 1 cm × 1 cm). Male and female superdonor pools were created to assure homogeneous odor samples within the experimental groups and to reduce effects of individual variations. This method has been successfully used in prior donation studies (Albrecht et al., 2011; Dalton et al., 2013), which utilized large donor sample groups. Control samples of odorless clean cotton pads (no odor samples without chemosignals) were created and treated like the chemosensory samples in terms of cutting and freezing. The samples were kept in re-sealable storage bags at −80°C (Lenochova et al., 2009) for no longer than 5 months. Thus bacterial decomposition of the samples was avoided.

Application Procedure

Raters were invited to three experimental application sessions (within-subject design) within 3 months (one session every 28 days). Females were scheduled to always participate in the same phase of their menstrual cycle. In a double-blind randomized design, participants were exposed to one of the three chemosensory samples (male chemosignals, female chemosignals, and neutral odor) per application session. Thirty minutes before application, quadrants of four donors were randomly chosen from the superdonor pool and put in cotton filter masks. At the beginning and at the end of each of the three application sessions, participants' mood was assessed via self-rating (Watson et al., 1988). The response options were adapted to a 100-point VAS (0 = not at all or very slightly, 25 = a little, 50 = moderately, 75 = quite a bit, 100 = extremely) and mood before and after exposure to the chemosensory samples was compared. After the fitting of the mask under the noses of the participants (Albrecht et al., 2011), a familiarization phase of five minutes was applied to avoid influences of imminent hormonal changes in association with the odor presentation onset that potentially modulate the participants' task performance (Wyart et al., 2007). The experimental tasks took 20–25 min and the odor mask was removed after exactly 30 min of odor exposure. Participants were instructed to breathe normally and rate the masculinity-femininity dimension of the chemosignals, of personality-attributes and of faces. All tasks were computerized. Upon successful completion of all three testing appointments, the raters were paid 45 Euros.

Odor Perception Tasks

A three-alternative forced-choice test was performed at the beginning of the first session to evaluate odor discrimination

capacities. Participants indicated blindly among three samples (two distractors vs. one target sample) the one sample smelling differently with three repetitions of all target and distractor combinations (four discriminations per odor condition and twelve discriminations in total).

At the end of the last session, participants performed an odor-rating task where the odor dimensions masculinity-femininity, intensity, and pleasantness were assessed. Hedonic ratings included assessment of intensity, pleasantness, and familiarity of the chemosensory samples (male chemosignals, female chemosignals and no odor sample), and were performed on 100-point VAS ranging from 0 (*not intense/pleasant/familiar at all*) to 100 (*extremely intense/pleasant/familiar*). The masculinity-femininity ratings of the chemosensory samples were performed using 100-point VAS ranging from the endpoint *masculine* (0), to neutral, (50) to the endpoint *feminine* (100).

Social Perception Tasks

For the adjective-rating task, participants rated the masculinity and femininity of 20 gender-neutral adjectives describing persons and personality traits on 100-point VAS.

The gender-neutrality of the personality attributes was identified in a pilot study with 20 male ($n = 10$) and female participants ($n = 10$) evaluating the neutrality of 60 adjectives (Pauly et al., personal communication). This sample included masculinity-related personality attributes (e.g., brutal) as well as femininity-related personality attributes (e.g., caring). The participants rated the gender of the words on a 5-point rating scale with the endpoints very masculine (−2) and very feminine (2). In total, 20 gender-neutrally rated adjectives describing personality attributes ($M = 0 \pm 1$ SD) were included in the task (e.g., friendly, childish, discrete).

For the rating of the faces, gender-neutral faces were constructed using the female and male face of the Averaged Karolinska Directed Emotional Faces repertoire (Lundqvist and Litton, 1998) that are not expressing emotions (picture codes: MNES and FNES). Then, hair in both pictures was masked so that only facial features were visible (**Figure 1**). Subsequently, the male and female facial stimuli were merged with three different proportions (40% male + 60% female, 50% each, 60% male + 40% female) using the software MorphX (http://www.norrkross.com/software/morphx/morphx.php). In total, each of those gender-neutral faces was presented five times to the participants.

Other tasks with faces and words were additionally presented during the experimental sessions; the results are discussed elsewhere (Moellers, 2015). As dependent variables, gender-neutral personality attributes and faces were rated using 100-point VAS ranging from the endpoint *masculine* (0), to neutral, (50) to the endpoint *feminine* (100) during application of the chemosensory samples.

Statistical Analyses

The software package SPSS Statistics 22 (Armonk, NY, USA: IBM Corp.) was utilized for statistical analyses. One-sample *t*-tests were performed to investigate odor discrimination performance. Differences of discrimination performance between the pairs of chemosensory stimuli (odor pairs: male–female, female–no odor, male–no odor) was assessed with the help of a repeated-measures ANOVA with the within-subjects factor odor pair and the between-subject factor sex of the rater.

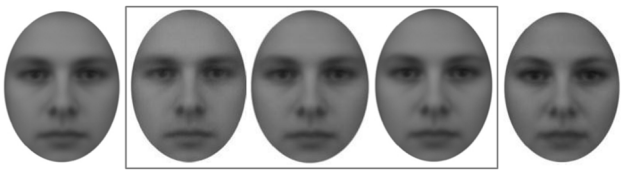

FIGURE 1 | Stimuli for the gender-neutral face rating task. The left face depicts a 100% masculine face template. The right face depicts a 100% female face template. Boxed faces in center depict merged gender-neutral experimental stimuli with different proportions (40% male + 60% female, 50% each, 60% male + 40% female). Reprint of facial stimuli with kind permission from Daniel Lundqvist. Taken and modified from Lundqvist and Litton (1998).

Normal distribution of the rating data was assessed by one-sample Kolmogorov–Smirnov-tests (all $p > 0.123$). Repeated-measures ANOVAs with the sex of the rater (male or female) as a between-subject factor and the sex of the donor (chemosensory samples: male chemosignals, female chemosignals, no odor sample) as a within-subject factor were utilized to analyze chemosensory communication of gender information in odor perception and social perception.

Bivariate Pearson correlations were used to assess associations between masculinity-femininity ratings and hedonic ratings (intensity and pleasantness) of the chemosensory samples. Violations of sphericity were adjusted via Greenhouse–Geisser correction and effect sizes were calculated for *F*-tests (η_p^2, partial Eta^2). Significant main effects and/or interactions were analyzed further using paired-comparison (*t*-tests for two samples and repeated samples) and corrected for multiple comparison using the Bonferroni method. *P*-values < 0.050 were considered significant.

RESULTS

Donation Exercise Intensity Analysis

In order to investigate general physical fitness, exercise intensity and associated sex differences, systolic and diastolic blood pressure as well as pulse were analyzed. Pulse varied significantly across measurements, $F(2,48) = 46.271$, $p < 0.001$, $\eta_p^2 = 0.658$, but not depending on the sex of the donor, $F(1,24) = 1.545$, $p = 0.226$, $\eta_p^2 = 0.060$. Systolic blood pressure varied significantly across measurements, $F(2,48) = 14.058$, $p < 0.001$, $\eta_p^2 = 0.369$, and depending on the sex of the donor, $F(1,24) = 5.352$, $p = 0.030$, $\eta_p^2 = 0.182$. Diastolic blood pressure varied significantly across measurements, $F(2,48) = 45.628$, $p < 0.001$, $\eta_p^2 = 0.655$, but not depending on the sex of the donor, $F(1,24) = 1.611$, $p = 0.217$, $\eta_p^2 = 0.063$. A significant interaction was found, $F(2,48) = 65.728$, $p = 0.037$, $\eta_p^2 = 0.128$.

No sex differences were found for pulse measures. As blood pressure is generally higher in normotensive men compared to

women (for a review: Lopez-Ruiz et al., 2008), sex differences in systolic and diastolic blood pressure were found prior to donation (**Table 1**). Physical fitness and exercise intensity after donation were comparable across sexes. In order to classify the strength of the physical activity during the donation, the heart rate (pulse in beats per minute; BPM) after the first donation session, male donors: $M = 104.21$, $SD = 19.42$; female donors: $M = 110.44$, $SD = 15.43$, and after the second donation session, male donors: $M = 107.52$, $SD = 19.84$; female donors: $M = 116.72$, $SD = 16.79$, can be classified as moderate and aerobic exercise zones (Fox et al., 1971).

Masculinity-Femininity Rating of the Chemosensory Samples

Masculinity-femininity rating of body odors varied significantly with sex of the donor (chemosensory samples: male chemosignals, female chemosignals or no odor sample), $F(2,58) = 9.526$, $p < 0.001$, $\eta_p^2 = 0.247$, and the sex of the rater (male or female), $F(1,29) = 9.866$, $p = 0.004$, $\eta_p^2 = 0.254$. Overall, exploratory comparisons revealed more feminine ratings of the no-odor samples, $M = 54.81$, $SD = 10.78$, than both female, $M = 40.77$, $SD = 15.6$); $t(30) = 3.707$, $p < 0.001$, and male chemosignals, $M = 44.42$, $SD = 13.42$; $t(30) = 3.709$, $p < 0.001$. Female raters accurately rated male chemosignals as more masculine than male raters, $t(29) = 3.599$, $p = 0.001$; whereas no sex differences were found for the rating of female chemosignals and no odor samples, all $p > 0.258$; **Figure 2**, **Table 2**.

Hedonic Ratings of the Chemosensory Samples

Intensity ratings were significantly different across chemosensory conditions, $F(2,58) = 11.580$, $p < 0.001$, $\eta_p^2 = 0.238$. No main effect of the sex of the rater can be reported, $F(1,29) = 0.003$, $p < 0.958$, $\eta_p^2 = 0.000$, but a significant interaction between sex of rater and donor was found, $F(2,58) = 4.059$, $p = 0.022$, $\eta_p^2 = 0.123$. Overall, paired-comparisons revealed no intensity differences between both the male, $M = 40.26$; $SD = 17.32$, and female, $M = 49.05$; $SD = 18.38$, chemosignals, $t(30) = 2.384$, $p = 0.072$, and the male chemosignals and the no odor sample, $M = 34.19$; $SD = 18.54$; $t(30) = 1.628$, $p = 0.342$. Only female chemosignals were perceived to be more intense than the no odor sample, $t(30) = 5.177$, $p < 0.001$. While female raters perceived male chemosignals, $M = 45.25$; $SD = 15.01$, to be as intense as female chemosignals, $M = 45.06$; $SD = 15.84$, $t(15) = 0.052$, $p = 0.959$, male raters perceived male chemosignals, $M = 34.93$; $SD = 18.49$, to be less intense than female chemosignals, $M = 53.30$; $SD = 20.43$; $t(14) = 3.218$, $p = 0.006$, **Figure 3**.

Pleasantness ratings significantly differed depending on the sex of the donor, $F(1,47) = 11.580$, $p < 0.001$, $\eta_p^2 = 0.285$, but not depending on the sex of the rater, $F(1,29) = 0.148$, $p = 0.704$, $\eta_p^2 = 0.005$. Exploratory paired-comparisons revealed

TABLE 1 | Sex-differences in physiological parameters pulse (in BPM) and blood pressure (in mm Hg) for male and female donors.

Physiological parameter		Sex of the donor		Sex difference
		Male	Female	
Pulse	Pre	86.74 (17.07)	94.67 (12.34)	0.135
	Post	107.52 (19.85)	116.72 (16.79)	0.219
Systolic blood pressure	Pre	121.40 (6.94)	112.56 (8.90)	0.008*
	Post	111.40 (8.77)	106.67 (6.24)	0.132
Diastolic blood pressure	Pre	80.69 (5.70)	74.21 (8.81)	0.031*
	Post	66.19 (6.93)	65.64 (4.69)	0.812

*Pre indicates measurements before physical activity, post indicates blood pressure after both 20 min donation sessions. Pairwise comparisons of sex differences of the donors (independent sample t-test) with Bonferroni-corrected p-values. *marks significant values p < 0.050.*

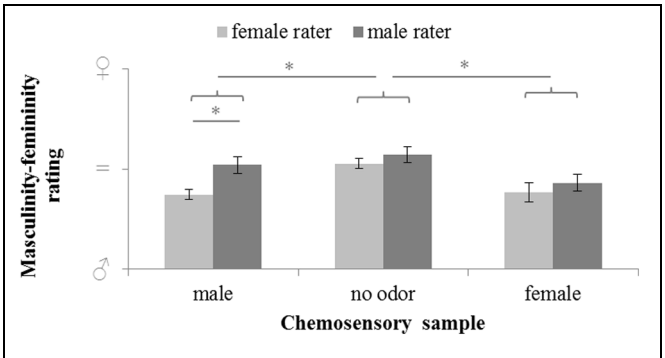

FIGURE 2 | Masculinity-femininity rating of chemosensory samples via a 100-point VAS by female and male raters (masculine ♂ = 0, neutral = 50; feminine ♀ = 100). * marks $p = 0.001$.

TABLE 2 | Mean values and standard deviations, M (SD), for the odor perception task (masculinity-femininity rating) and social perception tasks (gender-neutral personality attributes and faces) by sex of the rater (male and female) and chemosensory sample (no odor, male chemosignals and female chemosignals).

Chemosensory sample	Sex of the rater					
	Masculinity-femininity rating		Personality attributes rating		Face rating	
	Male	Female	Male	Female	Male	Female
Male chemosignals	52.00 (13.17)	37.31 (9.35)	52.39 (3.65)	51.75 (3.05)	55.46 (5.33)	47.05 (7.46)
Female chemosignals	43.17 (10.75)	38.53 (19.18)	50.85 (4.28)	52.64 (4.81)	55.17 (7.35)	46.45 (8.23)
No odor sample	57.10 (11.38)	52.66 (10.07)	53.58 (3.98)	53.40 (3.63)	56.60 (3.54)	42.01 (6.87)

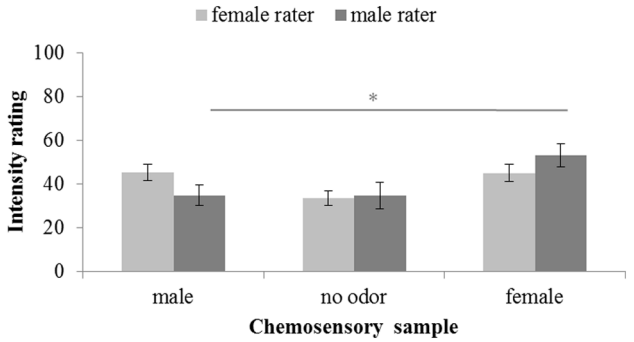

FIGURE 3 | Intensity rating of chemosensory samples via a 100-point VAS by female and male raters. * marks $p < 0.010$.

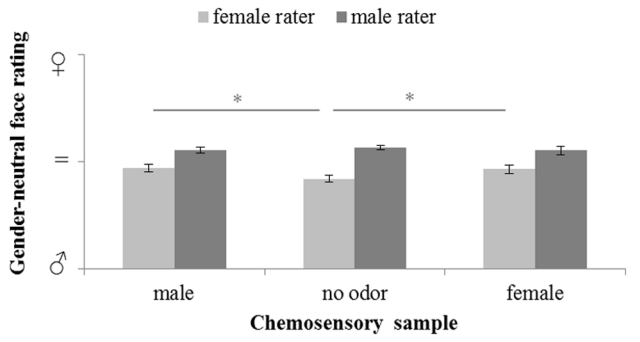

FIGURE 4 | Masculinity-femininity rating of gender-neutral faces via a 100-point VAS during exposure to chemosensory samples by female and male raters (masculine ♂ = 0, neutral = 50; feminine ♀ = 100). *marks $p < 0.050$.

that female chemosignals, $M = 38.48$; $SD = 17.04$, were perceived to be significantly less pleasant compared to male chemosignals, $M = 50.48$, $SD = 13.08$; $t(30) = 3.492$, $p = 0.006$, and compared to the no odor sample, $M = 52.05$, $SD = 8.89$; $t(30) = 3.945$, $p > 0.001$. Higher pleasantness of female chemosignals is associated with higher femininity, $r = 0.424$; $p = 0.018$, and lower intensity ratings, $r = -0.599$, $p < 0.001$. Odor ratings of male chemosignals were not significantly correlated, all $p > 0.438$. Familiarity ratings did not vary with the sex of the donor, $F(2,58) = 0.210$, $p = 0.811$, $\eta_p^2 = 0.007$, or the sex of the rater, $F(1,29) = 0.338$, $p = 0.565$, $\eta_p^2 = 0.012$.

Discrimination of Chemosensory Samples

During the discrimination task of the odors (twelve trials), the chance level of correct discrimination (33%) equals four correct out of twelve total trials (collapsed across odor conditions) and 1.33 correct out of four trials per odor condition. Participants correctly identified the target odor in 55.89% of all trials, $M = 6.71$, $SD = 1.95$; their general discrimination ability was significantly higher than chance level, one-sample t-test: $t(30) = 7.840$, $p < 0.001$. With regards to the different chemosensory conditions, participants were able to discriminate a number of pairs significantly higher than chance level in each odor condition, male-neutral: 58%, $M = 2.32$, $SD = 0.94$; male–female: 47.5%, $M = 1.90$, $SD = 1.19$; female-neutral: 63.75%, $M = 2.55$, $SD = 0.99$; all $t(30) \geq 2.68$, all $p \leq 0.012$. Discrimination capacity between the three different odor sample pairs did vary significantly, $F(2,58) = 3.32$, $p = 0.043$. Discrimination between the no odor sample and female chemosignals was significantly better than discrimination between male and female chemosignals, $p = 0.048$. No discrimination differences were found regarding a possible effect of the sex of the rater, $F(1,29) = 0.063$, $p = 0.804$.

Social Perception

The influence of male and female chemosignals on the perception of gender-neutral personality attributes revealed a main effect of the sex of the donors, $F(2,58) = 3.967$, $p = 0.024$, $\eta_p^2 = 0.120$, but not of the raters, $F(1,29) = 0.073$, $p = 0.789$, $\eta_p^2 = 0.003$. No difference was found between male and female chemosignals, $t(30) = 0.385$, $p = 0.703$. However, compared to the neutral sample, male and female chemosignals were both associated with more feminine adjective ratings, male: $t(30) = 2.716$, $p = 0.011$; female: $t(30) = 2.383$, $p = 0.011$, **Table 2**.

The influence of male and female chemosignals on the perception of gender-neutral faces (**Figure 4**) did not reveal a main effect of the sex of the donors, $F(2,58) = 1.685$, $p = 0.194$, $\eta_p^2 = 0.055$. However, a significant main effect of the sex of the raters, $F(1,29) = 27.152$, $p < 0.001$, $\eta_p^2 = 0.484$, and a significant interaction of the sex of the donors and raters, $F(2,58) = 4.892$, $p = 0.011$, $\eta_p^2 = 0.144$, was yielded. Paired-comparison revealed that male raters generally rated the faces as more feminine than female raters, all $p \leq 0.004$. Faces were perceived equally gender-neutral under exposure of female and male chemosignals, $t(15) = 0.402$, $p = 0.691$. While, for male raters, there were no rating differences across chemosensory conditions, all $p \geq 0.412$, female raters evaluated gender-neutral faces as significantly more feminine (**Table 2**) when exposed to male, $t(15) = 3.359$, $p = 0.004$, and female chemosignals, $t(15) = 3.010$, $p = 0.009$, compared to the no odor sample. Ratings of male and female chemosignals did not differ, $t(15) = 0.328$, $p = 0.747$.

Affective Introspection

For the PANAS subscales, no main effects of the sex of the donor or rater were found. Only sample- and sex-unspecific stabilizations of the raters' mood were found after the experimental procedure, both for positive mood, $F(1,28) = 15.220$, $p = 0.001$, $\eta_p^2 = 0.352$; pre: $M = 53.3$, $SD = 13.65$; post: $M = 48.57$, $SD = 15.91$, and for negative mood, $F(1,28) = 4.220$, $p = 0.049$, $\eta_p^2 = 0.131$, pre: $M = 10.89$, $SD = 8.13$; post: $M = 9.63$, $SD = 8.69$.

Chemosensory Induced Judgment Bias

The judgment bias induced by male and female chemosensory samples on odor perception and social perception is further investigated. *Post hoc*, judgment bias was calculated by

subtracting the rating of the no odor sample from the rating of the chemosensory sample (male or female chemosignal). Using this method, a masculinity bias was represented by a negative value and a femininity bias was represented by a positive value. A value not different from 0 represents no bias.

For the main odor perception and social perception tasks (i.e., masculinity-femininity rating of the chemosensory samples, gender-neutral personality attributes and faces rating tasks), judgment bias variables were calculated and chemosensory induced judgment bias was assessed via one sample *t*-tests (test value = 0; **Figure 5**).

For the odor perception task, a masculinity bias induced by male chemosignals was found in female raters, $M = -15.34$, $SD = 15.77$; $t(15) = -3.893$, $p = 0.001$, but not in male raters, $M = -5.10$, $SD = 14.03$; $t(14) = -1.408$, $p = 0.181$. A masculinity bias induced by female chemosignals was found in female raters, $M = -14.13$, $SD = 24.67$; $t(15) = -2.290$, $p = 0.037$, and in male raters, $M = -13.93$, $SD = 17.32$; $t(14) = -3.116$, $p = 0.008$.

The same analysis was computed for the two social perception tasks. For the rating of gender-neutral personality attributes, a femininity bias induced by female chemosignals was found in male raters, $M = 2.23$, $SD = 2.85$; $t(14) = 3.709$, $p = 0.002$, but not in female raters, $M = 0.75$, $SD = 4.72$; $t(15) = 0.637$, $p = 0.533$. A femininity bias induced by male chemosignals was found in female raters, $M = 1.64$, $SD = 3.01$; $t(15) = 2.182$, $p = 0.045$, but not in male raters, $M = 1.19$, $SD = 2.90$; $t(14) = 1.589$, $p = 0.134$.

For the rating of gender-neutral faces, a femininity bias induced by female chemosignals was found in female raters, $M = 4.44$, $SD = 5.91$; $t(15) = 3.010$, $p = 0.009$, but not in male raters, $M = -1.43$, $SD = 7.26$; $t(14) = -0.763$, $p = 0.548$. A femininity bias induced by male chemosignals was found in female raters, $M = 5.04$, $SD = 6.00$; $t(15) = 3.359$, $p = 0.004$, but not in male raters, $M = -1.14$, $SD = 5.22$; $t(14) = -0.846$, $p = 0.412$.

DISCUSSION

The present study aimed at establishing how humans communicate sex-specific information via body odors involving heterosexual male and female donors and raters. Healthy normosmic males and females were exposed to male and female chemosignals and no odor samples. First, a masculinity bias in human body odor perception was found in a masculinity-femininity rating, hedonic ratings and sensory-based discrimination of the chemosensory samples. Second, human body odor modulated the perception of gender-neutral faces and personality attributes toward a femininity bias.

Concerning the chemosensory communication of sex-specific information via male and female chemosignals in male and female raters, we found that female chemosignals are judged as rather intense and unpleasant by male raters. Although men and women are able to perceive sensory-based differences, the sex of the donor cannot be established from such stimuli. Both men and women seem to judge any body odor as rather masculine. Based on exploratory analyses, female raters are more accurate than male raters in assigning male body odor to a male donor, suggesting that mainly females detect the masculinity in male body odors.

Our result of a negative correlation between intensity and pleasantness of female body odor (i.e., the more intense, the more unpleasant the perception of female body odor) is in partial accordance with findings of Doty et al. (1978) where inversed pleasantness and intensity ratings were found for female and male axillary odor. This pattern is not restricted to body odor

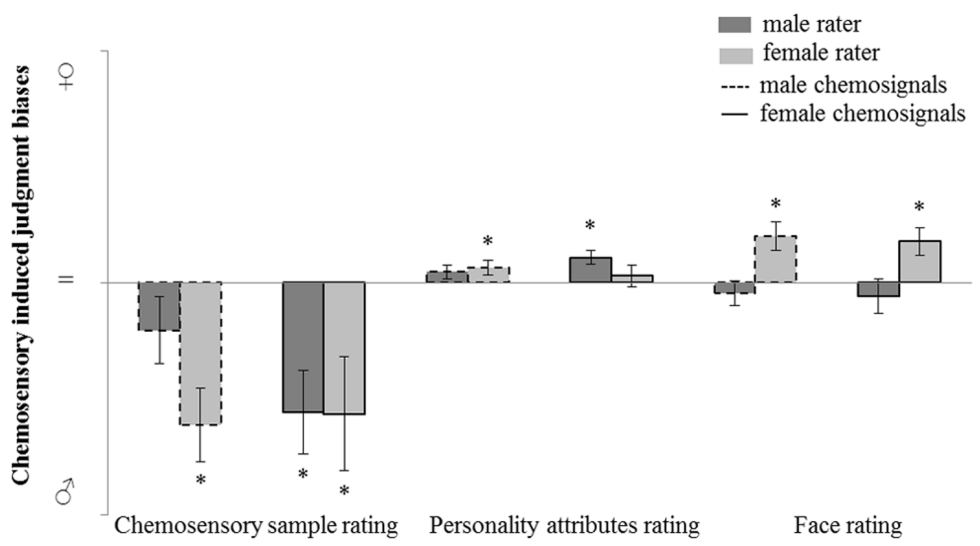

FIGURE 5 | Judgment bias in male and female raters induced by male and female chemosensory samples for odor perception (masculinity-femininity rating) and social perception (gender-neutral personality attributes and faces rating tasks). No odor-baseline controlled sample rating charted on a bias scale: masculinity bias (♂; chemosensory sample rating > no odor rating), no bias (=; chemosensory sample rating = no odor rating), femininity bias (♀; chemosensory sample rating < no odor rating). *marks $p < 0.050$.

and was also reported cross-culturally for food and everyday odorants (Distel et al., 1999). Besides the inability to establish masculinity or femininity features based on body odor alone, we conclude that the scent of human body odor seems to be closely associated to the male gender. Two reasons might explain why body odor is perceived as rather masculine. First, perceivable and intense body odor as a consequence of physical activity and strength might cue masculine gender stereotypes of dominance and power. A masculinity bias in sex identification might rely on a semantic tendency of strength being related to masculinity (Doty et al., 1978) – a tendency that might specifically affect female raters presenting higher olfactory abilities. Second, exposure to female body odor might be less frequent than exposure to male body odor. As females purchase and apply fragrances more often and perceive artificial fragrances as more arousing than males (Herz and Cahill, 1997), a diminished exposure to female body odor for both males and females might be the result. Along these lines, a decreased number of opportunities where determining body odor as originating from male or female donors might go along with an inhibited learning process of differentiating female and male body odor. Assuming that – in a social context – the source of a body odor is not clearly identifiable and the scent is less likely to have a female sender, the most adapted response would be to identify the scent as masculine. Another reason for diminished exposure to female body odor might be the decreased intensity compared to male body odor in relation to biological factors. Male body odor is often perceived as more intense and less pleasant than female body odor (Doty et al., 1978; Mitro et al., 2012), an effect that can be related to stronger axillary secretion (Sergeant, 2010) and a higher concentration of sweat-degrading skin bacteria (Jackman and Noble, 1983), steroid hormones, or axillary hair in men. In light of the previous studies and in accordance with our results, we conclude that an unequivocal sex identification based on body odor alone is unlikely to be performed by individuals from industrialized societies.

Investigating the possible modulating effects of chemosensory samples in male and female raters on social perception and self-perception, we aimed to clarify whether sex-specific information in body odors modulate the evaluation of ambiguous conspecifics (personality attributes and faces) rather than modulating affective introspection (mood rating). Supporting the idea that body odors – as social signals – affect the perception of conspecifics rather than introspection, we found that exposure to any body odor induced a femininity bias in social perception. No sex-specific chemosensory effect on a rater's mood was detected. When exposed to any body odor, men and women rated gender-neutral personality attributes as more feminine. Additionally, we found that women perceived gender-neutral faces to be more feminine. Body odors seem to facilitate social cognition and induce a femininity bias, which might be explained by the idea that chemosignals are representatives for social and emotional situations. Evidence arises from neuroimaging studies during which females exposed to chemosignals activate brain areas involved in the assessment of a human quality of chemosensory cues (Zhou and Chen, 2008). Furthermore, conspecifics' body odor is processed in the amygdala and insular regions, unlike other non-human odors (Lundström and Olsson, 2010). Additionally, a strong neural connection of chemosensory processing areas and emotionally relevant limbic areas exists (Albrecht and Wiesmann, 2006). These findings suggest that successful chemosensory communication with a conspecific requires an accurate assessment of emotional cues. Along this train of thought, the femininity bias might be a result activated by emotional sensitivity that is stereotypically associated with feminine referents. As females are more receptive to subtle emotional signals (Pause et al., 2004; Radulescu and Mujica-Parodi, 2013), we find here that the dominant visual signal is modulated more by the chemosensory signal than in male raters.

Taken together, we suspect task-related differences might have led to the sharp contrast of masculinity and femininity biases found. The masculinity bias was established in sex identification via masculinity-femininity ratings and evaluation of the chemosensory samples. The femininity bias was induced by the chemosignals in social perception tasks. Here, two different perceptive and cognitive processes (olfactory perception and evaluation versus multisensory integration and higher-order evaluation) are involved and might explain the opposing findings. While the masculinity bias becomes evident during evaluation of chemosensory information, the femininity bias appears when the olfactory information is a modulating source of information while performing a masculinity-femininity rating on ambiguous visual stimuli. We therefore assume that, besides sex and gender, task complexity might have played an important role on the gender-related biases in chemosensory information transmission.

Addressing the limitations of the present study, we acknowledge methodical limitations in relation to the donation method. Taking into account that different axillary glands are contributing to odorous secretions, we are aware that mainly thermoregulative eccrine glands were stimulated. However, based on knowledge of apocrine and eccrine hyperhidrosis, eccrine gland activity is involved in the transportation of odorous (sebaceous and apocrine) sweat (Hurley, 1989). Also, acknowledging the presence of apoeccrine glands (that are as high in number as apocrine and eccrine glands; Labows et al., 1999), we believe that a stimulation of apocrine and eccrine glands during the experimental set-up resulted in a complex mixture of chemical compounds. Chemosignals were grouped in donor pools characterized by one consistent characteristic: the sex of the donor. The benefit of the superdonor pool in homogenizing across entire group samples, however, represents the inconvenience of being unable to track the individual donor's quadrants that contributed to the chemosensory sample of each receiver. The donation method involving short periods of physical activity was chosen over continuous body odor collection throughout the day for two reasons. First, the entire donation in a laboratory setting assures that no uncontrolled psychological or emotional factors bias the chemosensory samples. Second, to assure that circadian hormonal variations between sexes do not influence the quality of the chemosensory samples (Mong et al., 2011), donation appointment times were kept short and constant for all donors. Additionally, while we controlled for the presence of axillary hair, we are not able to rule out that chemosensory samples might have differed

in association with the length of axillary hair (Kohoutová et al., 2012). We find an inverse intensity rating, and we think that the results might be influenced by axillary hair length.

We acknowledge that influences of hormonal contraception were controlled in female donors and raters and influences of menstrual cycle were kept constant in female raters, although not systematically studied. Menstrual cycle differences for female donors were controlled for by using pooled donor sets and, for every female rater, all testing sessions were placed in the same menstrual cycle phase. Female raters in fertile menstrual cycle phases show preferences for symmetrical (Gangestad and Thornhill, 1998) and dominant (Havlicek et al., 2005) body odor of male donors and exhibit an increased chemosensory sensitivity (Doty, 1981). Additionally, only pleasantness and preference ratings were affected by menstrual cycle, while across all cycle phases female raters did not report intensity differences in male body odor (Rantala et al., 2006). Male raters show a preference for ovulatory female body odor (Singh and Bronstad, 2001) only when female donors do not take hormonal contraception (Kuukasjärvi et al., 2004).

Formulating a distinct suggestion on the inclusion of male and female body odor donors in future chemosensory research, we would like to emphasize the importance of the inclusion of female chemosignals when performing chemosensory research on emotional, social, or sexual behavior in humans. To date, male chemosignals are most widely studied in emotional chemosensory communication research, with the argument that their body odor is not affected by menstrual cycle phases. While emotional introspection in a rater does not seem to be affected by the sex of a donor, emotional communication in female raters might still be biased. Observing a masculinity bias in body odor perception and a femininity bias introduced by chemosignals during social perception, we would like to encourage further research to disentangle the influence of cyclic fertility on chemosensory communication of sex and gender information in social perception.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception or design of the work. EM performed data acquisition. Data analysis and interpretation was performed by SM, EM, and JF. Drafting of the manuscript was performed by SM. Revising and approving the final version of the manuscript was accomplished by all authors.

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Chemosensory anxiety cues moderate the experience of social exclusion – an fMRI investigation with Cyberball

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Recent evidence suggests that the experience of stress can be communicated between individuals via chemosensory cues. Little is known, however, about the impact of these cues on neurophysiological responses during a socially threatening situation. In the current investigation we implemented a widely used paradigm to study social exclusion—Cyberball—to examine whether chemosensory cues signaling anxiety modulate the neuronal effects of ostracism. In a double-blind, within-subjects design, 24 healthy, normosmic participants were presented with chemosensory cues of anxiety (or control samples) and completed the Cyberball task while in a 3T fMRI scanner. Axillary sweat collected from male students awaiting an oral examination served as the anxiety cues while the chemosensory control stimuli consisted of sweat collected from the same individuals participating in an ergometer training session. The neuroimaging data revealed that under the control chemosensory condition, exclusion from Cyberball was associated with significantly higher orbitofrontal cortex and anterior cingulate cortex activity, which is consistent with previous studies in the field. However, when participants were primed with the anxiety sweat, the activity in these regions was not observed. Further, under exposure to anxiety cues during ostracism the participants showed deactivations in brain regions involved in memory (hippocampus), social cognition (middle temporal gyrus, superior temporal gyrus) and processing of salience (inferior frontal gyrus). These results suggest that successful communication of anxiety via the chemosensory domain may moderate the experience of social exclusion. It is possible that the anxiety signals make it easier for the individuals to detach from the group, pointing to the communicative role of chemosensory anxiety cues in enhancing adjustment mechanisms in light of a distressing situation.

Keywords: social exclusion, chemosignals, cyberball, ostracism, olfaction, anxiety

Introduction

Recent evidence suggests that the experience of stress can be communicated between individuals via chemosensory cues. It was proposed that upon activation of the sympathetic-adrenal medullary (SAM) system, operating closely with the hypothalamus-pituitary-adrenal (HPA) axis, individuals release sweat, which includes physiological markers of anxiety/fear (de Groot et al., 2015). In response to threat, ranging from situations inducing acute fear (such as skydiving, e.g., Mujica-Parodi et al., 2009) to situations inducing acute anxiety (such as anticipation of an oral examination, e.g., Prehn et al., 2006; Prehn-Kristensen et al., 2009), the release of "alarm" signals induces a partial fear state in those exposed to the chemosensory compounds (Prehn et al., 2006; Mujica-Parodi et al., 2009; de Groot et al., 2012). This includes fear-related behavioral and physiological outcomes such as improved cognitive performance (Chen et al., 2006), a bias toward identification of faces as more fearful (e.g., Zhou and Chen, 2009), increased visual field size, and enhanced sensory intake (de Groot et al., 2012), to name just a few. The successful transmission of the "alarm" signals is believed to serve the adaptive function of enhancing sensory vigilance, preparing the organism for environmental dangers (for a review see Stevenson, 2010).

Although current support for the role of chemosensory "alarm" signals in modulating specific emotional, cognitive and physiological processes is abundant (Stevenson, 2010; Pause, 2012), little is known about the impact of these signals on neurophysiological responses during an *actual* threatening context. Given that in everyday life, exposure to chemosignals rarely occurs without a relevant contextual background, it seems critical to assess the impact of chemosensory "alarm" signals during an actual threat. Thus, the current study investigated the impact of chemosensory cues of anxiety on one of the most distressing social situations, i.e., social exclusion. Social exclusion is considered a social danger, as it threatens the basic human need to belong, which is necessary for survival and well-being (Bowlby, 1969; Baumeister and Leary, 1995).

To examine whether chemosensory cues signaling anxiety modulate neuronal effects to social exclusion we first collected axillary sweat from donors in anticipation of an oral examination at a university, which has been linked to experiences of anxiety and the release of emotional chemosignals (e.g., Prehn-Kristensen et al., 2009). We then employed a widely used and well-validated paradigm to study social ostracism in the laboratory environment – Cyberball (Williams et al., 2000; Williams and Jarvis, 2006). In this task participants play a ball-tossing game with two individuals (in reality simulated by the computer) in which, after a series of inclusion trials, they are eventually excluded from the game. The exclusion from Cyberball has been shown to pose a threat to the basic human needs of belonging, feeling in control, maintaining self-esteem, and experiencing a meaningful existence (Williams, 2007; Eisenberger, 2012).

The central question of the current research was whether the communication of anxiety via chemosensory signals modulates the neuronal responses to social exclusion. The evidence suggesting that chemosensory "alarm" cues enhance salience of fear-related socio-emotional cues (Zhou and Chen, 2009) argues for augmented experience of social rejection following the exposure to the anxiety chemosignals. Similarly, studies pointing to emotion contagion via chemosensory "alarm signals" (Prehn et al., 2006; Mujica-Parodi et al., 2009; de Groot et al., 2012) suggest enhanced contagion of anxiety in the context of a distressing situation. Further, the results showing increased fear contagion when olfactory fear is paired with another modality (de Groot et al., 2014) also support this hypothesis. Cumulatively, the expected increased negative experience of social exclusion could be a collective result of chemosensory anxiety and a distressing context. Alternatively, given that chemosensory anxiety cues are potential social threat signals, it cannot be precluded that they are associated with enhancing reappraisal of social rejection to promote fitness. Specifically, from an evolutionary perspective, the successful chemosensory communication of another person's anxiety could be expected to lead to dissociation from the negative experience of social exclusion, in order to enhance productive coping mechanisms during the potentially harmful situation. This hypothesis is supported by an abundance of animal and human studies suggesting a key role for chemosensory "alarm" cues in preparing the organism to tackle a hazardous situation via boosting physiological arousal (Prehn et al., 2006; Inagaki et al., 2008; Pause et al., 2009) and enhancing sensory vigilance (Brown et al., 2004; Chen et al., 2006; Zhou and Chen, 2009; de Groot et al., 2012). These processes may be initiated in preparation to withdraw from the threatening situation (for a review see Pause, 2012).

Taken together, the current study assessed the neuronal implications of exposure to chemosensory anxiety cues in a threatening context of social exclusion. Does the smell of another's person anxiety make us more vulnerable to social exclusion or more prepared to cope with the difficult situation? Increased activity in regions typically involved in processing of social exclusion, including anterior cingulate cortex, medial orbitofrontal cortex, insula, and in regions previously reported to play a role in the processing of socio-emotional information, including the amygdala, hippocampus, and superior temporal gyrus, would support the first answer. By contrast, diminished activity in the regions typically implied in processing of social exclusion would argue for down-regulation of negative feelings associated with ostracism.

Materials and Methods

Ethics Statement

The local ethics committee at the Medical Faculty of RWTH Aachen University approved the current study. The experimental protocol was carried out in accordance with the provisions

of the World Medical Association Declaration of Helsinki. All participants gave written informed consent and were reimbursed for their time.

