Chemical Senses, 2018, Vol 43, 347–355
doi:10.1093/chemse/bjy021
Original Article
Advance Access Publication 30 March 2018

Original Article

Emotional Body Odors as Context: Effects on Cardiac and Subjective Responses

Jacqueline Ferreira[1,2,*], Valentina Parma[3,4,5,*], Laura Alho[6,7], Carlos F. Silva[1] and Sandra C. Soares[1,2,5]

[1]CINTESIS.UA, Department of Education and Psychology, University of Aveiro, Aveiro, Portugal, [2]Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Coimbra, Portugal, [3]William James Centre for Research (WJCR), Instituto Superior de Psicologia Aplicada (ISPA), Lisbon, Portugal, [4]International School for Advanced Studies (SISSA), Neuroscience Area, Trieste, Italy, [5]Department of Clinical Neuroscience, Division for Psychology, Karolinska Institutet, Stockholm, Sweden, [6]Lusófona University of Humanities and Technology, Lisbon, Portugal and [7]Instituto Universitário de Lisboa (ISCTE-IUL), CIS-IUL, Lisbon, Portugal

*These authors contributed equally to this work.

Correspondence to be sent to: Sandra C. Soares, Department of Education and Psychology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. e-mail: sandra.soares@ua.pt

Editorial Decision 20 March 2018.

Abstract

Many studies have indicated that the chemical cues from body odors (BOs) of donors experiencing negative emotions can influence the psychophysiological and behavioral response of the observers. However, these olfactory cues have been used mainly as contextual information for processing visual stimuli. Here, for the first time, we evaluate how emotional BO affects the emotional tone of a subsequent BO message. Axillary sweat samples were taken from 20 donors in 3 separate sessions while they watched fear, disgust, or neutral videos. In a double-blind experiment, we assessed the cardiac and subjective responses from 69 participants who were either exposed to negative emotional or neutral BOs. Our results showed a reduced cardiac parasympathetic activity (HF%)—indicating increased stress—when participants smelled the emotional BOs before the neutral BOs, compared to when they smelled neutral followed by emotional BOs. The intensity of the neutral odor also increased following the exposure to both negative BOs. These findings indicate that BOs contain an emotion-dependent chemical cue that affects the perceiver both at the physiological and subjective levels.

Key words: affective context, body odor, disgust, fear, heart rate variability, order of presentation

Introduction

Despite the widespread notion that humans have an impoverished sense of smell, humans seem to have olfactory skills comparable to those of other mammals as judged by detection studies (Laska 2017; McGann 2017). Beyond high sensitivity in absolute threshold tests (Parma et al. 2017), results further suggest that humans are able to detect social information from olfactory cues of body odors (BOs, Parma et al. 2017). For instance, humans are able to identify kinship (Porter et al. 1986), age (Mitro et al. 2012), sex (Penn et al. 2007), and detect emotional states from olfactory cues alone (e.g., de Groot et al. 2015). Moreover, humans can perceptually discriminate sickness from healthy odors at the very initial stages of infective processes (Olsson et al. 2014). Another study Regenbogen et al. 2017), although finding only nominal perceptual differences between sick and healthy body odors, showed that sick body odors activated a widespread network of brain areas and also tended to make individuals presented on photographs less likable. Furthermore, some studies have shown that BOs collected from negative or stressful contexts were rated as more intense and unpleasant compared to BOs collected in a control conditions (e.g., neutral and happy contexts, Ackerl et al. 2002; Zhou and Chen, 2009; Haegler et al. 2010).

The study of the emotional cues conveyed by BOs has mainly focused on negative emotions, such as fear and disgust (e.g., de Groot and Smeets 2017; Parma et al. 2017), to investigate one of the main functions of the sense of smell, namely protecting us from danger (Stevenson 2010). Studies have shown that chemosensory cues of hazardous stimuli receive a preferential cognitive processing (Li et al. 2008; Parma et al. 2015) and elicit behavioral and psychophysiological responses that operate to promote avoidance and improve the chances of survival (e.g., Pause et al. 2009). Though threat cues from odors may be detected and discriminated (e.g., de Groot et al. 2014; Mutic et al. 2016), effects of odor exposure are not necessarily dependent on a clear conscious percept during exposure (Lundström and Olsson 2010). Indeed, their effects have been revealed via the analysis of implicit measures including, among others, facial electromyography (EMG) and eye scanning (de Groot et al. 2012). As an example, smelling the BO of a person experiencing fear elicits fearful facial expressions and a correspondent increased muscle activity of the *medial frontalis*, whereas smelling the BO of a person experiencing disgust produces a disgust facial expression that magnifies the activity of the *levator labii* muscle. This suggests emotional contagion as one of the basic mechanisms regulating human chemosensory communication (see also the emotional complementarity approach, Mutic et al. 2016). Although several studies have used physiological measures for assessing the effects of emotional BOs, such as skin conductance responses (Adolph et al. 2010), EMG (e.g., Prehn et al. 2006; Pause et al. 2009; de Groot et al. 2012), and electroencephalogaphy (Pause et al. 2010; Rubin et al. 2012), only a handful of published studies investigated how common odors impact the cardiac response, a renowned marker of stress. Among the cardiac measures, the heart rate variability (HRV), which reflects the variation in time intervals between heartbeats (Task Force of the European Society of Cardiology 1996), has been used to reveal the stress states of individuals. Greater stress responses correspond to the reduction in the percentage of high frequency (HF%) of the total HRV, in the absence of concurrent reduction of total spectrum power. These parameters can be interpreted as a selective reduction of vagal activity, which corresponds to a heightened stress state (Hjortskov et al. 2004). For instance, unpleasant odors, such as isovaleric acid, are associated with heart rate increase (e.g., Alaoui-Ismaïli et al. 1997; Bensafi et al. 2002; Pichon et al. 2015). However, to date, and to the best of our knowledge, the only study using BOs to assess its effects on cardiac response is that of Albrecht and colleagues (2011), in which they showed that both neutral and anxiety odors decreased the recipients' heart rate over the time of exposure to the olfactory stimuli. Thus, it remains unclear how cardiac activity reflects the processing of olfactory negative stimuli.

