Human body odors of happiness and fear modulate the late positive potential component during neutral face processing: a preliminary ERP study on healthy subjects

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***Abstract*— Human body odors (HBOs) are powerful stimuli that can affect emotional, cognitive and behavioral processes. However, the characterization of the physiological response to HBOs is still to be fully investigated. Here, we analyzed the self-assessed emotion perception and the EEG event-related potentials (ERP) on 17 healthy young women during a simultaneous visual-olfactory stimulation. Particularly, we evaluated the effect of happiness and fear HBO on the amplitude of ERP waveforms elicited by neutral face processing. In addition, we evaluated the subjective valence and arousal perception of the presented neutral faces by means of the self-assessment-manikin test. We observed a significant increase in the amplitude of the late positive potential (LPP) for central left sites (i.e., C3) during the administration of HBOs with respect to clean air. On the other hand, we did not observe any significant change in the subjective valence and arousal scores as well as for the early components of the ERP (i.e., P100, N170, Vertex-Positive-Potential). Our preliminary results suggest that fear and happiness HBO can induce a protracted increase in the LPP, and possibly reflect an automatic and sustained engagement with emotionally significant content.**

I. INTRODUCTION

Recent advances in olfactory research have supported the hypothesis of a significant role played by human body odors (HBOs) in shaping our emotional, cognitive and behavioral processes [1]. Humans can transfer socially-relevant information (such as age, health status, and personality traits) and emotional states via HBOs [2], [3], [4]. Indeed, the exposure to body odors, collected from other individuals (i.e., senders) while feeling specific emotions, has shown a partial reproduction of the affective, perceptual, and behavioral state of the sender in the receiver: a phenomenon called "emotional contagion" [5]. Behavioral analyses highlight that happiness body odors can induce in the receivers a happier facial expression than neutral body odors and a global processing style, which is typical under positive mood conditions [6]. Moreover, stress-evoked HBOs have been able to modulate visual perception, for instance by reducing the perceptual acuity to happy facial expressions as well as by augmenting acuity to negative facial expressions [7]. However, it is worthwhile noting that this behavioral and perceptual modulation is even more relevant when the stimulus modulated (e.g., facial expression) is ambiguous [8]. Nevertheless, despite the ability of HBOs to convey or modulate emotional information, a characterization of the physiological response to such phenomenon is still to be fully investigated.

One of the most used and effective techniques to study the processing of emotional stimuli is the analysis of event-related potentials (ERP) obtained from electroencephalography (EEG). In this context, several studies of emotional processing have focused on the late positive potential (LPP), a prolonged positive deflection in the ERP, which arises in response to emotionally arousing stimuli. Indeed, it has been suggested that the modulation of the LPP responds to stimulus significance [7]. Recently, an ERP study involving simultaneous stimulation by means of visual and body odors stimuli has shown that HBO collected during anxiety state are able to modulate the processing of facial expressions at both an early (P1/N1, N170) and a late level (LPP) [9].

Given the role of odors in affecting the subjective perceptual experiences and the psychophysiological processing of emotional stimuli, this work aims to investigate whether happiness and fear body odors could modulate subjective and physiological processing of neutral facial expression in healthy individuals. To this end, we acquired EEG data from healthy young women during simultaneous visual-odor stimulation. HBOs of happiness and fear were extracted from sweat samples collected during happy and fearful emotional states induced by happy and scary movie clips, respectively [6], [10]. In this preliminary study, we analyzed ERP amplitude to infer significant changes in standard components of face processing. Specifically, we hypothesized that neutral faces would be processed differently based on HBO condition.

II. MATERIALS AND METHODS

A. Subject recruitment

We enrolled 17 non-smokers, right-handed, normosmic (tested through the Sniffin Sticks test [11]), medically healthy female subjects free from psychotropic medication, and assessed with an ad-hoc anamnestic interview. We purposely chose only female participants for two main reasons. On the one hand, it is known from the literature that gender influences the processing of HBOs [12], [13]. On the other hand, women tend to exhibit a greater preference for social emotional stimuli with respect to men [14], [15]. Participants with any condition that could affect olfactory functions were

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excluded from the study (e.g., subjects suffering of respiratory disease, illness, cold, allergy). All subjects signed an informed consent for participating to the study in accordance with the declaration of Helsinki. The experimental protocol was approved by the local Ethics Committee, University of Padua (prot. no. 3667).

B. Stimuli preparation

Odor stimuli consisted of clean air (CA), happiness HBO (h-HBO) and fear HBO (f-HBO). HBOs were prepared from sweat pads collected from the armpit of 4 men and 4 women all healthy heterosexual and Caucasian according to previous studies (e.g., [10]). These stimuli corresponded to sweat samples acquired during happy and fearful emotional states induced by happiness and scary video clips, respectively [6], [10]. The senders in the fear condition sat alone while watching the film clips, while those in the happiness condition sat in groups of three participants. Afterwards, they were asked to rate how angry, fearful, sad, happy, disgusted, neutral, surprised, calm and amused they felt on a 7-point Likert scale, which were used to indicate low/high arousal and positive/negative affect. Sweat pads were kept into separate vials and stored in a -80°C freezer [16]. None of the senders participated to the experiment as a receiver. Odors were administered with a 3-channel olfactometer purposely developed to delivery odors from pads at a slow flow rate (50ml/min).