Participants

All volunteers were part of a larger study on the effects of anxiety chemosignals on social cognition. Ten healthy males were recruited as sweat donors. Twenty-four healthy participants (14 men and 10 women) were recruited to take part in the Cyberball study, as sweat recipients. Only males were chosen to donate their sweat, as the apocrine glands in the male underarm area are known to be larger (Doty, 1981). Both genders were included in the fMRI study, as previous research indicated that male stress sweat induces similar neural responses in both gender recipients (Radulescu and Mujica-Parodi, 2013). All Cyberball participants (18–29 years, $M = 24.33$ years, $SD = 2.91$) were screened for fMRI contraindications. In addition, we included only right-handed (Edinburgh Inventory; Oldfield, 1971), non-smoking individuals, who did not suffer from any neurological nor psychiatric illnesses (as assessed via Structured Clinical Interview for DSM-IV, SCID, First et al., 1995), nor showed signs of depression (Beck Depression Inventory; Beck et al., 1961; $M = 2.9$; $SD = 3.9$). Participants' olfactory function, specifically odor identification, was assessed with Monex 40 (Freiherr et al., 2012). According to this task, all participants were normosmic (range: 27–36, $M = 31.54$, $SD = 2.90$).

Materials

Chemosensory Stimuli

The olfactory stimuli consisted of sweat samples collected from males undergoing an important oral examination (anxiety condition) and exercising at a stationery bicycle (sports condition). The sweat donors were invited to take part in the study if they anticipated an important oral examination about which they felt nervous. In addition, their participation was only possible if they reported to be: of Caucasian origin, heterosexual, non-smokers, aged between 18 and 40 years-old, healthy, physically fit and not taking any medication. Ten males (22–33 years, $M = 26.40$ years; $SD = 3.75$) who fulfilled these criteria were recruited to donate their sweat. They were asked to follow several rules starting 2 days prior to the sweat donation (consistently with, e.g., Zhou and Chen, 2009; de Groot et al., 2012). These included not going into the swimming pool or sauna, not consuming garlic, onion, asparagus, curry, and strongly spiced meals, not drinking alcohol and coffee, sleeping alone, not using deodorant, after-shave, scented creams, and perfumes. In addition, they were asked to use scent-less shampoo and soap provided to them by the experimenter starting 2 days prior to the donation as well as to wash their sheets with an odor-less detergent. Before the sweat donation session, they were asked to shower and wear clothes, which they had washed with an odor-less detergent provided by the experimenter. All the participants reported following these rules.

The experimental protocol for sweat collection was based on Prehn-Kristensen et al.'s (2009) design. Specifically, the olfactory stimuli were gathered from donors' underarm area with cotton pads attached with plasters for sensitive skin. In the anxiety condition, participants' sweat was collected during anticipation of an important oral examination for 60 min. In addition, at that time, participants' salivary samples were gathered to assess cortisol levels: 60 min before the examination (t0), 30 min before the examination (t1), right before the examination (t2), and right after the examination (t3) using salivettes (Sarstedt, Nuembrecht, Germany). Participants were further asked to complete a Self-Assessment Manikin (Bradley and Lang, 1994) and to evaluate the intensity of six basic emotions (anxiety, joy, surprise, anger, sadness, disgust; Ekman and Friesen, 1975) on visual analog scales, 5 min before the examination. Shortly before the examination the sweat pads were removed. The sports condition consisted of a 10-min introduction to the procedure, three sets of cycling on a stationary bike, 10 min in duration each, with 10 min breaks in between the sets. The salivary samples were collected right at the beginning of the session (t0) as well as 30 (t1), 60 (t2), and 90 min after the start (t3). The Self-Assessment Manikin and Basic Emotions questionnaire were administered after the last bicycle set. Shortly after that the sweat pads were removed. Upon completion of the sweat collection procedure the pads from both sessions were cut into 8 pieces each, blinded by an experimenter not directly involved in this research, sealed in odor-free freezer bags separately per condition and stored at −80° Celsius until the day of the examination. The four pieces of the pads per condition, originating from 4 different donors, were defrosted 30 min before the experiment and placed in odorless teabags. During the fMRI experiment, the olfactory stimuli were attached under participants' noses with an odorless strap for the duration of the experimental trial (see *Procedure* for more details). Care was taken to ensure that the chemosensory stimuli did not come into direct contact with participants' skin. Each chemosensory stimulus was prepared and used for one participant only.

Cyberball Task

Participants completed the Cyberball task (Williams et al., 2000), a virtual ball-tossing game developed to study social ostracism. The game consists of cartoon images representing other players in the upper corners of the screen and a hand representing the participant at the bottom of the screen, tossing the ball among each other (see **Figure 1A** for illustration). In the current version, the names of the players –"Dieter" and "Nora" – were displayed on the screen next to the animated cartoons. The participants were informed that they were connected to the other two individuals over the Internet, while in reality the game was simulated by the pre-set computer program. The game included two conditions: social inclusion and social exclusion. The participants completed two rounds of the game, one each under exposure to anxiety vs. control sweat cues, presented in a counterbalanced order (see **Figure 1B** for a schematic illustration of the session). For each odor, an inclusion condition was followed by an exclusion condition. Each condition started with a display indicating that the computer was connecting to the other players. The fixation cross was then presented for 15 s, after which the trial began. The participants could toss the ball to one of the two players, by pressing "left" with their index finger or "right"

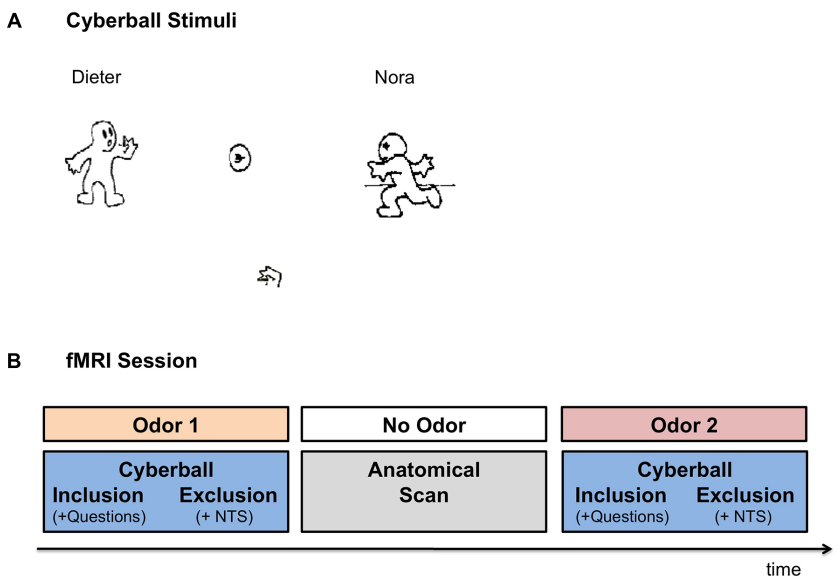

FIGURE 1 | Experimental Setup. (A) Cyberball game – the visual interface. Participants were presented with the icons via MRI compatible goggles. The participants were represented by the hand icon, at the bottom of the screen. Once the participant received the ball, they were allowed to toss it to one of the other two players (in reality simulated by computer). In the inclusion condition participants were part of the game throughout the trial. In the exclusion condition, the participants received the ball in the first 10 throws, after which they were excluded from the game for the rest of the trial (∼50–70 s). **(B)** Schematic Illustration of the FMRI session. In the fMRI session participants completed two runs of the Cyberball game under exposure to anxiety and sports chemosensory cues, presented in a counterbalanced order. In each Cyberball run the inclusion condition preceded the exclusion condition. Following the inclusion condition, participants were asked how angry they felt, how happy they felt, how much they liked Dieter and how much they liked Nora (i.e., Questions). Following the exclusion condition, participants completed the Need Threat Scale (NTS). In between the two runs of the Cyberball game the anatomical scan was carried out for approximately 10 min, in which no chemosensory cues were presented.

with their middle finger, on a response box. Each trial was set for 60 throws, with the opponents tossing the ball after 0.5–3.0 s of waiting time (determined randomly). In the inclusion condition, participants played with the two opponents throughout the trial. Each player received the ball on approximately 50% of the throws. In the exclusion condition, the participants received the ball for the first 10 throws, after which they were excluded from the game and did not receive the ball for the rest of the trial (∼50–70 s) while other players tossed the ball among themselves. Following the inclusion condition participants were asked how happy and how angry they were as well as how much they liked the female player and the male player. Following the exclusion condition, participants completed the Need-Threat scale (Williams et al., 2000, 2002) assessing participants' four fundamental needs: belonging (e.g., I felt I belonged to the group), self-esteem (e.g., I felt good about myself), meaningful existence (e.g., I felt non-existent), and control (e.g., I felt powerful). In order to maintain the cover story, the Need-Threat scale was administered to the participants only after the exclusion condition.

Procedure

Before and after the fMRI session participants were asked to evaluate the olfactory stimuli with respect to their valence, intensity and pleasantness. During fMRI scanning, the olfactory stimuli were attached under participants' noses for the duration of the Cyberball run (inclusion condition followed by an exclusion condition plus the accompanying questionnaires). The participants completed two rounds of the game under exposure to the anxiety vs. control sweat cues. The order of olfactory stimuli presentation was counterbalanced across the participants and both the participants and the experimenters were blinded to the nature of the olfactory cues. For a break between the two chemosensory stimulations, the two Cyberball trials were separated by a 10-min anatomical scan, during which no olfactory stimuli were presented. See **Figure 1B** for a schematic illustration of the fMRI session.

fMRI Data Acquisition

Functional MRI data were acquired in a three Tesla Tim Trio MR Scanner (Siemens, Erlangen, Germany) at the Department of Psychiatry, Psychotherapy, and Psychosomatics at the Hospital of the RWTH Aachen University. Functional images were collected with an echo-planar imaging (EPI) T2*-weighted contrast sequence sensitive to blood oxygenation level dependent (BOLD) changes (echo time [TE] = 28 ms, repetition time [TR] = 2 s, flip angle [α]: 77°, voxel size: 3 mm × 3 mm × 3 mm, 64 × 64 matrix, field of view [FOV]: 192 mm × 192 mm, slice thickness: 3.0 mm, gap: 0.75 mm, number of slices: 34 axial slices, whole-brain, slice acquisition sequence: ascending, 790 volumes per run).

Behavioral Data Analyses

The behavioral data were analyzed using IBM SPSS Statistics 20 (SPSS IC, Chicago, IL, USA). The effect sizes are reported

according to Cohen's *d* (1988) for paired-sample comparisons and all *post hoc t*-tests.

Sweat Donor Physiological and Questionnaire Data

The cortisol levels (in μg/dl) of the sweat donors were extracted at the diagnostic laboratory of the Medical Department of RWTH Aachen University from salivary samples (LDZ: Labordiagnostisches Zentrum Aachen). Three sweat donors were found to produce insufficient salivary amount necessary for the extraction of cortisol values at one of the collection points. The cortisol values of the remaining subjects ($n = 7$) were compared in a 2 (anxiety smell, sports smell) × 4 (time 0, time 1, time 2, time 3) repeated measures analysis of variance (rmANOVA). Follow up comparisons were carried out for each of the salivary sampling points (including the available salivary data for each time point) using Wilcoxon signed-rank test. In addition, the donors' emotional experiences (Basic Emotions and SAM emotions) in the anxiety and sports conditions were compared using Wilcoxon signed-rank test. The Wilcoxon signed-rank test was chosen for these data due to the small sample size of sweat donors ($n = 10$).

Odor Differentiation

In order to evaluate whether participants could differentiate the smells with respect to their pleasantness, intensity and valence, the participants' ($n = 21$) ratings of sweat stimuli against these criteria were compared in separate 2 (anxiety smell, sports smell) × 2 (time 1, time 2) × 2 (men, women) rmANOVAs. Three ratings of the participants were not recorded due to measurement errors.

Cyberball Behavioral Data

In the Cyberball game, participants' contentment was compared in the exclusion condition versus the inclusion condition (manipulation check) with a paired samples *t*-test, across chemosensory conditions. In addition, participants' evaluation of how much they liked the male and the female opponent in the game, how happy and how angry they felt following the inclusion trial were analyzed with separate 2 (anxiety smell, sports smell) × 2 (men, women) rmANOVAs. Similarly the ratings of the experience of fundamental needs of belonging, self-esteem, control and meaningful existence (NTS; Williams et al., 2000, 2002) following exclusion were analyzed with 2 (anxiety smell, sports smell) × 2 (men, women) rmANOVAs.

fMRI Data Analyses

The neuroimaging data were preprocessed and analyzed using SPM8 (Wellcome Trust Center for Neuroimaging[1]) implemented via Matlab 7.10 (MathWorks). Data from two participants who exhibited excessive motion (more than 3 mm in any direction) were excluded from the analyses: the final participant sample for the analyses consisted of 22 participants (12 men and 10 women).

Preprocessing

The fMRI data were preprocessed according to standard preprocessing steps (including realignment, coregistration, normalization, and smoothing). The functional scans were first realigned using a two-pass procedure. In this procedure, the first pass—the first scan, and the second pass—the mean scan, were substituted as reference image. Subsequently, the anatomical scans were coregistered to the mean EPI scan. The coregistered images were used for the estimation of spatial normalization parameters using unified segmentation approach (Ashburner and Friston, 2005). The normalization parameters applied to the images transformed them into the standard space as defined by the Montreal Neurological Institute (MNI) and resampled the images to a voxel size of 2 mm × 2 mm × 2 mm. Lastly, smoothing of the images was conducted with a Gaussian kernel of 8mm full-width-at-half-maximum.

First Level Analyses

In the first level analyses, the onset and duration vectors for separate Cyberball conditions (i.e., exclusion and inclusion blocks) under the two chemosensory conditions (i.e., anxiety and sports) were convolved with hemodynamic response function (HRF). In addition, the onsets and duration vectors for the sources of noise (i.e., instructions, questionnaires, and waiting time) were modeled out in a separate regressor of no interest. The mean across time for each voxel was modeled by a constant term and low-frequency drifts were removed using a high-pass filter with a cutoff period of 512 s. Temporal correlations were modeled by a first-order regression process as implemented in SPM.

Second Level Analyses

Second level analyses were conducted using GLM Flex, (extension to SPM8, see GLM Flex[2]) in which the experimental plan included the following factors: smell (anxiety, sports) and ostracism condition (inclusion, exclusion).

Whole Brain Analyses

Whole brain analyses targeted at examining the impact of anxiety chemosensory cues on the experience of social ostracism. We contrasted neural activity during exclusion relative to inclusion separately for: (a) the exposure to chemosensory control cues, (b) chemosensory anxiety cues, and (c) chemosensory anxiety cues – chemosensory control cues. Moreover, we conducted a whole brain smell (anxiety, sports) × condition (inclusion, exclusion) interaction. xJView[3], a viewing program for SPM, was used for exploring and processing of the contrasts and MarsBaR toolbox for SPM[4] was used for exploring and processing of the interaction. Additionally, the Anatomy toolbox for SPM (Eickhoff et al., 2005) and the xjView were used for anatomical localization.

Volume of Interest Analyses

In order to clarify which condition in our 2 (anxiety smell, sports smell) × 2 (inclusion, exclusion) design, drove the interaction, we identified activation clusters volumes of interest (VOIs) within socio-emotional regions, using MarsBaR toolbox from

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

[2]http://nmr.mgh.harvard.edu/harvardagingbrain/People/AaronSchultz/Aarons_Scripts.html

[3]www.alivelearn.net/xjview8

[4]http://marsbar.sourceforge.net/

all the significantly activated brain regions in the whole brain interaction. The VOIs included the areas previously implied in the Cyberball paradigm (i.e., orbitofrontal cortex and anterior cingulate) as well as the areas involved in social cognition and memory (i.e., superior temporal gyrus, hippocampus, inferior frontal gyrus). Although we are well-aware of the broad involvement of these areas in a wide range of functions, we chose the specified regions based on research (see below), suggesting an intimate relationship with measured processes, of which activation/deactivation patterns in the context of chemosensory anxiety could point to mechanisms by which chemosensory anxiety influences the experience of social exclusion. The VOIs included:

(1) Orbitofrontal cortex and anterior cingulate (associated with rumination and persisting negative affect, Kohn et al., 2013; as well as negative experience of social exclusion, Eisenberger, 2012);
(2) Right middle temporal gyrus and right superior temporal gyrus (linked to perception of familiar places and scenes, Tempini et al., 1998; Leveroni et al., 2000, and social cognition and theory of mind, Carrington and Bailey, 2009);
(3) Right hippocampus (involved in memory processes, Brewer et al., 1998; Wagner et al., 1998; Morris, 2007);
(4) Left inferior frontal gyrus (involved in response inhibition, emotion regulation, as well as processing of salience; Hampshire et al., 2010; Kohn et al., 2014).

For information about volume, center of mass, peak MNI coordinates, cluster size, and the peak intensity (T statistic), refer to **Table 3**.

The mean beta estimates (approximating the activation strength) values in the four clusters were extracted for each subject and each condition against the implicit baseline, and subjected to separate 2 × 2 × 2 rmANOVAs comprising factors: smell (anxiety, sports) × ostracism condition (exclusion, inclusion) × gender (men, women). *Post hoc* analyses were calculated using paired-samples t-tests. The comparisons of interest included: anxiety exclusion – anxiety inclusion, sports exclusion – sports inclusion and anxiety exclusion – sports exclusion.

Correction for Multiple Comparisons

To correct for multiple comparisons, we applied extent threshold correction as defined by Monte Carlo simulations (3DClustSim; implemented in AFNI; Cox, 1996). This procedure prevents false discoveries resulting from multiple testing. For a threshold at the voxel level of $p = 0.001$ uncorrected, and spatial properties of the current study, 10,000 simulations resulted in an extent threshold of 72 resampled voxels.

Results

Sweat Donors

Cortisol

Sweat donors showed higher cortisol levels when awaiting an oral examination than during ergometer training as revealed by a main effect of condition; $F = 8.774$, $p = 0.025$. The Wilcoxon Signed-rank test indicated that there was a significant difference in the cortisol values overall (average rank of 5.50 in the anxiety condition vs. average rank of 0.00 in the sports condition, $Z = -2.366$, $p = 0.018$), as well as specifically at time 0 (average rank of 5.50 vs. 1.00, $Z = -2.547$, $p = 0.011$), time 1 (average rank of 5.50 vs. 0.00, $Z = -2.805$, $p = 0.005$), time 2 (average rank of

TABLE 1 | Descriptive statistics for sweat donors' cortisol levels in the anxiety and sports conditions at times 0, 1, 2, 3, and overall [Mean (SD) and Median].

Condition	Time 0		Time 1		Time 2		Time 3		Overall	
	M (SD)	*Mdn*	*M (SD)*	*Mdn*	*M (SD)*	*Mdn*	*M (SD)*	*Mdn*	*M (SD)*	*Mdn*
Anxiety	0.68 (0.34)	0.61	0.59 (0.30)	0.60	0.54 (0.28)	0.41	0.65 (0.36)	0.65	0.62 (0.27)	0.61
Sports	0.46 (0.20)	0.50	0.36 (0.14)	0.33	0.34 (0.14)	0.32	0.34 (0.10)	0.32	0.37 (0.14)	0.39

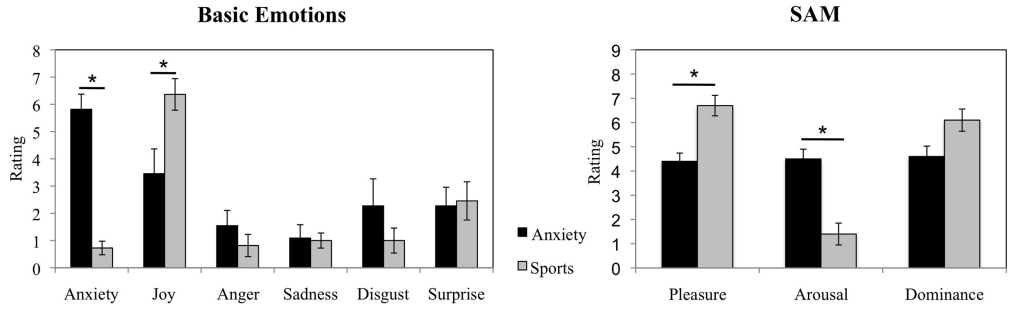

FIGURE 2 | Sweat donors' emotional responses in the anxiety vs. sports conditions. Sweat donors' evaluations of basic emotions and ratings in Self-Assessment Manikin during anticipation of an oral examination and during ergometer training. The figure depicts participants' mean ratings and standard errors. *$p < 0.05$.

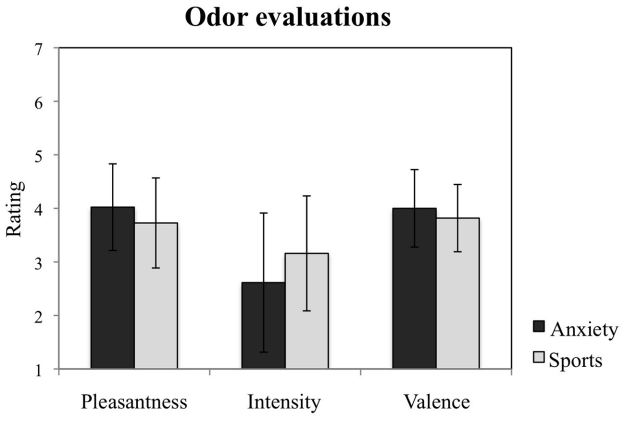

FIGURE 3 | Study participants' evaluations of the chemosensory cues. Participants' ratings of the odors from the anxiety and the sports conditions with regard to their pleasantness, intensity, and valence. The figure depicts participants' mean ratings and standard errors.

4.50 vs. 0.00, $Z = -2.521$, $p = 0.012$), and time 3 (average rank of 6.00 vs. 1.50, $Z = -2.310$, $p = 0.021$). No other main effects nor interactions were observed (all $p > 0.05$). See **Table 1** for an overview of participants' cortisol values.

Emotions

The Wilcoxon Signed-rank test revealed that the donors in the anxiety condition showed an increase in reported anxiety as compared to the sports condition (average rank of 5.50 vs. 0.00, $Z = -2.814$, $p = 0.005$). In addition, it revealed a decrease in experienced joy before the examination than during the ergometer training (average rank of 2.50 vs. 6.79, $Z = -2.053$, $p = 0.040$, see **Figure 2** for a visual depiction of mean rating differences). No significant differences in the experience of other basic emotions were observed (all $p > 0.05$). In addition, in the Self-Assessment Manikin, the donors reported feeling less pleasure while awaiting an examination (average rank of 0.00 vs. 5.50, $Z = -2.680$, $p = 0.007$) and more arousal while anticipating an examination as compared to the training (average rank of 5.50 vs. to 0.00, $Z = -2.831$, $p = 0.005$, see **Figure 2** for visual depiction of mean rating differences). The donors did not report differences in experienced dominance ($p > 0.05$).

Olfactory Samples

The participants exposed to the olfactory samples collected from the two situations reported no difference in odor characteristics with regard to pleasantness, $F = 3.775$, $p = 0.067$; intensity, $F = 2.234$, $p = 0.151$, and valence, $F = 1.055$, $p = 0.317$ (see **Figure 3**). No other main effects nor interactions with time nor gender for any of these measures were observed (all $p > 0.05$).

Cyberball

Manipulation Check

Across chemosensory conditions, the participants reported feeling significantly less contented in the exclusion condition ($M = 2.46$, $SD = 0.86$) as compared to the inclusion condition [$M = 3.29$, $SD = 0.79$; $t(23) = 3.815$, $p = 0.001$, $d = 1.01$, see **Figure 4**].

The Experience of Inclusion Under Chemosensory Cues

Following inclusion, under anxiety sweat as compared to sports sweat the participants did not report differences in the experience of happiness ($F = 0.750$, $p = 0.396$), nor anger ($F = 3.305$, $p = 0.083$), nor in how much they liked the male ($F = 0.013$, $p = 0.909$), nor the female participant ($F = 0.118$, $p = 0.735$). Further, the scores were not influenced by gender (all $p > 0.05$).

The Experience of Exclusion Under Chemosensory Cues – Need Threat Scale

The chemosensory cues of anxiety did not exert effects on the feeling of belonging ($F = 1.010$, $p = 0.326$), self-esteem ($F = 0.376$, $p = 0.546$), the experience of feeling "in control" ($F = 3.399$, $p = 0.079$), nor meaningful existence ($F = 0.583$, $p = 0.453$, see **Figure 4**). Further, the scores were not influenced by gender (all $p > 0.05$).

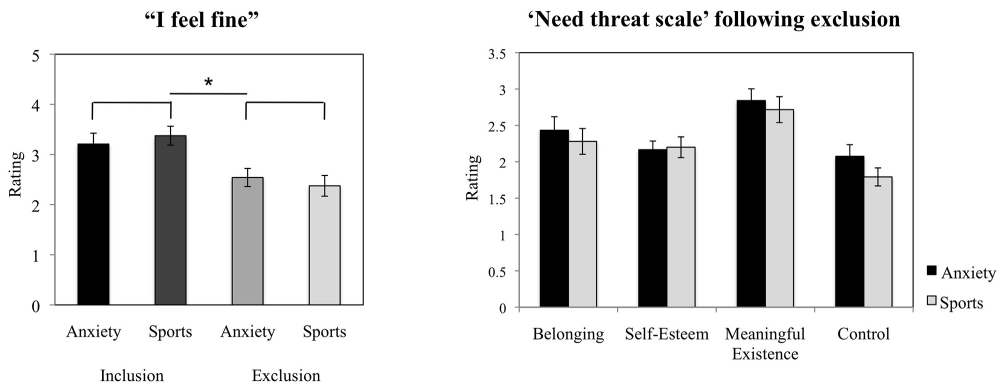

FIGURE 4 | Cyberball task. Participants' ratings of contentment in the exclusion condition compared to the inclusion condition (manipulation check), and evaluation of the experience of basic needs (NTS) following social exclusion. The figure depicts participants' mean ratings and standard errors. *$p < 0.05$.

fMRI

Neural Responses to Ostracism

Whole Brain Analyses

To assess the effect of chemosensory exposure on neural responses to social exclusion, we examined neural regions that differed in response to social exclusion (compared to inclusion) when participants were exposed to: (a) chemosensory sports (control) cues, (b) chemosensory anxiety cues, (c) chemosensory anxiety cues – chemosensory sports cues. Further, we conducted a smell (anxiety, sports) × condition (exclusion, inclusion) interaction.

Chemosensory sports (control) cues

We observed increased activity in the clusters encompassing: (1) rectal gyrus, superior orbital gyrus, anterior cingulate, and medial frontal gyrus; (2) anterior cingulate, rectal gyrus, and medial frontal gyrus, (3) superior occipital gyrus, angular gyrus and middle temporal gyrus (for details see **Table 2**; **Figure 5**).

Chemosensory anxiety cues

No significant suprathreshold activations were observed in the exclusion condition (as compared to inclusion condition).

Chemosensory anxiety cues – chemosensory sports cues

No significant suprathreshold activations were observed in the exclusion condition (as compared to inclusion condition).

Smell (anxiety, sports) × condition (exclusion, inclusion) interaction

Significant activations were observed in 11 clusters. For information about regions within the cluster, hemisphere, volume, center of mass, peak MNI coordinates, cluster size, and peak intensities of the volumes, please see **Table 3** and Supplementary Material for the figure depicting the interaction.

VOI Analyses

Four VOIs were selected due to their contextual importance to underlying processes (see methods section for further information) in order to disentangle the smell × ostracism interaction.

Orbitofrontal cortex/anterior cingulate

A significant main effect of ostracism condition ($F = 14.913$, $p = 0.001$) was identified in the VOI encompassing the superior orbitofrontal cortex and the anterior cingulate (Peak MNI coordinate region: −12, 44, −14, see **Figure 6**), with participants showing increased activity in this region in the exclusion condition ($M = 0.16$, $SD = 0.25$) and relative inhibition in the inclusion condition [$M = -0.01$, $SD = 0.24$, $t(21) = -3.990$,

TABLE 2 | Neural activations in the contrast Exclusion > Inclusion for: (a) sports chemosensory condition; (b) anxiety chemosensory condition; (c) anxiety chemosensory condition – sports chemosensory condition.

Contrast	Brain regions	Hemisphere	Peak MNI coordinates	*k*	Peak intensity
Control "sports" cues Exclusion > Inclusion	Rectal gyrus, superior orbital gyrus, anterior cingulate, medial frontal gyrus	L	−16, 46, −8	492	7.388
	Anterior cingulate, rectal gyrus, medial frontal gyrus	R, L	6, 34, −2	246	5.864
	Angular gyrus, superior occipital gyrus, middle temporal gyrus	L	−46, −82, 28	87	7.832
Anxiety cues Exclusion > Inclusion	No suprathreshold activation				
Anxiety – control Exclusion > Inclusion	No suprathreshold activation				

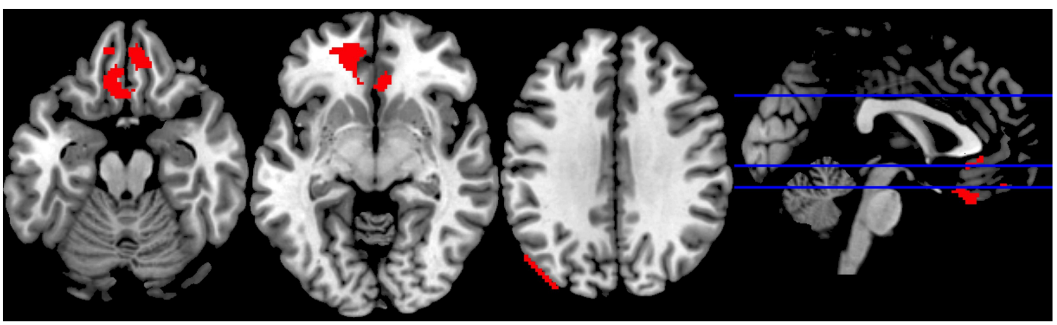

FIGURE 5 | Sports Exclusion > Sports Inclusion (Whole Brain analyses). Significant clusters at $p < 0.001$, with extent threshold = 72 voxels (corresponding to Monte Carlo correction) in the contrast Exclusion > Inclusion under the exposure to chemosensory sports (control) cues.

TABLE 3 | Information regarding brain regions, hemisphere, volume, center of mass, peak MNI coordinates, cluster size, and peak intensity (*T* statistic) for significantly activated clusters in the whole brain smell × condition interaction.

Cluster (regions)	Hemisphere	Volume (mm)	Center of mass	Peak MNI coordinates (X, Y, Z)	*k*	Peak intensity
***Orbitofrontal cortex, anterior cingulate, medial frontal gyrus**	L, R	5216.0	−3.9, 43.0, −14.9	−12, 44, −14	652	−7.2228
***Middle temporal gyrus, superior temporal gyrus**	R	1384.0	63.3, −38.5, −0.3	62, −40, 2	173	−5.5097
***Hippocampus**	R	1336.0	28.7, −36.4, −9.8	32, −38, −10	167	−6.6943
***Inferior frontal gyrus**	L	848.0	−45.8, 23.6, 15.7	−46, 26, 18	106	−5.79
Cerebellum, lingual gyrus, fusiform gyrus	L, R	16776.0	8.67, −52.4, −19.3	18, −52, −4	2097	−7.2425
Brainstem, midbrain, thalamus	L, R	4696.0	0.927, −20.6, −11.6	4, −24, −22	587	−7.2277
Transverse temporal gyrus, thalamus, superior temporal gyrus, insula, putamen	L	2320.0	−28.4, −22.7, 9.59	−34, −28, 10	290	−6.598
Caudate, putamen, thalamus	R	2128.0	21.2, −7.83, 11.7	22, −8, 20	266	−5.0169
Cerebellum	R	1456.0	47.4, −56.9, −38.7	52, −56, −40	182	−7.0123
Middle temporal gyrus, Broadmann area 21	R	992.0	50.3, −6.82, −19.4	58, 0, −26	124	−4.5159
Transverse temporal gyrus, superior temporal gyrus	R	800.0	40.8, 8.4, 7.3	44, −26, 4	100	−5.6465

The highlighted regions constitute the Volumes of Interest (VOIs) for which beta estimates were extracted for analyses aimed at disentangling the interaction.

$p = 0.001$, $d = 0.69$]. In addition, a significant smell × condition interaction ($F = 10.664$, $p = 0.004$) revealed that under the chemosensory control cues, the participants showed higher activation in the VOI in the exclusion condition ($M = 0.26$, $SD = 0.43$) than in the inclusion condition [$M = -0.09$, $SD = 0.38$, $t(21) = 4.278$, $p = 0.000$, $d = 0.86$, see **Figure 6**]. Importantly, the difference in the activity between exclusion and inclusion condition was not found for the anxiety cues [$t(21) = -0.165$, $p = 0.870$]. No other effects were significant (all $p > 0.05$).

Hippocampus

A significant smell × condition interaction ($F = 6.786$, $p = 0.017$) emerged in the hippocampal region (Peak MNI coordinate: 32, −38, −10, see **Figure 6**). *Post hoc* analyses indicated that under the anxiety smell, the difference between the relative inhibition in the hippocampus in the exclusion condition ($M = -0.15$, $SD = 0.34$), compared to the inclusion condition ($M = 0.02$, $SD = 0.26$) was significant [$t(21) = -2.206$, $p = 0.039$, $d = 0.56$ see **Figure 6**]. Under the control smell, the activity in the hippocampus was enhanced in exclusion ($M = 0.08$, $SD = 0.33$), whereas it was inhibited in the inclusion condition [$M = -0.07$, $SD = 0.20$, $t(21) = 2.476$, $p = 0.022$, $d = 0.55$ see **Figure 6**]. Additionally, in the exclusion condition, there was a significant difference between the inhibition in the hippocampus under the smell of anxiety ($M = -0.15$, $SD = 0.34$) and its activity under the chemosensory control smell [$M = 0.08$, $SD = 0.33$; $t(21) = -2.075$, $p = 0.05$, $d = 0.69$, see **Figure 6**]. The other differences were not significant (all $p > 0.05$).

Middle temporal gyrus/superior temporal gyrus

A significant smell × condition interaction ($F = 6.045$, $p = 0.023$) and a significant main effect of condition ($F = 12.505$, $p = 0.002$) were observed in the VOI encompassing the right middle temporal gyrus and the superior temporal gyrus (Peak MNI coordinate: 62, −40, 2, see **Figure 6**). As for the main effect of condition, the participants in the exclusion condition showed inhibition in the volume ($M = -0.04$, $SD = 0.50$) whereas in the inclusion condition they showed activity in this region ($M = 0.24$, $SD = 0.42$, $t(21) = -3.530$, $p = 0.002$, $d = 0.60$]. *Post hoc* comparisons disentangling the interaction revealed that under the anxiety smell, the difference between the relative inhibition in the region in the exclusion condition ($M = -0.32$, $SD = 0.66$) as compared to the activity in the inclusion condition ($M = 0.21$, $SD = 0.46$) was significant [$t(21) = -3.702$, $p = 0.001$, $d = 0.93$, see **Figure 6**]. Moreover, in the exclusion condition, there was a significant difference between the relative inhibition in the area under the smell of anxiety ($M = -0.32$, $SD = 0.66$) and the activity under the chemosensory control cues [$M = 0.24$, $SD = 0.84$; $t(21) = -2.344$, $p = 0.029$, $d = 0.74$, see **Figure 6**]. The other differences were not significant (all $p > 0.05$).

Inferior frontal gyrus

A significant smell × condition interaction ($F = 11.729$, $p = 0.003$) and a significant main effect of condition ($F = 19.928$, $p = 0.000$) were identified in the Inferior Frontal Gyrus (Peak MNI coordinate: −46, 26, 18, see **Figure 6**). Across chemosensory conditions, during exclusion condition the participants showed inhibition in the IFG ($M = -0.12$, $SD = 0.28$) whereas in the inclusion condition they showed activity in this region [$M = 0.08$, $SD = 0.24$, $t(21) = -4.438$, $p = 0.000$, $d = 0.77$]. Under the anxiety smell (but not sports), the difference between the inhibition in IFG in the exclusion condition ($M = -0.22$, $SD = 0.35$) as compared to the activity in the inclusion condition ($M = 0.15$, $SD = 0.34$) was significant [$t(21) = -5.887$, $p = 0.000$,

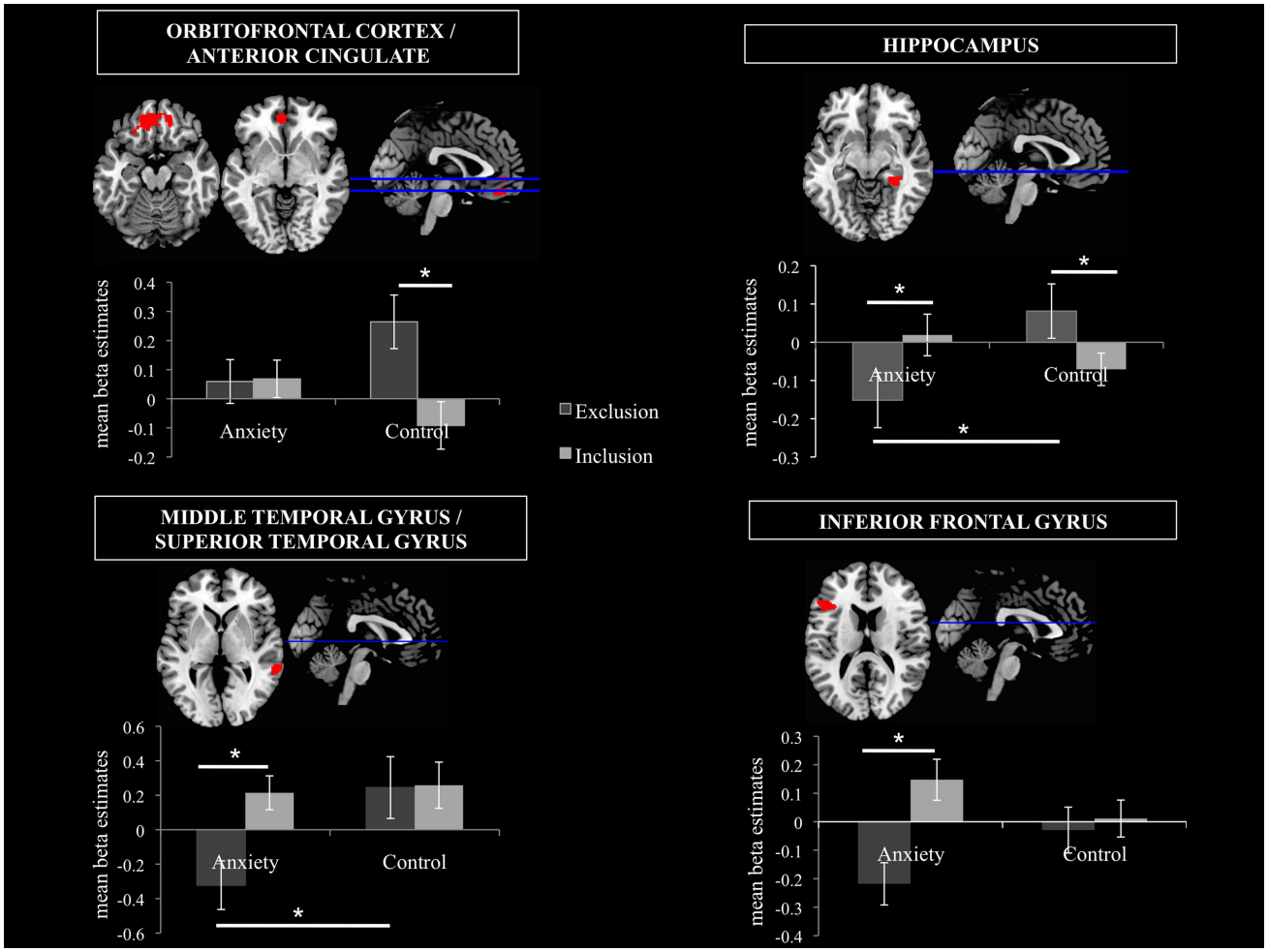

FIGURE 6 | Volumes of Interest (VOIs). Mean beta estimates *post hoc* comparisons in the 4 VOIs are displayed in order to disentangle the smell × ostracism condition interaction. The significant clusters are at $p < 0.001$, with extent threshold = 72 voxels (corresponding to Monte Carlo correction). *$p < 0.05$.