Considering that odors elicit emotional responses (see reviews, de Groot and Smeets 2017; Parma et al. 2017) and that the processing of chemical cues in general, and BOs in particular, is highly plastic and dynamically regulated by the context in which such stimuli are interpreted (Wilson et al. 2004); many studies have investigated how BOs serve as contextual information for other sensory modalities (preferentially visual). These studies have investigated the cognitive, behavioral, and psychophysiological modulations that odors induce in the processing of visual information (Pause et al. 2004; Zhou and Chen 2009; Zernecke et al. 2011), with the results showing that odors can indeed modulate the processing of other stimuli and events in a manner congruent with their valence (Smeets and Dijksterhuis 2014). For instance, smelling a fear BO while looking at faces biases the perception of the facial expression in a negative manner: happy faces are perceived as less happy than when exposed to a neutral context (Pause et al. 2004). Moreover, neutral or ambiguous faces are perceived as more fearful in the fear context than in the happy and neutral context (Zhou and Chen 2009). However, the role of BOs in serving as contextual stimuli for other sensory information is still in its infancy. The very same affective effects highlighted in the visuo-olfactory domain may emerge also when considering odor-olfactory stimulations. This would fit the idea that negative emotions, such as fear, have an adaptive function by facilitating the avoidant behaviors to potentially threatening stimuli in the environment (see review, Soares et al. 2017). Considering that olfactory stimulation makes these stimuli more long-lasting than visual stimuli (Yeshurun and Sobel 2010), the role of BOs as context in modulating other olfactory messages has yet to be determined.

Here, for the first time, we focus on how a BO affects the emotional tone of a subsequent BO, measured both subjectively and physiologically. Specifically, participants were asked to smell, in different orders, the BOs of individuals who donated their BO while either being in a fear, disgust, or neutral state. While smelling the odor, the cardiovascular activity was measured to reflect an individual's level of stress. After smelling, perceptual ratings of the odors were collected. This design allows us to test the effect of affective BO priming on the perception of another BOs. If the BO communication is indeed dynamic and highly dependent on contextual factors, we expect to observe a differential processing of emotional and neutral BOs depending on the order of the stimuli presentation. Specifically, we hypothesize that if fear and disgust BOs are presented first, increased perception of odor intensity and unpleasantness and a selective reduction of the cardiac parasympathetic activity will be observed when smelling the next odor, whereas the reversed pattern is expected when neutral BOs are presented first. Also, this design allows us to disentangle whether such effect occurs equally for BOs of negative valence (negative vs. neutral) or is emotion-specific (fear vs. disgust vs. neutral). If chemosensory cues of danger-related stimuli are emotion-specific, a differential pattern in psychophysiological responses should be found between fear and disgust BOs, in line with the categorical accounts of emotions (de Groot et al. 2012). If chemosensory cues of danger are instead valence based, that is, consistent with dimensional approaches (Barret 1998), we expect unspecific psychophysiological effects between fear and disgust, that is, an overall reduction in the HF% of the total HRV for both fear and disgust. Given that emotional chemosensory communication mostly occurs at low levels of conscious perception, no dissociations in the participants' perceptions of intensity and pleasantness between fear and disgust are expected.

Materials and methods

All the experimental procedures of this study were approved by the scientific council of the University of Aveiro and were performed in accordance with the Declaration of Helsinki and the standards of the American Psychological Association. Written informed consent was obtained from each donor and participant. Below, we will separately report the materials and methods for the Odor Sampling and the Transmission studies.

Study 1—Odor Sampling

Donors

Twenty donors came to the laboratory three times, 1 week apart for BO sampling, each time viewing one of three separate videos inducing either disgust, fear, or no emotion (neutral condition).

The donors (10 males: *M* = 21.30 years; *SD* = 2.36 years, range = 18–25; 10 females: *M* = 21.70 years; *SD* = 2.54 years, range = 19–28) were heterosexual, right-handed, and had no cardiovascular, respiratory, metabolic, psychiatric, or psychological disease nor were they taking any medication, during the three weeks of BO collection. However, all female donors were taking oral contraceptives to avoid possible fluctuations in odour quality during the regular cycle (Singh and Bronstad 2001; Havlíček et al. 2006). Donors completed the Trait Anxiety (STAI-T, Silva and Spielberger 2007) and Disgust Sensitivity (DS) questionnaires (Ferreira-Santos et al. 2011), both revealing no deviations from the average data which, therefore, granted their inclusion in the donors' sample.

Procedure

On the day before the BO collection, donors received a kit that included a cotton towel (Jumbo, Portugal) and a hypoallergenic bath gel (Lactacyd Derma, Omega Pharma Portuguesa) to be used for a shower at home before coming to the laboratory. The towels were washed with odorless soap (Blancotex, Jumbo, Portugal) and were packed separately in zip-lock bags prior to each BO collection (Alho et al. 2015).

Donors underwent dietary and hygienic restrictions before and during BO sampling to reduce sweat contamination with exogenous and endogenous odorants. Starting from the evening before the sweat collection to the end of the collection, donors were asked to refrain from eating odorous food (e.g., garlic, onion, cabbage, spices), to drink coffee and alcoholic beverages, to smoke, as well to engage in physical exercise (de Groot et al. 2014). On the day of the BO collection, the donors took a shower with the odor-free soap and were not allowed to use any other body hygiene and cosmetic products. In the laboratory, after they washed and dried their armpits one additional time, they wore a cotton t-shirt (SportZone, Portugal), with nursing pads positioned under both armpits (Mercurochrome Baby, Laboratoires JUVA, Portugal). The nursing pads were secured to the armpit area with a portion of medical tape (6 cm, Omnifix, Paul Hartmann LDA) placed on the external side of nursing pads (i.e., the side that was not in contact with the axillary area and never came in contact with the side of the pad in touch with the skin). To allow the collection of emotional BOs, the donors watched 25 min of disgust videos, containing sickening scenes ("Pink Flamingos," Rottenberg et al. 2007), fear videos containing horror scenes ("The Shining," Rottenberg et al. 2007), and a nature documentary for the neutral condition ("Easter Island-Solar Eclipse" National Geographic). The videos were presented in three separate sessions, a week apart, in a counterbalanced order. The disgust and fear videos have been successfully used in prior studies (Vianna and Tranel 2006; de Groot et al. 2012). Unlike fear and disgust videos, no study had used the neutral videos before. Therefore, 35 young adults pre-evaluated the neutral movie to confirm that the video was indeed innocuous and did not induce either disgust or fear (see Supplementary Table S1). Donors were presented with one video per session and instructed to avoid looking away from the monitor. The compliance to this rule was assessed visually by an experimenter. No donor had to be excluded due to this reason. Immediately after each video presentation, donors rated their perceived emotional experience, by using 2 separate 7-point Likert scales, and completed the Portuguese version of the Positive and Negative Affect Scale (Galinha and Pais-Ribeiro 2005). In order to control whether participants left the laboratory stressed after the emotional induction, following a 10-min pause, the donors rated once again their perceived fear and disgust subjective experience and, at the completion of the ratings, the cotton pads were removed from the t-shirt. Each of the 2 pads from each of the 3 sessions were cut into 4 equally sized quadrants (24 quadrants per donor), stored in sealed zip-locked bags, frozen at −20 °C, and defrosted 1 h before the beginning of the experimental session. The experimenter always used disposable gloves when handling the samples, in order to prevent contamination. Each cotton pad was used 5 times, balanced across conditions. This freezing procedure, as well as the reuse of the BO samples, has been adopted in other studies (e.g., Alho et al. 2015) and does not seem to change their hedonics characteristics (Lenochova et al. 2009). We accounted for potential differences between left and right sweat glands by asking participants to smell BOs from the left or the right armpits of single individuals. More specifically, in each block of 10 BOs, participants smelled 5 BOs from the left and 5 BOs from the right armpits, presented in a counterbalanced manner (Ferdenzi et al. 2009).

