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Original Article

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Comparing fear and anxiety chemosignals: Do they modulate facial muscle activity and facilitate identifying facial expressions?

Nuno Gomes[1], Bettina M. Pause[2], Monique A. M. Smeets[3], Gün R. Semin[1,3,*]

[1]William James Center for Research, ISPA – Instituto Universitário, Lisbon, Portugal
[2]Department of Experimental Psychology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[3]Faculty of Social and Behavioral Sciences, Utrecht University, Utrecht, the Netherlands

*Corresponding author. William James Center for Research, ISPA – Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041, Lisboa, Portugal. Email: gun.r.semin@gmail.com

Fear and anxiety are the most frequently studied emotional states in chemosignal research. Despite differences between these two emotional states, findings from research using fear and anxiety body odors (BOs) are often treated as part of a similar phenomenon. In this article, we examine possible similarities and differences between participants exposed to fear and anxiety BOs on 2 dependent variables commonly used in chemosignals' research: (1) the activation of facial muscles in displays of fear expressions (i.e. the *medial frontalis* and the *corrugator supercilii*); and (2) the time required to discriminate between negative emotional expressions (fear, anger, and disgust) and neutral ones. Our results show that fear (vs. rest) and anxiety (vs. exercise) BOs activate the *medial frontalis*, suggesting that both have a similar impact on receivers' facial muscles. However, we could not replicate previous findings regarding the influence of fear BOs in discriminating negative emotional faces from neutral ones. Two additional replication attempts failed to replicate the earlier results, indicating that the results reported in the literature with this specific paradigm should be interpreted cautiously. Suggestions for future research examining possible differences between fear and anxiety BOs are advanced.

Key words: fear, anxiety, body odors, facial electromyography, emotion discrimination, chemosensory communication

Fear and anxiety are the most frequently studied emotions in the chemosignal research (see de Groot and Smeets 2017). Using distinct methodologies (e.g. watching emotional clips; de Groot et al. 2012; participating in the Trier Social Stress Test for Groups; Meister and Pause 2021), these emotional states are often induced experimentally to collect emotion-related body odors (i.e. axillary sweat; BOs). These BOs are later presented to receiver participants to examine their behavioral and psychophysiological effects (e.g.Pause et al. 2010; de Groot et al. 2012; Lübke et al. 2017; Gomes et al. 2020). Numerous studies have examined the effects of fear and anxiety BOs independently, but their findings are often treated as part of a similar phenomenon (e.g. de Groot and Smeets 2017). However, despite their similarities, fear, and anxiety are different emotional states, with their BOs arguably triggering distinct behavioral repertoires in receivers. In this article, we planned to examine their possible similarities and differences by exposing receivers to fear and anxiety BOs. Below we first summarize the differences between fear and anxiety emotional states as well as the contexts where fear and anxiety BOs are sampled. We then note the differences between fear and anxiety BOs regarding the states they induce in their receivers. Subsequently, we provide an overview of the reported research.

Fear and anxiety are two aversive threat-related negative emotional states (Öhman and Wiens 2004), which disturb the body systems' homeostasis (McEwen 2007). From a functional perspective of emotions, they trigger appropriate adaptive responses to cope with potential hazards (e.g. Steimer 2002). Although both are high-alerting states, they occur in distinct situations. Fear is elicited in response to factual and acute sensory input, indicating that a potentially dangerous stimulus may threaten an individual's survival (e.g. Barlow 2002; LeDoux and Pine 2016). On the other hand, anxiety is seen as a generalized response to an uncertain/anticipated threat that is distal in space or time (e.g. Tovote et al. 2015; LeDoux and Pine 2016). It occurs not only in situations where the individual's survival may be threatened in the future (e.g. worries about dying) but also in anticipating situations involving, for instance, evaluation contexts where an individual's identity is potentially questioned (e.g. social and performance anxiety) (e.g. Barlow 2002).

Recently, many studies have shown that fear and anxiety states can be transferred between human beings through BOs with potential adaptive value for receivers (de Groot et al. 2017; Boesveldt and Parma 2021). These chemosignals seem to act as an alarm cue, leading receivers to a hypothetical preparedness state that facilitates coping with potentially dangerous stimuli in the surrounding environment. This preparedness state includes, among others, an increased sensory acquisition (see Susskind et al. 2008) that facilitates the exploration of the surrounding environment (see de Groot et

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al. 2012), facilitates withdrawal responses (i.e. startle reflex; Prehn et al. 2006), faster reactions to hypothetically threatening changes (Gomes and Semin 2021), and facilitates the processing of emotional faces (e.g. Kamiloğlu et al. 2018; Rocha et al. 2018; Silva et al. 2020).

Although fear and anxiety are both high-alerting states and the released chemosignals are thought to prepare receivers to cope with potentially stressful situations, there are remarkable differences between them that were not directly examined in the BOs literature. These potential differences are especially evident when we examine not only the conditions where the BOs are sampled but also their effects in modulating receivers' perception of emotional faces. Depending on the study design and goals, how emotional states are induced in the sweat donor can vary substantially (for a similar argument, see Lübke and Pause 2015). There are studies where donors are exposed to non-emotion-specific extreme stress conditions (involving positive states like joy and negative states like fear), such as first-time skydiving (Mujica-Parodi et al. 2009) or exercising on a high rope (Zernecke et al. 2011). Other studies involved emotion-specific collection methodologies. The present research report will be focused on the latter.

In the case of fear BOs sampling, the most common procedure is exposing donors to pre-piloted horror movies to induce fear (e.g. de Groot et al. 2012). In other words, donors are exposed to acute sensory inputs, vicariously experiencing dangerous stimuli that may threaten their well-being and, ultimately, their survival. The increment of the fear levels reported by donors during this emotion-induction methodology and the higher quantity of produced sweat (vs. a rest context, e.g. de Groot et al. 2015; Gomes et al. 2020) are thought to be a consequence of exposure to an actual stimulus that may constitute a source of danger. Congruently with what is observed in a fear state (see Susskind et al. 2008), receivers exposed to fear (vs. rest) BOs evidence psychophysiological and behavioral patterns of increased sensory acquisition, such as stronger activations of the *medial frontalis* and *corrugator supercilii* (e.g. de Groot et al. 2012; Gomes et al. 2020) (the muscles associated with fear faces which lead to widening eye apertures), increased sniffing volumes and faster visual exploration strategies (de Groot et al. 2012), and also facilitated processing of fear (but not other negative) facial expressions (e.g. Kamiloğlu et al. 2018; Silva et al. 2020; but see de Groot et al. 2018).

Contrastingly, anxiety sweat is collected from donors anticipating a possible threatening situation that does not challenge their survival but their identity. Donors, in this case, are about to participate in academic examinations or are instructed to give a public speech. These manipulations result in higher reported anxiety (e.g. STAI; Spielberger 1983) and cortisol levels (Pause et al. 2010; Meister and Pause 2021). Exposure to this kind of BOs primes receivers' defensive behaviors (i.e. increased amplitude of the startling reflex; Prehn et al. 2006), modulates the cardiac activity in congruence with stress responses (Rocha et al. 2018), intensifies pre- and post-attentive brain processing (Pause et al. 2010), and facilitates the processing of neutral and negative faces in general (i.e. not only fear faces as frequently observed during the exposure to fear BOs; e.g. Wudarczyk et al. 2016; Rocha et al. 2018).

Despite the described differences in the sampling procedures and the effects observed in receivers of these two BOs collected under overlapping but distinct emotional states, the obtained results are frequently treated as facets of the same phenomenon in the chemosignal literature, with no studies directly examining it. In fact, studies generally employ BOs collected either under fear or anxiety contexts but not both, making it difficult to examine the effects they elicit in the same receivers. Moreover, reviews of this research field treat the results as indicators of the aforementioned adaptive behavior without clearly examining why distinct behavior repertoires are sometimes observed between receivers exposed to fear and receivers exposed to anxiety BOs.