$d = 1.1$, see **Figure 6**]. No other significant differences emerged (all $p > 0.05$).

Discussion

The central question of the current study was focused on the neuronal implications of chemosensory anxiety signals in the context of social exclusion: do they deepen the negative experience of ostracism or alleviate it? We implemented a widely used paradigm to study social exclusion – Cyberball — while exposing participants to chemosensory cues signaling anxiety (versus control cues). The results revealed that exposure to anxiety cues: (1) modulates the activity in the brain regions involved in processing of negative experience of social exclusion and (2) down-regulates the neuronal areas involved in socio-emotional cognition. These results suggest that chemosensory anxiety signals might diminish the experience of social exclusion and facilitate withdrawal from others in the context of a stressful social situation.

Neural Responses to Ostracism under Chemosensory Anxiety Cues

Our results extend conclusions of previous studies in the area of chemosensory communication, by showing the association between the exposure to chemosensory anxiety signals and modulation of neural responses during an actual threatening situation of social ostracism. Specifically, during the episode of social rejection the presentation of chemosensory anxiety was not associated with increased activation of the regions previously implied in social rejection (e.g., Eisenberger et al., 2003; Eisenberger, 2012), and also observed in our control chemosensory condition (i.e., anterior cingulate, medial frontal gyrus, orbitofrontal cortices). The lack of activation in these regions, known to be a part of the pain matrix (Eisenberger et al., 2003; Eisenberger, 2012; although a recent meta-analysis by Cacioppo et al., 2013, challenged this perspective arguing that neural correlates of social pain are more complex than claimed by those earlier studies), might imply that chemosensory anxiety moderates

the experience of social rejection observed in the control situation.

Moreover, simultaneous deactivations in the brain regions involved in memory (hippocampus), social cognition (middle temporal gyrus, superior temporal gyrus) and salience processing (inferior frontal gyrus) might suggest that successful communication of chemosensory anxiety may be linked to enhancing the preparation of the individual to tackle a stressful episode (e.g., in line with Mujica-Parodi et al., 2009; Zhou and Chen, 2009). Previous studies demonstrated that anxiety signals are driven by the activation of the SAM system (de Groot et al., 2015), which plays a role in the initiation of the fight/flight response. If this state is at least partially communicated to the sweat recipients, it can be presumed that anxiety signals in the context of a distressing social situation may promote the emergence of mechanisms helping to address the hazardous scene e.g., via promotion of distance from the emotional state, or withdrawal from the rejection scenes (also see Koenigsberg et al., 2010; Premkumar, 2012). Accordingly, in the current experiment, upon presentation of the anxiety signals during exclusion, the relative deactivation of the hippocampal area, the region involved in memory, especially encoding processes (Brewer et al., 1998; Wagner et al., 1998), suggests diminished imprinting of negative events into the long term-memory. Given that the opposite pattern was observed in the control chemosensory condition, i.e., rejection led to stronger activity in the region, it appears that chemosensory anxiety plays a role in modulating this process in the context of a challenging situation such as ostracism. Correspondingly, the inhibition of the region encompassing middle temporal gyrus and superior temporal gyrus during the socially distressing episode under anxiety chemosensory cues implies decreased processing of social cues and diminished inclination for "theory of mind" or mentalizing processes, which could be related to increased detachment from the social experience altogether. Similarly, the modulation of inferior frontal gyrus activity in the anxiety (but not in the control chemosensory condition) suggests an influence of anxiety signals on salience processing: while being in a social situation with others (inclusion) the anxiety cues might add salience or arousal to the situation, as the chemosensory input may be perceived as an alarm signal. However, during dissociation from that situation (exclusion), the relative deactivation of the inferior frontal gyrus might be a result of the potential withdrawal from "social threat" signaled by anxiety sweat, which in turn lowers the inferior frontal gyrus activity.

Although, these results might appear counterintuitive in light of findings that anxiety cues enhance the salience of fear-related stimuli and the activation of the socio-emotional regions (Mujica-Parodi et al., 2009; Prehn-Kristensen et al., 2009) they suggest that anxiety signals in an actual, distressing context might override other functions and emphasize the primary role of the chemosensory "alarm" signals in the animal kingdom which is to initiate the organisms' withdrawal behavior from the situation appraised as threatening (e.g., Suh et al., 2004). This interpretation is also in line with a strong body of research showing that the participants exposed to sweat collected from stress-inducing social situation show other signs of preparedness for threat such as improved cognitive performance (Chen et al., 2006), enhanced sensory acquisition (sensory intake, increased visual field size, de Groot et al., 2012) and activation of the withdrawal systems (Prehn et al., 2006; Pause et al., 2009). It should be noted that the withdrawal in light of anxiety signals can be considered a positive outcome, as it facilitates the possible threatening stimulus that leads the sender of the signal to be alarmed in the first place. Future studies should investigate the interaction between the anxiety signals, processing of salience and withdrawal motivation. They should also further decode the mechanisms by which the anxiety signals might promote disconnection from the difficult social situation (e.g., what emotional or physiological tactics are employed by those exposed to the chemosensory cues). This is particularly important, because, given that the currently observed neural regions are involved in a wide range of processes, beyond social cognition and salience, it cannot be precluded, that other processes contribute to the observed results as well.

Lastly, we did not observe any gender effects in the current research, which suggests that the exposure to anxiety signals does not influence neural responses to social exclusion, as a function of gender. Although this is consistent with research showing that both males and females show similar neural activation patterns in response to the olfactory samples of male fear signals (Radulescu and Mujica-Parodi, 2013), studies with larger samples of men and women, designed to test for gender effects specifically are encouraged, to further explore possible differences in experience of social exclusion under influence of chemosensory cues in men and women.

Limitations

Although the commonly implemented Cyberball task offers a relatively high ecological validity, it suffers from several limitations when used in the fMRI scanner. Particularly problematic are: (1) the exclusion condition follows the inclusion condition and thus there is a risk of the neural responses being a result of expectancy violation (Somerville et al., 2006), and (2) the length of the blocks leads to a less-than-optimal signal-to-noise ratio. With regards to the current experiment, the choice of a within-subject design, although beneficial for measuring intraindividual variability, might have reduced the experience of social exclusion in the second round of the game (as the repeated rejection trial following inclusion trial appears less realistic). It has to be noted, however, that the order of odor presentation was counterbalanced across participants, such that a "lack of belief in the second exclusion" problem should not occur for different odors. Moreover, several studies have indicated that even a lack of belief in the cover story or knowledge of the exclusion being simulated by the computer is nevertheless associated with the automatic response typical for ostracism (Zadro et al., 2004; Sebastian et al., 2011, also in line with Williams, 2007). Further, the inclusion of a control chemosensory condition, in which non-social cues were presented, would be beneficial to drawing the conclusions regarding the social chemosensory nature of the effects. Lastly, the visual depiction in the differential contrast

sports exclusion > sports inclusion (**Figure 5**) suggests a possible motion artifact in the third panel. However, given that the motion parameters were included into our design (which should minimize such effects) and the extent of this cluster is in large part in the gray matter (with peak MNI coordinates within Superior Occipital Gyrus), it appears that the activation is not solely artifact related.

Conclusion

The current results suggest that successful communication of anxiety via chemosensory domain is associated with down-regulation of regions typically implied in the experience of social exclusion during social ostracism. Moreover, it suggests that chemosensory anxiety cues distance individuals from the group, by inhibiting social cognition, salience and memory formation regions during a distressing social event. Cumulatively, it is possible that the anxiety signals make it easier for the individuals to withdraw from the hazardous social situation, pointing to the communicative role of chemosensory "alarm" cues in enhancing adjustment mechanisms in light of distressing circumstances.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2015.01475

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Effect of fragrance use on discrimination of individual body odor

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Previous research suggests that artificial fragrances may be chosen to complement or enhance an individual's body odor, rather than simply masking it, and that this may create an odor blend with an emergent quality that is perceptually distinguishable from body odor or fragrance alone. From this, it can be predicted that a new emergent odor might be more easily identified than an individual's body odor in isolation. We used a triangle test paradigm to assess whether fragrance affects people's ability to distinguish between individual odors. Six male and six female donors provided axillary odor samples in three conditions (without fragrance, wearing their own fragrance, and wearing an assigned fragrance). In total, 296 female and 131 male participants selected the odd one from three odor samples (two from one donor, one from another; both of the same sex). We found that participants could discriminate between the odors at above chance levels in all three odor conditions. Olfactory identification ability (measured using Sniffin' Sticks) positively predicted discrimination performance, and sex differences in performance were also observed, with female raters being correct more often than men. Success rates were also higher for odors of male donors. Additionally, while performance was above chance in all conditions, individual odor discrimination varied across the three conditions. Discrimination rate was significantly higher in the "no fragrance" condition than either of the fragranced conditions. Importantly, however, discrimination rate was also significantly higher in the "own fragrance" condition than the "assigned fragrance" condition, suggesting that naturally occurring variance in body odor is more preserved when blended with fragrances that people choose for themselves, compared with other fragrances. Our data are consistent with the idea that fragrance choices are influenced by fragrance interactions with an individual's own body odor.

Keywords: deodorant, olfaction, body odor, identification, triangle test, smell

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Introduction

There is a wealth of evidence supporting the availability of various cues from human body odor. These cues concern a wide range of variables from emotion (Chen and Haviland-Jones, 2000; Fialová and Havlíček, 2012), menstrual cycle stage (Singh and Bronstad, 2001; Havlíček et al., 2006) through to health status (Moshkin et al., 2012). The aforementioned cues represent transitory changes in the perceptual qualities of body odor, and, despite these changes, individuals seem to maintain an underlying idiosyncratic quality to their odor which can be readily distinguished by others. Research has found that relatives can reliably discern the odor of a sibling from that of a stranger of the same age and sex (Porter et al., 1986), individuals can pick out a shirt worn by themselves out of 100 worn

by others (Lord and Kasprzak, 1989), and the odors of identical twins can be matched at above chance levels by human sniffers, even when the siblings are living apart (Roberts et al., 2005). These findings are further supported by research showing that humans have distinct and reproducible "fingerprints" comprised of specific volatile compounds in their body odor (Penn et al., 2007). Human body odors have also been found to contain cues to genetic similarity at the major histocompatibility complex (MHC), with research finding individuals to be capable of discriminating between MHC types, which may lead to adaptive mate choice for heterozygous offspring (Wedekind et al., 1995; Havlíček and Roberts, 2009).

There are a multitude of benefits incurred by an individual who can discriminate between conspecifics using olfactory information. For example it has been suggested that in the mother-infant relationship, odor recognition and detection are important for both the forming of an attachment, and for inducing feeding (Raimbault et al., 2007). It has been found that mothers can discriminate the smell of their own offspring from others (Porter et al., 1983; Ferdenzi et al., 2010), with neonates also reportedly being capable of discriminating between their own mother's axillary odors and that of an unfamiliar lactating female (Cernoch and Porter, 1985). Odor also appears to be important for human mate choice. Facial and body symmetry have been posited as reflecting an individuals' developmental stability; a potential indicator of genetic quality. This is therefore a potentially useful mate-choice relevant cue that varies across individuals. Studies have found that those who have higher levels of facial and body symmetry are rated as looking and smelling more attractive (Rikowski and Grammer, 1999; Thornhill and Gangestad, 1999).

Although these findings suggest that body odor discrimination is important, personal odor is often "modified" with the use of artificial fragrances (Roberts and Havlíček, 2012), with the conscious evaluation of body odor having a long history of negative connotations within numerous cultures (Schleidt et al., 1981). Reduction of ones' ability to detect individual characteristics of body odor would, at first sight, appear to be problematic given the information that can be gained from an individuals' odor and its influence in various social interactions. However recent research suggests that, rather than masking odor entirely, fragrances may in fact be chosen to complement and perhaps enhance the volatiles present in an individuals' body odor. For example, Milinski and Wedekind (2001) found that MHC genotype correlated significantly with an individuals' "liking" of a fragrance compound, which they argue suggests that humans choose fragrances to amplify genetic cues present in their odor. In keeping with this, Lenochová et al. (2012) found that mixtures of participants' body odor with their perfume of choice were perceived by female raters to be more pleasant than a mixture containing a randomly assigned perfume, even when controlling for the pleasantness of fragrances. This suggests that fragrances are chosen to work in tandem with individual body odor, potentially enhancing an individuals' personal olfactory fingerprint.

In light of this, the current study aimed to investigate the effect of fragrance use on the perceived individual quality of body odor, thus further investigating whether fragrances may mask or enhance idiosyncratic cues in body odor. To do this, odor samples were collected from individuals who were matched on deodorant brand use. In order to assess participants' ability to discriminate between these odors, triangle tests were conducted in which participants had to select the "odd one out" from three odors in which two were from the same individual. This test was conducted with both unfragranced body odor samples and, from the same individuals, blended samples of body odor and fragrance where the fragrance was the donor's usual brand of choice. The former allowed us to assess underlying ability for discrimination of body odors, while the latter allowed us to assess the impact of fragrance on idiosyncratic information available in that body odor. Finally, the test was repeated using samples containing body odor and a fragrance that was assigned to the donor by the experimenters (following Lenochová et al., 2012). This enabled us to investigate whether fragrance is specifically chosen by an individual in order to enhance their idiosyncratic biological information.

Based on previous findings showing that humans are capable of discriminating between individual odors, we expected that, at least in the unfragranced body odor condition, participants would be able to identify the odd one out at an above chance level. Similarly, in view of the findings of Lenochová et al. (2012), we predicted that performance would be at above chance levels for assessments of body odor and donors' own deodorant blends. Indeed, if body odor and fragrance do combine to form a new emergent odor, task performance might even exceed that of the no fragrance condition. In contrast, we hypothesized that participants would perform worse in the condition employing samples containing an assigned deodorant, as this fragrance had not been chosen by the donor and so might clash with the idiosyncratic body odor.

Materials and Methods

The study received ethical approval from the University of Stirling Psychology Ethics Committee.

Odor Collection

All donors provided informed consent. Odor samples were collected from six men (mean age $\pm$ SD $=$ 24.5 $\pm$ 5.24, range 19–32) and six women (mean age $\pm$ SD $=$ 21.17 $\pm$ 2.93, range 18–26), all of whom reported being heterosexual, non-smokers who regularly wore deodorant. As cyclical hormonal changes related to the menstrual cycle can affect the perceptual quality of body odors (Kuukasjärvi et al., 2004; Havlíček et al., 2006) we recruited only female donors who reported using hormonal contraception. Donors were additionally selected based on their current deodorant use, with all males reporting using the same commercially available fragrance (Lynx Africa—deodorant body spray). Female donors did not all use the same deodorant, but were selected so that there were two individuals each using the same deodorant (two using Sure Crystal Invisible, two using Nivea Pearl and Beauty and two using Dove Go Fresh Pomegranate and Lemon—all antiperspirant deodorants). This ensured that, for both men and women, triangle tests could be established utilizing donor pairs who used the same fragrance. All six female donors reported shaving their armpits during the study, whereas all male donors reported not shaving their armpits.

Each donor provided three axillary odor samples; one whilst wearing no deodorant (no fragrance), the second whilst wearing their own deodorant (own fragrance) and the third whilst wearing a deodorant provided by the experimenter (assigned fragrance). The assigned deodorant was chosen on the basis that it was not currently, or previously, used by any of the donors, with the six males receiving the same commercially available product which was designed for men (Adidas Ice Dive—a deodorant body spray), and the six female donors receiving the same commercially available deodorant which was designed for female use (Vaseline Active Fresh—an antiperspirant deodorant).

Odor collection took place on three consecutive days, with donors being instructed to shower before and between each session using fragrance free soap (Simple Pure™) which we provided. Donors were instructed to only use the soap provided, and the deodorants (only on the relevant days), and to avoid all other fragranced products. After showering, participants attached cotton pads to their armpits using surgical micropore tape. On the second and third days, after showering, participants were instructed to apply deodorant to both armpits (own deodorant on the second day, assigned deodorant on the third day), in their usual way, before attaching the cotton pads. These were left in place for 24 h, after which they were removed, placed in sealed plastic bags, and returned to the experimenter (within 2 h) where they were frozen at −30°C until use. Samples were removed from the freezer 2 h prior to test use, so that they could thaw, and placed back in the freezer at the end of each test session. Previous studies suggest that freezing and thawing of samples has little impact on perceptual qualities of odors (Roberts et al., 2008; Lenochová et al., 2009). In order to reduce the effect of any extraneous odors on the samples, and in line with previous research, participants were instructed to avoid being in smoky places, drinking alcohol, exercising, eating particularly strong smelling foods (e.g., curry, garlic), having sex and sharing a bed with another person starting from the day prior to odor collection and also during odor collection (Kohoutová et al., 2011; Lenochová et al., 2012).

Triangle Test Participants

All participants were visitors at the Centre for Life in Newcastle upon Tyne. The tests for male and female odor samples were completed by independent sets of participants. In total, 238 participants (65 men; mean age ± SD = 40.15 ± 16.15, range 16–76 and 173 women; mean age ± SD = 41.97 ± 13.36, range 17–79) completed the test with male odor samples. A set of 189 participants (66 men; mean age ± SD = 41.11 ± 14.75, range 16–76 and 123 women; mean age ± SD = 38.06 ± 14.83, range 16–78) completed the test with female odor samples.

Triangle Test Procedure

Participants provided informed consent and basic demographic information (age and sex). The nature of the task was explained in advance, and participants were told that they would be smelling samples of body odor and fragrance. Each participant was then presented with three 500 ml clear glass conical flasks, with aluminum foil caps, containing odor samples. Two of these odor samples were from the same individual, and the third was from a different donor of the same sex. For the donor who only presented one sample, the right axillary sample was used. Participants were informed that one of these was different from the rest, and they were instructed to remove the tinfoil covering and smell each flask before identifying the odd one out.

TABLE 1 | Donor pairings used in each triangle test.

Test session		Donors used in each condition		
		No fragrance	Own fragrance	Assigned fragrance
Male donor samples				
A	$n = 68$ mean age ± SD = 42.69 ± 13.45	1 and 2	3 and 4	5 and 6
B	$n = 74$ mean age ± SD = 41.62 ± 13.80	3 and 4	5 and 4	1 and 2
C	$n = 96$ mean age ± SD = 40.49 ± 14.97	5 and 6	1 and 2	3 and 4
Female donor samples				
D	$n = 59$ mean age ± SD = 42.76 ± 15.47	7 and 8	11 and 12	9 and 10
E	$n = 71$ mean age ± SD = 36.11 ± 14.43	9 and 10	7 and 8	11 and 12
F	$n = 59$ mean age ± SD = 39.10 ± 14.06	11 and 12	9 and 10	7 and 8

Each participant took part in one session, and were therefore exposed to all three conditions, with three odors in each (two of the same, one of a different donor), all of which were of the same sex. Consequently each participant was exposed to either all of the male donor samples OR all of the female donor samples. Mean participant age ± SD is shown for each test session.

Within each triangle test donor samples were paired so that each pair used the same deodorant (males paired with males and females paired with females). There were three odor conditions, with each triangle test having all three samples containing either no fragrance, own fragrance or assigned fragrance and participants were blind to these. Each participant took part in one session during which they completed one triangle test in each of the three odor conditions, with each test involving a different donor pair. Each session used either all male or all female donor samples, and consequently each participant was exposed to either all of the female or all of the male samples (see **Table 1**). After sample use each glass flask was cleaned using a fragrance free detergent (Neutracon, Decon Laboratories Ltd.) and allowed to dry prior to the next test session. Both male and female samples were used in three separate test sessions (**Table 1**) each of which was conducted over approximately a day and a half. This meant that samples were thawed and used for 5–6 h before being refrozen and thawed the next day where they were used for a further 2–4 h (depending on the number of visitors at the center). Samples were treated in the same way (i.e., time of use) across the three conditions. **Table 1** shows the number of participants who took part in each test session.

Additionally, each participant completed the Sniffin' Sticks Screening test. This is a 12-item cued odor identification test

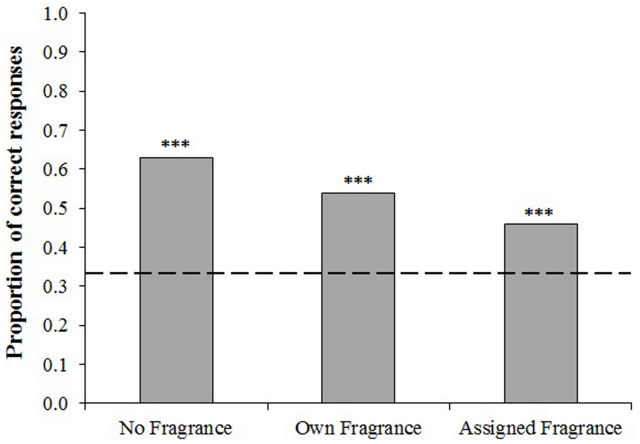

FIGURE 1 | Proportion of participants who correctly chose the odd one out on the triangle test. Dashed line indicates the proportion of correct responses which would be expected by chance (0.33). Binomial tests indicate significance above chance level ***$p < 0.001$.

(Hummel et al., 2007a) which assess ability to verbally label common odors. It employs the use of odor dispensing devices, shaped like pens. Participants sniff each of these and then must select the correct label for the odor from a choice of four words. The resulting score is the sum of correct answers. This was completed after the triangle test.

Results

Binomial tests were first conducted to compare the observed frequency of correct scores against that expected by chance (in this case 0.33). For each condition, participants were able to discriminate between the odors at a level significantly above chance (all p's < 0.001, **Figure 1**). A Chi-squared test indicated that there was a significant difference between the number of correct responses achieved in the three odor conditions, $\chi^2(2) = 23.87, p < 0.001$.

In order to investigate these differences further, a binary logistic regression was conducted. The dependent variable was the participants' response in each test (correct, incorrect) and we included five candidate predictor variables in the model; donor sex, participant sex, participants' scores on the Sniffin' sticks test, participants' age, and odor condition ("no fragrance," "own fragrance," "assigned fragrance"). Performance on the Sniffin' sticks test significantly and positively predicted participants' performance on the triangle tests, Exp (B) = 1.175, $p < 0.001$, as did participant sex, Exp (B) = 0.777, $p = 0.048$ (females having a higher proportion of correct responses, 0.57, compared to males, 0.48). The effect of donor sex was also significant, $p = 0.001$, Exp (B) = 1.503, such that there was a higher proportion of correct responses when assessing male samples (0.59) compared with female samples (0.49). Importantly, odor condition was found to be a significant predictor of test performance, $p < 0.001$. Orthogonal planned contrasts revealed that the proportion of correct responses was higher in the "no fragrance" condition than that of the two fragranced conditions, Exp (B) = 1.749, $p < 0.001$, and higher in the "own fragrance" condition than that of the "assigned fragrance" condition, Exp (B) = 1.375, $p = 0.03$. The model also revealed a significant interaction between odor condition and donor sex, $p < 0.001$, with participants returning more correct responses when assessing female samples in the "no fragrance" condition, Exp (B) = 0.175, $p < 0.001$, while the proportion of correct responses was higher in male samples in the "own fragrance" and "assigned fragrance" conditions, Exp (B) = 1.094, $p = 0.757$ (**Figure 2**). There was no significant interaction between participant sex and performance across the three conditions, $p = 0.603$. Interestingly, while it is well documented that olfactory ability declines with age (Hummel et al., 2007b) there was found to be no effect of participants' age on task performance, Exp (B) = 0.998, $p = 0.674$. We did, however, find that participants' age was significantly negatively correlated with performance on the olfactory identification test, $r = -0.207$, $n = 420$, $p < 0.001$, with older individuals performing worse than younger individuals.

Finally, in order to further investigate the significant interaction between odor condition and donor sex, we repeated the analysis separately for responses to male and female samples by male and female participants (**Figure 2**). Binomial tests indicated that, for female odor samples, men correctly discriminated the odors at proportions above chance in the no fragrance condition, $p < 0.001$ (0.68 correct), but not the own fragrance (0.38 correct) or assigned fragrance condition (0.30 correct), whereas women were correct at an above chance level in both the no fragrance, $p < 0.001$ (0.75 correct), and the own fragrance conditions, $p = 0.03$ (0.41 correct), but not the assigned fragranced condition (0.36 correct, see **Figure 2A**). However, performance was higher for male odor samples, with men performing at a significantly above chance level in both the no fragrance, $p = 0.001$ (0.52 correct), and the own fragrance conditions, $p < 0.001$ (0.58 correct), but not in the assigned fragrance condition (0.43 correct), and women performing above chance in all three conditions, no fragrance $p < 0.001$ (0.57 correct), own fragrance $p < 0.001$ (0.67 correct), and assigned fragrance $p < 0.001$ (0.61 correct; see **Figure 2B**).

Discussion

Our study aimed to investigate the impact of artificial fragrances on the perception of individual body odors, and in turn, to investigate whether fragrances might either mask or enhance idiosyncratic information available in odors. This was achieved using a triangle test paradigm, with participants identifying the "odd one out" from three odors, either with no fragrance, the donors' own fragrance, or an experimenter assigned fragrance. As expected, the discrimination rate was highest in the "no fragrance" condition, followed by the "own fragrance" and then the "assigned fragrance" conditions. Furthermore, participants' performance on the triangle test was mediated by their olfactory ability, as assessed using the Sniffin' Sticks identification task. Individuals with higher identification scores performed better in the triangle tests. We found no relationship between participants' age and their performance on the task, which might at first sight be surprising given that olfactory ability tends to decline with age (Hummel et al., 2007b). However, this is likely explained by the

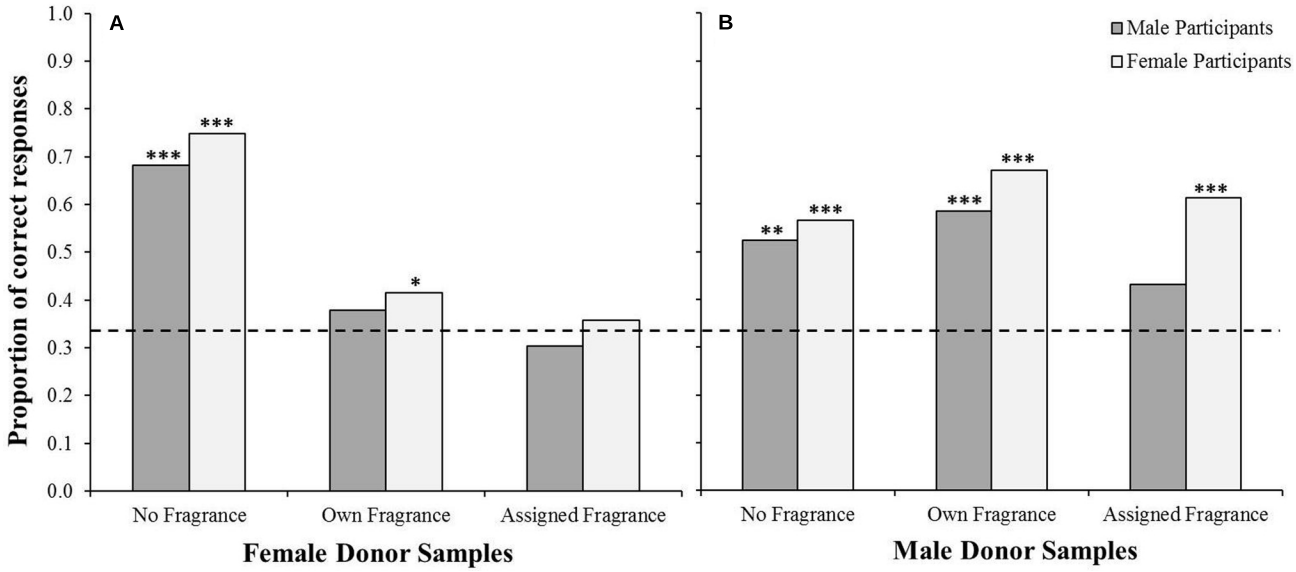

FIGURE 2 | Proportion of male and female participants who correctly chose the odd one out on the triangle test when using female samples (A) and male samples (B). Dashed line indicates the proportion of correct responses which would be expected by chance (0.33). Binomial tests indicated significance above chance $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

inclusion of scores from the Sniffin' Sticks task in the model. As would be predicted, these scores were negatively correlated with participants' age.

Our results also indicate that female participants performed better on the triangle tests than male participants did. This is perhaps unsurprising as it has repeatedly been reported that women tend to outperform men on various aspects of olfactory perception (Brand and Millot, 2001; Cardesín et al., 2006; Doty and Cameron, 2010). Additionally, previous work has also found women to outperform men in specific tasks of body odor identification (Schleidt, 1980) and self-recognition of body odors (Platek et al., 2001).

Irrespective of the participant sex differences reported, all participants were good at discriminating between odors, performing at a significantly above chance level in the no fragrance condition, supporting previous findings such as those of Lord and Kasprzak (1989). Furthermore, participants' performance was also at a significantly above chance level in both of the deodorant conditions, lending further support to the idea that fragrance does not mask information present in body odor. More importantly however, was the finding that performance was significantly better in the "own fragrance" condition compared to the "assigned fragrance" condition. This indicates that fragrance-body odor blends involving individually preferred fragrances are qualitatively different from blends involving randomly selected fragrances. Such findings further substantiate claims by Milinski and Wedekind (2001) and Lenochová et al. (2012) that fragrances may, perhaps unintentionally, be chosen to complement body odors. However, it does appear that, while participants' performance when assessing blends with the fragrance of choice was better than with assigned fragrances, it was poorer than when assessing body odor alone. This suggests that the emergent quality of the blend does not appear to actively enhance individuality, even though it does not appear to mask it either.

It must be noted, however, that the current study raised some interesting questions regarding differences in discrimination between odors when using male and female samples. For female odors the findings were largely consistent with the overall analysis, such that unfragranced samples were the easiest to discriminate, followed by own fragranced samples and then assigned fragranced samples, and with discrimination of assigned fragrance samples being at about chance levels (though performance in the two fragranced conditions was not significantly different). However, this pattern was not evident in male samples with participants performing in all conditions at a significantly above chance level, and with there being no significant difference between participants' performance across the three conditions.

It is possible that this finding was driven by the quality of the male odors. Male odors appear to be more intense and distinctive than female odors, and it may therefore be easier to discriminate between them even in the presence of a fragrance. In support of this, previous studies have suggested that discrimination between male and female odors is probabilistic, with sex classifications being related to the perceived intensity of the odors: stronger, more intense odors are more likely to be judged as male than weaker ones, regardless of the actual sex of the odor donor (Doty et al., 1978; Doty, 1981). An alternative, or contributory explanation is that the male fragrances used here were all deodorants, containing only fragrance and compounds which reduce the presence of odor causing bacteria, whereas the female fragrances used were all antiperspirant deodorants, and thus additionally contained compounds which inhibit the production of sweat. This may have also contributed to different levels of intensity in the male and female samples, but intensity was not assessed by our raters and we therefore cannot confirm this.

One further possible explanation is that the assigned fragrance for the male donors was in some way perceptually different than that given to the female donors, making discrimination of male odors easier. Either of these suggestions, in isolation or taken together, may provide an explanation for the improved performance with male samples, and future research should aim to investigate this further by including intensity ratings of the individual odors, with and without fragrances, as well as ratings of fragrance intensity in the absence of body odor, or perhaps by utilizing a unisex fragrance for the assigned condition.

Furthermore, due to the setting in which the experiment took place we were somewhat restricted as participants did not have time to complete more than three tests (taking approximately 10–15 min per participant). Conducting the study in this environment presented a trade-off between the number of participants completing the test and the number of tests they each completed, which allowed us to obtain a very good sample size with a large and representative age range. Importantly the odor conditions were balanced, with each participant completing a test in each odor condition, which is the critical element of the experimental design. It should also be noted that while we recruited a large sample of participants, there were only six donors of each sex, and future research should employ a larger number of donors in order to present a more representative range of odors.

Despite this, the current study benefits from adopting a more ecologically valid methodology than has previously been used. Previous research investigating the effects of fragrances on body odor tend to use perfumes as opposed to deodorants (Havlíček and Roberts, 2013). There is a good reason for this; perfumes are solely fragrance, whereas deodorants combine fragrance and odor suppressants. However, deodorants are widely used, with one study reporting that between 82.7 and 93.3% of 17,000 individuals sampled in the UK indicating they used a deodorant either daily or on most days (Rodriguez et al., 2013). Thus, assessment of the effects of deodorants, as well as perfumes, are important to understand the cultural effects of modern patterns of fragranced products on odor perception. It is also noteworthy that individual discrimination was possible despite the odor-suppressing qualities of deodorants and their anti-microbial action, and that because of this the current findings may actually underestimate discrimination rates. Furthermore, it was in the odor samples provided by women, who used antiperspirant deodorants, that identification was improved with the use of a chosen versus an allocated fragrance, lending additional support to the importance of fragrance/body odor blends in identification, rather than a reduction of sweat or body odor.

The findings from this study help to reveal just how complex the perception and holistic affective response to fragrance users by other individuals around us is in real-life interactions. As mentioned above, the majority of people wear some form of fragrance on a daily basis (Rodriguez et al., 2013). It is also likely to be the case that when entering a mate choice arena, for example when going on a date or for a night out in a nightclub, that an even larger proportion of individuals will be wearing fragranced products. Given this, it is most likely that encounters with new individuals in many social settings, and perhaps especially in a mate-choice context, will involve the perception of fragrance and body odor blends, rather than either the fragrance or body odor alone. This, coupled with the findings from the current study and those of Lenochová et al. (2012), highlights the potential importance of the fragrance choice decision that individuals make. It has been shown, for example, that fragrance preferences are linked to idiosyncratic genetic traits such as MHC (Milinski and Wedekind, 2001), but future research should focus on elucidating the fragrance choice process that individuals undergo, assessing the relative role of genetics but also other factors such as commercial advertising, which are likely to be influential in this process.

Clearly more work is needed to further elucidate the effects of fragrance on individual discrimination, as well as understanding the process related to fragrance choice, but the current study has provided some ground work which will be useful for directing future research in this area. The main findings are in keeping with previous literature discussed, supporting the idea that individual fragrance choice does not mask information present in body odor, though further research is needed to clarify the difference between odor discrimination of male and female odors. Finally, while we have found evidence to suggest that personal fragrance choice does not prevent the overall discrimination of an individual, further investigation must be carried out to ascertain whether fragrance use masks other kinds of information that may be available in body odor, such as emotions, health status and fertility status.

Author Contributions

CA and SR designed the study. CA collected the data and wrote the manuscript. CA, SR, and JH were all involved in analysis and interpretation of the data, as well as revising the manuscript.

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Body Odor Based Personality Judgments: The Effect of Fragranced Cosmetics

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People can accurately assess various personality traits of others based on body odor (BO) alone. Previous studies have shown that correlations between odor ratings and self-assessed personality dimensions are evident for assessments of neuroticism and dominance. Here, we tested differences between assessments based on natural body odor alone, without the use of cosmetics and assessments based on the body odor of people who were allowed to use cosmetics following their daily routine. Sixty-seven observers assessed samples of odors from 113 odor donors (each odor donor provided two samples – one with and one without cosmetic use); the donors provided their personality ratings, and the raters judged personality characteristics of the donors based on the provided odor samples. Correlations between observers' ratings and self-rated neuroticism were stronger when raters assessed body odor in the natural body odor condition (natural BO condition; $r_S = 0.20$) than in the cosmetics use condition (BO+cosmetics condition; $r_S = 0.15$). Ratings of dominance significantly predicted self-assessed dominance in both conditions ($r_S = 0.34$ for natural BO and $r_S = 0.21$ for BO+cosmetics), whereas ratings of extraversion did not predict self-assessed extraversion in either condition. In addition, ratings of body odor attractiveness and pleasantness were significantly lower in natural BO condition than in BO+cosmetics condition, although the intensity of donors' body odors was similar under both conditions. Our findings suggest that although olfaction seems to contribute to accurate first impression judgments of certain personality traits, cosmetic use can affect assessments of others based on body odor.

Keywords: body odor, olfaction, smell, personality assessment, cosmetics, perfume

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INTRODUCTION

Fragranced cosmetics can affect the way people are perceived by others, and this effect has been observed in several contexts. Fragrances have been shown to influence perceptions of attractiveness (Baron, 1981; Dematte et al., 2007). In the latter study, the authors provide evidence that faces were rated as significantly less attractive when presented with an unpleasant ambient odor in comparison to the no-odor condition. Marinova and Moss (2014) showed that the use of gender-congruent fragrances can increase the "halo effect" of certain socially desirable characteristics, such as intelligence. Consequently, fragrances may also modulate self-perception, including self-confidence, which may in turn influence the attractiveness of the person wearing the fragrance. This effect has been demonstrated in previous studies using video footage in which persons wearing a pleasant fragrance were judged as more attractive than those

who were not, despite the fact that raters could not perceive the odor (Higuchi et al., 2005; Roberts et al., 2009). Finally, there is some evidence suggesting that perfumes may affect impressions of people in professional contexts. For instance, Sczesny and Stahlberg (2002) showed that candidates using perfumes considered as typically masculine were perceived to be more suitable for a managerial position than those wearing a typically feminine perfume. However, Baron (1986) found that the effect of perfume on the impression conveyed by job applicants is modulated by other cues, such as their nonverbal behavior (perfumed applicants showing positive nonverbal cues were rated less positively by male interviewers than those with no perfume). Regarding the different genders, perfumed job candidates were evaluated especially favorably by female but not necessarily by male raters (Baron, 1983).

In Western cultures, natural body odor is generally perceived as unpleasant (Schleidt et al., 1981), and ratings of body odor pleasantness are on average relatively higher when participants use cosmetic products (Schleidt, 1980; Lenochová et al., 2012). Further, cosmetics may impede raters' ability to discriminate individual body odor (Allen et al., 2015) or, based on body odor samples, discriminate between men and women (Schleidt, 1980); presumably because artificial odorants modify the impression conveyed by body odor intensity and pleasantness. Although it might seem that perfumes may "mask" or "cover" the underlying natural body odor, some studies proposed that fragrances could be enhancing body odor attractiveness in a complementary fashion (Milinski and Wedekind, 2001). Indeed, Lenochová et al. (2012) found that attractiveness ratings of perfume-body odor blends varied among individuals, suggesting that perfumes in fact interact with natural body odor rather than simply mask it. This is consistent with an observation that, compared with randomly assigned fragrances, the discrimination rates are higher when individual body odors are blended with fragrances that people choose for themselves (Allen et al., 2015).