Dependent variables and data analysis

All data were analyzed via R using the lme4 (Bates et al. 2014) and BayesFactor packages (Morey and Rouder 2013). Considering that we could not base on the literature our hypotheses on the nature and magnitude of the effect size, we preferred to maintain the standard option, which is based on a default prior proposed by Jeffreys (1961), that is, the uniform distribution. To determine whether the videos were effective in inducing disgust and fear, respectively, we performed separate linear mixed models (LMMs) to analyze the subjective emotional ratings (fear and disgust), as well the positive and negative affect reported by the donors. The LMMs used for these analyses had the subjective emotional ratings, the positive or the negative affect as dependent variables, the Subject ID as a random factor and the following fixed factors: video condition (3 levels: fear, disgust, and control), session (2 levels: first or second odor presentation), order of presentation (2 levels: emotional to control and control to emotional), and sex (2 levels: males and females). Sex, group, and order differences are only discussed when significant, since this was not the main goal of the study. Results reported include the means (*M*) and the standard deviations (*SD* in brackets). To further determine the reliability of our analyses, we applied Bayesian statistics which, beyond determining potential differences between groups (as LMM), also provide evidence towards determining conclusions about a "no group difference," as well as informing on whether inconclusive evidence exists (i.e., data are not informative enough to provide support for either a difference or no difference between groups; Dienes 2016). Importantly, Bayesian analyses (ANOVABF with Subject as a random factor) allow to predict the likelihood of our hypotheses (a difference between the 2 groups exposed to the different odor conditions, as well as the direction of such difference). As a commonly accepted rule, a Bayes Factor (BF) value = 1 indicates no evidence of a difference, whereas BF between 3 and 10 indicated moderate evidence of difference between groups. BF comprised between 1 and 3 provide anecdotal evidence.

Results

The videos successfully induced disgust and fear experiences in the donors.

Donors reported significantly lower levels of positive affect after watching the disgust [*M* = 0.40 (0.70)] and the fear video [*M* = 0.80 (0.42)], as compared to when watching the neutral video [*M* = 1.75 (1.07)]. No significant differences in positive affect between the disgust and fear videos were observed. Similarly, donors reported significantly higher levels of negative affect after watching the disgust [*M* = 0.80 (0.63)] and the fear video [*M* = 1.50 (0.71)], as compared to when watching the neutral video [*M* = 0.10 (0.31)]. Notably, negative affect was significantly lower after viewing the disgust video compared to the fear video. To verify that the negative experience reported by the donors specifically reflected the emotional tone of each video, we evaluated the subjective ratings of disgust and fear before and after the vision of each video. Before watching the disgust, fear or neutral video the donors reported to experience similar levels of disgust and fear (Table 1). After seeing the disgust video, the donors reported significantly greater disgust [*M* = 3.18 (0.11)] than fear [*M* = 1.2 (0.11)], whereas the opposite pattern was revealed for the fear video, which elicited greater reports of fear [*M* = 2.6 (0.37)] than disgust feelings [*M* = 1.2 (1.9); Table 1]. In other words, we verified that the sweat samples were collected within-subjects by donors experiencing disgust (during the viewing of the disgust video) and fear (during the viewing of the fear video).

Study 2—Transmission Study

BO recipients

Ninety-two participants took part in this study as observers. None of them was included in the donation part of the study. Twenty-three participants were excluded, 20 due to technical issues with ECG and 3 for not having completed all questionnaires or for being older than 35 years. The remaining 69 participants (37 males: *M* = 22.76 years; *SD* = 4.16, range = 18–35; 32 females: *M* = 21 years; *SD* = 3.66 years, range = 18–31) followed the same selection criteria that were used for the donors. They were instructed to avoid using scented body products and to abstain from caffeine and physical exercise at least 12 h before the experimental session. Normal smell abilities were ensured using the odor identification subtest of Sniffin' Sticks test (Burghart Instruments, Wedel, Germany; Hummel et al. 2007). Only participants who scored 11/16 or above were included in the final sample. To control for potential individual differences across groups, we analyzed the STAI-T inventory and Disgust Propensity and Sensitivity Scale (Ferreira et al. 2016), instead of the Disgust Sensitivity (DS) questionnaire (Ferreira-Santos et al. 2011), given that the former is more sensitive to measure stable tendencies for experiencing disgust, hence more adequate to capture potentially subtle effects of odor-induced disgust. The results did not show any clinical or extremes signs of anxiety or disgust sensitivity and, as a result, no participant was excluded. Results are detailed in Table 2.