Nevertheless, in a meta-analysis (de Groot and Smeets 2017), differences between emotional states (e.g. fear and anxiety) under which the BOs were collected were identified as a possible source of study heterogeneity, but this could not be verified due to the small sample size. Hence, the aim of the first study reported in this article was to directly compare the effects of fear (vs. rest), and anxiety (vs. exercise) BOs with two dependent variables frequently used in the literature: (1) the activation of facial muscles (i.e. facial electromyography; fEMG) manifested in fear expressions (i.e. the *medial frontalis* and the *corrugator supercilii*; de Groot et al. 2014; Gomes et al. 2020) to examine whether the two BOs trigger similar/distinct facial changes—related with increased sensory acquisition—in receivers; and (2) processing negative and neutral facial expressions (e.g. Wudarczyk et al. 2016; Kamiloğlu et al. 2018; Rocha et al. 2018). To examine face perception, we relied on a paradigm developed by Kamiloğlu and colleagues (2018). The participant's task, guided by speed-accuracy instructions, was to discriminate between negative (i.e. anger, disgust, and fear) and neutral facial expressions gradually emerging from visual noise.

Regarding facial muscle activation, as observed in previous studies (e.g. de Groot et al. 2014; Gomes et al. 2020), we predicted that the exposure to fear (vs. rest) BOs would result in higher activation of the *medial frontalis* and the *corrugator supercilii*. Regarding anxiety (vs. exercise) BOs, it is difficult to predict what will be observed regarding the activation of the facial muscles as this has never been examined before. Consequently, the following hypotheses remain very exploratory. Concerning the *corrugator supercilii*, this muscle seems to respond to negative affect and increased arousal states (e.g. Cacioppo et al. 1986). Thus, the exposure to anxiety (vs. exercise) BOs is expected to also result in higher activations of the *corrugator supercilii*. Concerning the *medial frontalis*, this is thought to be a muscle more specific to fear (e.g., Ekman et al. 2002; Kaiser et al. 2017; Kamiloğlu et al. 2018). Hence, if anxiety BOs induce in receivers a distinct state from the one induced by fear BOs, higher activation of the *medial frontalis* is not expected when receivers are exposed to anxiety (vs. exercise) BOs.

Moreover, based on previous research, we hypothesized that fear (vs. rest) BOs would specifically speed up (reaction time; RT) the identification of specifically facial expressions of fear (vs. anger, disgust; see Kamiloğlu et al. 2018), while anxiety (vs. exercise) BOs would have a generalized speed-up effect in identifying all the facial expressions (i.e. anger, disgust, fear, and neutral) as negative or neutral (Wudarczyk et al. 2016; Rocha et al. 2018).

However, as we report (after detailing the methodology of study 1), these hypotheses were not confirmed. Results from study 1 indicate that both fear (vs. rest) and anxiety (vs. exercise sweat) BOs triggered stronger activations of the *medial*

frontalis. Moreover, we could not replicate Kamiloğlu and colleagues' (2018) results, namely that exposure to fear (vs. rest) BOs speed up the discrimination of only fear (vs. neutral) faces and not other negative expressions. This finding prompted us to perform studies 2 and 3, namely two additional replication attempts where newly collected as well as unanalyzed data from a previous project are examined, all failing to replicate Kamiloğlu et al.'s (2018) results.

Study 1

Method

Participants

Sixty university students from ISPA - Instituto Universitário (Portugal), aged 18–35 years (M_{Age} = 23.03 years; SD = 4.08), gave informed consent and participated voluntarily in study 1 (this study was preregistered in Open Science Framework: https://osf.io/cxhrt).

Following Kamiloğlu and colleagues (2018), all the participants were heterosexual, Caucasian, right-handed, non-smoking women with no reported neurological or psychiatric disorders, chronic respiratory diseases, and no allergic reactions, illnesses, or colds at the moment of the data collection. Only females were recruited due to their higher sensitivity toward emotional signals (Pause et al. 2020) and their superior sense of smell (relative to men; e.g. Brand and Millot 2001). Notably, only heterosexual participants were included because research shows that women perceive male sweat differently as a function of the donors' and their sexual orientation (e.g. Martins et al. 2005).

All the procedures were approved by the host institution's ethics committee and were conducted following the American Psychological Association standards and the Declaration of Helsinki guidelines.

Materials

Facial stimuli.

The facial stimuli used in this study were the same as those used by Kamiloğlu and colleagues (2018)—the original authors (2 of them also co-authored the present studies) kindly provided all their materials. These consist of grayscale pictures of the same 3 female and 3 male models displaying fear, disgust, anger, and neutral facial expressions—all retrieved from the Radboud Faces Database (Langner et al. 2010). Specifically, Kamiloğlu et al. (2018) generated a continuum of 30 images for each actor's facial expression by employing distinct Gaussian filters (varying linearly from 58 SDs to 0 SDs with 2 SDs decrements). In each continuum, the facial expression progressed from complete noise (i.e. totally blurred; 58 SD) to a clear image (0 SD) over 5000 ms. These continua constituted the stimuli presented to the participants in the study (Fig. 1).

Olfactory stimuli.

Four BO conditions (fear, rest, anxiety, and exercise BOs) were used in the present study.

Fear and rest sweat samples were collected at ISPA - Instituto Universitário (Portugal), following earlier procedures (e.g. de Groot et al. 2015; Gomes et al. 2020). Sweat was sampled from 24 heterosexual non-smoking Caucasian Portuguese males aged between 19 and 34 years (M_{Age} = 23.54 years; SD = 3.85), who gave their informed consent to participate in two sweat collection sessions (i.e. fear and rest induction sessions), each of 30 min duration.

Fear and rest sessions were performed in a counter-balanced order and took place with a week's interval between them. Fear and rest states were induced by exposing participants to two sets of pre-piloted film clips used in previously published research (Gomes et al. 2020; Silva et al. 2020). To induce fear, the clips were selected from the following terror films: The Nun (04 min 54 s), Mamma (07 min 40 s), Sinister (02 min 07 s), The Descent (02 min 41 s), The Grudge (02 min 10 s), REC 1 (02 min 53 s), Insidious (04 min 55 s), and A Tale of Two Sisters (07 min 30 s). For the rest condition, the clips were selected from documentaries: Solar eclipse (02 min 37 s), The Secret Life of Birds (04 min 25 s), The Transit of Venus (03 min 02 s), Equator: Battle for the light (02 min 12 s), Do we need the moon? (02 min 09 s), Discovery decade (01 min 42 s), Portugal Earth (03 min 08 s) and Wooly mammoth (03 min 36 s). Some nature sceneries retrieved from YouTube (11 min 40 s) were also used in the rest condition. Sweat was collected by placing non-woven absorbent pads (70% viscose, 30% polyester: Wells, Sonae SA, Portugal) in participants' armpits during the two emotion-induction sessions. As in previous studies (e.g. de Groot et al. 2015; Gomes et al. 2020), emotion manipulation was confirmed by: (1) asking participants to rate on 0–100 sliders their feelings (i.e. to what extent they felt angry, fearful, disgusted, sad, surprised, neutral, calm, happy, and amused); and (2) quantifying the sweat produced in each sweat collection by subtracting the pads initial weights from the weight of the pads after the sweat collection (for these data, please see the Supplementary Materials). Importantly, as in previous studies (e.g. de Groot et al. 2015; Gomes et al. 2020), to prevent sweat contamination, donors were instructed to follow a strict protocol involving several dietary, hygienic and social restrictions on the 2 days anticipating the sampling session (for a detailed explanation regarding the sweat collection, see Gomes et al. 2020). Importantly, at the beginning of each sweat collection session, the sweat donors gave the experimenter a daily journal where they reported their dietary hygienic, and social habits in the 2 days anticipating the session to confirm whether they accomplished the protocol or not. No participant reported deviations from the collection protocol. After completing all collections, sweat samples were chopped, homogenized, and pooled for each of the 2 donation conditions to reduce the possible effects of donors' interindividual variability. The 2 homogenized final "super-samples" were then weighed and packed into small pieces of ca. 0.1 g (super-donors) and stored at -80°C. The small pieces constituted the olfactory stimuli presented to the female participants. Notably, this "super-donors" preparation procedure is different from the one employed in previous research, such as in Kamiloğlu et al. (2018) (and also in de Groot et al. 2015; Gomes et al. 2020; Silva et al. 2020), where the authors combined four-eighths of pads from distinct sweat donors (2 from the left and 2 from the right armpit). This super-donor' preparation procedure was employed here to have more standardized sweat weights per "super-donor," increasing the homogeneity of the BO stimuli presented to participants. In other words, the method used to prepare the super-donors in previous research using fEMG relies on a small number of donors (e.g. N = 8) from which the sweat pads are equally divided in 8 pieces. Then, 4 pieces are chosen in a counterbalanced way to prepare each super-donor. However, this method is more