Previous studies have shown that natural body odor may also play a role in impression formation (Havlicek et al., 2005; Sorokowska et al., 2012; Sorokowska, 2013a,b). Body odor can generate spontaneous attributions of personality traits, with unpleasant odors generally associated with socially undesirable traits (McBurney et al., 1976; Sorokowska, 2013b). A recent series of studies found that people were able to assess certain personality characteristics based on natural body odor samples and, that in some domains, these attributes were congruent with self-assessed traits of body odor donors. In the first of these studies, perception of extraversion, neuroticism, and dominance ratings based on body odor samples were higher than the chance level (Sorokowska et al., 2012). The results of the second study (Sorokowska, 2013a) showed that assessments based on body odor by both children and adults were congruent with self-report in the case of neuroticism. Additionally, adults were able to assess dominance above the chance level (Sorokowska, 2013a). The third study corroborated previous findings concerning accurate assessment of neuroticism and dominance from body odor alone (Sorokowska, 2013b).

Which mechanisms might possibly link personality traits to the body odor? First, human physiology and personality might overlap, as both are associated with certain hormones and neurotransmitters (Gray et al., 1991; Cashdan, 1995; Zuckerman, 1995). However, this relates mainly to neuroticism and dominance. Second, some emotions might be perceived from body odors (see e.g., Chen and Haviland-Jones, 2000; Ackerl et al., 2002; for review see Fialová and Havlíček, 2012) and hence influence the body odors of people who often experience these emotions (Dalton et al., 2013; see Sorokowska et al., 2012 for a Discussion). For example, repeatedly, emotionally induced sweating resulting from elevated anxiousness and nervousness might modify the body odor of neurotic people. Previous studies indicated that judgments of agreeableness, openness to experience, and conscientiousness were not congruent with the self-assessed traits of odor donors (Sorokowska et al., 2012; Sorokowska, 2013a,b). This might be because no direct hormonal links between body odor and these traits exist. Further, conscientiousness, agreeableness, and openness to experience seem not to be closely related to emotions influencing the body odor composition. As it was suggested in one of the previous papers (Sorokowska, 2013a), it is possible that people might need more context-dependent information to accurately assess these characteristics.

The studies reviewed in previous paragraphs tested the effect of fragrance use on sex discrimination or attractiveness judgments. However, no study has examined whether fragrances affect personality attributions based on odor cues yet. Thus, the main aim of our study was to test the effect of cosmetics use on personality attributions. We also aimed to extend previous findings related to assessments of attractiveness, odor intensity, and pleasantness of natural body odor relative to a body odor–fragrance blend. To do so, we asked a panel of raters to assess neuroticism, extraversion, and dominance of others based on the samples of natural body odor and body odor collected from participants using cosmetics. Based on previous findings, we hypothesized that assessments of neuroticism (a characteristic typically considered socially undesirable) would better predict self-assessed neuroticism in the natural body odor condition than in the cosmetics use condition. In contrast, we predicted no significant differences between the two conditions in ratings of extraversion and dominance.

MATERIALS AND METHODS

Participants

Odor Donors

Odor donors were 113 individuals – 58 women aged between 17 and 33 years ($M = 23.17$, $SD = 3.0$) and 55 men aged between 20 and 34 years ($M = 24.58$, $SD = 3.81$). All donors provided informed consent prior to their inclusion in the study. They received a small gift (a set of cosmetics) for taking part in the study.

Odor Raters

Our rater sample comprised of 68 female students aged between 19 and 32 years ($M = 22.88$; $SD = 2.16$). None of the participants smoked or reported any olfactory-related impairment. Following

previous work (e.g., Sorokowska, 2013a), we did not control for menstrual cycle phase or contraception use. The study was conducted in accordance with the Declaration of Helsinki, and all aspects of the study were approved by the Institutional Review Board at the University of Wroclaw. All raters provided informed consent prior to their inclusion in the study. They received a small gift (a cosmetic product) for their involvement.

Procedure

Body Odor Sampling

We used armpit cotton pads to collect the body odor samples from odor donors. Such samples are less subject to possible odorous environmental contaminations relative to other methods (for details of the method see Sorokowska, 2013a). Body odor samples were collected twice from each donor: (i) without the use of cosmetics (i.e., natural body odor sample – natural BO condition) and (ii) while using cosmetics (BO+cosmetics). The odor donors were provided with two experimental sets each consisting of two 7 cm × 10 cm, 100% cotton pads, surgical hypoallergenic tape, unscented soap, a sterile 500 ml glass jar, and a new t-shirt.

For the collection of natural body odor samples, donors were asked to wash themselves with the unscented soap the morning of the experiment to attach the cotton pads under their arms with the surgical tape, put on the provided t-shirt (to avoid potential odor contamination from other clothes), and to wear the pads for twelve hours that day (Havlíček et al., 2011). The participants were asked to refrain from using scented cosmetics (e.g., fragrances, deodorants, and soaps), from consuming odorous foods (e.g., garlic, onions, or other spicy/odorous foods), and from drinking alcohol or smoking, beginning the day prior to the experiment (a standard procedure of studies that involve body odor assessment; e.g., Kohoutová, 2012; Roberts et al., 2013). Procedural instructions were provided in person and on a special instruction sheet that also included a questionnaire concerning the individual's activity during the body odor collection. No participant reported any major deviations from the procedure.

After 12 hours, the participants placed the pads in jars and returned them to the experimenter. The samples were then frozen overnight. Freezing of such samples has been shown to have no significant impact on perceived body odor quality (e.g., Roberts et al., 2008; Lenochova et al., 2009).

A similar procedure was repeated for the second collection of body odor samples. However, in this case, participants were free to use scented cosmetics.

Personality Assessment

After providing body odor samples, donors completed a self-description TIPI-PL personality questionnaire (Gosling et al., 2003; Polish adaptation by Sorokowska et al., 2014). The TIPI-PL is based on the Big Five personality model (Extraversion, Neuroticism, Openness to experience, Conscientiousness, and Agreeableness), and it consists of 10 pairs of adjectives, 2 pairs for each Big Five dimension (for example, Extraversion: "Extraverted, enthusiastic" and reversed "Reserved, quiet"). Our main motivation to use the brief personality assessment was to maintain the same procedure for the odor donors and odor raters in our study. We added two questionnaire items to assess self-perceived dominance. Participants were asked to rate how much they thought each scale applied to them on a 7-point scale (where 1 = definitely disagree and 7 = definitely agree).

Statistical Analyses

In the main experiment, we first run series of *t*-tests to compare the average ratings based on left- and right-sided samples for both men and women. To test the effect of sex and condition (natural vs. BO+cosmetics condition) on body odor assessment, we computed repeated measures ANOVAs. Ratings did not follow a normal distribution, however, ANOVA is robust to normality violation when employed on a sample size of $N > 100$. Therefore, we employed the parametric test. The study used a 2 (sex of the odor donor) × 2 (natural BO vs. BO+cosmetics conditions as repeated measure) design. Analyses were performed separately for each personality trait.

The congruence between self-assessments and ratings based on body odor was calculated in two ways. First, to test whether the congruence was higher for natural body odor samples or cosmetics use samples, we computed a "deviation from congruence", which was defined as the absolute difference between the self-assessment and rating (e.g., if self-assessed dominance was 5 and the rated dominance was 7, the "deviation from congruence" was 2). The lower the "deviation from congruence", the higher the congruence between the self-assessments and ratings based on odors. Second, we compared Spearman correlation coefficients for natural vs. cosmetics use odor samples. We used Spearman ranks because, according to the Shapiro-Wilk test of normality, none of the self-assessed traits were normally distributed (all $ps < 0.05$).

We tested the effects of sex and condition (natural BO vs. BO+cosmetics) on congruence of assessments using a repeated measures ANOVA. In the experiment, a 2 (sex of the odor donor) × 2 (natural BO vs. BO+cosmetics conditions as repeated measure) design was employed. The analysis was again performed separately for each personality trait.

Rating Sessions

Pilot Study

Methods used in previous studies of personality assessment based on body odor involved consecutive ratings of several personality characteristics based on a single odor sample (Sorokowska et al., 2012; Sorokowska, 2013a,b). Although this method decreases the possibility of olfactory adaptation, such a procedure may be more prone to the "halo effect", in which raters' assessments of various traits may not be entirely independent of one another (Nisbett and Wilson, 1977). Thus, prior to conducting the rating sessions, we tested for the possible presence of the "halo effect" by comparing two different procedures. In the first procedure, a group of 28 female judges (aged 19–22) assessed traits of a subset of odors "one by one", i.e., each odor sample was rated for perceived Neuroticism, Extraversion, Agreeableness, and Dominance using a single answer sheet. In the second procedure, a different group of 28 female raters (aged 19–22) assessed each characteristic on a separate answer sheet (the judges

first assessed the Neuroticism of all donors, then Extraversion of all donors, etc.; the sequence of samples was randomized). Each rater in our pilot study assessed 7 samples following one of the two procedures described above. In total, 49 samples of donors of both sexes were assessed. The samples that were used in the pilot study were not used in the main study (for example, if we used a sample from a right armpit of a given subject in the pilot study, in the main study we used a sample from the left armpit of the subject).

In the first procedure (consecutive assessments of traits), we observed significant correlations between rated Dominance and Neuroticism ($r = 0.32$, $p = 0.03$), Dominance and Extraversion ($r = 0.37$, $p = 0.01$), Dominance and Agreeableness ($r = -0.48$, $p < 0.001$), and Agreeableness and Neuroticism ($r = -0.43$, $p = 0.002$). In the second procedure (traits assessed separately), we observed a very similar pattern of correlations between Dominance and Extraversion ($r = 0.58$, $p < 0.001$), Dominance and Agreeableness ($r = -0.46$, $p < 0.001$), Agreeableness and Neuroticism ($r = -0.43$, $p = 0.002$), and Agreeableness and Extraversion ($r = -0.35$, $p = 0.002$). These correlations did not differ significantly between the two conditions for any of the traits assessed (test for difference between two correlation coefficients, *Statistica* software). Thus, we conducted the main study using consecutive assessments of traits. The main advantage of this procedure is that it is considerably less prone to olfactory adaptation as well as fatigue.

Main Experiment

In the main experiment, female raters were told to imagine a person connected to the scent they smelled, to rate his or her personality traits using a 7-point bipolar scale (the same which the donors had used to describe themselves), and to assess the sex of the person from whom the odor was taken (male/female). Following personality ratings, the judges rated the samples again, this time assessing the intensity, attractiveness, and pleasantness of the odor. Each woman rated the samples of six randomly selected odor donors (six samples of natural body odor and 6 samples of body odor with cosmetic use, both collected from the same odor donor).

RESULTS

Subjective Perceptual Differences: Effect of Condition and Sex

We found no significant differences between the average ratings based on the right- and left-sided samples for neither men nor women (all ps > 0.05). However, male odors were rated as more intense [$F(1,107) = 7.2$, $p < 0.008$, $\eta_p^2 = 0.06$], more pleasant [$F(1,106) = 7.0$, $p = 0.009$, $\eta_p^2 = 0.06$], and marginally more attractive [$F(1,106) = 3.1$, $p = 0.052$, $\eta_p^2 = 0.03$] than were female odors. Additionally, we found sex differences in attributed psychological traits. Men were rated as less Agreeable [$F(1,106) = 10.1$, $p < 0.002$, $\eta_p^2 = 0.09$], more Neurotic [$F(1,107) = 4.2$, $p < 0.05$, $\eta_p^2 = 0.04$], and more Dominant [$F(1,103) = 5.6$, $p = 0.02$, $\eta_p^2 = 0.05$] than were women. For assessments of Extraversion, the effect of donor sex was only marginally significant [$F(1,106) = 3.6$, $p = 0.06$, $\eta_p^2 = 0.03$].

We also found a significant effect of condition (see **Figure 1**). Body odor samples in the BO+cosmetics condition were assessed as more pleasant [$F(1,106) = 19.1$, $p < 0.0001$; $\eta_p^2 = 0.15$] and more attractive [$F(1,107) = 13.4$, $p < 0.001$, $\eta_p^2 = 0.11$] than were natural body odors, but there was no difference between the conditions in ratings of odor intensity ($F(1,107) = 2.7$, $p = 0.10$; $\eta_p^2 = 0.02$).

Further, there were no significant differences between conditions in personality judgments of body odor [Agreeableness ($F(1,107) = 0.6$, $p = 0.4$, $\eta_p^2 < 0.01$]; Dominance ($F(1,103) = 0.2$, $p = 0.60$, $\eta_p^2 < 0.01$; Neurotism ($F(1,107) < 0.01$, $p = 0.9$, $\eta_p^2 < 0.01$); Extraversion ($F(1,107) = 2.8$, $p = 0.10$, $\eta_p^2 = 0.025$)]. However, for Extraversion, we observed an interaction effect [sex-by-condition: $F(1, 107) = 8.1$, $p = 0.005$, $\eta_p^2 = 0.07$]. Extraversion ratings were higher for body odors in the BO+cosmetics condition than for natural body odors but only for female donors ($p = 0.007$; *post hoc* test with Bonferroni correction).

Congruence between Self-Assessments and Ratings Based on Body Odor Samples

We found no significant differences in congruence between the natural and cosmetic use odor conditions for Agreeableness [$F(1,107) = 0.4$, $p = 0.50$, $\eta_p^2 < 0.01$] and Extraversion [$F(1,107) = 2.9$, $p = 0.09$, $\eta_p^2 = 0.03$]. Congruence for Dominance and Neuroticism were significantly lower for cosmetics use body odor samples than for natural body odor samples [$F(1,103) = 5.9$, $p = 0.02$; $\eta_p^2 = 0.05$] and [$F(1,107) = 6.9$, $p = 0.01$; $\eta_p^2 = 0.06$; respectively].

Similarly to the previous analysis, correlations between self-assessments and ratings for Agreeableness were lower in the cosmetics use than natural condition (0.04 and −0.09, respectively). For Extraversion, the correlation increased from −0.12 to 0.10, for dominance, it decreased from 0.34 to 0.21, and for Neuroticism, it decreased from 0.20 to 0.15. However, none of these differences were statistically significant (two-tailed tests, all ps > 0.12). It is noteworthy that we replicated previous findings concerning congruence of self-assessments and assessments based on natural body odor for Neuroticism ($p < 0.04$) and Dominance ($p < 0.001$) and that ratings of dominance remained significantly congruent in the cosmetics use condition ($p < 0.04$; see **Table 1**).

DISCUSSION

The main aim of the current study was to test whether cosmetic use affects odor-based personality attributions. We corroborated previous findings demonstrating congruent perception between self-assessments and ratings of dominance and neuroticism based on natural body odors. In line with previous work, assessments of other personality traits (agreeableness, extraversion) did not correlate with self-reports. Critically, our results demonstrate that

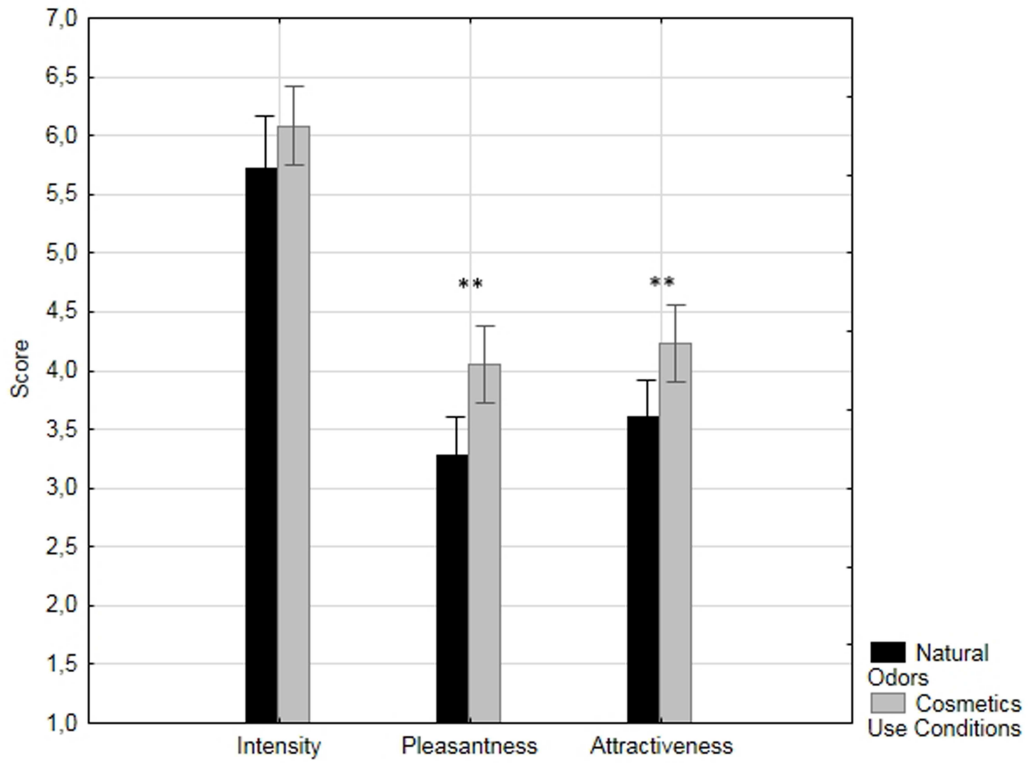

FIGURE 1 | Ratings of intensity, pleasantness, and attractiveness of body odor in the natural and cosmetics use conditions. Significant $p < 0.001$ is marked by **.

TABLE 1 | Correlations between self-assessed personality traits and body odor based personality judgments.

Personality trait	Natural condition	Cosmetics condition
	r_s	r_s
Agreeableness	0.04	−0.09
Neuroticism	0.20*	0.15
Extraversion	−0.12	0.11
Dominance	0.34**	0.21*

**$p < 0.01$; *$p < 0.05$.

when odor donors could use cosmetics, odor-based perceptions of their neuroticism no longer correlated with self-reports. In contrast, ratings of dominance significantly predicted self-assessed dominance in both the natural and cosmetics use conditions.

In agreement with the current findings, previous studies showed that neuroticism and dominance were relatively accurately assessed based on body odors (Sorokowska et al., 2012; Sorokowska, 2013a,b) and that dominance might influence the perception of body odor attractiveness (Havlicek et al., 2005). Thus, the effects reported for assessments of neuroticism and dominance from body odor appear to be robust, whereas assessments of extraversion are significantly associated with self-report in only one study (Sorokowska et al., 2012). The current study confirmed previous findings for natural body odor samples and further showed that ratings of dominance remain significant under more realistic conditions (i.e., when odor donors are permitted to follow their daily hygienic routine and use any cosmetics that they may normally use).

One may speculate about the contrasting effect of cosmetic use on neuroticism and dominance assessments. As the use of cosmetics appears to be a part of our self-presentation, people may use cosmetics in order to express themselves in a socially desirable manner. Personality traits vary in their social desirability, with neuroticism being considered rather undesirable in Western cultural settings (Konstabel et al., 2006). People may attempt to suppress neuroticism related cues with their fragrance choice. In contrast, people who tend to be dominant in social interactions might select perfumes that do not interfere with the personality impression based on their body odor. Perhaps they might even present themselves as being more dominant than they are in reality. Indeed, dominance cues appear to be a desirable characteristic of fragrances, and one that is frequently employed in advertisement of men's perfumes (Toncar and Fetscherin, 2012).

Consistent with previous studies (e.g., Lenochová et al., 2012), we observed increased ratings of attractiveness and pleasantness of body odor in the cosmetics use condition. However, there was no difference in personality attributions (with

the exception of extraversion ratings) between the cosmetics use samples and the natural body odors. Further, effect sizes (as assessed by partial eta squared) for the differences between the two conditions were quite low; suggesting that the effect of cosmetics use on mean values in personality attributions is rather modest.

There is robust evidence indicating that female body odor is on average considered more pleasant and less intense than male body odor is (McBurney et al., 1976; Hold and Schleidt, 1977). It also appears that more intense odors are stereotypically attributed as male, independent of the actual sex of the odor donor (Doty et al., 1978). Our study replicated past findings related to male body odor intensity, however – interestingly – we found that male body odors were rated as more pleasant than were female body odors. This result might be due to the fact that in our study, only female raters assessed the body odor samples. However, women are commonly found to be slightly more sensitive to various odors than men (Doty and Cameron, 2009), and they attach higher importance to olfaction in both sexual and non-sexual context (Havlicek et al., 2008). Also, previous studies regarding smell have shown that women more accurately recognize the sex of a donor on the basis of body odor (Hold and Schleidt, 1977) and that they are generally more accurate in their assessments of personality based on odor samples (Sorokowska et al., 2012). Although these are among the reasons that we employed female raters, future studies may test whether different results are obtained using male raters.

In the cosmetics use condition, participants were permitted to use fragranced cosmetics according to their personal routine. We did not control the quantity nor type of the cosmetics (i.e., deodorants, antiperspirants, perfumes) used by odor donors. The main rationale for this procedure was to collect the axillary odor samples under highly realistic conditions (i.e., to achieve high external validity). Also, Lenochová et al. (2012) showed that cosmetics selected by participants have higher effects on pleasantness and attractiveness ratings of body odor samples than do assigned cosmetics, which additionally suggests that assigned cosmetics might have differential effects on various body odor samples. In a similar line, it was recently reported that using your own fragrance compared to the assigned one increased success rate in individual discrimination of the fragrance-body odor blends (Allen et al., 2015). However, the procedure we used did not allow us to test the potential effect of different types of fragranced cosmetics. Thus, future studies should control for the type of cosmetics used by participants and investigate whether the cosmetics chosen by the participants compared to cosmetics assigned to them by researchers have different effects on how they are perceived. Future studies may also assess whether people are able to consciously modify the personality impression conveyed by the cosmetics they select. Finally, it would be of interest to examine whether different scents are chosen by participants depending on the social context (e.g., for a romantic meeting in contrast to a job interview).

It can be argued that some of the effects reported here might be attributed to the rating procedure. More specifically, raters were asked to assess all personality characteristics consecutively after smelling each odor sample. Although this procedure could potentially result in the "halo effect" (i.e., an impression made in one domain is transferred to an impression made in another domain in a stereotypic fashion; Nisbett and Wilson, 1977), the results of our pilot study indicated no major signs of the "halo effect" using this experimental paradigm for odor-based assessments. The main reason why we did not employ separate ratings of each trait is that it is considerably more time-consuming and, importantly, ratings might be affected by olfactory adaptation and fatigue. Another possible limitation of our study might be the use of the TIPI-PL scale both to measure the personality characteristics of the odor donors and to perform the ratings based on body odor samples. The TIPI is a very brief method (two items each consisting of two adjectives, i.e., four adjectives per personality characteristic), and its psychometric parameters are somewhat lower than those of longer inventories measuring the Big Five characteristics (Gosling et al., 2003; Sorokowska et al., 2014). However, thanks to the brevity of this tool, the raters could assess the samples using the same scales that the donors had used to describe themselves, and this enabled us to measure the congruence of self-assessment and odor-assessment sessions more precisely than in the case of the previous studies regarding the body odor and personality. Nevertheless, using the TIPI test could make both the self-assessments and ratings based on the odor samples slightly less reliable.

As discussed above, the congruent attribution of some personality domains based on body odor, namely neuroticism and dominance, appears to be robust. However, it is unclear whether people spontaneously employ these particular attributions when assessing others based on odor cues. Related research on personality attributions based on facial cues suggests that the most important dimensions are agreeableness and dominance (Oosterhof and Todorov, 2008). In the case of body odor, the hedonic dimension (pleasantness/attractiveness) and strength (intensity) seem to be among the most salient. As it was hypothesized in the previous studies (Sorokowska, 2013b), it is possible that the overall perceived pleasantness of odor samples might drive the personality-related judgments. However, it is also possible that some sex stereotypes might be additionally involved in this process, given that, like in our research, male and female body odor samples are generally rated differently. Additionally, research shows that unpleasant body odors are often associated with typically male characteristics (McBurney et al., 1976), which might create another link between sex stereotypes and these attributions. To understand the underlying cognitive processes related to personality assessments based on body odors, future studies should focus more on the overall impressions created by odor samples and investigate spontaneous associations generated by these odors.

To summarize, the current study tested the effect of cosmetic use on personality attributions. Our results showed that, when judging personality based on body odors of people using cosmetics, the raters were able to accurately assess the odor

donor's dominance but not neuroticism. It seems that cosmetics bias assessments of some important social cues and allow people to modify the impression they convey. People may employ cosmetics to be perceived in a socially desirable fashion and may attempt to cover cues that can lead to socially undesirable perception such as neuroticism. Future studies should explore how different types of cosmetic products such as deodorants and various perfumes specifically affect odor based personality judgments.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the study. AS collected the data. AS and PS analyzed the data. All authors drafted, critically revised, and approved the final version of the manuscript.

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Positive relationship between odor identification and affective responses of negatively valenced odors

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Hedonic ratings of odors and olfactory preferences are influenced by a number of modulating factors, such as prior experience and knowledge about an odor's identity. The present study addresses the relationship between knowledge about an odor's identity due to prior experience, assessed by means of a test of cued odor identification, and odor pleasantness ratings in children who exhibit ongoing olfactory learning. Ninety-one children aged 8–11 years rated the pleasantness of odors in the Sniffin' Sticks test and, subsequently, took the odor identification test. A positive association between odor identification and pleasantness was found for two unpleasant food odors (garlic and fish): higher pleasantness ratings were exhibited by those participants who correctly identified these odors compared to those who failed to correctly identify them. However, we did not find a similar effect for any of the more pleasant odors. The results of this study suggest that pleasantness ratings of some odors may be modulated by the knowledge of their identity due to prior experience and that this relationship might be more evident in unpleasant odors.

Keywords: food, smell, children, pleasantness, olfactory abilities, hedonic evaluation, odor preferences

Introduction

Preferences in adults can be described as "relatively stable evaluative judgments in the sense of liking or disliking a stimulus, or preferring it or not over other objects or stimuli" (Scherer, 2005). More specifically, olfactory preferences have been shown to have a profound impact on human psychology and behavior in varied aspects of life such as ingestion, environmental hazards, and social interactions (Stevenson, 2010). It is, therefore, important to understand the formation of these affective responses to odors and the effects of factors that may modulate them across the lifespan (for review see Rouby et al., 2009). The widely accepted view is that humans are not born with any fixed set of olfactory likes or dislikes and that affective responses toward odors are to a great extent shaped by evaluative conditioning (Herz, 2006), starting as early as in the pre- and peri-natal period (Marlier et al., 1998) and continuing in the context of everyday individual experience with odors within one's culture. Thus, certain odors are encountered more frequently than others in specific contexts and, as a result, are attributed with a locally specific meaning and hedonic value which people outside this cultural setting may not share. For example, in a cross-cultural study by Ayabe-Kanamura et al. (1998), significant differences in odor naming performance (also referred

to as "free identification") and ratings of pleasantness, edibility, and intensity between German and Japanese women were noted for many culture-specific odors, suggesting the crucial effect of odor familiarity on olfactory perception and ratings of pleasantness in particular.

Experience with odors constitutes a major factor modulating olfactory perception. It is thus frequently found that ratings of familiarity of a given odor are positively associated with ratings of pleasantness (Royet et al., 1999; Sulmont et al., 2002), although this finding does not invariably reach statistical significance (Savic and Berglund, 2000; Bensafi et al., 2002) or is not consistent across studies (Distel et al., 1999). Delplanque et al. (2008) have demonstrated that the strength of the association differs as a function of average odor pleasantness, with odors rated as pleasant exhibiting positive correlations with ratings of familiarity. However, no similar association was found for the unpleasant odors. This finding has recently been corroborated cross-culturally by Ferdenzi et al. (2013), who reported that the relationship between odor knowledge and affective response was generally asymmetrical and significant only for the pleasant odors, whereas the unpleasant ones seemed more resistant to cognitive modulation. In a similar vein, Konstantinidis et al. (2006) have demonstrated that identification of unpleasant odors (but not pleasant ones) was relatively independent of age. Finally, using the test of odor identification as a proxy for odor experience, Knaapila et al. (2007) have shown that some odors, which varied significantly in terms of mean pleasantness, were evaluated as more pleasant when correctly identified than when not. Overall, unpleasant odors tend to be less susceptible to cognitive and contextual effects.

The major body of evidence comes from studies with adult participants, who have already acquired substantial odor semantic knowledge, but this may be somewhat different in children. Indeed, although olfactory perception is extensively shaped by experience, affective responses to some biologically relevant odors appear to be independent of previous experience (Soussignan et al., 1997). As children have lower levels of odor semantic knowledge, their hedonic perception could be more influenced by the physicochemical properties of odors. Several previous studies have shown that odorant structure can predict hedonic perception (e.g., Khan et al., 2007; Mandairon et al., 2009) and this may occur in a manner that is dependent on the age of the participants. Specifically, Poncelet et al. (2010) measured hedonic response to odors in different age groups and reported a pronounced role of physicochemical properties in processing of odor hedonics in (prepubertal) children and elderly people, who, respectively, exhibit either a low level of, or a weak access to, odor semantic knowledge. This was in contrast to teenagers and young adults, who are characterized by higher levels of semantic odor representation. Among the physicochemical properties of odorants that can make an odor *a priori* unpleasant are those related to trigeminal stimulation (pungency; Herz, 2006), which triggers neurological protective reactions that help avert the organism from potentially harmful materials (for a review see Doty and Cometto-Muñiz, 2003).

The aim of the present study was to explore the relationship between knowledge of an odor's identity (assessed by means of performance on a cued identification task) and pleasantness ratings in a cohort of prepubertal children, who have less experience with odors than adults and in whom the process of odor knowledge acquisition is evident from their increase in odor identification scores with age (Ferdenzi et al., 2008). Although inclusion of preschool children would have been particularly informative, recruitment of slightly older children helped prevent several methodological issues related to limitations on young children's attention span and motivation. We hypothesized that an odor would be rated as more pleasant when identified correctly, aiming to assess whether the previously reported positive relationship between odor pleasantness and olfactory knowledge could be generalized to an age group that clearly exhibits ongoing olfactory learning. In so doing, we used a cued odor identification task on which Czech children perform well (Dudova et al., 2011; Hrdlicka et al., 2011) and for which individual odor identification rates as well as pleasantness ratings in the adult European population across the lifespan are well-established (e.g., Konstantinidis et al., 2006).

Materials and Methods

Participants

The participants were 91 children of Czech origin (36 boys, mean age 9.31 ± 0.73, range 8–11 years), who were third ($N = 44$; 15 boys) and fourth graders ($N = 47$; 21 boys) from two mixed-sex general education elementary schools. There was no significant difference in the proportion of boys and girls across grades in the sample, $\chi^2(1) = 1.12$, $p = 0.29$, and they did not differ in terms of mean age or age distribution, boys = 9.44 ± 0.82 and girls = 9.24 ± 0.67 years, respectively, $t(59.14) = 1.22$, $p = 0.23$. Two cases (boys) were not included in the analysis because the absolute distance of their ratings from the median exceeded the cut-off based on the median absolute deviation (Wilcox, 2010) for 8 out of 16 odors, and, at the same time, their ratings represented extremes in two out of the total of four plots in which outliers and extremes were visually detected.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). The study was approved by the IRB of the Charles University (Approval Number 2008/4). The children's parents provided written informed consent.

Olfactory Measures

Olfactory assessment included ratings of odor pleasantness and an odor identification test. We used the 16-item Sniffin' Sticks odor identification test, a psychophysical test of orthonasal chemosensory performance based on pen-like odor dispensing devices. The Sniffin' Sticks test has been widely used by clinicians and researchers across Europe to test olfactory abilities in adults (Hummel et al., 2007b) and children (Ferdenzi et al., 2008; Renner et al., 2009; Dudova et al., 2011; Hrdlicka et al., 2011). The identification test consists of odorants familiar to the general European population, such as orange, rose, garlic, and fish (full

TABLE 1 | Percentages of correct identifications and mean pleasantness for individual items of the Sniffin' Sticks identification test ($N = 89$).

Item	Percent identified	95% Confidence intervals	Mean ± SD Pleasantness		
			Overall	Correctly identified	Not identified
Orange	40.4%	30.9, 50.8	4.26 ± 1.05	4.22 ± 1.05	4.28 ± 1.062
Leather	47.2%	37.2, 57.5	2.90 ± 1.31	2.76 ± 1.34	3.02 ± 1.29
Cinnamon	78.7%	69, 85.9	3.94 ± 1.14	3.86 ± 1.17	4.263 ± 0.99
Mint	86.5%	77.9, 92.1	4.08 ± 1.07	4.03 ± 1.09	4.42 ± 0.90
Banana	89.9%	81.9, 94.6	4.16 ± 1.09	4.18 ± 1.08	4.00 ± 1.22
Lemon	32.6%	23.7, 42.9	3.53 ± 1.27	3.76 ± 1.09	3.42 ± 1.34
Liquorice	60.7%	50.3, 70.2	3.49 ± 1.28	3.48 ± 1.28	3.51 ± 1.29
Turpentine	31.5%	22.8, 41.7	2.51 ± 1.11	2.50 ± 1.26	2.51 ± 1.04
Garlic	75.3%	65.4, 83.1	2.08 ± 1.28	2.21 ± 1.31	1.68 ± 1.13
Coffee	77.5%	67.8, 85	1.99 ± 1.17	2.07 ± 1.20	1.70 ± 1.03
Apple	10.1%	5.4, 18.1	3.90 ± 1.18	4.22 ± 0.83	3.86 ± 1.21
Clove	73.0%	63, 81.2	2.07 ± 1.15	2.05 ± 1.18	2.13 ± 1.08
Pineapple	57.3%	46.9, 67.1	3.61 ± 1.35	3.61 ± 1.40	3.61 ± 1.31
Rose	55.1%	44.7, 65	4.08 ± 1.15	4.06 ± 1.21	4.10 ± 1.08
Anise	38.2%	28.8, 48.6	3.16 ± 1.22	2.85 ± 1.13	3.35 ± 1.25
Fish	69.7%	59.5, 78.2	1.66 ± 1.00	1.74 ± 0.94	1.48 ± 1.12

Note that pleasantness ratings have been recoded (1 = least pleasant, 5 = most pleasant).

list in **Table 1**). Cued identification is employed, in which participants select the name of the target odor from a candidate list of four. The resulting score is the sum of correct answers, which can vary between 0 and 16, with 4 as a chance score (Hummel et al., 1997). The same set of odorants was used to obtain category ratings of odor pleasantness, which copied the system of grading used in Czech schools (1 being the best grade achievable and 5 being the failing grade) to facilitate scale comprehension by this age group (1 = very pleasant odor, 5 = very unpleasant odor). The scores were subsequently recoded to 1 = very unpleasant, 5 = very pleasant.

Procedure

The children participated in individual testing sessions, which were scheduled for morning during school time, to avoid possible diurnal fluctuations in olfactory abilities. The testing took place in a quiet, ventilated room without strong ambient odors. The stimuli were presented in the order recommended by Hummel et al. (1997) for the standard procedure. The presentation of each stimulus took approximately 5 s. Subsequent stimuli were presented immediately after the participant selected a verbal label/pleasantness rating for the previous stimulus. Since a verbal label may affect hedonic perception (e.g., Herz, 2003), ratings of pleasantness were obtained first for all odors, followed by the task of odor identification. Subsequently, the participants were interviewed about their odor awareness using the COBEL questionnaire (Ferdenzi et al., 2008). The part on odor awareness has been published elsewhere (Saxton et al., 2014) and is not further reported here.

Statistical Analysis

All analyses were carried out with IBM SPSS 22.0. Normality of the raw data was checked for each odor separately. Firstly, we produced skewness and kurtosis values and their respective SEs, from which z-scores were computed and compared to the value of 1.96, as suggested by Field (2005). Secondly, we visually examined individual histograms of all relevant variables. Finally, we ran the Shapiro–Wilk's W test for each variable. Since the results of the Shapiro–Wilk's test, visual examination of the respective histograms, and skewness z-scores all indicated that the pleasantness ratings of each individual odor departed significantly from normality, non-parametric tests were employed where possible.

Descriptive Statistics

Based on the method proposed by Bonett and Price (2002), we computed 95% confidence intervals (95% CI) for median pleasantness of each odor. Confidence intervals for the proportions of correct identifications were computed following the method recommended by Newcombe and Altman (2000).

To test whether association between odor identification and pleasantness ratings is limited to unpleasant odors, we aimed to classify the odors on the basis of their median pleasantness. The median pleasantness values for each odor were entered into a two-step cluster analysis, in which we predefined three clusters in the solution and used default settings. Although a Shapiro–Wilk test showed that the assumption of normality was not met, $W = 0.862$, df = 16, $p = 0.021$, the procedure is considered fairly robust to violations of the assumption (IBM SPSS, 2012). Since the final solution may depend on the order of cases, to verify the stability of the solution, several trials with randomly ordered cases were run. The analysis repeatedly yielded a model of good cluster quality (average silhouette of 0.8). The group of pleasant odors included the odors of orange [median pleasantness rating of 5; 95% CI (4.49, 5.51)], apple, banana, cinnamon, lemon, liquorice, mint, pineapple, and rose [all with a median pleasantness rating of 4; 95% CIs (3.49, 4.51)]. The group of unpleasant odors consisted of the odor of fish (median pleasantness rating of 1), clove, coffee, and garlic [each with a median pleasantness

rating of 2, 95% CIs (−1.77, 3.39)]. The remaining odors (anise, leather, and turpentine) all received a median pleasantness rating of 3; 95% CIs (2.49, 3.51). The mean pleasantness values for the three groups are depicted in **Figure 1**.

The percentages of correct identifications and mean pleasantness ratings for each of the odors are given in **Table 1**.

Correlational Analyses of Odor Identification Scores and Pleasantness Ratings

To test for any overall association between individual children's performance scores on the odor identification test and their median pleasantness ratings given to the odors, Kendall's Tau correlations were performed. These analyses were performed on averages per participant of, firstly, all the 16 odors, secondly, the subset of nine pleasant odors (median pleasantness of 4), and thirdly, the subset of four unpleasant odors (median pleasantness of 2).

Odor-Specific Analyses: Odor Identification as a Predictor of Odor Pleasantness

Finally, to test whether the sought effect could be limited to certain individual odors, rather than spanning whole odor subsets, we performed odor-specific analyses. First, to determine whether children's pooled responses could be conceived of as a homogeneous sample, we tested for the effect of sex and age on odor identification performance and pleasantness ratings of the individual odors, respectively. Both of these variables are known to affect odor identification in children (Ferdenzi et al., 2008). To do this, we ran multiple Categorical Regression (CATREG) analyses using the IBM SPSS (2012) Optimal Scaling option. The independent variables of sex and age were treated as nominal and numeric, respectively, and the dependent variables of identification performance and pleasantness rating were scaled as nominal and spline ordinal, respectively. Both the nominal variables were categorized into groups of two, and the numeric and spline ordinal variables by ranking. A random initial configuration was selected, as recommended in cases in which at least one of the predictors has a nominal scaling level. The rest of the options were left to default settings. Subsequently, predictions of individual odor pleasantness with odor identification (a yes/no response) were modeled in the same manner, using identical settings.