Table 1 Disgust and fear ratings by donors before and after each video condition

Dependent variable				
Disgust rating on 7-point Likert scale				
	Pre	Pre	Post	Post
Disgust		−0.100 (0.070)		4.100*** (0.444)
Fear	0.100 (0.070)		−4.100*** (0.444)	
Neutral	−0.000 (0.060)	−0.100 (0.060)	−4.400*** (0.385)	−0.300 (0.385)
Constant	1.000*** (0.049)	1.100*** (0.049)	5.400*** (0.314)	1.300*** (0.314)
Observations	40	40	40	40
Log Likelihood	12.450	12.450	−56.049	−56.049
Akaike Inf. Crit.	−14.900	−14.900	122.099	122.099
Bayesian Inf. Crit.	−6.845	−6.845	130.154	10.154
Fear rating on 7-point Likert scale				
	Pre	Pre	Post	Post
Disgust		−0.500 (0.359)		−2.392*** (0.433)
Fear	0.500 (0.359)		2.392*** (0.433)	
Neutral	0.250 (0.311)	−0.250 (0.311)	−0.154 (0.366)	−2.546*** (0.366)
Constant	1.100*** (0.254)	1.600*** (0.254)	1.204*** (0.307)	3.596*** (0.307)
Observations	40	40	40	40
Log Likelihood	−48.177	−48.177	−55.189	−55.189
Akaike Inf. Crit.	106.354	106.354	120.377	120.377
Bayesian Inf. Crit.	114.409	114.409	128.432	128.432

The values reported in the first row of each cell represent beta values, whereas the values in brackets represent the standard errors. The constant values refer to the intercepts of the models considered. Empty cells represent the variables used as reference for the other calculations.

*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$

Procedures

The experimental session started with the signature of the informed consent and the questionnaires. To examine the effects of the BOs on the cardiac activity, 3 ECG Disposable Biopac EL503 Ag-AgCl snap electrodes were placed on the recipient's body following a standard lead II configuration (right arm, left leg, and right leg ground; (Berntson et al. 2007) and connected to a Biopac MP100, ECG Module (Biopac Systems, Inc.). Participants were then presented with blocks of 40 BOs (10 neutral BOs and 10 negative BOs, i.e., fear or disgust BOs, presented twice) in a double-blind, between-subject design. Participants were randomly assigned to one of the following BO conditions: neutral-disgust; disgust-neutral; fear-neutral; and neutral-fear. Each block of odors included BOs from 5 males and 5 females. Participants smelled each odor for 3 seconds and were then asked to rate the odor's perceived intensity and pleasantness levels using VAS scales (anchored to the extremes of 0 and 100). After each block of 10 odors, the observers rested for 5 min, before they were exposed to a new set of 10 odors. The recipients were instructed to sit quietly to avoid sudden movements and to sniff when the experimenter presented each odor in an open jar, positioned 2 cm away from the nostrils. Finally, the participants underwent the evaluation of olfactory functionality via the Sniffin' Sticks identification subtest (Hummel et al. 2007).

Dependent variables and data analysis

The analyses of odor intensity and pleasantness differences were conducted by separate LMM analyses. To fit the models, each rating was introduced as the dependent variable, the Subject ID was a random factor and the fixed factors were: Group [4 levels: Disgust-Neutral (DN), Neutral-Disgust (ND), Fear-Neutral (FN), Neutral-Fear (NF)], Order of presentation (2 levels: emotional-control and control-emotional), Session (2 levels: first vs. second odor presented), Odor Condition (3 levels: Disgust, Fear, Neutral), and Sex (2 levels, male and female)]. Additionally, to examine whether negative odors induced any stress response on the cardiac activity, we performed the frequency-and-time domain HRV analysis, using Kubios software (Tarvainen et al. 2014; University of Eastern Finland, Kuopio, Finland). The frequency-domain was calculated using the power spectrum analysis on the inter-beat-intervals (for more details, see Trinder et al. 2001). We considered the following measures: *total power* (reflecting total HRV, ms^2), High Frequency an index of pure

Table 2. Description of the recipients' sample

	Groups							
	DN		FN		ND		NF	
N	16		17		18		18	
Gender	8F		8F		8F		8F	
Age	21.50	(3.43)	24.12	(5.28)	20.63	(3.61)	22.39	(3.79)
STAI—Trait anxiety	36.75	(7.76)	31.82	(10.61)	31.61	(6.02)	34.56	(4.64)
Disgust propensity	18.13	(3.09)	17.94	(3.85)	17.50	(3.09)	17.78	(2.92)
Disgust sensitivity	14.87	(5.08)	12.76	(3.85)	12.11	(3.29)	13.11	(3.91)

The values reported means (SD).

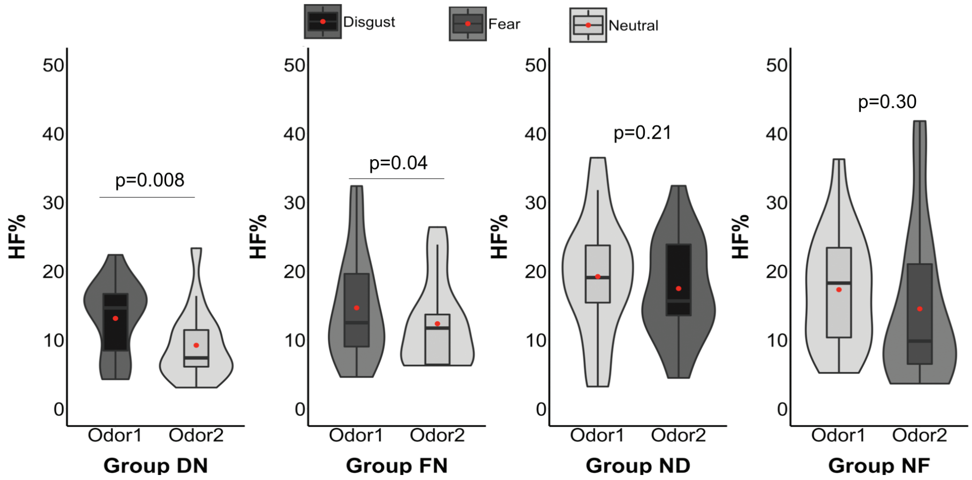

Figure 1. Graphical representation of the proportion of HF in each group per order of presentation of the odor (x-axis) and color coded by order of odor condition (emotional or neutral body odor).

vagal tone, expressed as absolute power in arbitrary units, *percentage of HF* (0.15–0.40 Hz) over total power. For a time-domain approach, we calculated the time interval between consecutive R-waves (RR), which reflects the myocardial contraction frequency (ms). To correct for non-normality, HRV variables were log-transformed (Task Force of the European Society of Cardiology 1996).