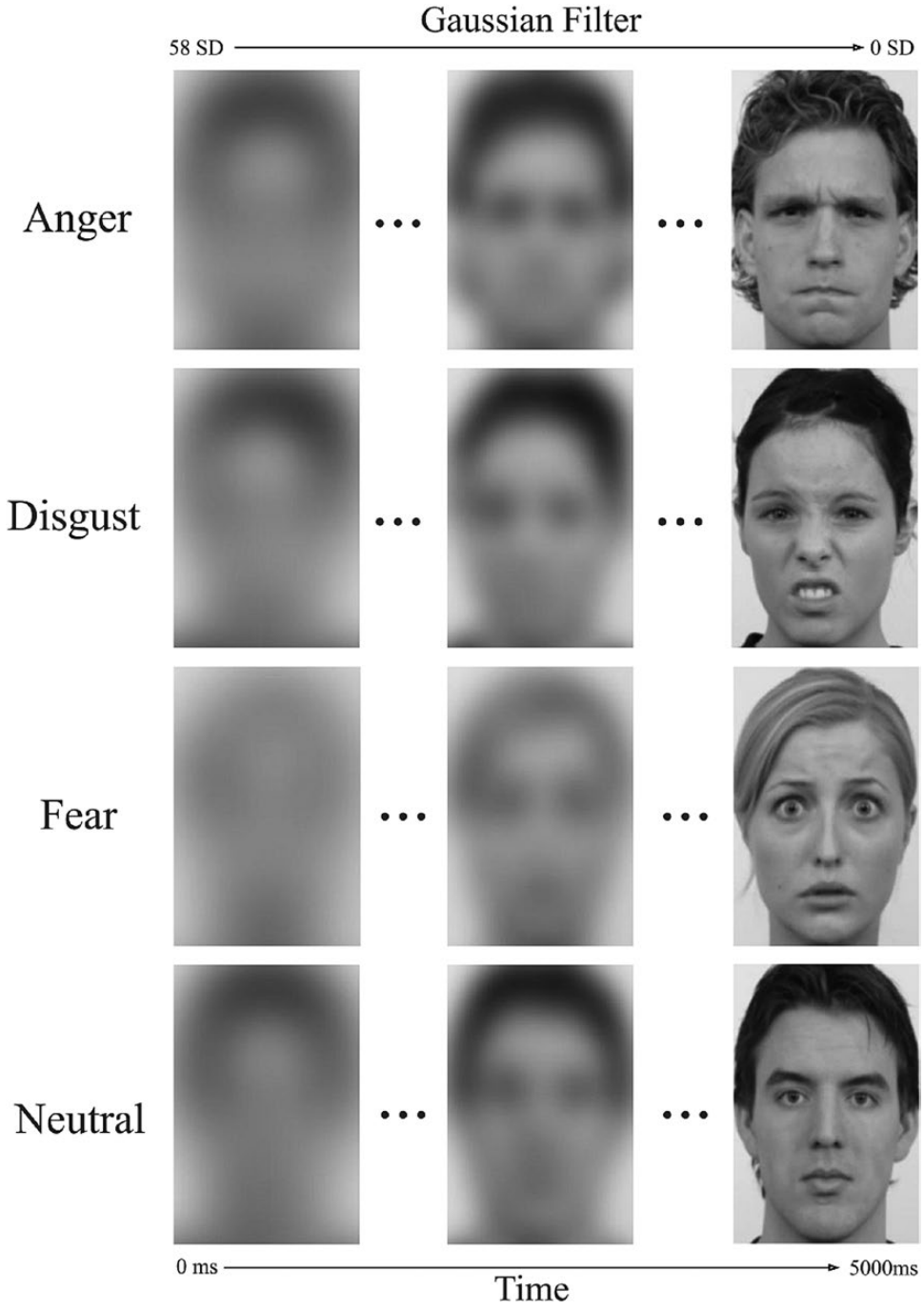

Fig. 1. Example of distinct face models displaying different emotions. Facial expressions progressed from complete noise (i.e. totally blurred; 58 SD Gaussian filter) to a clear image (0 SD Gaussian filter) over 5000 ms. The stimuli were retrieved from the Radboud Faces Database (Langner et al. 2010).

susceptible to donor-related specific effects (olfactory stimuli come from a small number of individuals), and does not control the quantity of sweat in each super-donor. By employing the super-donor preparation methodology described above, we randomly sampled our "super-donors" from a higher number of sweat donors, also controlling for the amount of sweat presented to each receiver across several conditions.

Anxiety and exercise BOs were collected at Düsseldorf University (Germany) using cotton pads (Ebelin dm-drogerie markt GmbH + Co. KG, Germany). The samples were obtained from 26 heterosexual non-smoking Caucasian European men aged between 18 and 32 years (M_{Age} = 23.37 years; SD = 3.45) over 2 h in two separate sessions: (1) an anxiety session following the protocol of a modified Trier Social Stress Test for Groups (TSST-G; von Dawans et al. 2011 here modified for evoking anxiety instead of stress); and (2) a control session including standardized ergometer training (i.e. bicycle). In brief, the participants underwent a mock assessment (anxiety session), with three participants per group performing in front of a female evaluator. The two tasks which are standard within a TSST-G, the job interview, and the mental arithmetic, were modified to three tasks, the original job interview, accompanied by a discussion of a politically controversial topic, and a defense speech (for further

information, see Supplementary Materials). In order to minimize variations of eccrine sweat production between sessions, the heart rates of sweat donors in the control session were controlled to match their heart rates in the anxiety session. That is, the heart rate of each participant in the anxiety condition was recorded. Then, the intensity of ergometer training (i.e. the exercise condition) was adjusted for the participant's heart rate to be comparable to the observed during the anxiety condition. Both sessions were scheduled at the same hour of the day. As in the fear and rest sweat collections, donors were instructed to accomplish a strict protocol involving several dietary, hygienic, and social restrictions to avoid sweat contamination (for a similar sweat collection procedure, see Meister and Pause 2021). Emotion manipulation was confirmed here by examining participants' salivary cortisol and self-ratings of mood, arousal, dominance, and anxiety (see Supplementary Materials). "Super-donor" samples were also prepared by chopping, homogenizing, pooling for the two-donation conditions, and then weighting small portions of ca. 0.1 g (the samples presented to the female participants) were also stored at −80°C.

Behavioral task.

Following the procedure from Kamiloğlu et al. (2018), the participants' task was to identify, as fast and accurately as possible, whether each of the facial continuums was a negative or a neutral facial expression (see Facial Stimuli section). Specifically, each trial started with a fixation cross in the middle of the screen and was displayed for 1 s. Then, a random facial continuum was introduced in the center of the screen, progressing from complete visual noise to a clear facial expression over a time window of 5000 ms. On a standard keyboard, participants were instructed to identify whether the emerging image was a neutral or a negative facial expression using 2 marked keys (counterbalanced between participants).

Procedure

As in Kamiloğlu et al. (2018), the experiment was conducted stand-alone. The experimenter was a female to avoid alterations in the female receivers' mood due to the presence of a male experimenter (Jacob et al. 2001). The sweat samples were removed from the freezer an hour before starting the data collection session. Each BO condition was identified by a code designated by another researcher who was not the experimenter. Thus, neither the experimenter nor the participants knew the BO condition (i.e. double-blind experiment). In contrast to the Kamiloğlu et al. (2018) experiment, four BO conditions (i.e. fear, rest, anxiety, and exercise sweat samples) were employed instead of just two (i.e. fear and rest sweat samples). Furthermore, participants were exposed to the BO conditions not by placing a vial under their noses but by means of an olfactometer connected to a nasal cannula.

After a general explanation of the procedure on arrival at the laboratory, participants' face was prepared for the EMG electrodes placed on the *corrugator supercilii* and *medial frontalis* (2 facial muscle associated with the facial expression of fear; Ekman et al. 2002). The experimenter cleaned the skin on the left side of the face (which displays stronger affective reactions than the right side on right-handed individuals; Dimberg and Petterson 2000), first with alcohol and then with an abrasive lotion (Lemon Prep; Mavidon). Two electrodes (Ag-AgCl) were then applied in a bipolar fashion over each of the target muscles, following the guidelines by Fridlund and Cacioppo (1986). A reference electrode was placed on the participant's forehead. The signal was acquired by a Bionex 8-channel chassis powered by BioLab (version 3.2.0; Mindware Technologies, Gahanna, OH, USA). During the data collection, the signal was online filtered using a 20–200 Hz bandpass filter. Before analyzing the data, the fEMG signal was rectified and smoothed with a 20 Hz low-pass filter using EMG Analysis software (version 3.1.5; MindWare Technologies, Gahanna, OH).