Results

Correlational Analyses of Odor Identification Scores and Pleasantness Ratings

Correlational analyses revealed no significant association between children's total identification scores and their mean pleasantness ratings for the complete set of odors, Kendall's Tau-$b = -0.07$, $p = 0.36$, $N = 89$ (**Figure 1**). That is, children who tended to correctly identify more odors than others did not exhibit any tendency toward higher ratings of pleasantness in

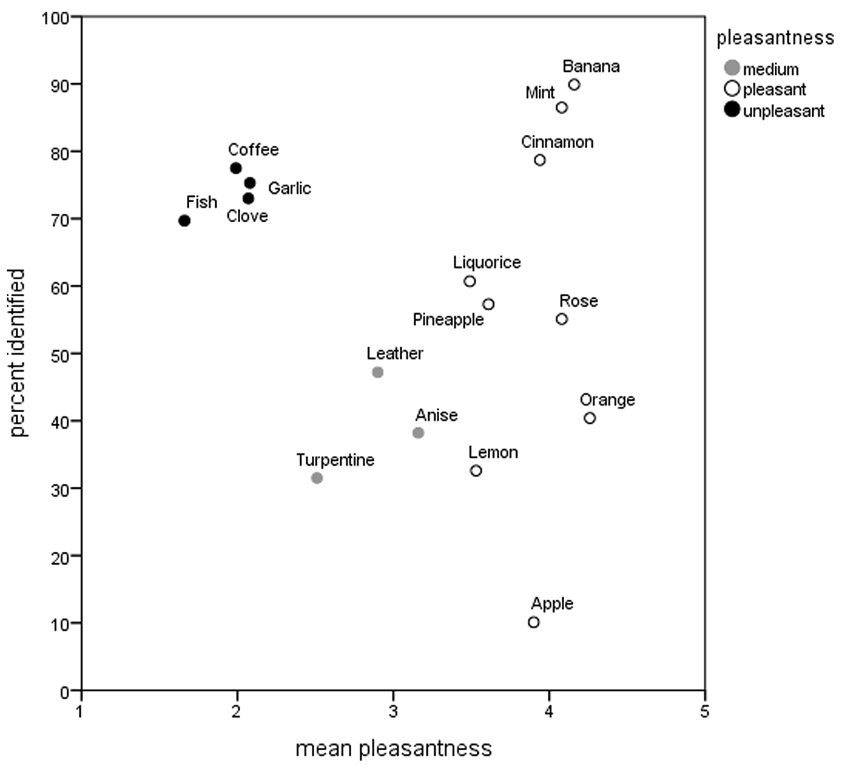

FIGURE 1 | Scatter plot of mean pleasantness ratings and percentages of correct identifications for the 16 odors of the Sniffin' Sticks odor identification test. The pleasant and unpleasant subsets are given in white and black, respectively, and the medium pleasant odors are given in gray.

general. Nor was there such an association found for the subsets of pleasant, medium, and unpleasant odors analyzed separately, Kendall's Tau-$b = 0.01$, Kendall's Tau-$b = 0.02$, $N = 89$, and 0.04, $N = 89$, all ps > 0.05, respectively. For exploratory purposes we also fitted a quadratic regression model to the data which however was not significant ($p > 0.1$). Relative frequencies of correct identification and mean pleasantness ratings for the individual odors can be found in **Table 1**.

Odor-Specific Analyses: Odor Identification as a Predictor of Odor Pleasantness

First, to test whether participant characteristics (sex and age) predicted odor identification and pleasantness ratings, multiple CATREG analyses were run. These showed that identification of the odor of orange was predicted by sex, $\beta = 0.25$, $F = 6.96$, $p < 0.01$, and age, $\beta = 0.21$, $F = 5.40$, $p < 0.05$, with girls and older children being more likely to correctly identify the odor. Also, sex (but not age) predicted pleasantness ratings of orange, $\beta = 0.23$, $F = 6.24$, $p < 0.05$, with girls (mean 4.35 ± 0.91 SD) rating the odor as more pleasant than boys (mean 4.12 ± 1.25 SD). However, both models only explained about 9% of the total variance in identification and pleasantness of orange, $R^2 = 0.095$, $F(2,88) = 4.50$, $p < 0.05$ and $R^2 = 0.093$, $F(2,88) = 4.39$, $p < 0.05$. Further, sex (but not age) also predicted pleasantness ratings of the odor of apple, $\beta = 0.24$, $F(1) = 5.76$, $p < 0.05$, again with girls (mean 4.09 ± 1.08 SD) giving higher pleasantness ratings to the odor than boys (mean 3.59 ± 1.28 SD). The overall model was significant but only explained 7.3% of the total variance in pleasantness ratings of the odor of apple, $R^2 = 0.07$, $F(2,88) = 3.37$, $p < 0.05$. Thus, for the odors of orange and apple, sex was included as a predictor in the subsequent analyses. There were no significant sex and age effects on identification or pleasantness ratings of any other odorants.

Second, and more importantly, identification significantly predicted odor pleasantness in two cases: firstly, in the odor of garlic, $\beta = 0.24$, $F = 7.75$, $p < 0.01$; $R^2 = 0.06$, $F(1,88) = 5.36$, $p < 0.05$, and, secondly, in the odor of fish, $\beta = 0.25$, $F = 6.97$, $p < 0.01$; $R^2 = 0.06$, $F(1,88) = 5.56$, $p < 0.05$. In both cases higher pleasantness ratings were given to these odors by children who correctly identified them (**Figure 2**). No significant relationship between odor identification and pleasantness was found for any of the other tested odors (**Table 2**).

Discussion

The key objective of the present study was to explore the relationship between children's knowledge of an odor's identity, assessed with a cued odor identification test, and pleasantness ratings given to these odors. The results show that identification success or failure only predicted odor pleasantness in the two cases of garlic and fish, both of which also happened to fall among the unpleasant odors. The two odors tended to be given higher ratings of pleasantness by children who could identify them correctly than by those who could not.

The Relation of Odor Identification and Pleasantness

In the study by Knaapila et al. (2007) with adult participants, the odors of cinnamon, lemon, rose, and banana were evaluated as more pleasant, and turpentine as less pleasant, by individuals who had identified them correctly compared with those who had not, suggesting that the association between knowledge of an odor's identity, assessed with an odor identification test, and odor pleasantness may take different directions for different odors. The positive relationship between odor identification

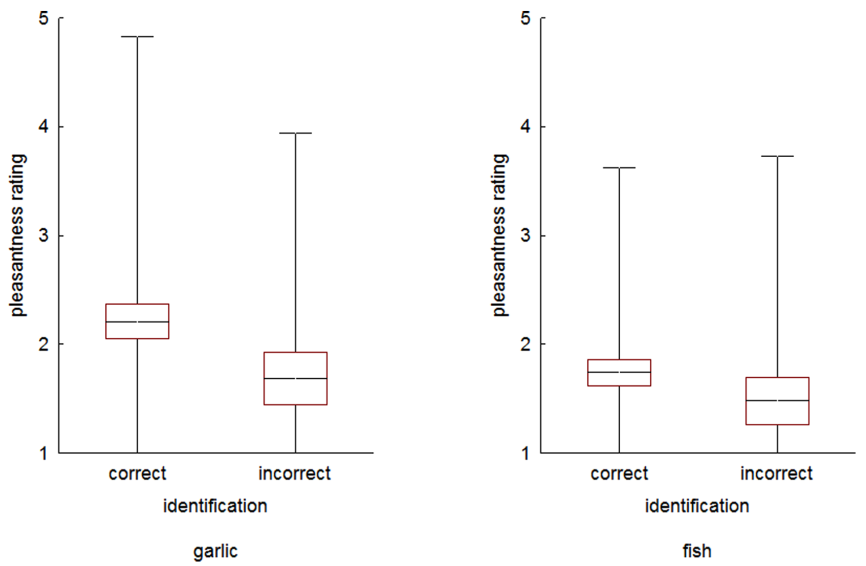

FIGURE 2 | Ratings of pleasantness in children who correctly identified and those who did not for the odors of garlic and fish. Middle line denotes mean, boxes ± SEM and error bars ± 2SD. The differences are significant at $p > 0.05$.

TABLE 2 | Categorical regression (CATREG) analysis for predicting odor pleasantness from identification (correct/incorrect) for the individual odors.

	Model			Identification		
	R^2	*F*	*p*	β	*F*	*p*
Orange	0.044	1.970	0.146	0.076	0.771	0.382
Leather	0.030	2.698	0.104	0.173	2.329	0.131
Cinnamon	0.027	2.407	0.124	0.164	3.229	0.076
Mint	0.026	2.295	0.133	0.160	3.569	0.062
Banana	0.013	1.188	0.279	0.116	1.438	0.234
Lemon	0.032	2.843	0.095	0.178	5.244	0.024
Liquorice	0.001	0.044	0.835	0.022	0.088	0.767
Turpentine	0.041	3.755	0.056	0.203	4.524	0.036
Garlic	**0.058**	**5.359**	**0.023**	**0.241**	**7.748**	**0.007**
Coffee	0.028	2.541	0.115	0.168	4.637	0.034
Apple	0.014	1.269	0.263	0.120	5.222	0.025
Clove	0.017	1.538	0.218	0.132	9.889	0.002
Pineapple	0.008	0.662	0.418	0.087	1.059	0.306
Rose	0.001	0.107	0.745	0.035	0.202	0.654
Anise	0.050	4.550	0.036	0.223	3.225	0.076
Fish	**0.060**	**5.559**	**0.021**	**0.245**	**6.965**	**0.009**

Odors for which both the model and predictor were significant at $p < 0.05$ are given in bold.

and pleasantness was reported for odors which were on average rated as relatively more pleasant (Knaapila et al., 2007). Similarly, Mennella and Forestell (2008) found in 5 to 8-year-olds higher identification rates in the odors they liked (bubble gum, strawberry, chocolate). However, the direct comparison with this study might be limited due to the differences in odor identification assessment (the former study employed a free odor identification task while in the present study we used a cued identification test). In contrast to both studies, in the present study a positive association was found for two of the four unpleasant odors. To further complicate this issue, Bensafi et al. (2007) showed that a shift in pleasantness ratings in correctly identified odors was limited only to those judged on average as neutral. The apparent discrepancies across the individual studies point to the complexity of the association between odor identification and pleasantness. This might be due to modulating factors which were not controlled for in the previous studies and, as a consequence, the association between odor identification and pleasantness might sometimes be limited to pleasant, neutral, or even unpleasant odors, as in the current study. Such modulating factors may include variation in pleasantness, familiarity, edibility, or pungency of the employed set of odorants. Researchers should address these issues while designing future studies to clarify reasons for these apparent discrepancies.

Furthermore, in our study, the positive relationship did not pertain to all odors rated as rather unpleasant but was limited to garlic and fish, whereas pleasantness ratings of the other two unpleasant odors (coffee and clove), which exhibited similar pleasantness ratings and percentages of correct identifications, were not related to identification success or failure. Consequently, this raises the question of how, besides the variables assessed within the present study, these two odors might differ from those of fish and garlic. One explanation may stem from the fact that the participants were children: unlike garlic and fish, coffee and clove may not be categorized as food odors by children. In the case of coffee, the obvious reason would be that most exposure to this odor in Czech children of this age group is through its presence in the children's close, everyday environment but not through direct consumption. Indeed, reports of coffee consumption in prepubertal children in various European countries show rather negligible values (Meltzer et al., 2008; Duffey et al., 2012; Ng et al., 2012) and a flavor preference study showed coffee to be amongst the least preferred in this age group, as well as in younger children (Liem et al., 2010). The odor of clove, in adults at least, tends to be associated with experiences at the dentist's rather than with food. For instance, in a study that assessed autonomic emotional responses to odors, it was found that the clove-smelling odorant eugenol, which is used in dentistry, was given very low pleasantness ratings and elicited autonomic reactions indicative of stress in participants who feared dental procedures (Robin et al., 1998, 1999). However, formation of this association in young children will be comparatively rare. Thus, the odors of coffee and clove may differ from the equally unpleasant odors of garlic and fish in that they may be less relevant to their everyday life. Unpleasant stimuli seem to constitute a unique odor category, e.g., they elicit faster and more accurate reactions since they may signal a potential danger (Boesveldt et al., 2010). It is for just this kind of odor that we would most expect to see changes in perception with increasing familiarity – where initial odor unpleasantness can be modulated by a learned association with food. Alternatively, but rather speculatively, since a major contributor to odor unpleasantness is trigeminal stimulation, and garlic and fish are arguably the most pungent stimuli in the set, it might be suggested as a mediating factor. However, at odds with this suggestion are the results for mint, which shows a relatively strong trigeminal component and yet was on average judged as rather pleasant. Thus, the validity of this suggestion should be addressed in future studies.

Another possibility is that the correct identification of fish and garlic is facilitated by pungency or odor intensity, as such distinctly perceptible odors may be less prone to confusion than others. However, all of the 4 unpleasant odors were identified at similar rates (see **Figure 1**) even though there is wide variation in their mean perceived intensity (see Konstantinidis et al., 2006). Furthermore, although garlic and fish are rated as relatively more intense than coffee and clove, there is no obvious relationship between intensity and identification across the 16 odorants used in the Sniffin' Sticks test (Konstantinidis et al., 2006). Hence, it seems relatively unlikely that intensity or pungency could have produced the observed pattern of results, compared with our suggested alternative regarding learning and familiarity.

Correct Identification Percentages for Individual Odors

In line with previous studies (e.g., Boesveldt et al., 2008; Haehner et al., 2009), significant differences were noted for the individual odors in the percentages of correct identifications (see **Figure 1**). There is ample evidence that across the population of European adults, the Sniffin' Sticks' odor of turpentine, along with apple, lemon, and sometimes anise, quite invariably tend to be misidentified (Eibenstein et al., 2005; Konstantinidis et al., 2008; Haehner et al., 2009; Catana et al., 2012; Orhan et al., 2012). The poor performance on some odors might be due to their less prevalent real-life significance or, possibly, less realistic sensory representation in the Sniffin' Sticks test. This could, at least, have been the case with apple, which was correctly identified by as few as one tenth of the participants. Another source of variation in cued odor identification tests is the nature of the distractor verbal labels provided. In some odors they might be more semantically or perceptually related to the target label than in other odors, which may, in turn, affect identification rates. Also, the unequal familiarity of the distractor verbal labels might have an impact on identification success rate as participants may use an exclusion heuristic to reach a correct answer without actually knowing the correct label. Although the Sniffin' Sticks test is a widely used instrument both in research and clinical settings, to our knowledge the equality of the distractor labels has not been systematically assessed.

The issue of age-appropriateness of the items employed is specifically relevant to the present study. The Sniffin' Sticks odor identification test has been successfully used with children before, including children as young as 3 years of age, with a success rate of 81% in children aged 6 years and over (Hummel et al., 2007a). In the olfactory tests deemed suitable for children, turpentine, and anise are not typically included but the other items have been successfully used in previous studies employing various other olfactory tests, both orthonasal and retronasal, with children as young as four-year-old (Richman et al., 1995; Monnery-Patris et al., 2009; Renner et al., 2009).

The effect of age on identification scores in our study was limited to only two odors (orange and apple). Taken at face value, this might be surprising as the effect of age is commonly reported in studies on odor identification in children (Richman et al., 1995; Ferdenzi et al., 2008; Monnery-Patris et al., 2009). The mostly negative findings reported here might be a consequence of the limited age range in our sample (8–11, with only three children being 11 years old). Further, in case of orange, which was the first item presented, the age effect might reflect a lack of concentration in the younger children at the beginning of the session.

Sex differences in odor identification, with women on average showing higher scores, have been repeatedly reported in adults (for reviews see Brand and Millot, 2001; Doty and Cameron, 2009) and some studies also found a similar pattern in prepubertal children (Richman et al., 1995; Ferdenzi et al., 2008; Monnery-Patris et al., 2009). Based on the current data, we found no significant differences in the overall identification score (data not shown, for details see Saxton et al., 2014). The negative results in our sample might be due to a limited statistical power as mean values were similar to those obtained by Ferdenzi et al. (2008) in French and Finnish children. When individual odors were analyzed separately, significantly higher scores in girls were found for the odor of orange. As identification scores in other odors showed no sex differences and the effect size in the case of orange was rather limited, we note that these results should be interpreted rather cautiously.

Identification as a Proxy for Prior Experience

In the present study, odor identification was employed as a proxy for prior experience in order to overcome developmental differences in children's use of various rating scales. In particular, younger children are more likely to respond at the extremes of rating scales (Chambers and Johnston, 2002) and, further, Berman et al. (1989) have suggested that even 8 to 10-year-olds tend not to assign ratings across the full range of the five-point rating scale. One might argue that for the sake of comparison, we could have collected both data on identification and familiarity ratings. However, we felt this was not achievable without compromising the quality of the collected data as attentional/perceptual capacity of the tested children is relatively limited.

However, the present approach also poses various methodological challenges. Most importantly, it is critical to consider the effect of the context provided by the odor label on olfactory perception and any subsequent ratings. Verbal labeling is known to modulate the perceived pleasantness of a given odor in adults and children alike (Bensafi et al., 2007), regardless of whether the identification has been correct or not (Ayabe-Kanamura et al., 1997), and whether or not the odor itself is actually presented (Herz, 2003). Therefore, in terms of the order of the tasks, we followed the procedure employed in previous studies (e.g., Distel et al., 1999; Degel et al., 2001; Sulmont et al., 2002) and obtained hedonic ratings first, before investigating what the participants knew about an odor's identity. Nevertheless, a covert, unprompted identification attempt may have occurred during ratings of pleasantness, well before the participants were instructed to do so. Besides this, participants might hold multiple hypotheses about this identity (Cain et al., 1998) and if this were the case, it would be impossible to know which actually affected the pleasantness ratings.

Finally, in the present study, odor identification performance was, on a given trial, only coded as a "success" (1) or "failure" (0). Although some responses classified as "incorrect" might have been less of a miss than others,

to be able to decide about the so-called near- and far-misses (Cain, 1979) one would have needed to know, among other things, the level of semantic similarity between the labels, as assessed specifically by this age cohort. Therefore, we caution that the reported correct identification percentages for the individual odors are not to be considered entirely synonymous with odor knowledge due to prior experience.

Conclusion

The present study aimed to explore whether the previously reported positive relationship between odor pleasantness and olfactory knowledge can be generalized to an age group that clearly exhibits ongoing olfactory learning, using a cued odor identification task as a proxy for prior experience with odors. We found a positive effect for two of the unpleasant odors, but not for any pleasant ones. In order to be able to make robust generalizations about the relationship between odor pleasantness and knowledge in children, future studies should employ a wider range of odors with contrasting pleasantness, and labels for which a degree of semantic similarity can be inferred, and should assess familiarity and intensity of the tested odors.

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Viewing Olfactory Affective Responses Through the Sniff Prism: Effect of Perceptual Dimensions and Age on Olfactomotor Responses to Odors

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Sniffing, which is the active sampling of olfactory information through the nasal cavity, is part of the olfactory percept. It is influenced by stimulus properties, affects how an odor is perceived, and is sufficient (without an odor being present) to activate the olfactory cortex. However, many aspects of the affective correlates of sniffing behavior remain unclear, in particular the modulation of volume and duration as a function of odor hedonics. The present study used a wide range of odorants with contrasted hedonic valence to test: (1) which psychophysical function best describes the relationship between sniffing characteristics and odor hedonics (e.g., linear, or polynomial); (2) whether sniffing characteristics are sensitive to more subtle variations in pleasantness than simple pleasant-unpleasant contrast; (3) how sensitive sniffing is to other perceptual dimensions of odors such as odor familiarity or edibility; and (4) whether the sniffing/hedonic valence relationship is valid in other populations than young adults, such as the elderly. Four experiments were conducted, using 16–48 odorants each, and recruiting a total of 102 participants, including a group of elderly people. Results of the four experiments were very consistent in showing that sniffing was sensitive to subtle variations in unpleasantness but not to subtle variations in pleasantness, and that, the more unpleasant the odor, the more limited the spontaneous sampling of olfactory information through the nasal cavity (smaller volume, shorter duration). This also applied, although to a lesser extent, to elderly participants. Relationships between sniffing and other perceptual dimensions (familiarity, edibility) were less clear. It was concluded that sniffing behavior might be involved in adaptive responses protecting the subject from possibly harmful substances.

Keywords: olfaction, motor response, affect, aging, hedonics

INTRODUCTION

One important characteristic of the human sense of smell is that it is a highly emotional sense. Affective responses to odors, and especially the most obvious ones such as attraction and disgust, serve important adaptive functions (Stevenson, 2010). They are involved in the regulation of behavioral response to events in the surrounding environment. Some particular smells can warn against toxic or dangerous substances (e.g., spoiled food, fire), enabling us to avoid serious environmental hazards. Other types of odor play a major role in sensory pleasure, modulating the ingestion of food, or contributing to social communication through attraction toward mates or attachment to kin. Such emotional responses to odors are expressed at different levels, from conscious and possibly verbalized subjective feelings to physiological changes and motor expression (e.g., Scherer, 2000). Measuring them thus requires differing methodological approaches, at the verbal (Churchill and Behan, 2010; Ferdenzi et al., 2013a), autonomic (e.g., Alaoui-Ismaïli et al., 1997; Bensafi et al., 2002a) and motor levels (such as sniffing behavior: Bensafi et al., 2003, 2007).

Research in animals and in humans has shown that sniffing, which is the active sampling of olfactory information through the nasal cavity, is of considerable importance in odor perception. The mere act of sniffing (whether or not an odorant is present) induces activation in the piriform cortex (Sobel et al., 1998), thus probably preparing the primary olfactory cortex for the arrival of olfactory information and detection of odors by the olfactory system. Laing, who was one of the first to investigate sniffing in humans, wrote (Laing, 1983, p. 99–102): "Perception of an [odor] in the environment usually initiates a sniffing episode [. . .]. Each sniff appears to be of shorter duration and to have a greater inhalation velocity than a normal breath" and "this [behavior] may enhance [odor] perception by increasing the amount and rate at which [odor] molecules reach the olfactory receptor epithelium." He also reported that sniff volume, duration and number during a sniffing episode decreased with increasing odor concentration, thus reducing the amount of inhaled odor when strong. Sniff volume and duration were also found to be inversely related to odor concentration in later studies (Warren et al., 1994; Walker et al., 2001; Johnson et al., 2003) and top-down accommodation to stimulus properties seems to occur very rapidly (160–260 ms) after onset of the first sniff (Johnson et al., 2003). This "concentration-dependent" characteristic of sniffing behavior was later exploited to set up a simple test of olfactory sensitivity based on the reduction in sniff volume and duration in presence of an odor compared to non-odorized air (Frank et al., 2003).

Although some authors have argued that other perceptual dimensions of odors such as hedonics occur too late in the neural cascade to have an influence on the nearly reflexive sniffing behavior (Johnson et al., 2003), there is now psychophysiological evidence that sniffing is modulated not only by odor intensity but also by subjective pleasantness. For example, breathed volume was visibly lower for the unpleasant odor of acetic acid than for the pleasant rose-like odor of phenylethanol (Warren et al., 1994). Similar findings were obtained comparing sniff volume in response to valeric acid compared with phenylethanol (Johnson et al., 2006), and to isointense odors of rotten egg (ammonium sulfide, unpleasant) compared with rose (phenylethanol; Bensafi et al., 2003) or strawberry (Bensafi et al., 2007), either perceived or imagined. In the latter comparison, differences extended to sniff duration, and notably, proved resilient, persisting in spite of instructions to maintain each sniff for a specific, constant duration. A pairwise comparison of groups of pleasant vs. unpleasant odorants provided similar conclusions (Prescott et al., 2010).

It is now clear that sniffing is part of the olfactory percept, since it (i) is influenced by stimulus properties, (ii) affects how an odor is perceived, and (iii) is sufficient in itself (with no odor present) to generate an olfactory percept and activate the olfactory cortex (Mainland and Sobel, 2006). However, the affective correlates of sniffing behavior, and in particular modulation of volume and duration as a function of odor hedonics, merit further investigation. Interpreting the motor expression of odor perception could, for example, be particularly informative in specific populations that are cognitively immature (children) or cognitively impaired (e.g., Alzheimer, Parkinson patients) and whose ability to verbally describe odor-related feeling is limited. However, to date many aspects of the relationship between sniffing behavior and odor hedonic valence remain unclear, in both these specific populations and the general population.

In this regard, several questions arise. Firstly, which psychophysical function best describes this relationship (e.g., linear, polynomial)? To date, only pairwise comparisons have been performed (between a pleasant and an unpleasant odor: (Warren et al., 1994; Bensafi et al., 2003, 2007; Johnson et al., 2006); or between a group of pleasant and a group of unpleasant odors: Prescott et al., 2010), which could not address this question. Secondly, does sniffing differentiate only clearly pleasant from clearly unpleasant smells, or can it discriminate between more subtle hedonic variations (e.g., slightly from strongly pleasant)? Thirdly, how sensitive is sniffing to other perceptual dimensions of odors such as familiarity or edibility? Fourthly, is the sniffing/hedonic valence relationship valid in other populations than young adults (e.g., in the elderly)? With regard to the possible use of sniffing measurement in the specific populations mentioned above, these four questions are essential and were addressed through four distinct experiments involving, for the first time, a very wide range of odorants. These aims were achieved through the use of an experimental sniffing measurement system developed in our laboratory.

MATERIALS AND METHODS

Participants

A total of 102 volunteers participated in 4 experiments (Experiment 1: 14 females, 6 males, mean age $\pm$ standard deviation $=$ 24.45 $\pm$ 1.63 years; Experiment 2: 16 females, 6 males, 23 $\pm$ 2.71 years; Experiment 3: 14 females, 16 males, 29.40 $\pm$ 1.05 years; Experiment 4: 16 females, 14 males, 67.37 $\pm$ 0.77 years). Participants were tested individually and

paid €16 for their participation. Exclusion criteria included self-reported olfactory impairment and/or neurological disease. All participants claimed normal sense of smell. The study was conducted in accordance with the Declaration of Helsinki and experimental procedures were approved by the local Lyon Sud-Est II review board.

Odorants

Forty-eight odorants were used in Experiment 1, and 20 in Experiment 3 and 4 (19 of which were also used in Experiment 1; see **Table 1**). These stimuli were chosen to represent a wide range of perceived pleasantness. All odorants (molecules provided by Sigma-Aldrich) were diluted in mineral oil and presented in 15 ml flasks (opening diameter: 1.7 cm; height: 5.8 cm; filled with 5 ml solution). Stimuli were absorbed on a scentless polypropylene fabric (3 × 7 cm; 3 M, Valley, NE, USA) to optimize evaporation and air/oil partitioning.

In Experiment 2, 16 complex aromas were used (see **Table 1**). These stimuli were chosen because they represent subtle variations within the positive pole of the pleasantness scale. They were used to further investigate (after Experiment 1) the link between sniffing and pleasantness with a different, more evocative, set of odorants. All odorants (provided by Firmenich SA) were diluted in odorless dipropyleneglycol to obtain similar subjective intensities (see Delplanque et al., 2008). Solutions (4 ml) were injected into the absorbent core of cylindrical felt-tip pens (14 cm long, inner diameter 1.3 cm, Burghart, Germany).

Sniffing Measurement Apparatus

Sniffing was recorded using a custom-built system composed of four modules (**Figure 1**): (1) an electronic USB device (multiple function board), (2) an airflow sensor to measure participants' nasal respiration, (3) a response box to collect subjective evaluations of odors and response times (not used in this study), and (4) dedicated software.

(1) The multiple function board (National Instruments, NI-USB6009, TX, USA) was used to acquire signals from the respiratory airflow sensor and response box. It can also send output signals (Transistor-Transistor Logic: TTL) to external devices (psychophysiology or EEG recording systems, for example).
(2) The airflow sensor (AWM2100V, Honeywell, MN, USA) allowed acquisition of both inhalation and exhalation phases. It was connected to a nasal cannula (Cardinal Health, OH, USA; 2.8 mm inner diameter), comprising two small tubes positioned in the participant's nostrils.
(3) The custom-built response box comprises 5 buttons in a finger-wise arrangement. Box size is 178 × 127 mm. Each button is a keyboard-like switch closing a 5 V circuit.
(4) The software, for the use of the experimenter, took the form of a multi-panel graphic interface. A "Participant" panel was dedicated to subject identification (participant's code and other related information) and to selecting files dedicated to implementation of the experiment. Here, all the information concerning the experimental trials and conditions (sequences of events, instructions, and questions

TABLE 1 | List of the odorants used in Experiments 1, 2, 3, and 4.

Odorant	CAS number	Concentration (volume/volume)	Experiments
(−)-Fenchone	7787-20-4	0.67	1
(+)-Fenchone	4695-62-9	0.67	1
1,8-Cineol	470-82-6	0.17	1
1-Butanol	71-36-3	0.04	1
1-Propanol	71-23-8	0.07	1
2,3-Butanedione	431-03-8	<0.01	1
alpha-Ionone	127-41-3	29.36	1
alpha-Pinene	7785-26-4	0.1	1
alpha-Terpinene	99-86-5	0.19	1
Benzyl acetate	140-11-4	1.47	1
cis-3-Hexenylacetate	3681-71-8	0.25	1
Citral	5392-40-5	1.65	1
Citronellal	106-23-0	1.27	1
Citronellol	106-22-9	17.81	1
D-Carvone	99-49-0	1.92	1
Ethyl phenylacetate	101-97-3	4.93	1
Ethyl salicylate	118-61-6	5.48	1
Isobutyric acid	79-31-2	0.1	1
Isovaleric acid	503-74-2	0.19	1
Linalool	78-70-6	2.16	1
Myrcene	123-35-3	0.15	1
p-Cresol	106-44-5	1.84	1
Pentanol	6032-29-7	0.03	1
Propionic acid	79-09-4	0.03	1
R-(+)-limonene	5989-27-5	0.2	1
S-(-)-limonene	5989-54-8	0.2	1
Terpinen-4-ol	562-74-3	15.97	1
trans-2-Hexenylacetate	2497-18-9	0.16	1
trans-Anethole	4180-23-8	4.24	1
1-Decanol	112-30-1	33.74	1,3,4
1-Heptanol	111-70-6	0.91	1,3,4
3-Hexanol	623-37-0	0.08	1,3,4
Acetophenone	98-86-2	0.56	1,3,4
Allyl caproate	123-68-2	0.55	1,3,4
Benzaldehyde	100-52-7	0.15	1,3,4
beta-Ionone	14901-07-6	30.6	1,3,4
Dodecanal	112-54-9	27.74	1,3,4
Ethyl butyrate	105-54-4	0.01	1,3,4
Eugenol	97-53-0	13.12	1,3,4
Geraniol	106-24-1	21.26	1,3,4
Guaiacol	90-05-1	2.09	1,3,4
Heptanal	111-71-7	0.07	1,3,4
Hexanoic acid	142-62-1	3.63	1,3,4
Isoamyl acetate	123-92-2	0.03	1,3,4
Isoamyl phenylacetate	102-19-2	59.14	1,3,4
L-Carvone	99-49-0	2.37	1,3,4
Methyl anthranilate	134-20-3	12.65	1,3,4
Phenyl ethanol	60-12-8	2.66	1,3,4
Diphenyloxide	101-84-8	13.55	3,4

(Continued)

TABLE 1 | Continued

Odorant	CAS number	Concentration (volume/volume)	Experiments
Beer	NA	20	2
Fig flower	NA	10	2
Flower	NA	20	2
Fruit	NA	10	2
Laundry soap	NA	1	2
Lavender flower	NA	10	2
Leather	NA	5	2
Lilac flower	NA	10	2
Magnolia flower	NA	20	2
Melon	NA	50	2
Pineapple	NA	10	2
Raspberry flower	NA	50	2
Shampoo	NA	10	2
Violet flower	NA	10	2
Wood	NA	5	2
Yogurt	NA	10	2

NA, Not Applicable.

such as olfactory dimensions to be evaluated) were read from an input ASCII file. Once the fields of this panel were filled in, the experimenter had the possibility of running an acquisition test through the "Calibration" panel, so that the respiratory signal that would be recorded during the experiment had enough amplitude without saturating. A graphic display of the signal was provided on this panel, so that the user could monitor the participant's respiratory signal in real time. Once calibration was completed, the experiment could be launched on the "Run" panel. Finally, the user could set some additional parameters and options (e.g., acquisition frequency, thresholds and scales) through the "Parameters" panel. Sniffing data, subjects' responses via the button box and related information such as response times were stored in an output ASCII results file.

Experimental Procedures

In all four experiments, participants read the instructions and provided written informed consent to the procedure before starting the experiment. Testing was performed in an experimental room designed specifically for olfactory experiments. The experimenter presented the odorants 1 cm below the subject's nose, for about 3 s. Participants were instructed to sniff at each stimulus presentation and rate hedonic valence (in all experiments), odor intensity and familiarity (in Experiments 2, 3, and 4), and edibility (in Experiments 3 and 4) on scales from 1 (not at all pleasant, intense, familiar, edible) to 9 (very pleasant, intense, familiar, edible). Odorants were presented every 20–30 s. In order to familiarize the participants with the experimental setting, they were first trained with a sequence of 1–3 non-odorized trials.

Data Analysis

For the purpose of the experiments presented here, the physiological signal was digitally recorded at 100 Hz. Sniffs were pre-processed by removing baseline offsets, and aligned in time by setting the point where the sniff entered the inspiratory phase as time zero. Maximum sniff flow rate, sniff duration and volume were calculated for the first sniff of each trial. The endpoint for volume and duration calculation was the point where the sniff returned to zero flow (end of the inspiration phase).

The relationship between hedonic ratings and sniffing behavior was analyzed with linear and degree-two polynomial regressions, with pleasantness as predictive variable and sniff characteristics as dependent variables. Where necessary, similar analyses were conducted between the other ratings (intensity, familiarity, edibility) and sniffing characteristics, and Pearson correlations were computed between pleasantness and the other ratings (intensity, familiarity, edibility). When one of these other ratings was related both to pleasantness and to a sniffing characteristic, partial correlation was conducted to determine to what extent the relationship between pleasantness and sniffing could be due to this third variable. Given the relatively large number of tests performed, it was chosen not to consider marginal effects with significance level between $p = 0.05$ and $p = 0.10$ and to give limited importance to effects with probability between $p = 0.01$ and $p = 0.05$. All statistical analyses were conducted with Statistica v.12 (Tulsa, OK, USA).

RESULTS

Experiment 1 (Relationship Between Pleasantness and Sniffing for a Wide Range of Odors, from Unpleasant to Pleasant)

As expected, the mean pleasantness ratings of the 48 odorants were relatively well spread out along the possible range from 1 to 9: mean pleasantness was 4.5 ± 1.4, ranging from 1.5 (for Isovaleric acid) to 7.0 (for alpha-Terpinene). Checking for outliers, defined as values greater or less than three standard deviations from the mean, found one outlier (sniff duration < M-3SD); conclusions excluding the odor in question (results in brackets) remained unchanged. There was a significant linear relationship between pleasantness and sniff volume ($R^2 = 0.46$, $p < 0.0001$), and sniff duration ($R^2 = 0.51$, $p < 0.0001$; without outlier: $R^2 = 0.45$, $p < 0.0001$), but not maximum sniff flow rate ($R^2 = 0.04$, $p = 0.191$). Coefficients were even higher when a degree-two polynomial model was used to test the relationship between pleasantness and sniff volume ($R^2 = 0.62$, $p < 0.0001$) and between pleasantness and sniff duration ($R^2 = 0.68$, $p < 0.0001$; without outlier: $R^2 = 0.60$, $p < 0.0001$); the relationship between pleasantness and maximum sniff flow rate remained non-significant ($R^2 = 0.10$, $p = 0.100$; see **Figure 2**). The shape of the relationship suggests that these results were due to a significant positive relationship for unpleasant odors (the more unpleasant, the smaller and shorter the sniffs), with no or maybe a converse relationship for pleasant odors. This possibility was tested by dividing the odorants into two groups: unpleasant (average pleasantness <5; $N = 28$ molecules) and pleasant (average pleasantness >5; $N = 20$ molecules) and running the same analyses again on these subgroups. No linear regressions

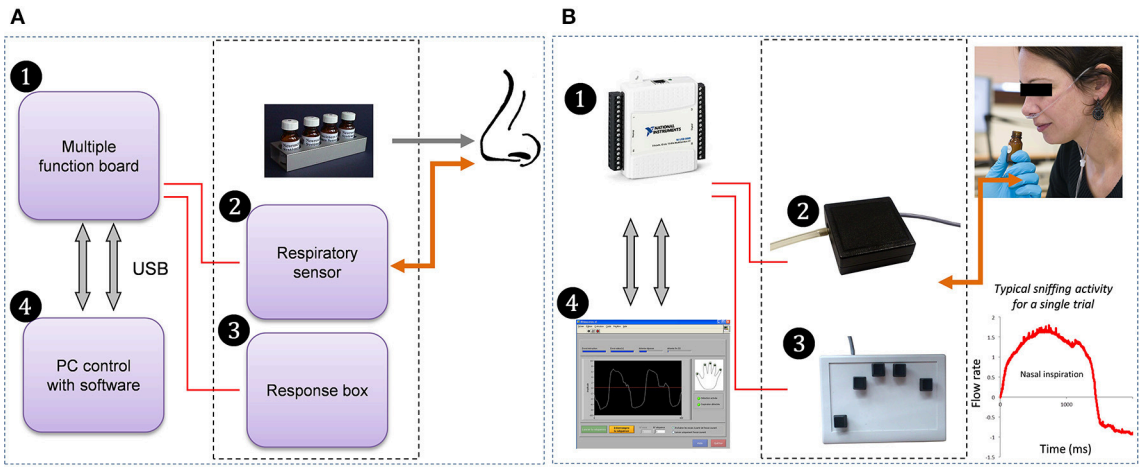


FIGURE 1 | Custom-built sniffing measurement apparatus: flow chart (A) and corresponding devices (B). The multiple function board (1) is used to acquire signals from the respiratory airflow sensor (2), which is connected to a nasal cannula positioned in the participant's nostril, allowing acquisition of respiratory signal, and from the 5-button response box (3) activated by the participant's fingers. The software (4) is used to set up the experimental parameters, launch the sessions and store responses.

were significant for the pleasant odors ($R^2s < 0.23$, $ps > 0.110$), whereas they were for the unpleasant odors ($R^2s > 0.71$, $ps < 0.0001$ for sniff volume, and for duration with or without outlier).