Results

The cardiac parasympathetic activity is selectively reduced when participants smell the negative odors before the neutral odor

The results of HRV measures, time- and frequency-domain, revealed no significant differences between groups at the baseline, when no odor was presented (Supplementary Table S2). Considering that the duration of the exposure to the odor is different across groups (see Supplementary Table S3 with the results of the model and Supplementary Figure S1), we included the duration of the session as a covariate in the model assessing whether HRV variables are different based on the group and the odor condition. The only measure reaching the level of significance was the percentage of cardiac parasympathetic activity. As depicted in Figure 1, the high frequency proportion (HF%) of that activity was significantly reduced when participants smelled the negative BO as compared to the neutral BO. Although no differences were evident between DN and FN groups, they both showed reduced HF%, as compared to ND and NF. Please refer to Supplementary Table S4 for details on the other measures.

Smelling a negative BO increases the perceived intensity of a subsequently presented neutral BO

The LMM analysis on intensity ratings revealed no significant main effect of Group [X^2 (3, N = 2760) = 4.89, P = 0.18] and any interaction involving this factor ($P > 0.05$). A significant main effect of Odor Condition [X^2 (2, N = 2760) = 18.00, P = 0.0001] was found. Post hoc contrasts indicated that the neutral odor was perceived as more intense than the disgust [Z = 3.06, P = 0.007, CI (2.71–5.33)], but not the fear odor [Z = 2.01, P = 0.13, CI (2.26–6.71)]. Importantly, a significant main effect of Odor Presentation Order [X^2 (1, N = 2760) = 6.24, P = 0.01] was retrieved. The second odor was perceived as more intense than the first odor presented. As evident from Figure 2, this pattern is significant for the DN (neutral = 47.63; disgust = 40.33, $P < 0.004$) and the FN groups (neutral = 45.63; fear = 39.23, $P < 0.02$), but not for the ND and NF groups. This entails that the neutral BO was perceived as more intense following the presentation of a negative BO, irrespective of the emotional characterization of the odor itself (disgust or fear). The Bayesian analysis confirmed that there was no evidence of a difference across the intensity ratings of the groups (BF = 0.21 ± 0.88%). For the full results, please refer to Supplementary Table S5. Considering that the distribution of intensity ratings is bimodal within each group, equally for both odor conditions, and across groups, we believe this reflects a different sensitivity to odors by the participants. By calculating the local minima within the 2 maximal peaks of the overall density distribution (Intensity: 35.02), we split the sample in low-sensitivity group (those who rate the odors as having intensities lower than 35.02) and high-sensitivity group, those who rate the odors as having an intensity above 35.02.

Within group, the neutral and emotional odor conditions are perceived as iso-pleasant

The LMM on pleasantness ratings did not reveal significant main effect or interactions ($P > 0.05$, Table 3 for full model details), besides the effect of Group [X^2 (3, N = 2760) = 12.28, P = 0.006]. However, post hoc contrasts revealed that the NF group rated the fear samples as more pleasant than the FN group [Z = 3.66, P = 0.007, CI (6.84–11.97)], as showed in the Figure 3. The Bayesian analysis support only up to anecdotal evidence of Group differences in the pleasantness ratings (BF= 1.04 ± 0.66%). For the full results, please refer to Supplementary Table S6.

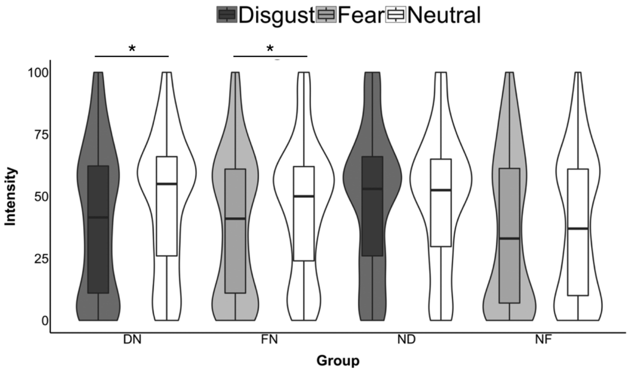

Figure 2. Intensity ratings across groups and odor conditions. *$P < 0.05$.

Table 3. Results of the mixed model pleasantness ~ Group*Odor+Session

	Pleasantness odor		
	B	*CI*	*P*
Fixed			
(Intercept)	38.76	33.51 to 44.01	**<0.001**
Group: DN	0.31	−5.16 to 5.78	0.911
Group: FN	−3.67	−8.69 to 1.34	0.152
Group: NF	4.35	−0.60 to 9.31	0.086
Odor: Disgust	−1.16	−4.52 to 2.19	0.497
Odor: Fear	0.58	−1.38 to 2.53	0.563
Session	1.38	−0.58 to 3.33	0.168
Group: DN*Odor: Disgust	3.44	−2.14 to 9.01	0.228
Random			
σ^2	347.523		
$\tau_{00, \mathrm{ID}}$	40.114		
N_{ID}	69		
$\mathrm{ICC}_{\mathrm{ID}}$	0.103		
Observations	2760		
$\mathrm{R}^2 / \Omega_0^2$	0.139/0.136		

The ND group represents the reference group. For S3, Supplementary Table S3. Linear mixed-effects model fit by REML: Duration~ Group*Odor. The ND group represents the reference group.

Discussion

The goal of the current research work was to investigate the ability for an emotional or neutral BO to act as context for the decoding of a subsequently presented BO, as reflected in subjective and psychophysiological responses. First and foremost, the analysis of the cardiac activity revealed that smelling the negative emotional odors before smelling the neutral odor reduced the cardiac parasympathetic activity measured during the presentation of the second block of odors (the neutral BOs, in this instance). Instead, when participants smelled the negative emotional odors preceded by the neutral odor, such reduction in the cardiac parasympathetic activity did not emerge when the second odor block (in this case, the negative emotional odors) was presented. This is expected as the emotional tone of the odor is negative, whether it is fear or disgust (e.g., de Groot and Smeets 2017; Parma et al. 2017). No specific modulation was retrieved based on the specific emotion transmitted, supporting the idea that BOs presented first and serving as context for BOs presented subsequently may be based on the communication of the valence of the stimulus but not its specific emotional tone (e.g., Chen et al. 2006; Prehn et al. 2006). This result suggests that being merely exposed to negative chemical cues can influenced the HRV response of subsequent neutral stimuli. In the animal kingdom, many studies have demonstrated that fear chemosignals act as warning signals by affecting the physiological responses of the recipients and increasing their level of vigilance for environmental cues (Wyatt 2003). For example, Horii and colleagues (2013) conducted a study where they investigated the effect of the order of odor exposure on the Autonomic Nervous System (ANS) activity in rats. They found that an unpleasant odor (i.e., the odor of a predator) induced a stress response in the recipients. They questioned whether the subsequent exposure to a pleasant odor (linalool) would facilitate the stress recovery process. Instead, they showed that the subsequent exposure to a pleasant odor amplified the ANS-mediated stress response. According to the authors, exposing the rats to aversive stimuli, in this case smelling the presence of a predator, generates a hyper-alert state, which facilitates behavioral responses that promote escape or avoidance.