Participants were asked to place their heads on a chin rest placed 50 cm away from the screen and to wear the nasal cannula that was connected to a computer-controlled 4-channel olfactometer (see Lundström et al. 2010), employing a flow rate of 2.4 L min^{-1}, that was used to control the BO conditions presentation. During each BO condition, 95% of the air introduced into participants' nostrils traveled over the sweat pads, while the remaining 5% of the flow came from a clean air channel.

Due to the high number of BO conditions employed here, this experiment was divided into 12 blocks (3 in each BO condition). After reading the experimental instructions on the screen, participants started with a training block while exposed to clean air. Then, they performed the 12 blocks of the main experiment. Each block started with an instruction at the beginning of the block, followed by a fixation cross for 8 s. The BO condition was introduced 2 s after the onset of the fixation cross. The EMG signal started collecting at 600 ms before the introduction of the BO into the participants' nostrils until the end of the fixation cross (600 ms baseline followed by 6 s of target signal). Participants were then asked to perform 18 trials of the behavioral task. The 18 faces (3 fear, 3 anger, 3 disgust, and 9 neutral faces; i.e. 9 negative and 9 neutral faces) were randomly selected per block from the set of facial stimuli and presented in and random order to the participant. Importantly, each emotional face was present the same number of times to each participant across the 12 blocks (i.e. 6 times). The same applies to neutral faces (18 times for each participant). Between the different facial stimuli, a fixation cross was displayed for 500 ms. Each block took approximately 2 min. After each block of the facial discrimination task, a 1-min washout period (only clean air being introduced into participants' nostrils) took place, and a new block started. BO conditions were randomly presented following just one constraint: the same BO condition was not present consecutively. Please note that the washout period used in this study (i.e. 1 min) is shorter than the one typically used in research involving BOs and fEMG (i.e. around 5 min; see de Groot et al. 2015; Gomes et al. 2020; Silva et al. 2020). The washout period was reduced to keep the experiment shorter due to the higher number of BO conditions used here compared to previous studies (e.g. Kamiloğlu et al. 2018). This reduction of the washout duration was possible because, different from previous experiments, an olfactometer was used to directly deliver the BO into participants' nostrils, providing a more controlled experimental environment where no longer breaks between conditions are needed to make sure that participants and experimental room are clean from the previous BO condition.

After the main task, participants, in a counterbalanced order, rated the hedonic value (pleasantness) and intensity of the sweat samples using 7-point Likert scales (ranging from

"very unpleasant" to "very pleasant" and "very weak" to "very strong," respectively).

Participants were then thanked, debriefed, and received monetary compensation or course credit. The entire experimental procedure took approximately 60 min.

The experiment was created using the PsychoPy builder interface (version: 2021.2.1; Peirce et al. 2019).

Statistical analysis

fEMG.

The fEMG data were collected to assess whether fear and anxiety BOs triggered higher activations of the facial muscles involved in expressing fear (i.e. *medial frontalis* and *corrugator supercilii*) compared to their direct control BO conditions (rest and exercise sweat, respectively). When the data were inspected, one participant had to be excluded from the fEMG analysis due to a software error resulting in no recorded signal. Please note that, in the fEMG analysis, we slightly changed the preregistered analysis plan to allow model conversion.

Although fEMG data were continuously collected during the experiment, only 6.6 s per block (0.6 s before the exposure to the BO plus 6 s after it) were extracted and analyzed (3 blocks per BO condition). EMG data were averaged in intervals of 200 ms and checked for artifacts. For each participant, muscle, BO condition, and block values higher than 2.5 median absolute deviations (MAD; Leys et al. 2013) units were marked as artifacts (see Gomes et al. 2020). Participants' facial video recordings were used to identify these artifacts as results of non-odor-related movements (e.g. sneezing). When the artifacts were confirmed, this signal portion was deleted. Otherwise, it remains untouched (4.88% of the data points of *corrugator supercilii* and 4.25% of the *medial frontalis* were identified as artifacts). Blocks where baselines present artifacts were also removed because noisy baselines can comprise the entire 6 s of the target signal (13.70% of the blocks for *corrugator supercilii* and 13.56% of the blocks for the *medial frontalis*). The first 3 intervals (of each block) of 200 ms were averaged to serve as the baseline. EMG data were then baseline corrected by subtracting from each 200 ms segment the mean activity of the corresponding muscle's baseline. Then, the corrected fEMG signal from distinct blocks was averaged per BO condition, participant and muscle.

fEMG data were analyzed using 2 linear mixed models (one for each muscle), including participant ID as a clustering factor, the muscle activity as the dependent variable, time (i.e. 200 ms time intervals) as a continuous independent variable, and BO condition as the predictor to the model. As in Gomes et al. (2020), time was centered on easing the parameter interpretation. We considered the BO condition, time, and their interaction as fixed effects. Contrary to previous work (Gomes et al. 2020), regarding *medial frontalis*, we did not include a quadratic effect of time in the model because there was no significant main effect of quadratic time nor an interaction between quadratic time and BO condition revealed in the present data set. In the *corrugator supercilii*, the quadratic effect of time was kept in the analysis. As random effects, we consider, in both models, random intercepts per subject as well as random slopes per odor condition. The model was estimated using restricted maximum likelihood, and a Satterthwaite approximation of the degrees of freedom was considered (see West 2009).

Data were analyzed using the GAMLj module (Gallucci 2019) powered by jamovi (The jamovi project 2022).

Behavioral task.

For replication purposes, the pre-registered analysis plan was changed and the analysis of the data retrieved from the behavioral task (i.e. reaction times in classifying the continua as negative or neutral emotional faces) followed the same procedure as Kamiloğlu and colleagues (2018).

After averaging the data per design cell and conducting an initial data inspection, one participant was excluded due to a software error that resulted in no recorded data, and 5 participants were excluded due to low accuracy in one or more design cells (accuracy rates between 0 and the chance level). The data analysis regarding the reaction time (RT) data was based on correct responses only (i.e. negative button was pressed when a negative continuum was presented, and the neutral bottom was pressed when a neutral face was displayed) (Table 1).

The RT data were first checked for outliers identified as values exceeding 2.5 median absolute deviations (Leys et al. 2013). Values identified as outliers (3.36%) were then altered to be one unit above the next extreme score on that variable (according to Field 2014).

A 4 BO conditions (fear, anxiety, rest, and exercise) × 4 face conditions (fear, anger, disgust, and neutral) repeated measures ANOVA was conducted to examine the effects of BO and face condition on the RT in identifying the continua as negative or neutral facial expressions. In case of a significant interaction, we follow the procedure employed by Kamiloğlu and colleagues (2018), testing the specific interaction hypotheses evidenced by the authors. First, we would calculate "delta RTs" by (1) subtracting the RT scores under rest odor from the RT scores under the fear odor; and (2) by subtracting the RT scores under exercise odor from the RT scores under the anxiety odor. Please note that fear and rest odor, anxiety, and exercise odors were collected from distinct populations involving distinct sampling procedures (see Methods section). Subsequently, planned contracts were run to test whether the different expressions were classified faster than the others.

Pairwise comparisons were corrected using the Holm-Bonferroni procedure. As a measure of the effect size, we reported the $\eta 2_p$.

Results and discussion

fEMG data

Medial frontalis.

The results from the linear mixed model (LMM) analysis ($R2_{conditional} = 0.47$) for *medial frontalis* activity are summarized

Table 1. Mean accuracy rates per BO and face conditions in study 1.