Experiment 2 (Relationship between Pleasantness and Sniffing for Odors Ranging from Neutral to Pleasant)

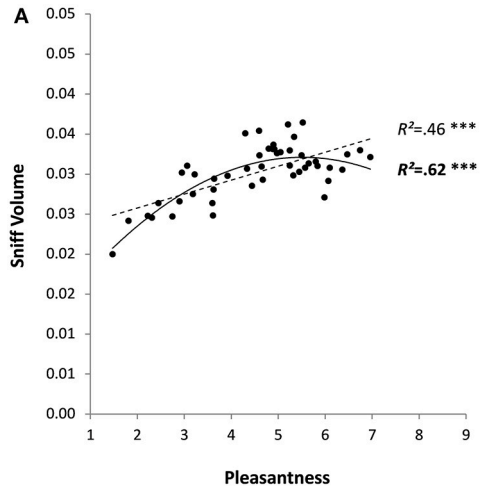
In the second experiment, odors were rated as rather pleasant on average (5.8 ± 1.4, ranging from 3.8 for Leather to 7.6 for Shampoo). No outliers were found for any of the analyzed variables. In agreement with the results obtained in Experiment 1 on the pleasant sub-group of odorants, Experiment 2 found no significant relationships (linear or quadratic) between pleasantness and any of the sniff parameters ($R^2s < 0.03$, $ps > 0.110$; see **Figure 3** for all R^2s and ps). Pleasantness was unrelated to perceived intensity (Pearson Correlation: $R = 0.27$, $p = 0.319$) and positively correlated with familiarity ($R = 0.85$, $p < 0.0001$). No significant linear or quadratic relationships were found between perceived intensity or familiarity and the sniff parameters (**Table 2**).

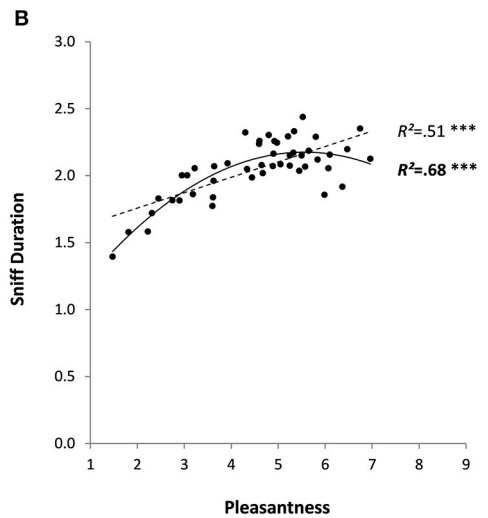
Experiment 3 (Relationship between Several Perceptual Dimensions and Sniffing in Young Adults)

The 20 odorants used in this experiment were relatively varied in pleasantness: mean pleasantness was 4.9 ± 1.4, ranging from 2.1 (for Hexanoic acid) to 7.0 (for Isoamyl acetate). No outliers were found for any of the analyzed variables. The detailed results of the linear and quadratic regressions between pleasantness and sniff parameters are shown in **Figure 4** (left column) and are fully in line with the conclusions of Experiment 1 on prediction of sniff volume and sniff duration by odor pleasantness. In contrast with Experiment 1, however, maximum sniff flow rate linearly increased with increasing pleasantness ($p < 0.05$). Only one relationship was significant for prediction of sniff parameters by familiarity and edibility (both of which correlated strongly with pleasantness: $R = 0.76$ and $R = 0.84$, respectively, $p < 0.001$): increasing familiarity was linearly associated with increasing sniff volume (**Table 2**). The partial correlation between pleasantness and sniff volume revealed a slight decrease in R-value and significance level ($R = 0.67$ instead of 0.73 and $p < 0.01$ instead of 0.001) when familiarity was a covariate, suggesting that familiarity is involved, although moderately, in the relationship. Again in this experiment pleasantness and intensity were independent ($R = -0.11$, $p = 0.653$), but this time intensity predicted sniff duration (significant linear and quadratic relationships, with sniffing duration decreasing with increasing intensity; **Table 2**).

Experiment 4 (Relationship between Several Perceptual Dimensions and Sniffing in Older Adults)

As in Experiment 3, the 20 odorants received relatively varied pleasantness ratings in a group of elderly participants: mean pleasantness was 5.1 ± 1.0, ranging from 2.5 (for Hexanoic acid) to 6.7 (for L-Carvone). No outliers were found for any of the analyzed variables. The detailed results of the linear and quadratic regressions between pleasantness and sniff parameters are shown in **Figure 4** (right column) and are in line with the conclusions of Experiments 1 and 3 on the prediction of sniff volume and sniff duration by odor pleasantness. Although the predictions appeared to be more moderate and had lower levels of significance than in Experiment 3 with younger adults (maximum level of significance: $p < 0.05$), computation of the difference between the two age-groups' Rs using the r-to-Fisher-z transformation revealed no significant difference ($ps > 0.276$ for the linear predictions, and $ps > 0.104$ for the quadratic predictions). When considering the prediction





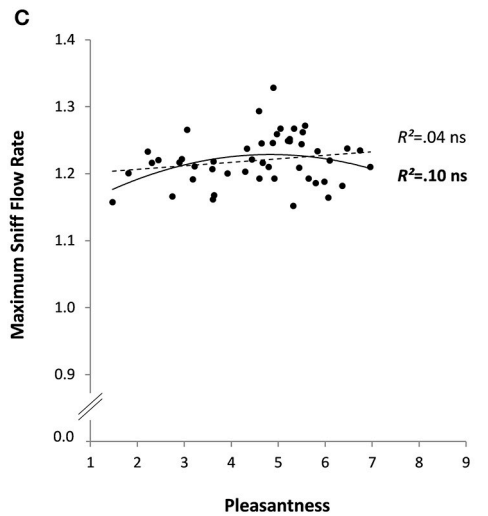
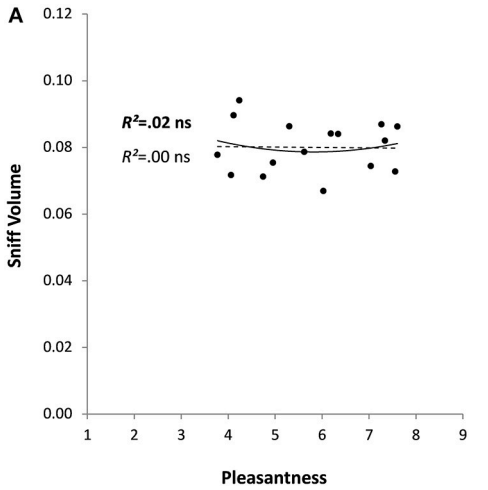
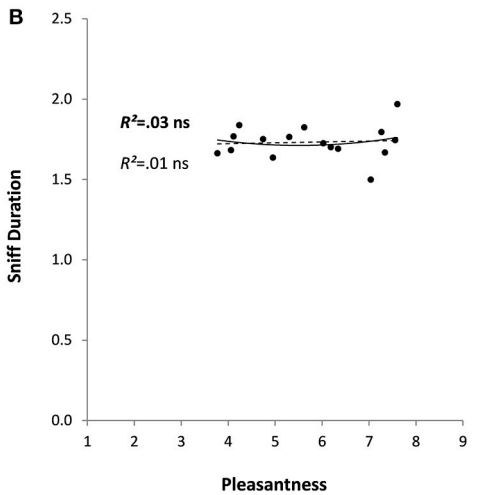


FIGURE 2 | Sniff characteristics (A: volume, B: duration, and C: maximum flow rate) as a function of odor pleasantness for 48 odorants in Experiment 1. Linear and quadratic relationships are represented by trend curves, R^2 and level of significance (***$p < 0.001$; ns: non-significant or $p > 0.05$; linear: dashed line and regular font; quadratic: continuous line and bold font).





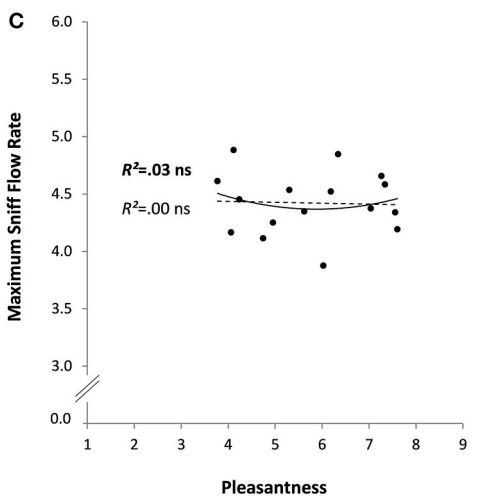


FIGURE 3 | Sniff characteristics (A: volume, B: duration, and C: maximum flow rate) as a function of odor pleasantness for 16 odorants in Experiment 2. Linear and quadratic relationships are represented by trend curves, R^2 and level of significance (ns: non-significant or $p > 0.05$; linear: dashed line and regular font; quadratic: continuous line and bold font).

TABLE 2 | Results of the linear and quadratic regressions illustrating the prediction of sniff parameters (volume, duration, and maximum flow rate) by perceptual variables other than pleasantness (familiarity, intensity, edibility) in Experiments 2, 3, and 4.

			Sniff volume		Sniff duration		Maximum sniff flow rate	
			R^2	p	R^2	p	R^2	p
Experiment 2	Intensity	Linear	0.06	0.343	0.00	0.925	0.13	0.174
		Quadratic	0.08	0.565	0.00	0.993	0.15	0.338
	Familiarity	Linear	0.01	0.670	0.00	0.903	0.00	0.861
		Quadratic	0.04	0.751	0.00	0.990	0.00	0.985
Experiment 3	Intensity	Linear	0.18	0.060	**0.36**	**<0.01**	0.02	0.553
		Quadratic	0.18	0.180	**0.38**	**<0.05**	0.03	0.743
	Familiarity	Linear	**0.21**	**<0.05**	0.08	0.234	0.19	0.056
		Quadratic	0.26	0.080	0.14	0.287	0.21	0.138
	Edibility	Linear	0.18	0.062	0.12	0.134	0.13	0.114
		Quadratic	0.20	0.150	0.18	0.185	0.14	0.271
Experiment 4	Intensity	Linear	0.10	0.181	**0.21**	**<0.05**	0.02	0.536
		Quadratic	0.16	0.233	0.23	0.114	0.19	0.175
	Familiarity	Linear	0.11	0.153	0.05	0.337	0.12	0.128
		Quadratic	0.11	0.363	0.13	0.321	0.13	0.297
	Edibility	Linear	**0.22**	**<0.05**	**0.21**	**<0.05**	0.14	0.110
		Quadratic	0.23	0.110	0.28	0.064	0.14	0.270

Significant relationships ($p < 0.05$) are in bold.

of sniff parameters by familiarity and edibility (both, as in Experiment 3, correlating strongly with pleasantness: $R = 0.78$ and $R = 0.89$, respectively, $p < 0.001$), only edibility was linearly associated with increasing sniff volume and duration (**Table 2**). Partial correlations between pleasantness and sniffing volume revealed a marked decrease in R-values and significance levels (sniff volume: $R = 0.21$ instead of 0.50 and $p = 0.387$ instead of <0.05; sniff duration: $R = 0.29$ instead of 0.53 and $p = 0.228$ instead of <0.05) when edibility was a covariate, suggesting that edibility strongly mediated the relationship. As in Experiment 3, pleasantness did not correlate with intensity ($R = 0.18$, $p = 0.448$), and intensity predicted sniff duration (significant linear relationship, with sniffing duration decreasing with increasing intensity; **Table 2**).

DISCUSSION

The aim of the series of experiments presented in this paper was to better understand the relationship between sniffing behavior and odor perceptual characteristics. Including a wide range of odorants spread over the hedonic continuum and repeating the experiment in different groups of participants allowed us not only to confirm previous conclusions that participants sniff unpleasant odors less, in volume and duration, than they do with pleasant odors (Warren et al., 1994; Bensafi et al., 2003, 2007; Johnson et al., 2006), but also to more finely describe these relationships. Especially, it was shown that (i) sniffing is sensitive to the distinction between pleasantness and unpleasantness, and to subtle variations in unpleasantness, but not in pleasantness, and (ii) the more unpleasant the odor, the smaller the spontaneous sampling of olfactory information through the nasal cavity.

Stevenson (2010, p. 14) argued that "odors are especially adept at eliciting negative emotions in humans." In line with this, and assuming that unpleasant odors are associated with harmful substances (but see below for a discussion on this point), the present results confirmed that sniffing behavior may in some cases have adaptive value of protection against toxic substances. Firstly, sniffs of reduced duration and volume decrease the amount of inhaled odor, thus limiting the organism's exposure to a potential threat. Similar reduction of stimulus input when the stimulus is harmful has been shown in other sensory modalities (e.g., defensive responses such as blinking in response to bright light or tactile stimulation of the eye; Ongerboer de Visser, 1980). Secondly, it may also be that stimuli of high ecological value, such as unpleasant odors, are processed more quickly than stimuli with lower survival value. Top-down accommodation to stimulus properties after sniff onset (Johnson et al., 2003) may be faster when the stimulus is unpleasant, allowing adaptive behavioral response—such as initiating termination of odor sampling—to occur as soon as possible. Again, faster processing of threatening stimuli has been shown in studies in olfaction (Bensafi et al., 2002b; Jacob et al., 2003; Jacob and Wang, 2006) and other sensory modalities (e.g., emotional face processing: Calvo et al., 2006). Regarding the pleasant pole, it cannot be excluded that the ecological value of the odors we chose was not high enough to demonstrate a relationship between sniffing behavior and degree of pleasantness. Future studies should be conducted with other sets of odors including food odors with higher reward value (such as highly appetitive chocolate or vanilla, for example), and with participants in a state of hunger (a factor of great importance both in determining the current reward value of an odor—see (Small et al., 2001)—and in influencing sniffing behavior—see (Prescott et al., 2010)—but that was not controlled

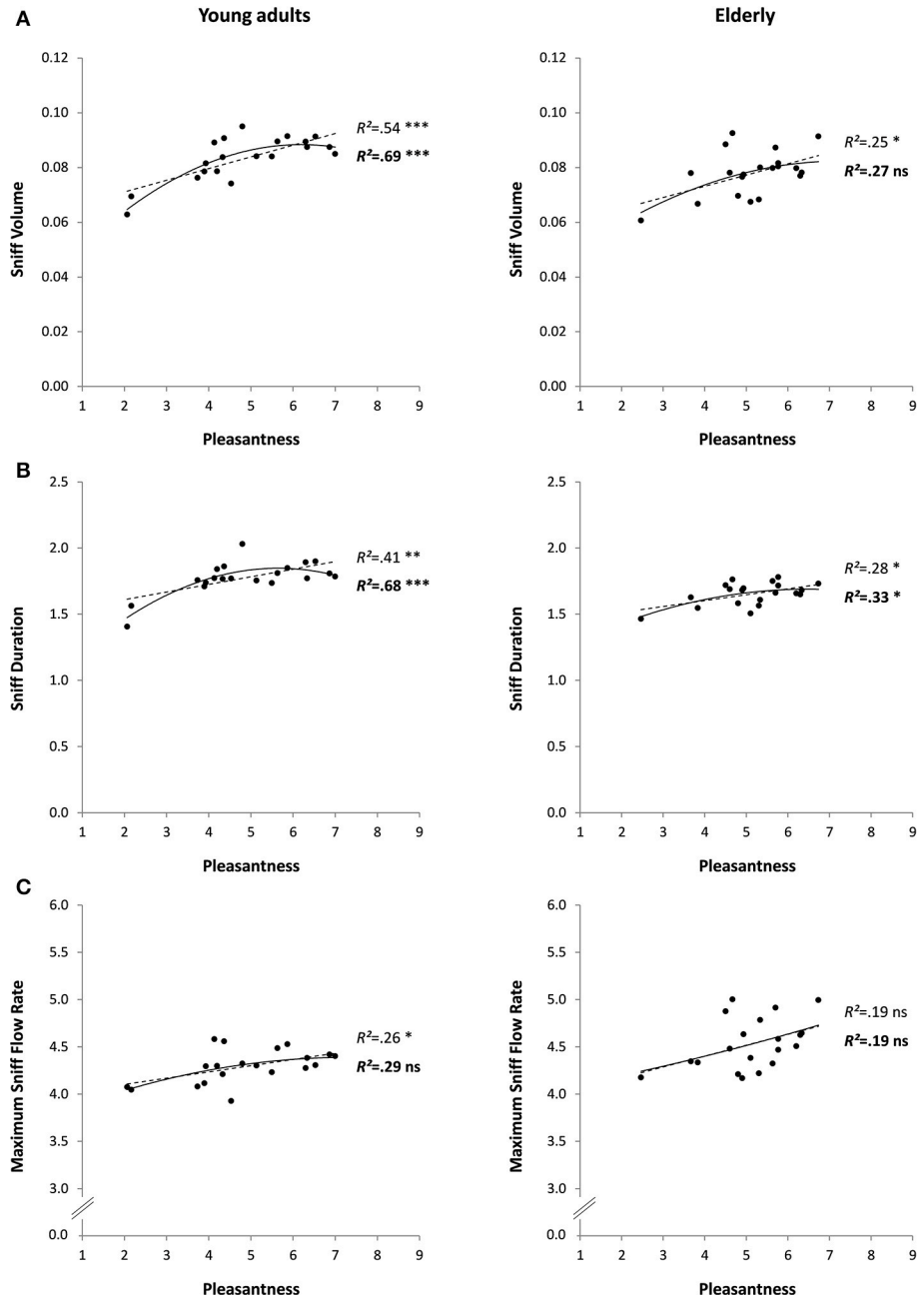


FIGURE 4 | Sniff characteristics (A: volume, B: duration, and C: maximum flow rate) as a function of odor pleasantness for 20 odorants in Experiment 3 (young adults: left column) and Experiment 4 (elderly adults: right column). Linear and quadratic relationships are represented by trend curves, R^2 and level of significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: non-significant or $p > 0.05$; linear: dashed line and regular font; quadratic: continuous line and bold font).

for in the present study); it may be that these odors will be sampled in greater amounts than more moderately pleasant odors. Our interpretation of the adaptive function of sniffing behavior should, however, be qualified, since not all unpleasant odors come from noxious sources and some harmful substances (toxic flowers such as lily of the valley or fruits such as manchineel apple) may have pleasant smells. Sniffing may constitute an early basic component of the adaptive response to smells, while higher-level processing components, occurring later in time, refine the response according to the individual's past experience and culturally shaped mental representation of the odor. For example, the sniffing response to the offensive odor of a ripe cheese may be reduced compared to a pleasant odor of, say, vanilla, but in the end the odor source will be approached and even ingested because learning has shown it to be highly appreciable.

In the light of these results, it can be hypothesized that sniffing behavior is a motor compound of the human affective processes that allows the individual to adjust to environmental conditions or events by displaying adapted behavior (Scherer, 1994; Keltner and Gross, 1999). In the olfactory modality specifically, affective responses to smells are involved in several major adaptive functions, including threat detection, ingestion and social communication (Stevenson, 2010). Some affective responses have been shown to be recurrent across cultures, which is consistent with the idea that they have an adaptive value for humans in general, independently of individual or environmental variations (Ferdenzi et al., 2013a). The present experimental setting suggested a significant involvement of olfactomotor response in at least the first function. If this is true, it should be the case for any human being, independently of individual variation such as age. And indeed it actually is the case, since we showed that the relationship between pleasantness and sniffing behavior was conserved during normal aging (Experiment 4), even though the magnitude of the effect appeared, but not significantly, to be reduced. This is consistent with a recent study comparing young and old adults, in which sniffs were larger and longer for pleasant vs. unpleasant odors, independently of age (Joussain et al., 2013), and with studies in other modalities (e.g., face perception) showing that adaptive threat detection is unimpaired in older adults (Mather and Knight, 2006).

Also in agreement with the idea that sniffing behavior might be involved in adaptive response to smells, it was shown that pleasantness predicted sniffing behavior better than other perceptual odor dimensions, such as intensity, edibility or familiarity. Although a link between intensity and sniffing parameters has been reported several times (Laing, 1983; Warren et al., 1994; Walker et al., 2001; Johnson et al., 2003), relationship was moderate in the present study, probably because intensity was not varied and odors were supposed to be comparable for intensity. Edibility and familiarity—although much more variable across odorants than intensity—only occasionally and moderately predicted sniff volume or duration in any of the four experiments. For edibility, the weakness of the link can be explained by the fact that whether an odor comes from an edible source is not the sole criterion for determining whether it is relevant to the individual and for letting it reach the nasal mucosa without restriction (e.g., social odors are highly relevant despite being non-edible; Lundström et al., 2008). Edibility was more influential in the elderly than in younger adults, suggesting that this olfactory property may be processed differently in old age (as is hedonicity, for example: Joussain et al., 2013). For familiarity, a stronger link with sniffing was expected. Indeed, novelty of an olfactory stimulus is processed even earlier than pleasantness (Delplanque et al., 2009) and it would also seem reasonable that unfamiliar (or novel) odors might induce wariness, and thus limitation of odor sampling. This was true in one instance in the present study, but familiarity and pleasantness are not a perfect match (see Ferdenzi et al., 2013b; Delplanque et al., 2015) and the latter seems to be a stronger and more reliable predictor of sniffing. In sum, pleasantness is a very prominent perceptual criterion (Engen, 1982; Yeshurun and Sobel, 2010) that individuals use to adjust their olfactomotor behavior to the environment's odorous stimulations in an adaptive fashion.

Finally, the robustness of the relationship between pleasantness and sniffing behavior could also be seen in its persistence despite variations in sniffing pattern. Sniff volume and flow rate were lower in Experiment 1 than in Experiments 2–4; this could be due to several differences between the experiments. Firstly, odors were less pleasant on average in Experiment 1 (4.5 vs. 5.8, 4.9, and 5.1 in Experiments 2, 3 and 4, respectively). Secondly, in Experiment 1 participants had to smell more than twice the number of odors presented in the other experiments, and they may have needed to protect themselves from overstimulation and subsequent sensory adaptation (Dalton, 2000), a phenomenon that makes the rating process more difficult. Thirdly, as only one judgment was performed in Experiment 1 (pleasantness) vs. 3–4 judgments in the other experiments, the amount of sensory information needed by the participants to provide their answers may have been less in Experiment 1. The fact that the prediction of sniffing volume by pleasantness was replicated in these different experiments—in spite of these behavioral differences—makes it even more significant. It is thus likely that this relationship also exists in real life, when subjects are not specifically asked to make judgments about randomly encountered odors; but this should be tested in the future with more ecological methods.

In summary, the present study offers new insights into the link between olfactomotor response and odor perception, highlighting the privileged role of hedonics in the modulation of sniffing behavior. This behavior seems, in humans of all ages, to have adaptive value in limiting the entry of potentially harmful substances into the nasal cavity. The present results suggest that sniffing measurement could be a reliable proxy for hedonic response to smells, at least for discriminating pleasant from unpleasant smells and between smells of various degrees of unpleasantness, in populations in which verbal evaluation of hedonic responses is not possible or reliable.

AUTHOR CONTRIBUTIONS

MB designed the research; MT and GC performed data acquisition; CF, GC, AF, and MB analyzed and interpreted the data; CF and MB wrote the paper.

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The inability to self-evaluate smell performance. How the vividness of mental images outweighs awareness of olfactory performance

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To rate one's individual olfactory performance is difficult and in many cases differs clearly from validated objective olfactory performance measures. This study aimed to investigate the basis for this measurement drift between objective and subjective olfactory performance evaluation. In absence of an actual odor, one may imagine an olfactory stimulus to evaluate his subjective olfactory performance. Therefore, the impact of the vividness of mental images on self-evaluation of smell performance in patients with mild to severe olfactory dysfunction and healthy controls was investigated. Fifty-nine patients with peripheral olfactory dysfunction ranging from reduced olfactory function (hyposmia) to complete loss of olfactory perception (anosmia) and 16 healthy controls were included. Olfactory performance was assessed using the Sniffin' Sticks battery, the vividness of olfactory mental images was evaluated using the vividness of olfactory imagery questionnaire (VOIQ). Decreased vividness of odor images was obtained for anosmic patients, and a trend of poorer odor imagery was determined in hyposmic patients. Multiple regression analyses revealed the VOIQ score as significant predictor for olfactory self-evaluation for hyposmic patients and healthy controls. In contrast, for anosmic patients, the only significant predictor for self-rating of olfactory performance was the threshold-detection-identification (TDI) score, measuring overall olfactory performance. The results of this study indicate that sensory perception and mental images are closely related to each other. Furthermore, subjects who were able to perceive odors, even to a smaller extent, rely on the vividness of their mental odor images to evaluate their olfactory performance. In contrast, anosmic patients rather trust in their knowledge that they are not able to perceive odors. We are therefore able to subjectively rate our olfactory performance levels, if we are not able to perceive odors, but not if we are able to perceive olfactory input.

Keywords: olfaction, self-evaluation, olfactory dysfunction, olfactory imagery

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Introduction

Previous studies on the ability of a self-assessment of overall olfactory function provided ambiguous results. It has been shown that patients with smell loss are often unaware of their olfactory deficits (Nordin et al., 1995; Murphy et al., 2002). However, some results suggest that patients with severe

smell loss are more aware of their dysfunction compared to hyposmic patients (Schöpf and Kollndorfer, 2015). Also healthy controls seem to be challenged by self-ratings of their olfactory performance (Landis et al., 2003). Thus far, little is known on the origins of difficulties in the self-evaluation of olfactory function. The major question regarding the challenge of self-evaluation of olfactory abilities is the absence of current odors during self-evaluation. One possible strategy of self-rating one's own olfactory abilities without a current odor as basis of assessment is the retrieval of mental odor representations perceived in the past.

Mental odor representations—or mental images—are defined as the creation of mental representations in absence of an external stimulus (Freeman, 1981). Mental imagery has been well documented in a broad range of sensory systems: visual (Farah, 1989; Kosslyn et al., 2001), auditory (Halpern and Zatorre, 1999), and motor system (Jeannerod and Frak, 1999). The evidence for the ability to form mental odor representations without any olfactory stimulus has been discussed controversially. Even though some researchers suppose inability to form mental odor representations (Engen, 1987; Herz, 2000), support for olfactory imagery is available from research in olfactory hallucinations (Arguedas et al., 2012), dreams (Stevenson and Case, 2005a), and volitional imagery (Djordjevic et al., 2004). Mental imagery is often assessed by vividness ratings. In these questionnaires subjects are instructed to create mental representations for a certain sensory system and to evaluate the degree to which the mental representation equals the perceptual experience (Sheehan, 1993; Gilbert and Kemp, 1996). However, it has already been reported that the imagination of odors occurs less frequently and is less vivid than the imagination of other senses, e.g., sights or sounds (for review, see Stevenson and Case, 2005b; Arshamian and Larsson, 2014). Thus far, less is known about the vividness of mental odor representations in patients with olfactory dysfunction.

Therefore, the present study aimed to investigate the impact of the vividness of mental odor representations on the ability to evaluate one's own olfactory performance. Therefore, we investigated patients with mild to severe olfactory dysfunction and healthy controls. We hypothesized that the ability to generate vivid olfactory representations is reduced in patients with olfactory dysfunction, as supposed by the perceptual theory. Furthermore, we assume that the vividness of mental representations influences self-ratings of olfactory performance.

Materials and Methods

Subjects

Ninety-two patients with olfactory dysfunction were initially included in this study. To avoid interference with memory, or other cognitive impairment in patients with traumatic brain injury, only patients with smell loss due to sinonasal diseases or idiopathic olfactory dysfunction were included in our final sample. Our cohort therefore consisted of 59 patients with olfactory dysfunction (34 female, 25 male) and 16 healthy controls (nine female, seven male). Information on this control groups olfactory performance has already been presented in Krajnik et al. (2014). Detailed sociodemographic data, is presented in **Table 1**. The study was designed as a retrospective data analysis study investigating vividness of olfactory imagery on a selected group (olfactory dysfunction) of a large study population that was acquired in a different context, but with the questionnaires necessary for the present paper. All subjects had no history of neurologic or psychiatric diseases. The study was approved by the Ethics Committee of the Medical University of Vienna. All subjects were informed about the aim of the study and gave their written informed consent prior to inclusion.

Olfactory Performance

Olfactory performance was assessed using the Sniffin' Sticks test battery (Burghart Instruments, Wedel, Germany). This test battery includes three subtests that assess nasal chemosensory function: detection threshold; odor discrimination; and odor identification. The Sniffin' Sticks battery uses pen-like devices for odor presentation (Kobal et al., 1996, 2000; Hummel et al., 1997). The odor detection threshold of *n*-butanol was identified using a single-staircase, three-alternative, forced-choice procedure. In the second subtest, odor discrimination ability was determined using 16 triplets of odorants (two pens contained the same odorant; the third pen contained an odd odorant). The participants were asked to detect the odd pen in a forced-choice procedure. The odor identification task consists of 16 common odors using a multiple-choice answering format, with a list of four descriptors for each odor, again in a forced-choice procedure. The scores for the detection threshold range from 1 to 16, and, for the other two subtests, a score between 0 and 16 may be achieved. The results of all three subtests were summed to obtain the threshold-detection-identification (TDI) score. Normosmia, or normal olfactory performance, is characterized by a TDI score of at least 31, and hyposmia (reduced olfactory function) is defined as a TDI between 17 and 30.75. A TDI-score of less than 17 is categorized as anosmia (Kobal et al., 2000). In addition, all participants were asked to evaluate the 16 odors of the identification test regarding their intensity of the odor (1 = very weak; 9 = very intense). Furthermore, all subjects were asked to evaluate their sense of smell on a nine-point scale (1 = good sense of smell; 9 = poor sense of smell).

Olfactory Imagery

The capability for olfactory imagery was assessed with the *vividness of olfactory imagery questionnaire* (VOIQ; Gilbert et al., 1998),

TABLE 1 | Sociodemographic data of the study sample.

	Olfactory dysfunction		Healthy controls
	Anosmics	Hyposmics	
	Mean (SD)	Mean (SD)	Mean (SD)
Number of participants (male/female)	43 (19/24)	16 (6/10)	16 (7/9)
Age	54.09 (13.60)	56.13 (8.62)	30.63 (6.98)
Duration of smell disorder (in years)	9.43 (10.05)	12.97 (14.07)	–

translated into German (see supplementary materials). The participants were instructed to mentally retrieve 16 odors of four different categories: personal hygiene (bath); food-related (barbecue); tobacco; and vehicles (car). In each category, the subjects were verbally presented with four specific odors and were asked to imagine (e.g., "The odor of unlit tobacco—a cigarette, cigar, or pouch of pipe tobacco."). For each specific situation, the participants had to evaluate the vividness of their imagination on a five-point Likert scale (1 = perfectly realistic and as vivid as the real odor; 5 = No odor at all, you only "know" that you are thinking of an odor). All 16 items were summed to a total score, with low values reflecting good odor imagery abilities, and high values representing poor olfactory imagination abilities. In addition, total values were calculated for each category.

Data Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois), version 20.0. For all test scores, mean and standard deviation (SD) were calculated. To investigate the impact of olfactory impairment on odor imagery, anosmic and hyposmic patients were compared with healthy controls. All variables fulfilled requirements for parametric testing, thus Pearson's correlation, and one-way ANOVA were calculated. *Post hoc* Tests were Bonferroni-corrected to deal with alpha-inflation. Equality of variances was calculated using the Levene-Test. Group differences in self-evaluation of olfactory performance were calculated using the non-parametric Kruskal–Wallis test. Correlations between self-evaluation and TDI scores were computed using Spearman's rho. For all reported variables, variances did not differ significantly. Multiple regression analyses were computed to figure out potential predictors for self-evaluation of olfactory performance for all three groups separately. The alpha level for all statistical tests was set to $\alpha = 0.05$.

Results

Sociodemographic Data

The sample was tested for significant differences in gender distribution and educational background. For all three groups, anosmics, hyposmic, and normosmics no differences for gender ($\chi^2 = 0.223$; $p = 0.895$) and educational background ($\chi^2 = 6.541$; $p = 0.365$) were determined. With regard to age, significant group differences were determined between healthy controls and patients with olfactory dysfunction [$F(2,72) = 27.374$; $p < 0.001$]. *Post hoc* analysis revealed no difference in age between anosmic and hyposmic patients ($p = 0.999$).

Olfactory Performance

Data analysis revealed a mean TDI score for anosmic patients of 11.97 (SD 2.74). Participants with reduced olfactory function achieved a mean TDI score of 24.25 (SD 3.71). For the healthy control group, a mean TDI score of 35.80 (SD 2.23) was obtained. Mean TDI values of the three groups differed significantly [$F(2,72) = 423.48$; $p < 0.001$; $\eta_p^2 = 0.922$]. Detailed olfactory performance results and subjective evaluation of olfactory performance are summarized in **Table 2**.

TABLE 2 | Results of olfactory performance measures.

	Olfactory dysfunction		Healthy controls	
	Anosmics Mean (SD)	Hyposmics Mean (SD)	Mean (SD)	*p*-value
Odor threshold	1.45 (0.89)	4.44 (2.69)	9.05 (1.78)	<0.001
Odor discrimination	5.567 (1.91)	9.68 (1.58)	12.94 (1.69)	<0.001
Odor identification	4.97 (1.96)	10.13 (3.36)	13.81 (1.42)	<0.001
TDI score	12.97 (2.74)	24.25 (3.71)	35.80 (2.23)	<0.001
Subjective olfactory performance	8.51 (0.77)	6.93 (1.69)	3.06 (1.79)	<0.001
Intensity rating	1.72 (1.63)	4.05 (1.61)	7.22 (1.14)	<0.001

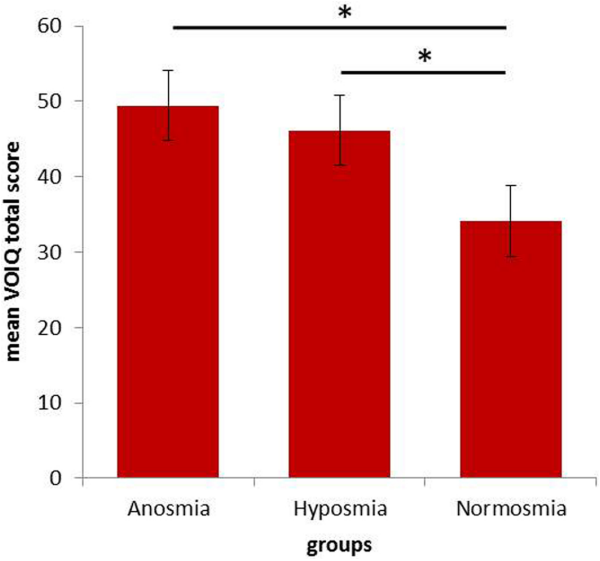

FIGURE 1 | Mean total VOIQ scores for all three groups [anosmic patients (n = 43), hyposmic patients (n = 16), and healthy controls (n = 16)]. Significant group differences ($p < 0.05$) are marked with an asterisk. Error bars reflect the standard error.

Olfactory Imagery

Analysis of the VOIQ total score revealed significant group differences [$F(2,72) = 6.667$; $p = 0.002$; $\eta_p^2 = 0.156$]. *Post hoc* analyses showed significantly higher VOIQ scores in patients with olfactory dysfunction compared to healthy controls (see **Figure 1**). A detailed overview of olfactory imagery performance is presented in **Table 3**. Hyposmic patients did not differ significantly from the two other subject groups in their VOIQ score. However, a trend of poorer vividness of mental representations in hyposmic patients compared to healthy controls ($p = 0.065$) was observed. Even though the healthy control group was significantly younger compared to anosmic and hyposmic patients, neither age ($r = 0.149$, $p = 0.203$) nor gender ($r = 0.067$, $p = 0.566$) influenced the VOIQ performance significantly. Investigating the influence of duration of olfactory dysfunction, no significant correlation was determined for patients with olfactory dysfunction ($r = 0.115$, $p = 0.387$).

TABLE 3 | Results of olfactory imagery questionnaire for vividness (VOIQ).

	Olfactory dysfunction		Healthy controls	
	Anosmics Mean (SD)	Hyposmics Mean (SD)	Mean (SD)	*p*-value
VOIQ—bath	12.60 (4.56)	11.75 (3.62)	8.13 (3.16)	0.002
VOIQ—barbecue	12.72 (4.28)	12.00 (3.81)	10.13 (3.60)	0.098
VOIQ—tobacco	11.26 (4.83)	10.94 (4.37)	7.38 (3.14)	0.006
VOIQ—car	12.88 (3.89)	11.44 (3.54)	8.81 (3.60)	0.013
VOIQ—total	49.46 (15.04)	46.13 (13.24)	34.44 (11.92)	0.002

Self-Evaluation of Olfactory Performance

The three groups differed significantly in their self-evaluation ($H = 47.002$; $p < 0.001$). *Post hoc* analyses showed significant differences between all three groups. Anosmic patients reported poorest olfactory abilities (mean 8.51; SD 0.77), whereas healthy controls reported highest olfactory abilities (mean 3.06; SD 1.79). Self-reporting data revealed that healthy controls rated themselves significantly better than hyposmics, and hyposmics rated themselves significantly better than anosmic patients. However, self-reporting data was not correlated with objective olfactory performance measurement (TDI score) in healthy controls ($\rho = -0.221$, $p = 0.411$) and hyposmic patients ($\rho = 0.126$, $p = 0.643$). Only for anosmic patients, a significant correlation between self-reporting and olfactory performance measures was obtained ($\rho = -0.373$; $p = 0.014$).

Multiple Regression

The multiple regression model was set up to investigate the predictors of olfactory imagery performance in more detail. In a first step, potential predictors of the dependent variable were included into the model. Following potential predictors were included in the model using stepwise iterations in multiple regression analyses: VOIQ total, TDI, score, gender, and age. Interestingly, computed statistical models differed between patients with complete smell loss and subjects who were still able to perceive odors (hyposmic patients and healthy controls). For anosmic patients, the results of the regression revealed the TDI as the only statistically significant predictor for self-evaluation of olfactory performance [$R^2 = 0.10$; $F(1,41) = 4.274$; $\beta = -0.307$; $p = 0.045$; see **Figure 2**]. For the other two subject groups, not the TDI but the VOIQ score was determined to significantly predict olfactory self-rating [hyposmics: $R^2 = 0.33$; $F(1,14) = 6.905$; $\beta = 0.575$; $p = 0.020$; healthy controls: $R^2 = 0.29$; $F(1,14) = 5.738$; $\beta = 0.539$; $p = 0.031$; see **Figure 2**].

Discussion

The present study aimed to investigate the impact of vividness of olfactory imagery on self-assessment of olfactory performance in patients with peripheral impaired olfactory function, compared to healthy controls. Results revealed significantly reduced olfactory imagery abilities in anosmic patients and a trend of poorer vividness of mental representations in the hyposmic subject group. Furthermore, different predictors for self-evaluation were obtained. In hyposmic patients and healthy controls the VOIQ score was determined as a significant predictor for olfactory self-rating. In contrast, in the anosmic patient group, the TDI score, measuring overall olfactory performance, was the only variable, that significantly predicted olfactory self-rating.