Previous studies that investigated odor contextual effects using fear-related cues within the visual domain in humans showed similar results. In the context of fear-related cues, the observers seem to act with more caution (Pause et al. 2004; Zhou and Chen 2009). For instance, female recipients when exposed to fear chemosensory stimuli (compared to a neutral sweat and a control condition) performed better in a word association task, showing higher accuracy and shorter response times on the meaningful word conditions, compared to conditions where words displayed an ambiguous content

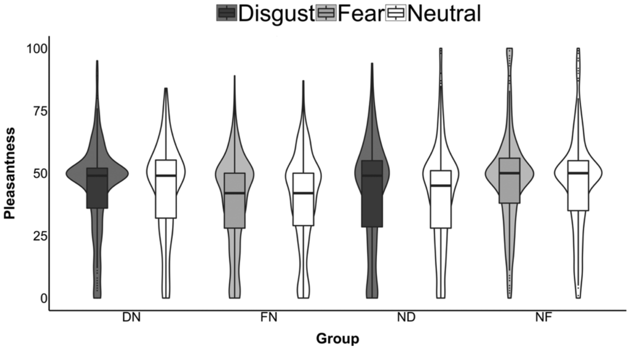

Figure 3. Pleasantness ratings across groups and odor conditions.

(Chen et al. 2006). At the neural level, fear-related cues are encoded in the same way as other biologically relevant and threatening stimuli. As Mujica-Parodi and colleagues (2009) demonstrate, exposure to stress cues increases the BOLD responses in the amygdala, an area which preferentially responds to relevant and threatening information (Sander et al. 2003). Accordingly, research has also shown that BOs associated with disgust prompt the mobilization of the organism to avoid potential contaminants or diseases. Disgust BOs are known to induce disgusted facial expressions (de Groot et al. 2012), which involve slightly narrowed brows, decreased eye and nasal aperture (nose wrinkling), a facial structure which favors the limitation of the incoming sensory input, reflecting the motivation to avoid or reject pathogenic agents (e.g., Susskind et al. 2008). Indeed, humans are able to detect BO cues from sick individuals (Olsson et al. 2014), with this effect being enhanced when BOs and faces are combined (Regenbogen et al. 2017). Hence, as evident for animals, also humans are affected by negative emotional cues, such as fear and disgust, as indicators of threat in the environment and implement adaptive responses evident at the physiological level that prepare the organism to deal with dangerous situations. Moreover, this effect seems to be enhanced when the odor serves as a contextual information presented in parallel with visual information, such as socially relevant stimuli (faces; e.g., Zhou and Chen 2009; Zernecke et al. 2011). Our study adds new evidence to the existing literature, in which BOs are presented in cross-modal paradigms, by showing that these adaptive responses are extended to olfactory intramodal contexts.

Besides a cardiac response compatible with a stress-induced response, the subjective ratings of odor intensity additionally confirmed that the negative BOs act as cues to the presence of threatening information. This is evident when we evaluate the intensity ratings performed on the neutral odor. Indeed, the perceived intensity of the neutral odor was greater after having previously being exposed to one of the 2 negative BOs (i.e., fear and disgust). On the contrary, when the neutral odor was rated during the first session, then no difference in the intensity of the BOs were retrieved. These results suggest that the negative BOs may have induced an increased attentive state that facilitates the detection of threatening stimuli, which is reflected at the perceptual level with greater ratings of intensity. Indeed, this hypothesis would be in line with evidence obtained via conditioning paradigms, which reveal that the sensitivity to an odor paired with a threatening stimulus is increased after the association is made (see Parma et al. 2015). Contrary to other accounts which suggest that the association of an odor with a threatening stimulus also produces a change in the quality of the stimulus (Li et al. 2008), we were not able to retrieve any changes in the pleasantness of the BOs, irrespective of whether they acted as a prime or a subsequent stimulus. The lack of significant differences in the pleasantness ratings across groups and odor conditions as well as sessions, suggests that the odor conditions, irrespective of the emotional tone expressed, were all neutral to mildly unpleasant, as expected from BO samples from donors who did not wear any fragrance (Lenochová et al. 2012). The comparison with the threatening effect revealed in the intensity ratings suggests that the exposure to negative BOs versus neutral BOs sensitizes individuals to the presence of the BO, but does not change the quality of the BO, as suggested by the pleasantness ratings.

One may argue that the lack of emotional specificity in the psychophysiology and subjective ratings in the observers may depend on an inefficient emotional induction in the donors. However, this is highly unlikely given that our donors subjectively rated their experience as selectively congruent with the emotional tone of the videos they were exposed to. This method has been supported already by many accounts (e.g., de Groot et al. 2012). However, we cannot exclude that with other psychophysiological tools an emotional-specific effect would have been retrieved. Indeed, as previously demonstrated by de Groot and colleagues (2012), EMG is able to differentiate the stress responses of different negative emotions, which suggests that relevant information (danger detection) may be gathered redundantly with many systems. Future studies should combine these measures and include additional ones to map which are the most effective measures reflecting the decoding of odor as context in chemosensory communication.

A potential limitation of our study is the lack of positive stimuli for a full account of the valence dimension. Therefore, to extend the comprehension of chemosensory modulation on physiological response, we encourage futures studies to examine the effects of positive emotions (e.g., happiness) using a within-subject design. Our results suggest that being exposed to negative human cues can affect the HRV response of subsequent neutral stimuli. However, we did not measure the duration of such effect future studies could directly test this research question. Moreover, to fully test the extent to which intramodal BOs contextual effects emerge, all the options not included in the present design (e.g., neutral-neutral, fear-fear, disgust-disgust conditions) should additionally be investigated. Furthermore, since the results from Mutic et al. (2016) study showed that chemical cues of aggression can indicate a specific type of information, the intention to harm, we also encourage further studies to include this negative emotion, in order to investigate whether its effects on cardiac and subjective response is specific or valence based. Another limitation of our study is that we only included women participants who were taken the hormonal contraception and, therefore, our results cannot be generalized to women that are not undertaking hormonal contraception.