	BO condition			
Face condition	Anxiety BO	Exercise BO	Fear BO	Rest BO
Anger	76.75%	77.78%	78.40%	76.34%
Disgust	97.94%	98.77%	96.50%	97.53%
Fear	96.30%	94.44%	96.30%	90.95%
Neutral	89.99%	90.47%	89.16%	89.92%

in Fig. 2. First, a significant main effect of BO condition ($F(3, 57.9) = 3.27$, $P = 0.027$) was revealed. Interpreting the parameters estimates for fixed effects, it is possible to observe that, overall, anxiety BOs ($M = 0.10$ μV, SE = 0.04) activate the *medial frontalis* significantly more than its direct control (i.e. exercise chemosignals) ($M = 3.96 \times 10^{-3}$ μV, SE = 0.04; $B = 0.10$, $t(58) = 2.18$, $P_{uncorrected} = 0.033$). Regarding fear BOs ($M = 0.08$ μV, SE = 0.04), they also triggered an overall stronger activation of the *medial frontalis* when compared with its direct control (i.e. rest BOs; $M = 4.46 \times 10^{-4}$ μV, SE = 0.03) that almost reaches significance, in the hypothesized tendency ($B = 0.08$, $t(57.7) = 2.04$, $P_{uncorrected} = 0.057$). However, it is important to note that overall, statistically significant differences between BO conditions can be observed only when uncorrected *P*-values for multiple comparisons are considered—a Holm-Bonferroni correction procedure results in all *P*-values greater than 0.05. This may occur because the data have sufficient power to reveal a significant main effect but not significant ad hoc comparisons (which, due to the correction for multiple comparisons, demands a much lower *P*-value to be significant). This suggests that the overall effect of the BO condition is small in magnitude. Additionally, even considering the uncorrected *P*-values, we observed only a marginally significant difference between fear and rest BOs. These observed weak effects for fear (vs. rest) BOs somehow contradict previous research showing stronger activations of the *medial frontalis* in response to these stress-related BOs (e.g. de Groot et al. 2012, 2014, 2015; Gomes et al. 2020). After inspecting the methodology to create "super-donors" employed in this study and comparing it with previous research from our lab, we observed that here the amount of sweat pad per super-donor (0.1 g) is about five times smaller than in other studies where four eights of a pad were used in each "super-donor" (e.g. around 0.5 g used in Gomes et al. 2020). This may explain the weak effects observed *medial frontalis* in response to fear odors and could also contribute to explaining the small magnitude of the main effect of the BO condition. Another critical point is that BOs were delivered directly into participants' noses using a continuous flow olfactometer in the present study. In contrast, in previous studies measuring fEMG, participants sniffed the BOs at their own pace from a vial placed under their noses. It is possible that the olfactometer, due to its constant airflow, has impacted participants' facial muscle activity (i.e. the air being introduced into the participants' nostrils may have led to facial movements or specific facial muscle contractions). Nevertheless, future research is necessary to understand the effects of olfactometers and distinct air flows on the activity of participants' facial muscles.

Additionally, a main effect of time was also evidenced ($F(1, 6172.9) = 14.54$, $P < 0.001$). However, as it is irrelevant to this research, it will not be further explored. Importantly, of high importance for the present study, the LMM analysis also revealed a significant interaction between BO condition and time ($F(3, 6173.2) = 7.42$, $P < 0.001$). Interpreting the parameter estimates for fixed effects and directly comparing anxiety BOs with its direct control—i.e. exercise BOs—it is possible to observe that, although the exposure to both conditions triggered in participants a growing tendency of *medial frontalis* activation over time, this tendency is significantly higher during the exposure to anxiety BOs ($B = 2.57 \times 10^{-3}$, $t(6170.40) = 2.20$, $P = 0.028$). These results evidence for the first time that anxiety BOs trigger a higher activation of medial frontalis than exercise BOs. Moreover, as predicted and following earlier literature, fear BOs also triggered a growing tendency of the *medial frontalis* activity in participants. At the same time, the exposure to their direct control—i.e. rest BOs—resulted in a decreasing tendency ($B = 2.46 \times 10^{-3}$, $t(6175.96) = 2.09$, $P = 0.036$). Notably, the results contradict the prediction that exposure to anxiety BOs would not result in stronger activation of the *medial frontalis* because this was expected to be a fear-specific muscle. This data pattern suggests that anxiety and fear BOs may trigger similar processes in receivers, at least when the activation of facial muscles is considered.

Notably, an analysis including intensity and pleasantness ratings did not modify the observed pattern of results, ruling out the role of these two dimensions in the observed data pattern.

Corrugator supercilii.

Regarding *corrugator supercilii*, contrary to our predictions, the LMM analysis ($R2_{conditional} = 0.54$) did not reveal a main effect of the BO condition ($F(3, 64.2) = 0.69$, $P = 0.564$) nor a significant interaction between BO condition and time ($F(3, 6081.6) = 1.21$, $P = 0.303$) or between BO condition and time squared ($F(3, 6076.3) = 2.13$, $P < 0.094$). Only a main effect of time ($F(1, 6081.4) = 9.11$, $P = 0.003$) and time squared ($F(1, 6076.1) = 11.75$, $P < 0.001$) were observed, which is not relevant for this research and therefore not explored further.

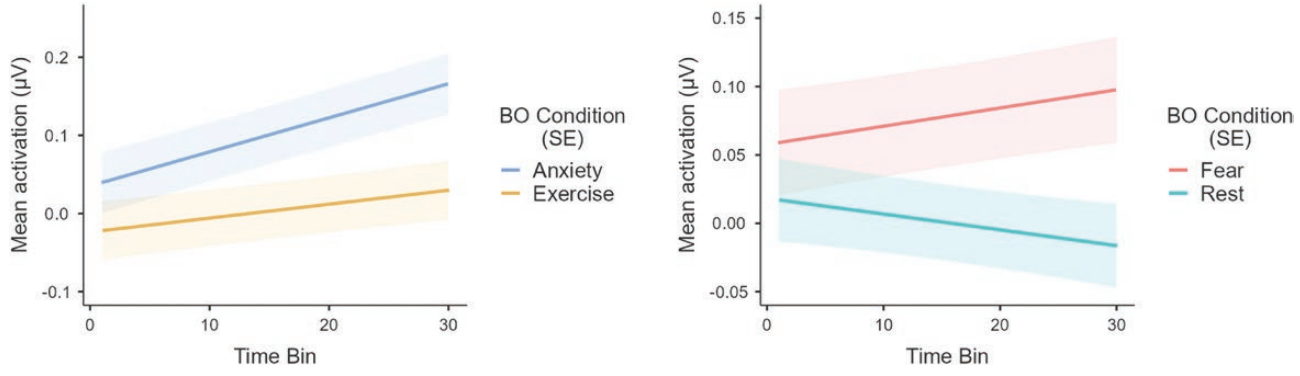

Fig. 2. Mean activation of the medial frontalis in microvolts (μV), per pair of BO conditions. Each time point represents a 200 ms time bin (6 s, 30 time bins of 200 ms each). The shaded area represents the standard error around the mean.

These results indicate that the distinct BO conditions did not significantly modulate the activity of the *corrugator supercilii*. As aforementioned, it is possible to speculate that using an olfactometer or the specific methodology in preparing the "super-donors" may have resulted in the absence of the typical effects observed in earlier literature comparing fear and rest BOs.

Moreover, as observed for the *medial frontalis*, adding the intensity and pleasantness subjective ratings as covariates to the model did not change the previously described data pattern.

Behavioral task

The results from the behavioral task are summarized in Fig. 3.

First, the repeated-measures ANOVA revealed a main effect of the face condition ($F(2.37, 125.43) = 587.08$, $P < 0.001$, $\eta 2_p = 0.92$), which will not be explored further because it is not central to the research question addressed in the present study.

Additionally, contrary to what was hypothesized, the repeated-measure ANOVA did not reveal a main effect of BO condition ($F(3, 159) = .68$, $P = .565$, $\eta 2_p = 0.01$) or an interaction between BO condition and face condition ($F(6.15, 326.08) = 1.18$, $P = 0.314$, $\eta 2_p = 0.02$). Follow-up analyses on delta RT were therefore not conducted. Hence, the obtained results did not replicate what was evidenced by Kamiloğlu and colleagues (2018), showing that fear (vs. rest) BOs sped up the identification of fear (but not other negative) facial expressions as negative ones (For the sake of comparison with Kamiloğlu et al. (2018), we conducted an additional repeated measures ANOVA considering just two BO conditions—i.e. fear and rest BOs. This ANOVA also revealed no main effect of the BO condition [$F(1, 53) = 1.62$, $P = 0.208$, $\eta^2_p = 0.03$], nor an interaction between BO and face conditions [$F(2.33, 159) = 1.02$, $P = 0.373$, $\eta^2_p = 0.02$].). Moreover, as the effects reported in the literature using this paradigm are not replicated, no conclusions are possible to make in this study regarding the influence of anxiety BOs on the perception of emotional faces.