Decreased mental imagery abilities in patients with sensory loss have already been determined in visually impaired patients. Various case studies investigating cortically blind patients found impaired visual mental imagery (Farah et al., 1988; Chatterjee and Southwood, 1995; Policardi et al., 1996). These deficits in building mental images have not only been found in patients with complete sensory loss, but also in patients with impaired sensory perception. In patients with peripheral visual impairment who were still able to perceive stimuli Palermo et al. (2013) investigated the vividness of visual mental images and revealed that the presence of a visual defect, even if correted by lenses, corresponded to a decrease in the vividness of mental images. These findings are in line with our study, in which anosmic patients revealed statistically significant poorer vividness of mental representations and the hyposmic group showed a trend of reduced vividness of olfactory representations compared to healthy controls. No correlation between disease duration and vividness of olfactory imagery was obtained in patients with smell loss. We assume that the ability to imagine odors is disturbed, in patients with a decreased olfactory sensory input. Even though it is assumed that odor representations are stored predominantly in long-term memory (Herz and Engen, 1996), a continuous sensory stimulation may be required to sustain the trace of the representation. Previous studies indicate that olfactory memory is not based on internal mental representations of odors (Köster et al., 2014a). Moreover, the authors assume that olfaction is hardly comparable to other senses, such as vision due to the different functions of these senses for human beings. Whereas vision provides information on spatial orientation, olfaction is directed at warning as well as the detection of unknown and potential dangers. Therefore, the visual model of memory and recognition may not be appropriate to describe olfactory memory. This assumption is supported by a study investigating food memory. In contrast to the traditional view on visual memory as a reactivation of previous experiences, food memory is rather targeted at detecting novelty and change (Morin-Audebrand et al., 2012).

In a study which investigated how olfactory imagery is represented neurally in patients with acquired olfactory loss, a decrease in the vividness of olfactory imagery in patients with olfactory dysfunction was detected (Flohr et al., 2014). As no differences in the ability to create visual mental images were determined, compared to healthy controls, the authors concluded that regular exposure to sensory-specific stimuli is necessary to maintain the capability for mental imagery. The study sample investigated by Flohr et al. (2014) included patients with various causes of olfactory loss, with the majority of causes being traumatic brain injuries. As traumatic brain injury could not only impair the perception of odors, but also the olfactory memory, our study included only patients with peripheral olfactory dysfunction, to investigate a study sample as homogeneous as possible.

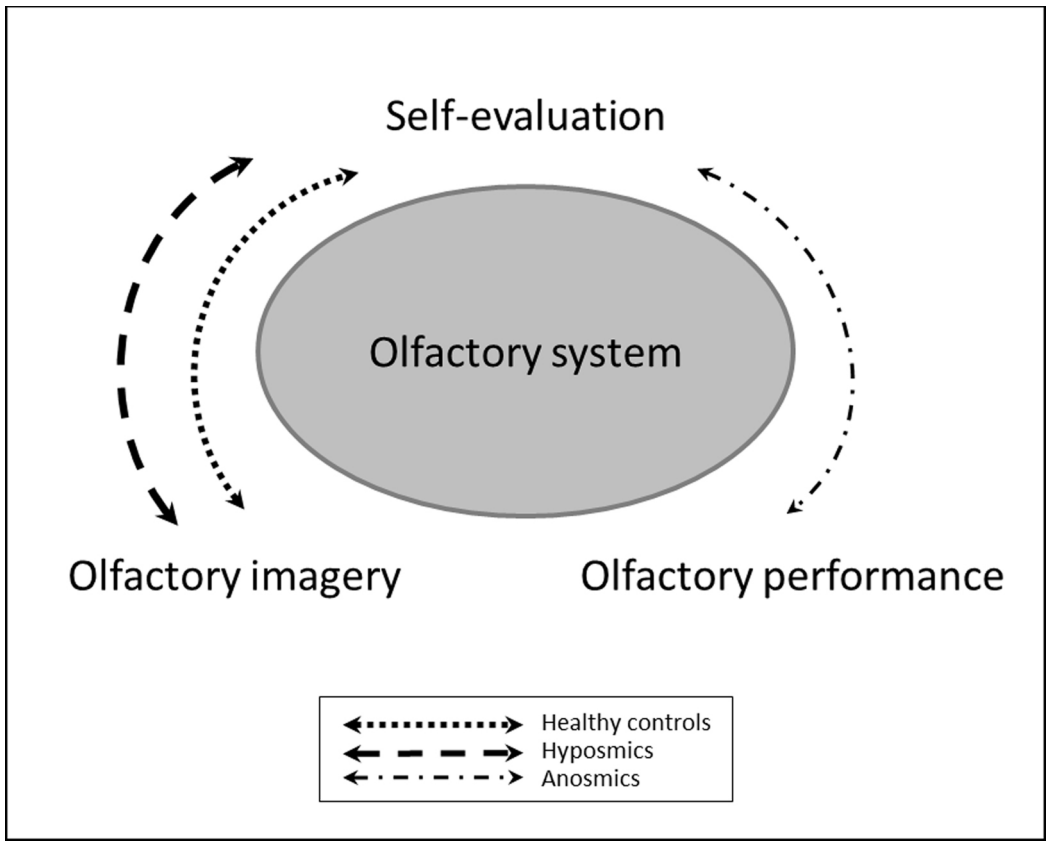

FIGURE 2 | Schematic representation of the multiple regression models for the three subject groups. Healthy controls and hyposmic patients seem to rely on their ability to create mental representation of odors to self-evaluate their olfactory performance in absence of a current odor. In contrast, anosmic patients, rather trust in their knowledge that they are not able to perceive odors.

Mental odor imagery has been discussed controversially in the past. Previous studies claimed that the creation of mental representations of odors by non-experts is not possible at all (e.g., Herz, 2000). In contrast, recent investigations using functional imaging methods indicate the ability to imagine odors not only in experts (Plailly et al., 2012) but even in non-experts, as they observed an activation of the piriform cortex, the major primary olfactory area (Djordjevic et al., 2005; Bensafi et al., 2007). However, Royet et al. (2013) noted that the activation of the piriform cortex may be caused by other reasons than olfactory imagery: First, the activation of the piriform cortex may arise due to sniffing activities during an olfactory imagery task. Second, activation of the piriform cortex may be caused by drawing attention to odors in the environment of the subject. And third, the activation of the piriform cortex may be a result of cross-modal associative learning (Gottfried et al., 2002).

The sensory system is a closed mechanism, in which different variables and factors interact with each other. Rather than investigating the effect of a single parameter in an isolated way, there is the need to explore the whole system to seek understanding of its mechanisms. We therefore used multiple regression analyses in which we included measures that may influence self-evaluation. This systematic investigation revealed different predictors for self-evaluation of olfactory performance for anosmic patients compared to hyposmic patients and healthy controls. Anosmic patients, who suffer from a complete loss of their sense of smell, use the information that they perceived no odors to evaluate their own olfactory performance. In contrast, participants who are still able to perceive odors, even in smaller dimension, rather rely on their ability to imagine odors to assess their own olfactory performance. We can therefore hypothesize that if a person, who is able to perceive odors, is asked to self-evaluate their olfactory function, they will try to assess a concrete stimulus. If no odor is actually available, they might rely on the vividness of odors retrieved from long-term memory. Patients with a complete smell loss are usually aware of their inability to perceive odors and therefore trust in their knowledge of poor olfactory performance.

A potential limitation of this study is the subjective assessment of the vividness of olfactory imagery. In this study patients with olfactory dysfunction were included; therefore a comparison with actually presented odors was not possible. Previous studies claimed to assess olfactory imagery objectively (Djordjevic et al., 2004). However, imagery is always subjective, as it is a person's rating of vividness or comparability to presented odors. Previous research (Bensafi and Rouby, 2007) has argued that the self-reporting questionnaire used in this study, is a valid measure of olfactory mental images. Furthermore, no visual imagery test was included to determine whether the difficulties

in patients with olfactory dysfunction were sensory-specific or general problems with mental imagery. Based on previously published literature (for review, see Arshamian and Larsson, 2014), it can be assumed that the reduced vividness of olfactory imagery in patients with olfactory dysfunction is sensory-specific.

Another limiting factor of the present work is the healthy control group, which is significantly younger compared to anosmic and hyposmic patients. However, age and gender were neither significantly correlated with olfactory imagery nor with self-evaluation. We therefore assume that these differences do not influence the results of the present study.

Conclusion and Future Directions

The results of our study demonstrate that the retrieval of olfactory mental representation is affected by individual olfactory performance. This study revealed that patients with peripheral smell loss show a decreased vividness of olfactory representations. Furthermore, we were able to define different predictors for olfactory self-ratings in anosmic patients compared to hyposmic patients and healthy controls. Whereas the first seem to rely rather on the fact that they do not perceive any odor to assess their own olfactory performance, the latter two subject groups tend to rely on their odor imagery abilities to evaluate their smell performance. Previous studies have already shown that olfactory training may induce significant improvements in olfactory performance (Hummel et al., 2009; Damm et al., 2014). Future studies could investigate the alterations in olfactory imagery as well as their basis of olfactory self-ratings in anosmic patients after completing olfactory training, to determine whether alterations of olfactory performance induced by such a training program are accompanied by changes in the ability to imagine odors. Although it is assumed that only about one third of general population is able to create mental odor representations, and this ability does neither improve odor identification nor odor naming abilities (Köster et al., 2014b), it can therefore be speculated, that it is unlikely that an olfactory training may force olfactory imagery abilities. However, this may be part of future investigations.

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Unilateral Resection of the Anterior Medial Temporal Lobe Impairs Odor Identification and Valence Perception

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The anterior medial temporal lobe (TL), including the amygdala, has been implicated in olfactory processing, e.g., coding for intensity and valence, and seems also involved in memory. With this background, the present study evaluated whether anterior medial TL-resections in TL epilepsy affected intensity and valence ratings, as well as free and cued identification of odors. These aspects of odor perception were assessed in 31 patients with unilateral anterior medial TL-resections (17 left, 14 right) and 16 healthy controls. Results suggest that the anterior medial TL is in particular necessary for free, but also cued, odor identification. TL resection was also found to impair odor valence, but not intensity ratings. Left resected patients rated nominally pleasant and unpleasant odors as more neutral suggesting a special role for the left anterior TL in coding for emotional saliency in response to odors.

Keywords: anterior medial temporal lobe, amygdalo-hippocampectomy, olfactory perception, odor valence, brain lateralization, temporal lobe epilepsy (TLE)

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INTRODUCTION

The medial temporal lobe (TL) is the main host for brain areas involved in both memory, emotional, and olfactory processing in the mammalian brain (van Hartevelt and Kringelbach, 2012; Lehn et al., 2013; Hudry et al., 2014).

Several cortical areas located within the medial TL, among others the piriform cortex, the amygdala, the entorhinal cortex as well as the hippocampal formation, have been linked to olfactory processing due to their close anatomical connectivity to the olfactory receptor neurons (Room et al., 1984; Carmichael et al., 1994). Work on a variety of animal models contributed significantly to our functional understanding of the olfactory neural pathway (Wilson, 2001) and the vast development of functional imaging techniques in the last decade finally succeeded in transferring this knowledge also to human olfaction by showing activation of these areas upon a variety of olfactory tasks (cf. Lundström et al., 2011).

Among all brain structures residing in the medial TL, amygdala has proven strong involvement in olfactory processing. It is located within a monosynaptic projection from the olfactory receptor neurons and has been associated with several aspects of odor processing (as reviewed in Seubert et al., 2013), odor recognition (Jung et al., 2006), odor-association learning (Gottfried et al., 2002), odor intensity coding (Anderson et al., 2003) but also with combined coding of both olfactory

intensity and valence (Winston et al., 2005). Due to this vast variety of functional involvement, the specific contribution of amygdala in different olfactory tasks is still difficult to define. Thus, human lesion studies may contribute valuable knowledge because they suggest for example that intensity and quality judgments are functionally separated within the medial TL. Lesions to the medial TL, formed by either resection or reoccurring epileptic activity, impair humans' ability to assess the identity or quality of odors, while leaving the ability to detect odors and perform odor intensity-scaling tasks intact (Eichenbaum et al., 1983; Jones-Gotman and Zatorre, 1988).

Less is known about the functional relevance of the dense bulbar and piriform projections to the entorhinal cortex and hippocampus (Room et al., 1984). Corresponding to their traditional role in memory processing, they are commonly associated with identification and retrieval of odor qualities (Kjelvik et al., 2012; Lehn et al., 2013). However, emerging data promote the notion that the entorhinal cortex and temporal pole act as an amodal hub, subserving modality-selective regions formation and retrieval of semantic knowledge (Patterson et al., 2007; Baxter, 2009; Suzuki and Baxter, 2009). As such, these areas have been proposed to act as key relay for the retrieval of olfactory memories including recognition of complex perceptual configurations rather than odor processing *per se* (Biella et al., 2007; Kerr et al., 2007; Chapuis et al., 2013).

Investigations in patients suffering from neural insult restricted to medial TL have contributed considerably to better understanding of the functional relevance of these brain areas for olfactory processing. Patients undergoing epilepsy surgery with resection of anterior TL have been shown to have impaired performance for odor discrimination (Eskenazi et al., 1983; Zatorre and Jones-Gotman, 1991), identification (Jones-Gotman and Zatorre, 1988, 1993; Jones-Gotman et al., 1997), odor matching and recognition (Abraham and Mathai, 1983; Eskenazi et al., 1986; Jones-Gotman and Zatorre, 1993; Dade et al., 2002; Buchanan et al., 2003).

Lateralization of olfactory functions has been investigated with different methods, e.g., using monorhinic odor presentation in healthy individuals, utilizing the fact that the olfactory pathway is primarily unilateral. Other studies have investigated non-operated and operated patients with unilateral TLE. Right dominance has been reported in odor matching (Abraham and Mathai, 1983), recognition (Jones-Gotman and Zatorre, 1993; Broman et al., 2001; Olsson and Cain, 2003) and discrimination (Zatorre and Jones-Gotman, 1991) and left dominance in odor memory tasks (Lee et al., 2002; Buchanan et al., 2003) and recently also in odor valence processing (Hudry et al., 2014). Interestingly, the study by Hudry et al. (2014) reported altered valence evaluation (in the sense of a shift toward unpleasant) in left TLE patients in comparison to both right TLE patients and healthy controls. This latter finding corresponds with early reports on left hemispheric dominance for odor valence processing in healthy participants (Royet et al., 2000) but leaves open the question as to what degree epilepsy contributes to such specific left hemispheric impairment of odor valence ratings. We thus wanted to investigate odor valence perception in patients with TLE after resection of the anterior medial TL.

The aim of the current study was twofold. First, we investigated whether anterior medial TL-resection in TLE patients, as compared to healthy controls, affected odor identification, intensity and valence ratings. Second, we attempted to replicate recent reports of left hemispheric dominance in processing of odor valence compared to odor intensity. To this end we compared patients with TLE after unilateral resection of the left and right anterior medial TL. An odor rating task requiring intensity and pleasantness judgments for a group of two pleasant and two unpleasant odors each in weak and strong concentrations was implemented, as well as an identification test of everyday odors that was provided with and wihtout verbal cues. Because we focused on the question of hemispheric dominance in these olfactory tasks, we investigated two patient groups with unilateral right and left lesions using consecutive, unilateral odor stimulation of each nostril. To control for general differences in evaluation of sensory stimuli between TLE patients and healthy controls, a visual gray-scale rating task was included to the procedure.

MATERIALS AND METHODS

Subjects

Forty-five individuals participated in the study, 14 healthy controls (6 male) and 31 patients (13 male) with TLE. All patients had undergone surgery with unilateral anterior medial temporal resection (ATR) including amygdala and hippocampus at Uppsala University hospital (Spencer et al., 1984). In four patients, post-operative MRI examination showed incomplete resection of the amygdala. Time for surgery was on average 6.9 years ($SD = 4.6$) before study participation. Neuropsychological functions were tested and have been reported elsewhere (Åhs et al., 2010, 2013). In the patient group, 28 were right and 5 left-handed, controls were all right-handed. The patient group was subdivided based on side of resection and left- and right ATR subgroups were matched for age, seizure duration, clinical outcome, and neuropsychological performance (Åhs et al., 2010).

All 45 participants (31 patients) completed the first two parts of the study including an odor identification task (cued and free odor identification) and a visual gray-scale rating task. A subgroup of 25 of 31 patients also performed a third task, being an odor intensity and pleasantness rating task including four odors (two pleasant, two unpleasant) in two concentrations each (weak, strong). Demographic data of patient and control group are given in **Table 1**. An independent samples *t*-test was used to compare age between the groups, showing that patient groups did not differ from each other, neither in the whole sample with $N = 31$ [$t_{(29)} = -0.6$, $p > 0.5$] nor in the subsample with $N = 25$ that performed the odor intensity and pleasantness tasks [$t_{(23)} = -0.8$, $p > 0.3$]. However, the group of 14 healthy

TABLE 1 | Mean age of participant groups in the odor identification and odor rating task.

	Odor identification		Odor rating	
Group	***N***	**Mean (*SD*)**	***N***	**Mean (*SD*)**
Left ATR	19	44.6 (10.7)	14	43.2 (7.4)
Right ATR	12	47.2 (11.1)	11	46.5 (10.8)
Both ATR	31	45.5 (10.5)	25	44.6 (9.3)
Control	14	33.6 (9.17)	14	33.6 (9.17)

ATR, anterior medial temporal lobe resection; N, number of participants; SD, Standard deviation.

controls was younger than the patients [$N = 31$; $t_{(43)} = -3.6$, $p < 0.01$].

Odor Identification Tests

Testing usually lasted about 50 min and took place in a well-ventilated room at Uppsala University. Odor stimuli for the identification task were taken from the "*Sniffin' Sticks: Identification – Extended Test*" (Hummel et al., 2007). Sixteen available odors, all commonly rated as very familiar, provided in pen-like devices, were grouped into two sets A (orange, leather, cinnamon, peppermint, banana, lemon, licorice, turpentine) and B (garlic, coffee, apple, clove, pineapple, rose, aniseed, fish). Both odor sets were presented monorhinally to all participants with order of odor set and nostril being balanced over participants and over patient groups (resection side) and all odor testing was performed with participants' eyes closed.

Each participant was presented with both odor sets (A/B) at one nostril each, whereas the other nostril was held closed by the participant. After presentation of the first odor set to the first nostril, odor set and side of exposure were changed, randomized between individuals. Each odor was presented for about 4 s in 2–3 cm distance from the exposed nostril. "Free identification" of the odor (FID) was assessed first. Following their response (or acknowledgment that they did not know the answer), participants chose from four possible answers (translated to Swedish from the "*Sniffin' Sticks*" test) that were presented simultaneously during 5 s as a "cued identification" (CID) test. Four of these answer alternatives where replaced with similar ones to prevent priming effects for upcoming odor presentations. Answers from FID and CID were coded with one point in case of correct identification and zero for no or clearly incorrect answer. Half a point, was given for correctly identified category (e.g., "fruit" for orange) in FID test. Sum of correct answers was analyzed using analysis of variance (ANOVA) including the between-group factor *Group* (left resected, right resected, control) and the within-group factor *Presentation side* (left, right nostril). Fisher's *Least Significant Difference* was used for *post hoc* test.

Odor Rating Tests

Two pleasant (citral and peach) and unpleasant (valeric acid, butyric acid) odorants were purchased from Sigma–Aldrich and prepared in weak and strong concentrations by blending to 10 ml with mineral oil. Volume concentrations, calculated as odorant volume divided by target volume 10 ml, are given in brackets for weak and strong blends, respectively: citral (0.01, 1), peach (0.01, 0.5), valeric acid (0.02, 0.1), and butyric acid (0.0067, 0.1). Stimuli were prepared in 160 ml wide mouth (62 mm) opaque glass jars with screw cap and presented by experimenter in 3–4 cm distance from the participant's nostril for about 4 s. A series of all eight odors was presented monorhinally and each odor was given twice to each nostril of each participant ($8 \times 2 \times 2$ stimulations) with 1 min break between concentration series at same nostril and longer breaks between presentations at different nostril. All odor testing was performed with participants' eyes closed. This exposure procedure was pre-tested in a pilot study in order to warrant iso-intensity of odor blends within the respective groups 'weak' and 'strong' and to optimize inter-stimulus interval to allow for headspace saturation with the given odorant and glass jar volumes. Odor series was: valeric acid -weak, -strong; peach -weak, -strong; citral -weak, -strong; and butyric acid -weak, -strong with second presentation in reversed order. After each odor presentation, participants used two nine-point scales, one ranging from nothing (0) to maximal (9) to rate odor intensity, and the second ranging from very bad (−4) via neutral (0) to very good (+4), to rate odor pleasantness. Intensity and valence data were investigated separately using mixed model designs. In a first step, we compared odor rating performance in healthy participants and whole group of ATR patients, i.e., regardless of side of resection, thus defining the between-group factor *Group* (control, ATR). In a second step, we addressed the question if left and right ATR have different impact on evaluation of odor intensity and valence by comparing both patient groups with each other, thus using the between-group factor *Patient Group* (left ATR, right ATR). The within-group factor *Stimulation Side* (left, right nostril) was included in all analyses. The within-group factor *Concentration* (weak, strong) was included in analyses of intensity ratings whereas the within-group factor *Valence* (pleasant, unpleasant) was included in analysis of pleasantness ratings in order to confirm that our manipulations of odor intensity and pleasantness were perceived accordingly by all groups.

Visual Gray Scale

Eight squares ranging in shades of gray between 20 and 90% white content (steps of 10%, created in Microsoft's Powerpoint) were selected as visual stimuli and their grayness was rated by the participants. The task was selected to be independent of both olfactory as well as semantic processing. This task was presented to the participant on a color calibrated computer screen in a self-paced manner. To become acquainted with the stimulus material, participants were shown all eight squares of different grayness simultaneously at beginning of the task, followed by successive presentation of each square for "grayness" rating using a visual nine-point scale ranging from 1 = 'completely white,' through 5 = 'intermediate,' to 9 = 'completely black.' Thus, the participants' task was to rate square grayness, it was not required to match stimuli with rating scale options. A random series of visual stimuli was presented twice to each participant and mean grayness-ratings given by each participant were analyzed using a mixed model analysis including the between-group factor *Group* (control, left ATR, right ATR).

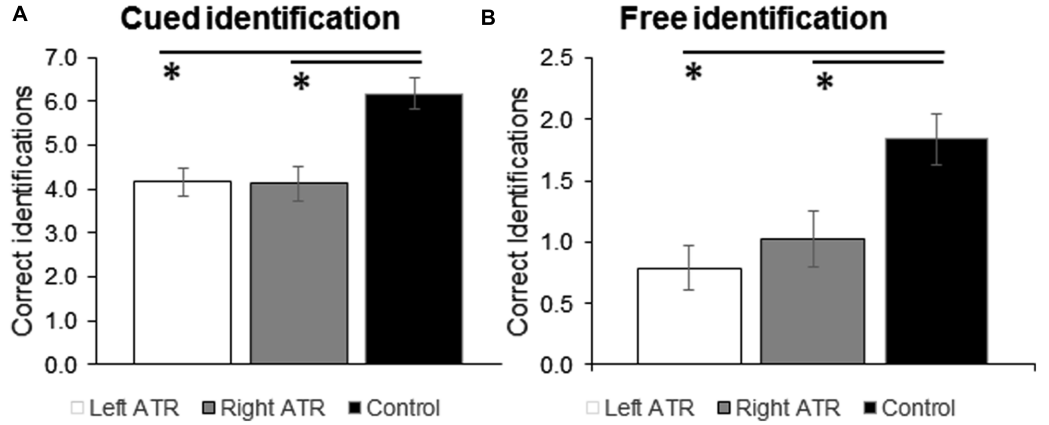

FIGURE 1 | Mean ratings and standard error of means (SEM) are shown for correct answers given in the cued (A) and free odor identification task (B). Data are shown separately for the experimental groups left ATR, right ATR, and control. ATR, anterior medial temporal lobe (TL) resection. *$p < 0.05$ for comparison against control group.

This study was carried out in accordance with the recommendations of the local ethics committee. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

RESULTS

Free Odor Identification and Cued Odor Identification

Results from two-way ANOVAs (*Group* × *Stimulation Side*) are given in **Figures 1A,B**. Groups (control, Left or Right ATR) differed in performance in the free [$F_{(2,42)} = 7.6$; $p = 0.002$] and the cued odor identification task [$F_{(2,42)} = 10.8$; $p < 0.001$]. *Post hoc* test revealed that both groups of patients were significantly worse in odor identification than the control group in both FID and CID (all $p < 0.02$). Left and right resected patients, however, did not differ from each other in free ($p = 0.427$) or cued odor identification task ($p = 0.948$). Presentation side did not have a significant effect on performance in the FID or CID task (both $p > 0.2$).

Odor Intensity

Odor intensity ratings are given in **Table 2**. Mixed Model analysis (*Group* × *Stimulation Side* × *Concentration*) showed a significant main effect for the factor *Concentration* [$F_{(1,1238)} = 88$, $p < 0.001$] indicating that both groups (ATR, controls) correctly differentiated between weak and strong odors by showing significantly lower intensity ratings for the weak as compared to the strong stimuli. No other significant main effects [*Group*: $F_{(1,1238)} = 1$, $p = 0.3$; *Stimulation Side*: $F_{(1,1238)} = 0.1$, $p = 0.7$] and no significant interactions between the three factors were found (all $F_{(1,1238)} < 0.9$, $p > 0.7$). Thus, we can conclude that the group of ATR patients did not differ from controls in perception of odor intensity.

In a second mixed model analysis of odor intensity ratings, we wanted to investigate whether left and right ATR have differential effects on odor evaluation. We therefore focused on comparing only the patient groups with each other. Included to the analysis were the between-group factors *Patient Group* (left ATR, right ATR) and *Concentration* (weak, strong) as well as the within-group factor *Stimulation Side* (left, right nostril), results are given in **Table 2**. As in the first analysis of patient vs. control groups, a significant main effect of *Concentration* [$F_{(1,790)} = 53.6$, $p < 0.001$] was found indicating perceptual discrimination between weak and strong odors. No further significant main effects [*Group*: $F_{(1,790)} = 0.7$, $p = 0.39$; Stimulation Side: $F_{(1,790)} = 1.9$, $p = 0.16$] and no significant interactions between the three factors were found [all $F_{(1,790)} < 0.8$, $p > 0.3$]. These results indicate that resection side did not modulate the perception of odor intensity in left and right ATR patients differently.

Odor Valence

Mixed model analysis (*Group* × *Valence* × *Stimulation Side*) comparing control group with ATR patients irrespective of resection side showed a significant main effect for the factor *Valence* [$F_{(1,1240)} = 828{,}4$, $p < 0.001$], thus confirming successful manipulation of odor valence, which was indicated by positive

TABLE 2 | Mean values and standard error of means (in brackets) are given for odor intensity and grayness ratings for control group ($N = 14$) and patient group ($N = 25$).

	Intensity ratings		Grayness ratings
Group	**Strong**	**Weak**	
Control	5.6 (0.1)	4.4 (0.1)	6 (0.19)
Patient	5.5 (0.1)	4.3 (0.1)	
Left ATR	5.4 (0.2)	4.3 (0.2)	6.1 (0.17)
Right ATR	5.7 (0.2)	4.3 (0.2)	6.1 (0.17)

ATR, anterior medial temporal lobe resection.

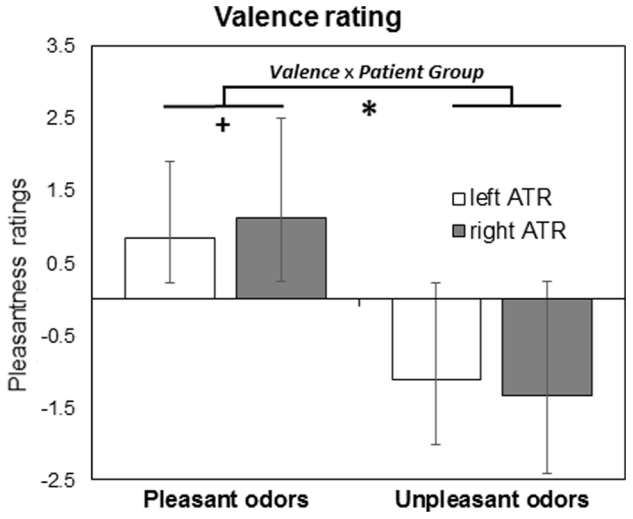

FIGURE 2 | Results from valence rating task are given as mean and standard error of means for patient groups with left (*N* = 14) and right (*N* = 11) anterior medial temporal lobe resection (ATR). $^{*}p < 0.05$ for Valence × Patient Group interaction, $^{+}p < 0.1$ for pairwise comparisons.

($M = 1.3$, $SEM = 0.6$) and negative ($M = -1.3$, $SEM = 0.6$) pleasantness ratings for nominally pleasant and unpleasant odors, respectively. Furthermore, a significant main effect of the factor *Group* was found [$F_{(2,1240)} = 7.8$, $p = 0.005$], showing that control group rated odorants as more pleasant than patient group ($M = 0.13$, $SEM = 0.07$ and $M = -0.12$, $SEM = 0.06$, respectively). A significant *Group* × *Valence* interaction [$F_{(1,1240)} = 24.5$, $p < 0.001$] was explained by more extreme un/pleasantness ratings in control subjects that differed significantly from patient ratings for pleasant ($p < 0.001$) but not for unpleasant odors ($p = 0.13$) as tested with pairwise comparisons. No other significant main effect [*Stimulation Side:* $F_{(1,1240)} = 0.4$, $p = 0.5$] or other significant interaction between the three factors were found [both $F_{(1,1240)} \leq 0.15$, $p \geq 0.7$].

In a second analysis, we investigated whether left and right ATR patient groups differed in odor valence perception. Results of a three-way Mixed Model analysis (*Patient Group* × *Valence* × *Stimulation Side*) are given in **Figure 2**, revealing a significant effect of *Valence* [$F_{(1,792)} = 332$, $p < 0.001$] but not *Patient Group* [$F_{(1,792)} = 0.1$, $p = 0.8$] or *Stimulation Side* [$F_{(1,792)} = 1.6$, $p = 0.2$]. A significant *Patient Group* × *Valence* interaction [$F_{(1,792)} = 4.3$, $p = 0.038$, **Figure 2**] reflected weaker differentiation between positive and negative odors in left ATR group irrespective of stimulation side. Pairwise comparison between left and right ATR groups showed a trend for pleasant odors ($p = 0.09$) but not for unpleasant odors ($p = 0.21$). No other significant interaction was found [$F_{(1,792)} < 0.4$, $p > 0.5$].

Visual Gray Scale

Data from gray scale test are shown in **Table 2**. Performance showed reasonable test-retest reliability (correlating performance in first and second half of visual gray scale test) ranging between $r = 0.71$ and $r = 0.99$ in patient groups and between $r = 0.78$ and $r = 0.99$ in the control group, thus indicating that patient and healthy controls were as reliable in their use of numbers to rate their perception. Grayness ratings were not significantly modulated by the factor *Group* as shown by the mixed model analysis [$F_{(2,36)} = 0.03$, $p = 0.97$].

DISCUSSION

Aims of this study were to investigate whether anterior medial TL-resection in TLE patients affected odor processing and to specifically estimate the role of left ATR in odor evaluation. Our results showed impaired odor identification and odor valence ratings, but not odor intensity ratings, in ATR patients as compared to healthy controls and confirmed special deficit in odor valence rating in patients with left ATR.

The study showed that free odor identification was about twice as good, and cued odor identification about 50% better in healthy controls as compared to ATR patients. With reference to normative data on cued odor identification (Hummel et al., 1997), this difference is larger than what would be expected from age difference between patients and controls alone. CID accuracy levels in control group lie at 75% which is below 85% reported by Hummel et al. (1997) but corresponds with accuracy levels reported by Olsson and Cain (2003) who also used monorhinal odor presentation.

Odor intensity ratings did not differ between control and patient groups (**Table 2**), thus confirming isointense perception between groups. *Stimulation Side* did not modulate control group performance in any of the olfactory tasks. Earlier studies have reported inconsistent results using monorhinic odor presentation in healthy volunteers. Broman et al. (2001) found no difference for odor identification whereas Herz et al. (1999) found left side advantage. Herz et al. (1999) also observed higher pleasantness ratings following right side presentation. Such findings from monorhinic odor presentations are often interpreted as related to hemispheric dominance. However, human imaging studies investigating olfactory hemispheric dominance seem to indicate a special role for the left hemisphere and especially left amygdala in valence perception (Zald and Pardo, 1997; Royet and Plailly, 2004). We will further discuss hemispheric lateralization in odor valence perception when discussing findings in our left ATR patient group.

With regard to valence ratings, healthy controls rated odors (across positive and negative odors) as more pleasant than ATR patients. The lower valence ratings, in absence of lower intensity ratings after ATR, may reflect compromised emotional processing as well as faulty semantic processing. Indeed, it has been demonstrated that valence ratings of familiar or namable odors to a great extent are dependent on the name given to the odor object rather than the odor itself (Djordjevic et al., 2008). Thus, odor identification deficits in ATR patients and related shortage of semantic odorant information may have contributed to observed differences in odor valence perception. Another plausible explanation to differences in valence ratings between

patients and controls concern rating tendencies. To control for individual differences in rating tendencies, participants rated grayness of visual stimuli. No group differences in consistency or level of ratings could be detected, thereby suggesting that the observed difference in valence ratings reflects a difference also in valence experience rather than just rating behavior.

Comparison between patient groups showed that left ATR group perceived odorants as more neutral than right ATR group. Reports of hemispheric lateralization of odor valence processing are far from consistent but tend to support left dominance (Zald, 2003; Royet and Plailly, 2004). Such left laterality seems especially true for amygdala activation, which correlates with odorant aversiveness (Zald and Pardo, 1997) and thus, our data could be interpreted as reflecting impaired emotional odorant processing. Alternatively, it has been suggested that odor valence ratings are dominated by processing in the left TL by virtue of its dependence on semantic representation of the odor object (Royet et al., 1999, 2001). However, the more neutral valence ratings in left ATR, as compared to right ATR, was not paralleled by worse odor identification, thus indicating that the observed lateralization of valence perception may have been more driven by compromised emotional rather than semantic processing. Altogether these results extend recent findings of an extensive study of TLE patients (Hudry et al., 2014) to patients after ATR. Hudry et al. (2014) report lower (in the direction of less pleasant) valence in left TLE across pleasant and unpleasant odors, which is paralleled by reduced pleasantness ratings following left nostril stimulation when comparing healthy controls with patient groups regardless of TLE lateralization. Interestingly, in the latter comparison Hudry et al. (2014) report the same pattern of more neutral pleasantness ratings for patient group irrespective of TLE lateralization. Thus, both the Hudry et al. (2014) study and our findings suggest left TL involvement in odor valence processing. Possible reasons for differences between these two studies may be related to the fact that patients in our study were assessed post-surgery and also several years after resection. The olfactory system has a large plasticity, from the receptor to the cortical level, thus the mainly perceptual process of odor intensity ratings may have recovered due to a functional reorganization, similarly to what was recently demonstrated in hippocampal processing in patients with TLE (Banks et al., 2012).

CONCLUSION

This study shows that epilepsy patients with anterior medial TL resection have compromised olfactory cognition. This is particularly true for free odor ID but also cued odor ID. In addition, an altered valence perception does not seem to depend on a general change in rating behavior or odor intensity perception. The left anterior medial lobe shows a special role for valence perception in line with previous findings. According to our pattern of results, patients with left ATR experienced odors to be less emotionally salient, possibly reflecting an absent or deficient left amygdala.

AUTHOR CONTRIBUTIONS

Contribution of authors were as follows: concept and design of work, acquisition, analysis of data: EK, MF, MG, MO. Analysis, interpretation of data, drafting, and revising of the final manuscript: SJ, JL, FÅ, MO.

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In special this includes that we or our institutions did not receive payment or services from a third party for any aspect of the submitted work at an times.

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Odor Perception in Children with Autism Spectrum Disorder and its Relationship to Food Neophobia

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Atypical sensory functioning in Autism Spectrum Disorder (ASD) has been well documented in the last decade for the visual, tactile and auditory systems, but olfaction in ASD is still understudied. The aim of the present study was to examine whether children with ASD and neuro-typically (NT) developed children differed in odor perception, at the cognitive (familiarity and identification ability), sensorimotor (olfactory exploration) and affective levels (hedonic evaluation). Because an important function of the sense of smell is its involvement in eating, from food selection to appreciation and recognition, a potential link between odor perception and food neophobia was also investigated. To these ends, 10 children between 6 and 13 years old diagnosed with ASD and 10 NT control children were tested. To compare performance, 16 stimuli were used and food neophobia was assessed by the parents on a short food neophobia scale. Results revealed that (i) significant hedonic discrimination between attractive and aversive odors was observed in NT ($p = 0.005$; $d = 2.378$) and ASD children ($p = 0.042$; $d = 0.941$), and (ii) hedonic discrimination level was negatively correlated with food neophobia scores in ASD ($p = 0.007$) but not NT children. In conclusion, this study offers new insights into odor perception in ASD children, highlighting a relationship between odor hedonic reactivity and eating behavior. This opens up new perspectives on both (i) the role of olfaction in the construction of eating behavior in ASD children, and (ii) the measurement and meaning of food neophobia in this population.

Keywords: autism, olfaction, food neophobia, hedonic evaluation, exploratory behavior

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INTRODUCTION

According to the American Psychiatric Association's Diagnostic and Statistical Manual, Fifth Edition (DSM-5), Autism Spectrum Disorder (ASD) is characterized by both (i) deficits in social communication and social interaction and (ii) stereotyped, restricted, repetitive patterns of behavior, interest or activity (including atypical speech and movement, resistance to change, and atypical sensory behavior). These symptoms are present in early childhood and combine to limit and impair everyday functioning.

Atypical sensory functioning in ASD has been well documented in the last decade for the visual (Simmons et al., 2009), tactile (Puts et al., 2014) and auditory (Hitoglou et al., 2010; O'Connor, 2012) systems (Marco et al., 2011); for instance, it has been shown that orientation toward social

sounds is impaired in ASD children (Dawson et al., 2004). On the other hand, olfaction and taste in ASD are still understudied despite the fact that experimental proof of the importance of environmental odor cues for the social and cognitive development of ASD children was provided by two recent studies. In the first, Parma et al. (2013) showed that automatic imitation – a prominent social skill that is impaired in ASD – in a reach-to-grasp action task is induced in ASD children when the object to be grasped is paired with the smell of their own mother, suggesting that a familiar body odor may promote imitation in ASD children. In the second study, Woo and Leon (2013) exposed 3–12 year-old ASD children to either daily olfactory/tactile stimulation along with sensory and cognitive exercises (enrichment group), or to only standard care (control group); after 6 months of enrichment, the severity of autistic traits (assessed on the Childhood Autism Rating Scale, Schopler et al., 1980) was significantly lower in the enrichment group than in controls.

Besides these 2 promising scientific attempts, the few clinical and scientific reports available that characterized olfactory function in this population suggest that individuals with ASD have atypical responses to olfactory stimuli (reviewed in Schecklmann et al., 2013 and Martin and Daniel, 2014), although results have not often been concordant: odor detection ability was equivalent between adults with ASD and controls in three studies (Suzuki et al., 2003; Tavassoli and Baron-Cohen, 2012; Galle et al., 2013), whereas in another study odor detection was better in ASD patients (Ashwin et al., 2014). Odor identification was impaired in two studies (Suzuki et al., 2003; Galle et al., 2013). Studies in ASD children also showed lack of consensus: Bennetto et al. (2007) reported lower odor identification ability in ASD patients than controls, whereas Dudova et al. (2011) found lower odor detection but no difference in identification between ASD children and healthy controls.