To sum up, the use of the cardiac response allows us to gather insights into the olfactory-induced stress/relaxation responses and to reveal that odor-odor presentation order can affect the subsequent decoding of the message. Therefore, trial by trial analyses are warranted when several emotional BO are compared.

Supplementary material

Supplementary material are available at *Chemical Senses* online.

Funding

This research was supported by a doctoral grant from the Portuguese Foundation for Science and POPH/FSE Program [grant reference SFRH/BD/85376/2012] to J.F., by FEDER through the operation POCI-01-0145-FEDER-007746 funded by the Programa Operacional Competitividade e Internacionalização—COMPETE2020 and by National Funds through FCT—Fundação para a Ciência e a Tecnologia within CINTESIS, R&D Unit. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Ackerl K, Atzmueller M, Grammer K. 2002. The scent of fear. *Neuro Endocrinol Lett*. 23:79–84.

Adolph D, Schlösser S, Hawighorst M, Pause BM. 2010. Chemosensory signals of competition increase the skin conductance response in humans. *Physiol Behav*. 101:666–671.

Alaoui-Ismaïli O, Robin O, Rada H, Dittmar A, Vernet-Maury E. 1997. Basic emotions evoked by odorants: comparison between autonomic responses and self-evaluation. *Physiol Behav*. 62:713–720.

Albrecht J, Demmel M, Schöpf V, Kleemann AM, Kopietz R, May J, Schreder T, Zernecke R, Brückmann H, Wiesmann M. 2011. Smelling chemosensory signals of males in anxious versus nonanxious condition increases state anxiety of female subjects. *Chem Senses*. 36:19–27.

Alho L, Soares SC, Ferreira J, Rocha M, Silva CF, Olsson MJ. 2015. Nosewitness identification: effects of negative emotion. *Plos One*. 10:e0116706.

Barret LF. 1998. Discrete emotions or dimensions? The role of valence focus and arousal focus. *Cogn Emot*. 12:579–599.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 67:1–48.

Bensafi M, Rouby C, Farget V, Bertrand B, Vigouroux M, Holley A. 2002. Autonomic nervous system responses to odours: the role of pleasantness and arousal. *Chem Senses*. 27:703–709.

Berntson GG, Quigley KS, Lozano D. 2007. Cardiovascular psychophysiology. In: Cacioppo JT, Tassinary LG, Bernston GG, editors. *Handbook of psychophysiology*. New York: Cambride University Press. p. 182–210.

Chen D, Katdare A, Lucas N. 2006. Chemosignals of fear enhance cognitive performance in humans. *Chem Senses*. 31:415–423.

de Groot JHB, Semin GR, Smeets MAM. 2014. Chemical communication of fear: a case of male–female asymmetry. *J Exp Psychol Gen*. 143:1515–1525.

de Groot JHB, Smeets MAM. 2017. Human Fear Chemosignaling: Evidence from a Meta-Analysis. *Chem Senses*. 42:663–673.

de Groot JHB, Smeets MAM, Kaldewaij A, Duijndam MJA, Semin GR. 2012. Chemosignals communicate human emotions. *Psychol Sci*. 23:1417–1424.

de Groot JHB, Smeets MAM, Rowson MJ, Bulsing PJ, Blonk CG, Wilkinson JE, Semin GR. 2015. A sniff of happiness. *Psychol Sci*. 26:684–700.

Dienes Z. 2016. How Bayes factors change scientific practice. *J Math Psychol*. 72:78–89.

Ferdenzi C, Schaal B, Roberts SC. 2009. Human axillary odor: are there side-related perceptual differences? *Chem Senses*. 34:565–571.

Ferreira J, Soares S, Bem-Haja P, Alho L, Rocha M, Madeira N, Silva C. 2016. Portuguese version of the disgust propensity and sensitivity scale-revised: preliminary data. *Eur Psychiatry*. 33:S223–S224.

Ferreira-Santos F, Martins EC, Sousa P, Mauro C. 2011. *Escala de sensibilidade ao Nojo–revista, versões Português de Portugal (DS-R-pt) e Português do Brasil (DS-R-br)*. LabReport No. 4.

Galinha IC, Pais-Ribeiro JL. 2005. Contribuição para o estudo da versão Portuguesa da positive and negativeaffect schedule (PANAS): II-estudo psicométrico. *Análise Psicológica*. 23:219–227.

Haegler K, Zernecke R, Kleemann AM, Albrecht J, Pollatos O, Brückmann H, Wiesmann M. 2010. No fear no risk! human risk behavior is affected by chemosensory anxiety signals. *Neuropsychologia*. 48:3901–3908.

Havlíček J, Dvořáková R, Bartoš L, Fleg J. 2006. Non-advertized does not mean concealed: body odour changes across the human menstrual cycle. *Ethology*. 112:81–90.

Hjortskov N, Rissén D, Blangsted AK, Fallentin N, Lundberg U, Søgaard K. 2004. The effect of mental stress on heart rate variability and blood pressure during computer work. *Eur J Appl Physiol*. 92:84–89.

Horii Y, Nagai K, Nakashima T. 2013. Order of exposure to pleasant and unpleasant odors affects autonomic nervous system response. *Behav Brain Res*. 243:109–117.

Hummel T, Kobal G, Gudziol H, Mackay-Sim A. 2007. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur Arch Otorhinolaryngol*. 264:237–243.

Jeffreys H. 1961. *Theory of probability*. 3rd ed. Oxford (UK): Oxford University Press.

Laska M. 2017. Human and animal olfactory capabilities compared. In: Buettner A, editor. *Springer handbook of odor*. Cham: Springer International Publishing. p. 675–686.

Lenochova P, Roberts SC, Havlicek J. 2009. Methods of human body odor sampling: the effect of freezing. *Chem Senses*. 34:127–138.

Lenochová P, Vohnoutová P, Roberts SC, Oberzaucher E, Grammer K, Havlíček J. 2012. Psychology of fragrance use: perception of individual odor and perfume blends reveals a mechanism for idiosyncratic effects on fragrance choice. *PLoS One*. 7:e33810.

Li W, Howard JD, Parrish TB, Gottfried JA. 2008. Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. *Science*. 319:1842–1845.

Lundström JN, Olsson MJ. 2010. Functional neuronal processing of human BOs. *Vitam Horm*. 83:1–23.