Importantly, considering that the fEMG results did not show a strong and clear difference between exposure to fear and rest BOs (as observed in previous research; e.g. de Groot et al. 2012; Kamiloğlu et al. 2018; Gomes et al. 2020), one could argue that the communication through emotional BOs was compromised in the present study. This situation could have occurred due to the aforementioned methodological differences in the preparation of "super-donor" pads (e.g. a lower amount of sweat pad) and BO delivery (i.e. via an olfactometer instead of placing a vial under participants' noses). Consequently, the impaired emotional communication could have led to results that do not replicate the ones reported by Kamiloğlu and colleagues (2018). To address these arguments, we started by conducting a further study, also employing an olfactometer, where the amount of sweat pad per "super-donor" was increased and just fear and rest BOs (as in Kamiloğlu et al. 2018) were used (study 2). Furthermore, we also examined unanalyzed data from earlier unpublished research (studies 3), where Kamiloğlu et al.'s (2018) "super-donors" preparation and BO delivery methodology were used. These 2 additional replications are reported below.

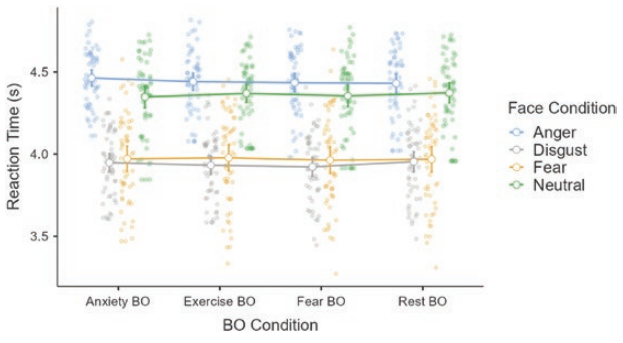

Fig. 3. Mean reaction time in classifying anger, fear, disgust, and neutral facial expressions as negative or neutral under exposure to fear or rest BOs (study 1). Error bars represent 95% CI.

Study 2

After observing that the results from study 1 did not replicate the ones reported by Kamiloğlu and colleagues (2018), we conducted an additional study to exclude the possibility that the reduced sweat amount per "super-donor" in study 1 (when compared with previous research) was precluding us from observing the effect of fear (vs. rest) BOs on the perception of emotional faces. Hence, study 2 relied on the same behavioral paradigm as study 1, and on the same odor delivery method (i.e. an olfactometer). However, only 2 BO conditions were employed (i.e. fear vs. rest BOs), and "super-donors" were prepared as in study 1 but using higher pad weights (instead of 0.1 g, here 0.5 g was used—i.e. the weight of the pad pieces per "super-donor" was increased to ca. 0.5 g).

Method

Participants

Thirty female students from ISPA—Instituto Universitário (Portugal) were recruited. One participant was excluded due to a software problem during data collection. This resulted in a final sample of 29 participants aged between 18 and 31 (M_{Age} = 21.34 years; SD = 2.70). The same inclusion criteria as in study 1 were applied here.

Materials

Facial stimuli.

The same as in study 1.

Olfactory stimuli.

Different from study 1, only 2 BO conditions were employed in study 2: fear and rest BOs. They were prepared from the same "super" fear and rest samples as in study 1, using the same "super-donors" preparation method. However, the weight of the pad pieces per "super-donor" was increased to ca. 0.5 g.

Behavioral task.

The same as in study 1.

Procedure

As in Kamiloğlu et al. (2018) and study 1, the experiment was conducted in a stand-alone manner by a female experimenter. Sweat samples were removed from the freezer an hour before starting the data collection session. Each BO condition was

also identified by a code conceived by a researcher other than the experimenter. Thus, neither the experimenter nor the participants were aware of the odor condition (i.e. double-blind experiment).

Different from study 1 and Kamiloğlu et al. (2018), no fEMG measurements were taken in study 2. Thus, after a general explanation of the procedure on arrival at the laboratory, participants were asked to wear nasal cannulas connected to an olfactometer with the same characteristics as in study 1. Then, they were asked to place their heads on a chin rest 50 cm away from the screen. The instructions for the behavioral task were displayed on the screen, and the participant was then instructed to perform 12 training trials.

After concluding the training phase, participants started the experimental phase. The first BO (either fear or rest BOs) was then introduced to the participants' nostrils. After 5000 ms looking at a fixation cross, participants performed 72 trials of the behavioral task (36 consisting of negative expressions and another 36 involving neutral expressions; random order). After concluding the first phase, they had a mandatory 5-min break (i.e. a washout period) exposed just to clean air. The procedure was then repeated for the other BO condition.

Notably, the presentation order of BO conditions, the hand used to give the answers, and the order of the keys (negative, neutral) were counterbalanced between participants.

When the behavioral task was concluded, participants were instructed to evaluate the hedonic value (pleasantness) and the intensity of the sweat samples (counterbalanced order) on a 7-point Likert scale (ranging from "not at all" and "very much").

At the end of the experiment, participants were debriefed about the study's main goal and received monetary compensation.

Statistical analysis

As in Kamiloğlu et al. (2018) and study 1, the RT data analysis was based only on correct responses (see Table 2). Table 2 shows the accuracy rates per BO and face condition.

The RT data were also checked for outliers identified as values exceeding 2.5 MAD (Leys et al. 2013). Values identified as outliers (3.02%) were then altered to be one unit above the next extreme score on that variable (same procedure as in Kamiloğlu et al. 2018).

Then, a 2 BO conditions (fear, rest) × 4 face conditions (fear, anger, disgust, and neutral) repeated-measures ANOVA was conducted to examine the effects of BO condition and face condition on the RT in identifying the continua as negative or neutral facial expressions. As in study 1, if a significant interaction was found, we followed the procedure employed by Kamiloğlu and her colleagues (2018), testing the specific interaction hypotheses reported by the authors. We first calculated the RT differences for each facial image condition (fearful, angry, disgusted, or neutral) by subtracting the RT scores under rest BO from the RT scores under the fear BO (delta RTs). Then a planned contrast analysis was run to test whether fear expressions are classified faster than other facial expressions.

Pairwise comparisons were corrected using the Holm-Bonferroni procedure. As a measure of the effect size, we reported the $\eta 2_p$.

Table 2. Mean accuracy rates per BO and face conditions in study 2.

	BO condition	
Face condition	Fear BO	Rest BO
Anger	62.78%	61.11%
Disgust	93.33%	97.22%
Fear	87.22%	91.67%
Neutral	82.04%	86.85%

Results and Discussion

The obtained results are summarized in Fig. 4. As in study 1, the repeated measure ANOVA revealed a main effect of the face condition ($F(2.37, 66.33) = 137.39$, $P < 0.001$, $\eta_p 2 = 0.83$) that will not be further explored because it is not relevant for the present study.

Moreover, also as in study 1, the repeated-measure ANOVA did not reveal either a main effect of BO condition ($F(1, 28) = 0.07$, $P = 0.796$, $\eta_p 2 = 0.00$) or an interaction between BO condition and face condition ($F(1.99, 55.74) = 0.53$, $P = 0.593$, $\eta 2_p = 0.02$), not supporting, once again, the data pattern evidenced by Kamiloğlu and her colleagues (2018).

Study 3

After observing that the result from studies 1 and 2 did not replicate the ones reported by Kamiloğlu and colleagues (2018), we examined unanalyzed data from a previous project where the same paradigm was used. In study 3, an exact replication of Kamiloğlu et al. (2018) was conducted (e.g. BOs were presented to the participant by placing a vial under their nose and not by using a continuous air flow olfactometer) in the same laboratory (Utrecht University). One difference should be noted: fear and rest BOs were sampled from Portuguese donors instead of Dutch sweat donors. This study's fEMG results confirmed that exposure to fear (vs. rest) BOs results in stronger activations of the *medial frontalis* and the *corrugator supercilii*, which were reported in Gomes et al. (2020). Below we report the behavioral results obtained with Kamiloğlu et al.'s (2018) paradigm collected in the same study.

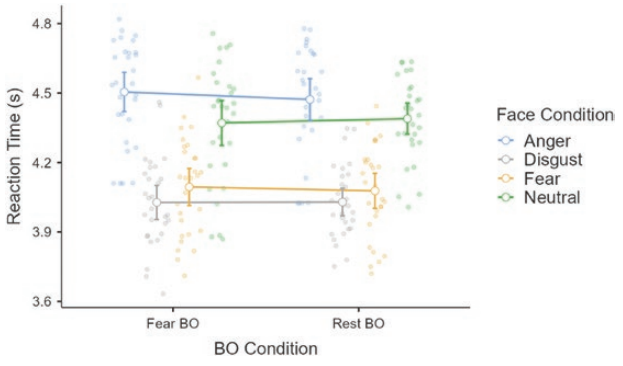

Fig. 4. Mean reaction time obtained in study 2 in classifying anger, fear, disgust, and neutral facial expressions as negative or neutral under exposure to fear or rest BOs. Error bars represent 95% CI.