However, olfaction has important functions involving other abilities than just detection and identification, and these functions have been understudied in ASD patients. Firstly, the sense of smell constitutes an early warning system against odorant molecules that may, for example, signal toxic food to be avoided. Secondly, it plays a major role in hedonic pleasure, especially regarding food. Hrdlicka et al. (2011) showed that ASD children perceived the odors of pineapple and cinnamon (among 16 odors) as less pleasant than controls; but how hedonic ratings is changed for pleasant odors and unpleasant odors in ASD children remains unclear. Are these important functions of olfaction (attraction to and avoidance of smells) enhanced/maintained/impaired in this disorder? The general aim of the present study was to characterize olfactory function in ASD children at both the cognitive (odor familiarity and odor identification ability: **objective 1**) and sensorimotor and hedonic levels (**objective 2**), by considering the positive and negative hedonic value of smells. To this end, a pleasant and an unpleasant odor (at various concentrations) selected from a previous study (Joussain et al., 2013) were presented to ASD children and controls. The odors were embedded in a series of 16 stimuli including a non-odorized stimulus and odorant compounds that included both mixtures of molecules and their individual components. Whereas no hypothesis was tested for the mixtures and the individual components, for pleasant and unpleasant stimuli, we tested the bidirectional hypothesis that affective reactivity to odors is reflected by (i) hypo-emotionality (decreased pleasantness of attractive odors; decreased unpleasantness of aversive odors) or (ii) hyper-emotionality (increased pleasantness of attractive odors; increased unpleasantness of aversive odors). Verbal responses were collected, accompanied by behavioral quantification of nasal olfactory exploration using video tools.

An important function of the sense of smell is its involvement in eating, from food selection to appreciation and recognition. Eating is a multifactorial mechanism involving three main sources of variability: the eater (with his/her food history and sensations), the object (food and its characteristics) and the context (physical and social environment Rozin and Tuorila, 1993; Meiselman and MacFie, 1996; Renner et al., 2012). Eating activities have become more complex over the course of evolution and the determinants of food choice are multiple (Köster, 2009). Eating well (or normally) can be learned. The construction of children's dietary behavior requires sensorimotor, social and psychological skills (de Suremain and Razy, 2012). The process is sometimes difficult: eating disorders affect 13–50% of neuro-typically (NT) developed children, but more than 80% of children with ASD (Ledford and Gast, 2006; Nadon et al., 2013). In particular, selectivity is by far the most common problem encountered by children with ASD (Sharp et al., 2013; Cermak et al., 2014; Rastam and Wentz, 2014).

Although the term "food selectivity" has been understood in different ways in *ad hoc* studies of ASD children, there is some consensus that it restricts the number of accepted foods (Cermak et al., 2014; Rastam and Wentz, 2014). The primary objective of food learning is to widen the diversity of foods accepted by children, so as at least to cover their vital needs. This opening strengthens and widens during childhood and adolescence (Nicklaus, 2009). Many intrinsic and extrinsic factors influence the acceptance of new foods by children, such as parental behavior or sensory processes (Blissett and Fogel, 2013). A major hindrance to widening food diversity and the acceptance of new foods is **food neophobia,** defined as a reluctance to consume or tendency to reject foods considered new by the eater (Loewen and Pliner, 1999; Dovey et al., 2008). Food neophobia was found to be associated with sensory experience (Aldridge et al., 2009; Shim et al., 2011), sensory functioning (Cooke, 2007) and anxiety (Galloway et al., 2003).

One of the main causes of greater food selectivity in children with ASD may lie in their particular sensory functioning (Matson and Fodstad, 2009; Beighley et al., 2013; Cermak et al., 2014). Notably, olfactory alterations may jeopardize acceptance of food and dangerously restrict variety of diet in ASD children (Demattè et al., 2014). Therefore, the third objective (**objective 3**) of the present study was to examine the relationship between hedonic response to pleasant and unpleasant odors and behavioral attitudes toward food (i.e., food neophobia).

MATERIALS AND METHODS

Participants

This preliminary study, approved by the *Commission Cantonale Valaisanne d'Ethique Médicale* institutional review board (IRB number: CCVEM 022/14), tested 10 children diagnosed with ASD (all boys; age range, 6–13 years) and 10 NT control children, matched for age (±6 months) and gender. The ASD group was composed with children considered as eligible for the Swiss ASD Observatory and children officially diagnosed by the Autism Diagnostic Assessment Centre of Lyon. No data were available on IQ and language level. With regard to ASD symptom, six were announced with ASD or with pervasive developmental disorder and four as Asperger. The NT control participants had normal school performance, without any known behavioral or psychological disorder. All participants and their legal guardians agreed to participate in the study by signing a consent form.

Food neophobia was assessed by the parents on a standard 10-item questionnaire (the French adapted food neophobia scale: AFNS) with good internal consistency (Reverdy et al., 2008). For each item, parents were required to indicate to what extent the corresponding statement was true, on a 7-point scale from "Very true for me" to "Not at all true for me."

The 10 items were: (1) My son is very particular about the foods he will eat (reversed scoring); (2) My son likes foods from different countries; (3) My son doesn't trust new foods (reversed scoring); (4) My son likes to try unusual foods; (5) When my son has the choice between different flavors for a certain food (for example, ice-cream or sweets), he likes to choose a flavor that he doesn't not know; (6) My son will try a dish, even if he doesn't not know what's in it; (7) The foods my son knows are sufficient for him (reversed scoring); (8) My son is willing to eat anything that is offered; (9) My son is afraid to eat things he has never had before (reversed scoring); and (10) My son will not taste a food when he doesn't know what it is (reversed scoring). For questions 2, 4, 5, 6, and 8, the highest score (7 points) was given to the response "Very true for my son" and the lowest (1 point) to "Not at all true for my son"; for questions 1, 3, 7, 9, and 10, the scores were reversed. The food neophobia score was obtained by adding the scores for the 10 questions (range: 10–70); the higher the score, the higher the neophobia grade.

There was no significant difference between groups in terms of age in years (mean ± SEM; NT: 9.97 ± 0.80, ASD: 9.58 ± 0.83; Mann-Whitney test: $z = 0.680$, $p > 0.05$) or food neophobia score (NT: 48.8 ± 4.27, ASD: 42.4 ± 4.75; Mann–Whitney test: $z = 0.869$, $p > 0.05$).

Stimuli

In order to compare hedonic reactivity to pleasant and unpleasant food odors in ASD and NT children, 4 concentrations of a pleasant mint odor (L-Carvone, CID = 439570, 1%, 2.37%, 5% and 10%) and unpleasant fishy odor (Trimethylamine, CID = 1146, 10, 25, 50, and 100%) were presented to the participants. In addition, three binary mixtures (50/50%) containing respectively the smells of (rose + grass), (vanilla + cocoa) and (rose + cocoa), and their individual components ("vanilla": ethyl vanillin, CID = 8467, 100%; "cocoa": isobutyl phenylacetate, CID = 60998, 28%; "rose": phenyl ethanol, CID = 6054, 2.65%; and "grass": cis-3-hexenol, CID = 5281167, 0.21%) were also presented. All odorants (Sigma–Aldrich) were diluted in mineral oil. They were presented in 15 ml flasks (opening diameter: 1. 7cm; height: 5.8 cm; filled with 5 ml dilution) and absorbed on scentless polypropylene fabric (3 cm × 7 cm; 3 M, Valley, NE, USA) to optimize evaporation and air/oil partitioning. Finally, an empty jar containing only an odorless solvent (mineral oil) served as control stimulus. A total of 16 stimuli (15 odorous and 1 control) were thus used.

Protocol

One important aspect of children's involvement in the study was that they were prepared for the experimental sessions a few weeks before. They had been informed in advance by their teacher and parents that they would take part in a sensory study involving olfaction. Experiments were performed in the cities of Sion and Sierre (Switzerland), in specially adapted rooms.

The experimenter started with a detailed explanation of the procedure to the child. Participants were required to sit on a chair, either on the right or left side of the experimenter, in front of a table (or if not possible, with a box on their knees). They were videotaped by two digital camcorders (one in front of the participant, and the other oriented toward his left or right profile) during the experimental session. The experiment started as soon as the participant was installed, and included two phases.

Phase 1 consisted in familiarizing the children with olfactory exploration. Sixteen trials were presented in randomized order (Hasard software). The experimenter opened a jar and gave it to the child, who was asked to smell the odor, without touching the odorant jar with his nose, and to put the jar back on the table or in the box once smelled. Stimulus-onset asynchrony varying from 20 to 30 s was used.

Phase 2, the experimental phase, was conducted the same day, at least 30 min after phase 1. Verbal and behavioral responses to the same 16 stimuli were characterized in ASD and NT children using implicit (video recording of olfactory exploration) and explicit (verbal response) approaches. As in phase 1, each trial started as soon as the experimenter presented the jar to the child, telling him: "You have to smell this jar without touching it with your nose." The child's task was to answer the following questions: (1) "Do you like this odor?"; (2) "Do you know what is it?"; and (3) "Can you tell me what it is?". Stimulus-onset asynchrony from 20 to 90 s was used, depending on the child's verbal production.

Data Analysis

Verbal Data

The first question ("Do you like this odor?") enabled analysis of hedonic response, scored as follows:

"1" for a "Yes" or nod of the head or any positive response such as "It's ok," "It's good," etc.; "−1" for a "No" or any negative response such as "Not so much," "Not really," "Not too much," etc.; or "0" for an unclear or non-hedonic response such as

"I don't know," "Medium," "Strong," "So-so," "Quite strong," "Strong, medium," etc.

The second question ("Do you know what it is?") enabled analysis of odor familiarity, scored as "1" for a "Yes," and "0" otherwise.

Finally, the third question ("Can you tell me what it is?") enabled analysis of identification ability, coded by conformity with a veridical label (vl). One or several vls were defined for each odor, with a score of "1" if any of the vls was used; however, if the participant did not use the vls, but used a semantically related word, then 0.5 point was affected: (1) L-Carvone (four concentrations; vl = "Mint," but "Toothpaste" accepted); (2) Trimethylamine (four concentrations; vl = "Fish," but "Pooh," "Anchovy," or "Cat-food" accepted); (3) Phenyl ethanol (vl = "Rose," with 0.5 points for "Lavender" or "Herbs," as being semantically close); (4) Cis-3 hexenol (vl = "Grass," with 0.5 points for "Grape" or "Crushed flowers"); (5) Ethyl vanillin (vl = "Vanilla" and/or "Caramel," with 0.5 points for "Sugar"); (6) Phenyl acetate isobutyl (vl = "Chocolate" or "Cocoa"); (7) Phenyl ethanol + Cis-3-hexenol (vl = "Rose," "Flower" or "Grass," with 0.5 points for "Grape," "Leaf" or "Herbs"); (8) Ethyl vanillin + Phenyl acetate isobutyl (vl = "Vanilla," "Caramel," "Chocolate" or "Cocoa," with 0.5 points for "Honey"); (9) Phenyl ethanol + Ethyl vanillin (vl = "Flower," "Rose," "Caramel" or "Vanilla"), (10) solvent (no vl).

Behavioral Data

The profile video sequence recorded for each participant was divided into 16 segments, corresponding to each odorant condition, using appropriate software (Volcan®, Lyon, France; see Rinck et al., 2011). For each segment, olfactory exploration of the jar was quantified, starting when the participant moved the jar in front of his nose/lip, or even earlier if a strong focus of the odor was observed (e.g., head movement or marked diminution of the approach movement), and ending when the participant moved the jar away from his nose. Four variables were analyzed: (i) number of olfactory explorations per stimulus; (ii) total duration of olfactory exploration; (iii) mean duration of olfactory exploration (total duration/number of explorations); and (iv) duration of the first olfactory exploration.

Statistical Analyses

For statistical analyses of verbal and behavioral data, five parameters were calculated for each participant and each variable (verbal variables: odor identification, odor familiarity, and odor pleasantness; behavioral variables: number of olfactory explorations, total duration of exploration, mean duration of exploration, and duration of first exploration): (1) mean value for all 15 odors (m_g); (2) mean value for the four trials of L-Carvone ($m_{L-Carvone}$); (3) mean value for the four trials of Trimethylamine ($m_{Trimethylamine}$); (4) mean value for all simple mixture components (m_{simple}); and (5) mean value for all mixtures ($m_{mixture}$). It is important to note here that 50% of the ASD children were not able (or did not agree) to perform the whole study (see Results), so that, because the experimental design was randomized, the mean value calculated for each odor category (carvone or trimethylamine, for example) was not necessarily based on the same number of trials; consequently, the effect of odor concentration for carvone and trimethylamine could not be assessed.

Two types of statistical comparison were performed: (1) inter-group comparison between NT ASD groups for the parameters m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple} and $m_{mixture}$ used Mann–Whitney *U* tests for all verbal and behavioral variables; (2) intra-group comparison of $m_{L-Carvone}$ vs. $m_{Trimethylamine}$ and m_{simple} vs. $m_{mixture}$, in the NT group on the one hand and in the ASD group on the other hand, used Wilcoxon tests.

Finally, to relate odor hedonic perception with food neophobia, two types of correlation analysis were performed: (i) between pleasantness ratings of both pleasant and unpleasant odors on the one hand, and food neophobia score on the other hand; and (ii) between a hedonic categorization index (the absolute value of the difference between the mean hedonic score for L-Carvone ($m^h_{L-Cavone}$) and the mean hedonic score for Trimethylamine ($m^h_{Trimethylamine}$) (i.e., $m^h_{L-Cavone} - m^h_{Trimethylamine}$) on the one hand and food neophobia score on the other hand.

For all analyses, the level of statistical significance was set at 0.05. Analyses were performed using SPSS software (version 22 for Windows).

RESULTS

Firstly, as regards the experiment itself, NT children were able to perform the whole experimental session (16 odors), whereas ASD children were not able to experience all the odorant stimuli during the session (mean ± SEM; 12.8 ± 1.21; trend on Mann–Whitney test: $z = 1.890$, $p = 0.058$). The interruption was made at the child's request, for the following reasons: one child decided from the outset to test only eight odors; one child could no longer concentrate; and three children expressed emotional reactions such as disgust, preventing them from continuing.

All statistics (z and p values) for identification, familiarity, pleasantness and behavioral data are presented in **Tables 1** and **2**.

Regarding verbal data, analysis of odor identification performance (**Table 1**; **Figures 1A–C**) revealed no significant effect of group for m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple}, or $m_{mixture}$. On intra-group comparison, a trend toward better identification of the pleasant odor Carvone than the unpleasant odor Trimethylamine was observed in the NT but not in the ASD group, while comparison between mixtures and their individual components was not significant in either NT or ASD children.

Regarding familiarity ratings (**Table 1**; **Figures 1D–F**), no significant difference between groups was found for m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple}, or $m_{mixture}$. Moreover, Carvone and Trimethylamine did not differ in familiarity in the NT or ASD group; nor did the mixtures and their individual components.

With regard to odor pleasantness (**Table 1**; **Figure 2**), no significant effect of group was observed for m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple}, or $m_{mixture}$, but intra-group comparison revealed that Carvone was rated as significantly more pleasant than Trimethylamine by NT children, and by ASD children. To

TABLE 1 | Inter-group and intra-group comparison for odor identification, familiarity and pleasantness: sample size (*N*), observed *z*-value and *p*-value for each parameter and each variable.

	Identification score			Familiarity ratings			Hedonic value		
	N	***z*-value**	***p*-value**	***N***	***z*-value**	***p*-value**	***N***	***z*-value**	***p*-value**
m_g	18	−0.535	0.593	18	−0.936	0.349	18	−0.089	0.929
$m_{L-Carvone}$	18	−1.331	0.183	18	−0.552	0.581	18	−1.115	0.265
$m_{Trimethylamine}$	18	−0.320	0.749	18	−1.206	0.228	18	−0.239	0.811
m_{simple}	18	−0.183	0.855	18	−0.819	0.413	18	−0.584	0.559
$m_{mixture}$	17	−0.872	0.383	17	−0.253	0.800	17	−0.546	0.585
$m_{L\text{-}Carvone}$ vs. $m_{Trimethylamine}$									
NT	10	−1.697	**0.090**	10	−1.491	0.136	10	−2.816	**0.005**
ASD	8	−0.216	0.829	8	−0.184	0.854	8	−2.032	**0.042**
m_{simple} vs. $m_{mixture}$									
NT	10	−0.831	0.406	10	−0.211	0.833	10	−1.472	0.141
ASD	7	−0.422	0.673	7	−1.355	0.176	7	−1.892	**0.058**

p-values < 0.1 are highlighted.

TABLE 2 | Inter-group and intra-group comparison for number of olfactory explorations, total duration of exploration, mean duration of all explorations and duration of first exploration: sample size (*N*), observed *z*-value and *p*-value for each parameter and each variable.

	Number of olfactory explorations		Total duration of exploration		Mean duration of all explorations		Duration of first exploration	
	N	***z*-value**	***p*-value**	***z*-value**	***p*-value**	***z*-value**	***p*-value**	***z*-value**	***p*-value**
m_g	19	−1.643	0.100	−0.653	0.514	−1.388	0.165	−1.306	0.191
$m_{L-Carvone}$	19	−0.178	0.859	−0.163	0.870	−1.061	0.288	−1.143	0.253
$m_{Trimethylamine}$	19	−1.464	0.143	−1.143	0.253	−1.715	**0.086**	−1.960	**0.050**
m_{simple}	19	−1.152	0.249	−0.041	0.967	−0.408	0.683	−0.327	0.744
$m_{mixture}$	18	−1.274	0.203	−0.889	0.374	−1.510	0.130	−0.933	0.351
$m_{L\text{-}Carvone}$ vs. $m_{Trimethylamine}$									
NT	10	0.0001	0.999	−0.153	0.878	−0.255	0.799	−0.255	0.799
ASD	9	−1.089	0.276	−0.533	0.594	−1.599	0.110	−1.836	**0.066**
m_{simple} vs. $m_{mixture}$									
NT	10	−0.368	0.713	−0.153	0.878	−0.357	0.721	−0.357	0.721
ASD	8	−1.270	0.204	0.0001	0.999	−0.420	0.674	−0.420	0.674

p-values < 0.1 are highlighted.

assess the magnitude of this effect in each group, we performed an effect size analysis using Cohen's *d* for paired samples. Results obtained with a classical bootstrap procedure (1000 resamples for each group) showed that effect size was greater in NT (Cohen's *d*: 2.378; Percentile Bootstrap 95% Confidence Interval or CI: 1.709–4.487) than in ASD (Cohen's *d*: 0.941; Percentile Bootstrap 95% CI: 0.503–1.881), although the two CI overlapped slightly.

Moreover, whereas mixtures and their individual components did not differ in pleasantness in the NT group, there was a trend toward lower pleasantness for mixtures than the components in the ASD group.

Regarding behavioral data (**Table 2**): for the variable "total duration of exploration" (**Figures 3A–C**), no significant effect of group was found for m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple}, or $m_{mixture}$ and intra-group comparison did not show any significant difference between Carvone and Trimethylamine in the NT or ASD group. Moreover, no significant difference between mixtures and their individual components was observed in the NT or ASD group.

Analysis of "number of olfactory explorations" (**Figures 3D–F**) found no significant effect of group for m_g, $m_{L-Carvone}$, $m_{Trimethylamine}$, m_{simple}, or $m_{mixture}$. Intra-group comparison found no significant difference between Carvone and Trimethylamine, or between mixtures and their individual components, in the NT or ASD group.

For "mean duration of all explorations" (**Figures 4A–C**), there was a trend toward a lower value for $m_{Trimethylamine}$ in ASD children than NT children, but analysis did not show any significant influence of group for m_g, $m_{L-Carvone}$, m_{simple}, or $m_{mixture}$. Moreover, intra-group comparison did not show any significant difference between Carvone and Trimethylamine, or between mixtures and their individual components, in the NT or ASD group.

Finally, for "duration of the first exploration" (**Figures 4D–F**), a significant effect of group was observed for $m_{Trimethylamine}$, reflecting shorter exploration duration in ASD children than NT children, while no effect of group was observed for m_g, $m_{L-Carvone}$, m_{simple} or $m_{mixture}$. Intra-group comparison revealed no significant differences between Carvone and

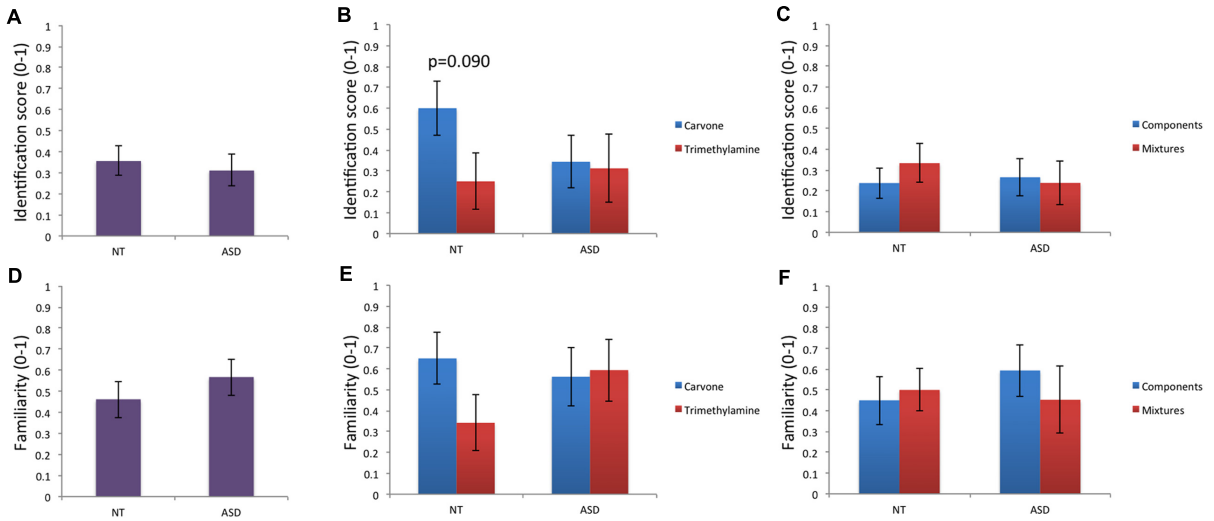

FIGURE 1 | Odor identification and odor familiarity. (A–C) Odor identification performance did not differ between groups or between odor conditions within groups. A trend ($p < 0.10$) toward better identification of the pleasant odor (Carvone) than the unpleasant odor (Trimethylamine) was observed in the NT group. **(D–F)** For familiarity ratings, no significant effect of group or odor condition (within group) was observed.

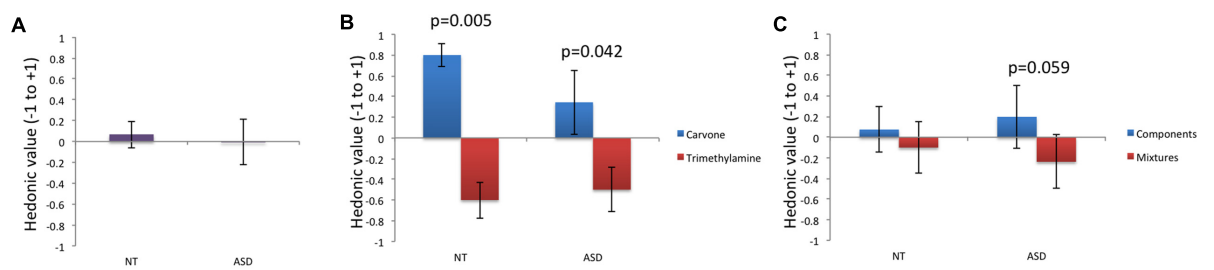

FIGURE 2 | Odor pleasantness. For odor pleasantness ratings, no difference was observed between groups when considerign all odors **(A)**. A significant effect of valence was observed in the NT group: the smell of Carvone was perceived as significantly more pleasant than the smell of Trimethylamine ($p = 0.005$). A similar effect of valence was observed in the ASD group, although the magnitude of the effect was lower ($p = 0.042$) **(B)**. It noteworthy that a trend toward lower pleasantness of odor mixtures than the individual components was observed in the ASD group ($p = 0.059$) **(C)**.

Trimethylamine in the NT group, but a trend for ASD children to exhibit a shorter sniff in response to Trimethylamine than Carvone. No significant difference was observed between mixtures and their individual components, in the NT or ASD group.

Thirdly, results regarding a link between odor pleasantness and food neophobia revealed no significant relationship between pleasantness ratings of unpleasant odors and food neophobia scores in NT ($r = -0.27$, $p = 0.438$) or ASD children ($r = 0.33$, $p = 0.420$). However, although there was no significant relationship between pleasantness ratings of pleasant odors and food neophobia scores in NT children ($r = 0.28$, $p = 0.424$), a trend toward a negative relationship was observed in ASD children ($r = -0.65$, $p = 0.081$): ASD children who perceived "attractive" odors as less pleasant had higher neophobia scores. This relationship between odor pleasantness and food neophobia in ASD children was confirmed by analysis taking account of the odor hedonic categorization index presented above: a significant negative relationship between odor hedonic categorization index and food neophobia score was observed in ASD ($r = -0.85$, $p = 0.007$) but not NT children ($r = 0.42$, $p = 0.226$): ASD children who had difficulty in hedonically categorizing smells (low index) had higher neophobia scores (**Figure 5**).

DISCUSSION

The aim of the present study was threefold: to examine whether ASD and NT children differed in odor perception, at both cognitive level (familiarity and identification ability) (objective 1) and sensorimotor (olfactory exploration) and hedonic levels (objective 2), and to assess a potential link between atypical odor perception and behavioral attitude toward food (food neophobia) (objective 3).

Regarding the **first objective**, the study provides very minor support for impaired odor identification in ASD children compared to controls: the only inter-group difference was that identification of the pleasant odor tended to be better

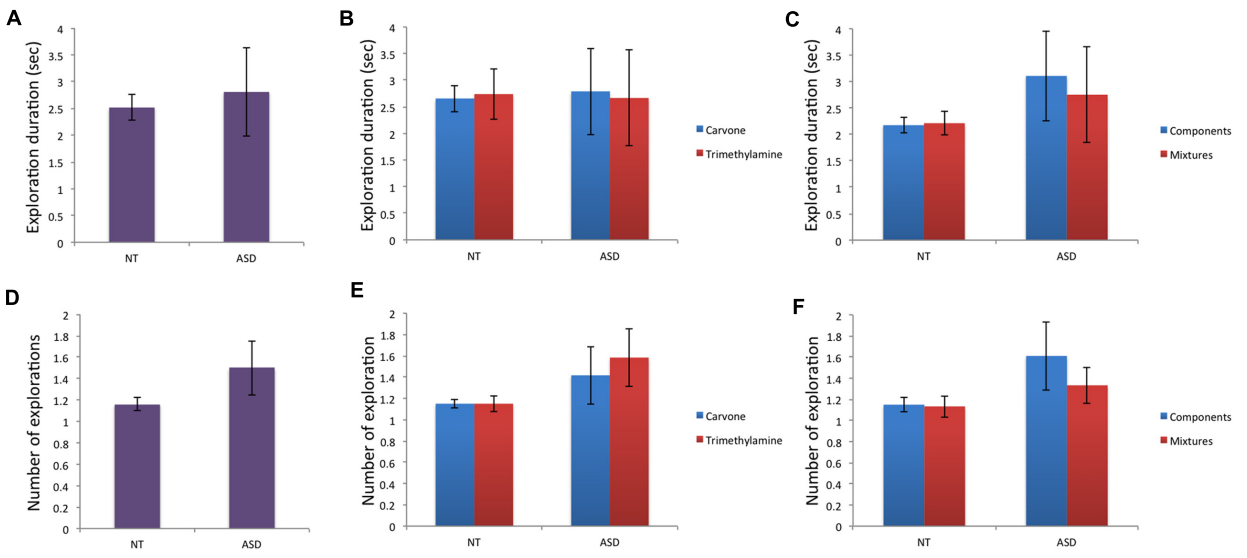

FIGURE 3 | Behavioral data (i): total duration of explorations and number of olfactory explorations. (A–C) No significant effect of group or of odor conditions within groups was observed for total duration of exploration. **(D–F)** For number of olfactory explorations, no significant effect of group or odor condition (within group) was observed.

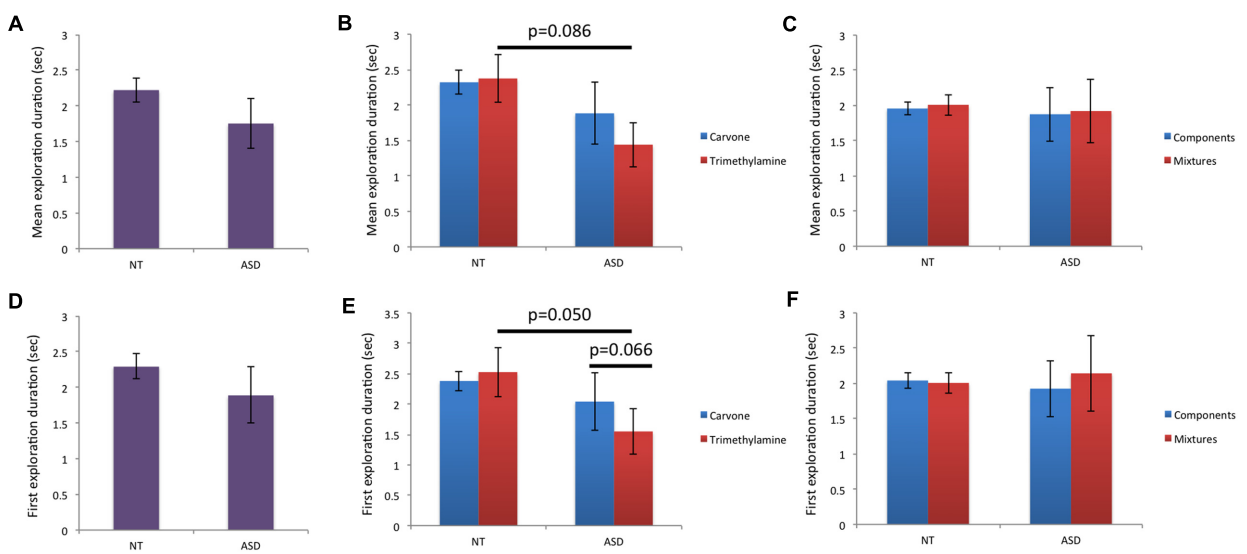

FIGURE 4 | Behavioral data (ii): mean duration of all explorations and duration of first exploration. (A–C) For mean duration of all explorations, a trend toward shorter mean duration for the unpleasant smell (Trimethylamine) in ASD than NT children was found ($p = 0.086$). The other inter-group or intra-group comparisons were non-significant. **(D–F)** For duration of first exploration, a significant effect of group was observed for Trimethylamine: ASD children explored this unpleasant odor less than NT children ($p = 0.050$). Moreover, a trend toward shorter exploration duration for Trimethylamine than Carvone was observed in the ASD group ($p = 0.066$).

than for the unpleasant odor in NT but not ASD children. Although studies have reported much evidence for impaired odor identification in ASD, findings have sometimes been inconsistent between studies. For example, Suzuki et al. (2003) measured odor detection and odor identification abilities in adult patients with Asperger's syndrome and matched control subjects; compared to controls, patients exhibited intact odor detection levels but impaired odor identification ability. In another study, Galle et al. (2013) measured several aspects of olfactory perception (detection, discrimination, identification and ratings for intensity, pleasantness and familiarity) in ASD adults (including both classical autism and Asperger's syndrome) and controls; whereas olfactory thresholds, odor discrimination and intensity, pleasantness and familiarity ratings did not differ between groups, odor identification ability was lower in autistic subjects than in both control and Asperger's syndrome subjects.

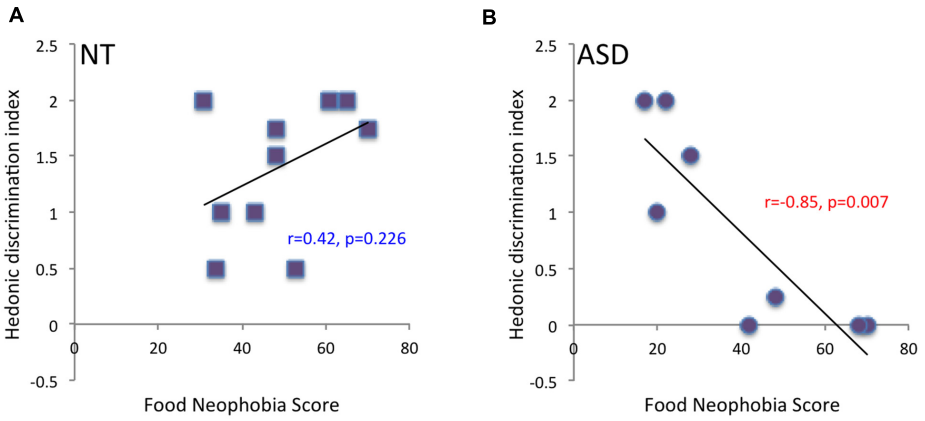

FIGURE 5 | Correlation between hedonic categorization index and food neophobia score. A significant negative correlation between odor hedonic categorization index and food neophobia score was observed in ASD children ($p = 0.006$) **(B)**, but not in NT children **(A)**.

Studies in ASD children reported inconsistent results. Bennetto et al. (2007), found that odor identification ability in ASD patients aged from 10 to 18 years old was lower than in controls. In a longitudinal study of ASD children, May et al. (2011) reported that odor identification ability improved with age (from 7 to 11 years) in ASD children as in controls. Dudova et al. (2011) reported that ASD children (mean age, 10 years) were impaired in odor detection as compared with matched controls, but not in identification ability (although ASD children identify the smell of orange better and the smell of cloves worse). Thus, identification ability does not seem to be clearly impaired in children with ASD, in line with the weak, non-significant difference in the identification performance between ASD and NT children in the present study. Nevertheless, it is worth mentioning here that it is not unlikely that both linguistic and cognitive factors characterizing the ASD group may have accounted for our findings. For example, language capacities were not measured and one cannot discard the possibility that odor identification performances in ASD children may depend on their level of language. Moreover, our group included Asperger's syndrome participants whose performance could enhance the overall performance of the ASD group as suggested for adults by Galle et al. (2013).

With regard to the **second objective**, studies reported some minor differences in odor pleasantness in ASD children. For example, Hrdlicka et al. (2011), assessed differences in odor hedonic ratings in ASD children vs. controls. Odor hedonic ratings were measured on a 5-point scale using the smells contained in the identification part of the Sniffin Sticks test (see: Hummel et al., 1997; Kobal et al., 2000). The ASD children undervalued 2 of the 16 smells compared to controls, perceiving the odors of pineapple and cinnamon as less pleasant. It is worth noting that in a study with only ASD children, Dudova and Hrdlicka (2013) found no significant correlation between autism severity and odor detection, odor pleasantness ratings or odor identification ability. In the present study, whereas significant hedonic discrimination measured by verbal response (pleasantness of the attractive versus the aversive odor; **Figure 2B**) was observed in both groups, behavioral data (duration of first exploration) showed that ASD, unlike NT children, discriminated the unpleasant from the pleasant odor, the former being less explored (**Figure 4E**). This inconsistency between verbal reports and behavioral and implicit measures of olfactory processing was also noted by Legiša et al. (2013), who tested ASD children and matched controls (aged 8–14 years) and examined how emotional responses to odors were reflected in peripheral nervous system responses (facial and autonomic responses); the two groups showed very similar facial and autonomic emotional responses to smells but, comparing peripheral responses and verbal reports, ASD children seemed less likely to verbally express an affective state corresponding to their facial expression.

The **third objective** was to examine to what extent odor hedonics could be related to behavior toward food (i.e., food neophobia) in ASD children. Allowing for the limits related to the exploratory nature of the study, it emerged that less contrasted odor hedonic categorization was negatively correlated with food neophobia scores in ASD children: the less they discriminated hedonically (especially for pleasant odors), the more neophobic they were. Similarly, previous studies showed that difficulty in categorizing an object (e.g., food) was closely linked to its likability: the pleasantness or likability of foods that were difficult to categorize was diminished (Yamada et al., 2012). In the same study, food neophobia level was related to food likability. In agreement with such a link between odor hedonics and food neophobia, Raudenbush et al. (1998) showed that neophobic individuals evaluated smells as less pleasant and sniffed them less vigorously. In the present study, although food neophobia scores were similar in both groups, they were associated with different hedonic judgments between the two. It is known that children eat what they like and like what they know (Cooke et al., 2007). Therefore, given the significant influence of emotion on mnemonic processes (Kensinger, 2009a,b) and eating behavior (Aldridge et al., 2009), one hypothesis may be that the hesitation (or uncertainty) between a positive or negative judgment for emotional smells exhibited by certain ASD children influenced

both acceptance of foods and neophobic construction. Although the present study does not provide significant proof of causality between differences in olfactory hedonics and food neophobia, our findings open up a new avenue of research in the field, considering the role of the olfactory function in understanding food neophobia construction in children with ASD. In addition, another future development regarding this issue could be the use of measurements that do not rely strongly on language and social capacities. Besides the behavioral characterization of children's perception used here (number and duration of nasal explorations), it would be interesting to record physiological variables like sniffing, heart rate, respiratory rate, in order to strengthen our understanding of the relationship between food neophobia and affective perception of smells in ASD children.

While the present study provides new information about the olfactory function in ASD children, some of the methodological issues require discussion. For example, since most odorant molecules selected in the present study induce trigeminal sensations, one cannot discard the possibility that some differential effects between ASD children and controls are due to the stimulation of the fifth cranial nerve. Furthermore, it is important to note that this exploratory study comprised a small sample of subjects (10 per group). For practical reasons, it was not possible to include more participants in the study. Moreover, among ASD children, only 50% were able to complete the whole olfactory session. Differences between ASD children who could perform the entire study and those who could not, rely on cognitive, verbal and affective processing: (i) ASD children of our sample vary in their attentional abilities, some children being able to concentrate during the entire experimental task, and other not, (ii) one child who could not perform the entire study was non-verbal, (iii) some ASD children had strong emotional reactions following odor exposure, especially marked by disgust and aversion to some smells. These issues of exclusion of participants (two children for concentration and verbal problems), and missing data from 3 other children (particularly those who could not complete the entire task due to strong affective reactions to the smells) have an unknown impact on the study findings that extends beyond sample size and power limitations. Since not all children were able to test all 16 stimuli, additional analyses of the influence of odor intensity on odor pleasantness could not be assessed. Nevertheless, this issue provided important information about the number of stimuli that ASD children can experience in a reasonable amount of time (10 odorant conditions seems adequate according to the present findings). Another sample bias that may have affected some of the null findings is sample heterogeneity since our ASD group included six typical ASD children and four Asperger syndromes. It is likely that the use of a larger and less heterogeneous sample could have converted the few trends observed into significant effects. For example, the ability to identify an odor seems to be related to the degree of neophobia (Dematte et al., 2013), and this relationship deserves to be investigated further in larger groups of children. In particular, degree of neophobia is likely to be higher in ASD than NT children (Martins et al., 2008), which did not emerge in the present study likely because of lack of power.

In summary, notwithstanding the above, the present study offers new insights into odor perception in ASD children, highlighting a relationship between odor hedonic reactivity and eating behavior.

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