McGann JP. 2017. Poor human olfaction is a 19th-century myth. *Science*. 356:eaam7263.

Mitro S, Gordon AR, Olsson MJ, Lundström JN. 2012. The smell of age: perception and discrimination of body odors of different ages. *Plos One*. 7:e38110.

Morey RD, Rouder JN. 2013. Bayesfactor: computation of bayes factors for common designs. R package version 0.9.4. Available from: http://CRAN.R project.org/package=BayesFactor.

Mujica-Parodi LR, Strey HH, Frederick B, Savoy R, Cox D, Botanov Y, Tolkunov D, Rubin D, Weber J. 2009. Chemosensory cues to conspecific emotional stress activate amygdala in humans. *PLoS One*. 4:e6415.

Mutic S, Parma V, Brünner YF, Freiherr J. 2016. You smell dangerous: communicating fight responses through human chemosignals of aggression. *Chem Senses*. 41:35–43.

Olsson MJ, Lundström JN, Kimball BA, Gordon AR, Karshikoff B, Hosseini N, Sorjonen K, Olgart Höglund C, Solares C, Soop A, *et al*. 2014. The scent of disease: human body odor contains an early chemosensory cue of sickness. *Psychol Sci*. 25:817–823.

Parma V, Ferraro S, Miller SS, Åhs F, Lundström JN. 2015. Enhancement of odor sensitivity following repeated odor and visual fear conditioning. *Chem Senses*. 40:497–506.

Parma V, Gordon AR, Cecchetto C, Cavazzana A, Lundström JN, Olsson MJ. 2017. Processing of human BOs. In: Buettner A, editor. *Springer handbook of odor*. Cham: Springer International Publishing. p. 127–128.

Pause BM, Adolph D, Prehn-Kristensen A, Ferstl R. 2009. Startle response potentiation to chemosensory anxiety signals in socially anxious individuals. *Int J Psychophysiol*. 74:88–92.

Pause BM, Lübke K, Laudien JH, Ferstl R. 2010. Intensified neuronal investment in the processing of chemosensory anxiety signals in non-socially anxious and socially anxious individuals. *PLoS One*. 5:e10342.

Pause BM, Ohrt A, Prehn A, Ferstl R. 2004. Positive emotional priming of facial affect perception in females is diminished by chemosensory anxiety signals. *Chem Senses*. 29:797–805.

Penn DJ, Oberzaucher E, Grammer K, Fischer G, Soini HA, Wiesler D, Novotny MV, Dixon SJ, Xu Y, Brereton RG. 2007. Individual and gender fingerprints in human body odour. *J R Soc Interface*. 4:331–340.

Pichon AM, Coppin G, Cayeux I, Porcherot C, Sander D, Delplanque S. 2015. Sensitivity of physiological emotional measures to odors depends on the product and the pleasantness ranges used. *Front Psychol*. 6:1821.

Porter RH, Balogh RD, Cernoch JM, Franchi C. 1986. Recognition of kin through characteristic body odors. *Chem Senses*. 11:389–395.

Prehn A, Ohrt A, Sojka B, Ferstl R, Pause BM. 2006. Chemosensory anxiety signals augment the startle reflex in humans. *Neurosci Lett*. 394:127–130.

Regenbogen C, Axelsson J, Lasselin J, Porada DK, Sundelin T, Peter MG, Lekander M, Lundström JN, Olsson MJ. 2017. Behavioral and neural correlates to multisensory detection of sick humans. *PNAS*. 114:6400–6405.

Rottenberg J, Ray RD, Gross JJ. 2007. Emotion elicitation using films. In: Coan JA, Allen JJB, editors. *Handbook of emotion elicitation and assessment*. London: Oxford University Press. p. 9–28.

Rubin D, Botanov Y, Hajcak G, Mujica-Parodi LR. 2012. Second-hand stress: inhalation of stress sweat enhances neural response to neutral faces. *Soc Cogn Affect Neurosci*. 7:208–212.

Sander D, Grafman J, Zalla T. 2003. The human amygdala: an evolved system for relevance detection. *Rev Neurosci*. 14:303–316.

Silva DR, Spielberger CD. 2007. *Manual do Inventário de Estado-Traço de Ansiedade (STAI)*. California (USA): Mind Garden, Inc.

Singh D, Bronstad PM. 2001. Female body odour is a potential cue to ovulation. *Proc Biol Sci*. 268:797–801.

Smeets MAM, Dijksterhuis GB. 2014. Smelly primes–when olfactory primes do or do not work. *Front Psychol*. 5:96.

Soares SC, Maior RS, Isbell LA, Tomaz C, Nishijo H. 2017. Fast detector/first responder: interactions between the superior colliculus-pulvinar pathway and stimuli relevant to primates. *Front Neurosci*. 11:67.

Stevenson RJ. 2010. An initial evaluation of the functions of human olfaction. *Chem Senses*. 35:3–20.

Susskind JM, Lee DH, Cusi A, Feiman R, Grabski W, Anderson AK. 2008. Expressing fear enhances sensory acquisition. *Nat Neurosci*. 11:843–850.

Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. 2014. Kubios HRV–heart rate variability analysis software. *Comput Methods Programs Biomed*. 113:210–220.

Task Force of the European Society of Cardiology. 1996. Heart rate variability: standard of measurement, phyiological interpretation, and clinical use. *Circulation*. 93:1043–1065.

Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, Kim Y. 2001. Autonomic activity during human sleep as a function of time and sleep stage. *J Sleep Res*. 10:253–264.

Vianna EP, Tranel D. 2006. Gastric myoelectrical activity as an index of emotional arousal. *Int J Psychophysiol*. 61:70–76.

Wilson DA, Best AR, Sullivan RM. 2004. Plasticity in the olfactory system: lessons for the neurobiology of memory. *Neuroscientist*. 10:513–524.

Wyatt TD. 2003. *Pheromones and animal behaviour: communication by smell and taste*. UK: Cambridge University Press.

Yeshurun Y, Sobel N. 2010. An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annu Rev Psychol*. 61:219–241.

Zernecke R, Haegler K, Kleemann AM, Albrecht J, Frank T, Linn J, Brückmann H, Wiesmann M. 2011. Effects of male anxiety chemosignals on the evaluation of happy facial expressions. *J Psychophysiol*. 25:116–123.

Zhou W, Chen D. 2009. Fear-related chemosignals modulate recognition of fear in ambiguous facial expressions. *Psychol Sci*. 20:177–183.