Method

Participants

Thirty-two female right-handed non-smoking students from Utrecht University (the Netherlands) were recruited to participate in the first study. However, 1 was excluded due to an ethnic background other than Caucasian. Thus, this replication attempt relied on data from 31 participants aged between 19 and 34 (M_{Age} = 22.32 years; SD = 3.15). The same inclusion criteria as in study 1 were applied here.

Materials

Facial stimuli.

The same as in studies 1 and 2.

Olfactory stimuli.

The sweat collection procedure was similar to the one reported in studies 1 and 2. Moreover, as in study 2, only two BO conditions were employed: fear and rest BOs. As we already reported in Gomes et al. (2020) (A study consisting of the same BO samples and the same receiver participants as in studies 3 and 4 but focused just on the fEMG measurements collected at the beginning of the behavioral task was reported in Gomes et al. (2020).), sweat samples were obtained from 8 heterosexual non-smoking Caucasian Portuguese males aged between 21 and 35 years (M_{Age} = 27.5 years; SD = 4.87) who gave their informed consent to participate in 2 sweat collection sessions (one for inducing fear and another to induce a rest state). Like study 1, each session lasted 30 min, separated by a week's interval. In each session, participants were exposed to one of two film sets (the same used in study 1). Sweat was collected using non-woven absorbent pads (70% viscose, 30% polyester; Wells, Sonae SA, Portugal) at ISPA – Instituto Universitário (Portugal). The same emotion manipulation checks as in study 1 were used here. These data can be consulted in Gomes et al. (2020). Importantly, as in study 1, donors accomplished the same strict protocol involving several dietary, hygienic, and social restrictions to prevent sweat contamination. For a detailed explanation of the BOs collection, see Gomes et al. (2020).

Then, to reduce the possible effects of donors' interindividual variability, we combined pad pieces from 4 different donors (randomly selected) to create a "super-donor." In other words, distinctly from studies 1 and 2—where all the pads were chopped, homogenized, and pooled for each of the 2 donation conditions—in study 3, we followed the same procedure used by Kamiloğlu et al. (2018). Each pad (in total 16 pads per donation condition; 2 pads collected from each donor) was cut into 8 pieces, and then 4 pieces from 4 different donors (2 from the right and 2 from the left armpits) were randomly combined in an amber vial to create each "super-donor." Each receiver was exposed to the same combination of donors in both sweat conditions (i.e. fear and rest). Sweat samples were conserved at −80°C degrees and then sent in dry ice to the Netherlands, where they were used.

Behavioral task.

The same as in studies 1 and 2.

Procedure

Once again, as in Kamiloğlu et al. (2018) and study 1, a female experimenter conducted the experiment in a stand-alone manner. The amber vials containing the sweat samples were removed from the freezer an hour before starting the data collection session. Each BO condition was also identified by a code conceived by a researcher other than the experimenter. In such a way, neither the experimenter nor the participants were aware of the odor condition (i.e. double-blind experiment).

As in study 1, after a general explanation of the procedure on arrival at the laboratory, participants' face was prepared for the EMG electrodes placed on the *corrugator supercilii* and *medial frontalis* (2 facial muscle associated with the facial expression of fear; Ekman et al. 2002).

Participants were then asked to place their heads on a chin rest 50 cm from the screen. A closed amber vial containing one of the two BO conditions was placed 2 cm away from the participant's nose. With the vial still closed, the instructions for the behavioral task were displayed on the screen, and the participant was then instructed to perform 12 training trials. The training phase started with a 5000 ms fixation cross displayed in the center of the screen.

After concluding the training phase, participants wore a nose clip to prevent preliminary sniffs. Then the nose clip was removed at the same time that the experimental phase started. Once again, this phase started with a 5000 ms fixation cross, the time interval in which the EMG signal was acquired. As in the previously reported studies, participants then performed 72 trials of the behavioral task, followed by a mandatory 5-min break with no vial placed under their noses. A new vial containing the other BO condition was then placed under their nose, and the procedure was repeated during the second experimental phase.

Once again, the presentation order of BO conditions, the hand used to give the answers, and the order of the keys (negative, neutral) were counterbalanced between participants.

When the behavioral task was concluded, participants were instructed to (1) evaluate the hedonic value (pleasantness) and the intensity of the sweat samples (counterbalanced order) on a 7-point Likert scale (ranging from "not at all" and "very much"); (2) perform 4 trials of a 2-alternative forced-choice reminder task (see de Groot et al. 2015) to assess their ability to distinguish between sweat samples. For the data regarding the hedonic value (pleasantness), the intensity of the sweat samples, and participants' ability to distinguish between sweat samples, please see Gomes et al. (2020).

At the end of the experiment, participants were debriefed about the study's main goal and received monetary compensation.

Statistical analysis

The same as in study 2. In the case of study 3, the mean percentage of replaced outliers was 3.45%. Two additional participants were excluded due to a high number of outlier values (>75%). Table 3 shows the accuracy rates per BO and face condition.

Results and discussion

Results from study 3 are summarized in Fig. 5.

First, a main effect of face condition was found ($F(3, 84) = 169.47$, $P < 0.001$, $\eta 2_p = 0.86$). Once again, this effect will not be further explored because it is not relevant to the goal of the present study.

Table 3. Mean accuracy rates per BO and face conditions in study 3.

	BO condition	
Face condition	Fear BO	Rest BO
Anger	85.48%	79.30%
Disgust	98.66%	94.35%
Fear	94.62%	91.40%
Neutral	94.27%	91.49%

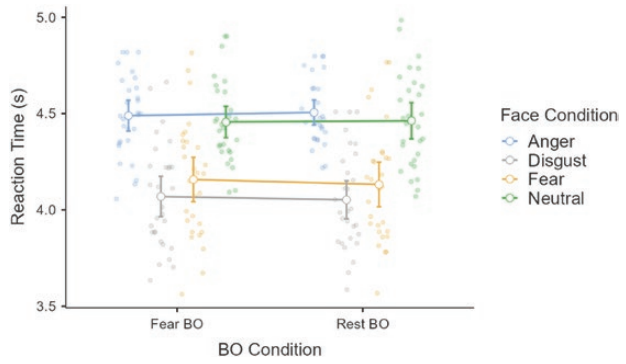

Fig. 5. Mean reaction time obtained in study 3 in classifying anger, fear, disgust, and neutral facial expressions as negative or neutral under exposure to fear or rest BOs. Error bars represent 95% CI.

Moreover, as in studies 1 and 2, the repeated-measure ANOVA did not reveal either a main effect of BO condition ($F(1, 28) = 0.03$, $P = 0.858$, $\eta 2_p = 0.00$) or an interaction between BO condition and face condition ($F(3, 84) = 0.62$, $P = 0.606$, $\eta 2_p = 0.02$). No further analyses on Delta RT were conducted. As observed in study 1, these results suggest that contrary to what was evidenced by Kamiloğlu and her colleagues (2018), fear (vs. rest) BOs do not speed up the identification of fear facial expressions as negative.

General discussion

The aim of the first reported experiment (study 1) was to compare the effects of fear (vs. rest) and anxiety (vs. exercise) BOs on receivers, using two dependent variables commonly employed in research with fear BOs: (1) the activation of facial muscles involved in displaying fear facial expressions (i.e. the *medial frontalis* and the *corrugator supercilii*; de Groot et al. 2014; Gomes et al. 2020), and (2) the processing of negative and neutral facial expressions (e.g. Kamiloğlu et al. 2018; Rocha et al. 2018; Silva et al. 2020).

Despite the differences between these two stress-related emotional states (especially when the emotion induction procedures for sweat sampling are considered), the fEMG results revealed a similar activation pattern of the *medial frontalis* muscle during the exposure to fear (vs. rest) and anxiety (vs. exercise) BOs. Contrary to what was hypothesized, the exposure to both fear and anxiety BOs triggered in receivers a growing activity of this fear-specific muscle over time (please note that regarding the main effect of the BO condition, only non-robust and marginally significant differences were observed between conditions; see the "Results and Discussion" section of study 1). These results show for the first time that anxiety BOs activate the receivers' facial musculature differentially and suggest that, despite the differences between fear and anxiety, their BOs may activate similar facial muscle configurations (i.e. the activation of *medial frontalis*).

Although contrary to our initial predictions, these results can be interpreted in light of a functional perspective of emotions (e.g. Adolphs and Andler 2018). Fear is a stress-related, high-vigilance emotional state (e.g. LeDoux and Pine 2016). When in a fear state, individuals contract specific facial muscles, such as the *medial frontalis* (and the *corrugator supercilii*), widening their eyes and nasal apertures. Consequently, their visual field is expanded, and their sniffing volume is increased, resulting in a state of increased sensory acquisition hypothesized to have evolved to facilitate the detection and coping with potential threat sources (see Susskind et al. 2008). Interestingly, a (chemo) signal of fear emitted by a fearful donor seems to have an alarm function, activating an adaptive state of increased sensory acquisition in receivers. This, in turn, facilitates the exploration of the surroundings (de Groot et al. 2012) and potentially the consequent detection of threat sources (e.g. Gomes and Semin 2021). Importantly, anxiety also involves high vigilance (e.g. Tovote et al. 2015; LeDoux and Pine 2016). Hence, the exposure to an anxiety (chemo) signal may also work as an alarm system, facilitating receivers' detection and coping with potential threat sources. This argument is supported by research showing facilitated defense responses (i.e. startle reflex) in individuals exposed to anxiety (vs. exercise) BOs (Prehn et al. 2006). Thus, the results of study 1 showing that anxiety BOs trigger an increased activity of a fear-related muscle (i.e. *medial frontalis*) constitutes additional evidence of this alarm function—it may increase receivers' sensory acquisition at the function of facilitating the detection and coping with potential hazardous events.

Regarding the *corrugator supercilii*, the results did not reveal a distinct pattern of activation triggered by anxiety (vs. exercise) BOs, nor replicate the typical effect of fear (vs. rest) BOs in activating this muscle. Hence, no strong conclusions can be reached regarding how anxiety BOs influence *corrugator supercilii*. As mentioned in the "Results & Discussion" section of study 1, this may have happened due to the distinct methodology used to prepare the "super-donors," or by the fact that BOs exposure was performed through an olfactometer instead of using the typical procedure when fEMG is collected, which is placing a vial containing the BO condition under the participants' noses (e.g. de Groot et al. 2014; Gomes et al. 2020). Hence, further research is needed to disentangle the effects of anxiety BOs on the activity of the corrugator supercilii.

Importantly, these results constitute the first direct evidence that anxiety and fear chemosignals may activate similar processes in their receivers regarding facial musculature configuration. This suggests that, although fear and anxiety are emotional states elicited in distinct situations, the released chemosignals may carry similar information, leading receivers to states of increased vigilance in both cases. Additionally, one may also speculate that, as fear and anxiety BOs trigger similar changes in receivers' facial muscles, they may present similar chemical compositions as well. However, the analysis of BOs' chemical composition is still in its infancy (e.g. Smeets et al. 2020), and, to the best of our knowledge, no study has examined these possible differences so far. Hence,

this argument remains purely speculative, needing future research to address it.

Moreover, several limitations of these results should be highlighted, with special emphasis on the ones related to the results' generalizations and ecological validity. These findings relied on sweat collected from a limited sample of donors with very specific inclusion criteria (e.g. non-smoking young Caucasian heterosexual males) undertaking a very strict protocol to avoid sweat contamination (e.g. dietary restrictions). The receiver participants were also selected following a specific set of inclusion criteria (e.g. non-smoking young Caucasian heterosexual females with no severe olfactory impairments). Moreover, the BOs were collected in pads from donors' armpits and introduced directly into receivers' nostrils using an olfactometer. Considering all these methodological constraints, it is difficult to know whether these findings generalize to, for instance, other age groups and ethnic backgrounds or even occur when sweat is collected from female donors or male receivers are used. It is also hard to predict whether they occur in social situations in non-laboratory contexts (for similar arguments, see e.g. Roberts et al. 2022). Future research may consider addressing such limitations.

Additionally, a face discrimination task (Kamiloğlu et al. 2018) was employed in study 1 to explore whether exposure to fear (vs. rest) and anxiety (vs. exercise) BOs trigger distinctly facilitated face perception patterns in receivers. Research so far has been suggesting that exposure to fear BOs explicitly enhances the processing of fear (vs. other negative) facial expressions (Kamiloğlu et al. 2018; Silva et al. 2020; de Groot et al. 2021 but see de Groot et al. 2018), while anxiety (vs. exercise) BOs enhance the processing of negative and neutral facial expressions in general (e.g. Wudarczyk et al. 2016; Rocha et al. 2018). However, study 1's results do not support the effects of anxiety BOs on face perception, nor does it replicate Kamiloğlu et al.'s (2018) results that demonstrated fear BOs to enhance the discrimination of fear facial expressions (but no other emotional faces) as negative.

Two additional replication studies could also not replicate the findings reported by Kamiloğlu et al. (2018), suggesting that the authors' results should be interpreted cautiously. Please note that, as a limitation, the sample sizes in these replication attempts, although comparable to the one in Kamiloğlu et al. (2018), are not justified based on a power analysis. Hence, a pre-registered replication of the authors' results is needed. Without further research, any argument about the reason behind the observed absence of emotional BOs' effects on face perception remains pure speculation. Nevertheless, a possibility that could be considered is that the research paradigm is not suitable to capture the perceptual changes induced by being exposed to the two distinct BOs. This may be due to the procedure used to introduce noise in the visual stimuli. Kamiloğlu and colleagues (2018) utilized several Gaussian filters to "degrade" the facial stimuli. However, these filters change the stimuli's spatial frequency (SF) properties (for more information regarding the filters and their potential impact on image visual processing, see Perfetto et al. 2020). SF has been proven to play an important role in face perception (e.g. Vuilleumier et al, 2003), and filtering some of its bands always impairs the visual processing (e.g. higher reaction times in detecting faces filtered for high or low SF bands when compared to intact pictures; Stein et al. 2014). Using Gaussian filters, Kamiloğlu et al. (2018) did not simply introduce noise in the facial stimuli; they also filtered some of the SF components of the stimuli that may be crucial for observing the influence of emotional BOs on face perception. Future research should consider introducing noise in the images using methodologies that leave their low-order visual properties, such as SF, intact or controlled to prevent potential confounding (e.g. Random image structure evolution; see Sadr and Sinha 2004). Notably, several studies have evidenced that fear and anxiety BOs have a modulatory role in face perception using ambiguous emotional morphed faces (e.g. faces containing fear and happiness, fear and anger, or fear and disgust features; Mujica-Parodi et al. 2009; Zhou and Chen 2009; Zernecke et al. 2011; Wudarczyk et al. 2016; de Groot et al. 2021). Future research examining the differences and similarities between fear and anxiety BOs on face perception might consider this methodology. It "degrades" the emotional information on the faces (i.e. make them ambiguous, giving space for the influence of BOs in their processing; Damon et al. 2021), keeping their low-order visual properties almost intact.

In conclusion, we have shown that anxiety BOs, as fear ones, activate in receivers the *medial frontalis*—a facial muscle associated with an increased sensory acquisition. Additionally, aiming to uncover similarities and differences in face perception under the exposure to fear and anxiety BOs, we employed a face discrimination task developed by Kamiloğlu and colleagues (2018) and could not replicate their findings. This suggests that the authors' results should be interpreted cautiously, highlighting the need for a pre-registered replication of their results. Nevertheless, future research examining face perception under exposure to fear and anxiety BOs should consider more carefully controlled paradigms to avoid potential confounds related to stimuli's low-order visual properties.

Supplementary material

Supplementary material is available at *Chemical Senses* online.

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Author contributions

BMP, MAMS, and GRS planned the research. NG executed the research, analyzed the data, and wrote the final paper. All the authors read and revised it.

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Conflict of interest

We have no conflicts of interest to report regarding the findings of these studies.

Ethical approval

The host institutions' ethics committee approved all sweat collection and exposure procedures. They were conducted following the standards of the American Psychological Association and the guidelines of the Declaration of Helsinki.

Consent to participate

All participants gave their informed consent before participating in the study. At the end of the data collection session, all participants were debriefed about the goals of the different studies.

Consent for publication

All participants gave their informed consent to publish their data.

Data availability

The raw data and the databases used for the analyses reported in the paper are available online: https://osf.io/r8fw5. All data preprocessing steps are available, upon request, from the corresponding author. All data, the analyses output, and the code are available online: https://osf.io/r8fw5.

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