Visual stimuli consisted of 336 neutral expressive faces from the Chicago Face Dataset [17]. More specifically, based on the ratings provided in [17], images were distributed across the odor blocks to match for physical facial features, actors' age, attractiveness, femininity, masculinity, trust- worthiness and for the seven levels of emotional expressiveness.

Odor and visual stimuli delivery were synchronized among them as well as with the physiological data acquisition device by means of an e-prime system and custom scripts.

C. Experimental protocol

In Fig. 1, we report a schematic representation of the stimulation protocol. Odor and images were presented in a randomized block design. The odor stimulation session consisted of 21 blocks (7 displaying CA, 7 displaying h-HBO, 7 displaying f-HBO). In each block, lasting on average 36s, only one odor and 6 images were presented. Each picture was presented for 2s and preceded by a 2s grey interval during which a white fixation cross was displayed at the center of the screen. The inter-stimulus-interval was in the range (1-3) s. At the end of each block, the images were presented again to the subjects who rated them in terms of valence and arousal level by means of a computerized version of the 9-point Valence and Arousal scales of the Self-Assessment Manikin (SAM) [18]. During the rating blocks only clean air was presented.

D. EEG acquisition and preprocessing

We acquired EEG signals with a 256-channel high-density Geodesic EEG System 300 from Electrical Geodesics, Inc. (EGI) at a sampling rate of 500Hz. EEG was analyzed offline with EEGLAB [19] and MATLAB custom scripts (the Mathworks Inc.). All signals were band-pass filtered between 0.1Hz and 45 Hz and downsampled to 100Hz. Bad channels were removed if flat for more than 5s or if poorly correlated with their adjacent channels ($\rho < 0.8$). Removed channels were recovered through spherical spline interpolation. Preprocessed signals were then re-referenced to the numeric average of all channels. Afterwards, we obtained epochs by segmenting the signals from -1000ms to 2000ms around visual stimulus onset. For each epoch the subtractive baseline ranging from -1000ms to 0ms was removed. After a visual inspection, the epochs contaminated by abrupt signal changes were removed. Cleaned epochs were decomposed into sets of independent components (ICs) by means of independent component analysis [20]. ICs corresponded to statistically independent time series associated with static spatial maps, that could represent sources of brain activity or different type of artifacts (e.g., ocular and other sources of noise). Components were automatically associated to brain activity or artefact with the IC label EEGLAB plug-in [21], and EEG signals were reconstructed with the only contribution of brain ICs.

E. ERP analysis

ERPs were analyzed in the whole epoch range at several regions-of-interest (ROI), selected based on specific hypotheses to reduce the number of comparisons. Each ROI was made of a set of electrodes of interest and their adjacent neighbors. Specifically, we focused on occipital (O1/2) and parietal electrodes (P3/4, P7/8), for studying standard VEP potentials (i.e., P100, N170, P200 and N250 components [22]) and on central (C3/4, Cz; [23]) and central-parietal (CP5/6; [24]) for the vertex positive potential (VPP) and the late positive potential (LPP), respectively. To match the electrodes' position between the geodesic net and the international 10-10 positions we used the approximate correspondence reported in [25]. Then, we averaged the time-courses of the electrodes contributing to each ROI and obtained average ERPs for each ROI.

F. Statistical analysis

SAM scores were analyzed with a 1x3 ANOVA with odor condition as a factor (i.e., happiness vs. fear vs. neutral). We performed two separate analyses for the Valence and Arousal ratings.

Analogously, a 1x3 ANOVA with odor condition as a factor was employed to analyze ERP differences in the time

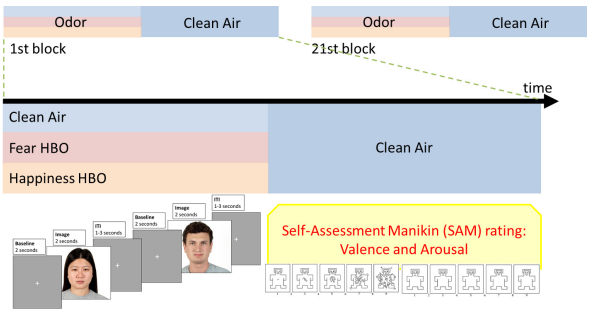

Fig. 1. Experimental protocol. For each block one among CA, h-HBO or f-HBO odor and 6 neutral images were presented.

range from 0 to 2000ms after stimulus onset. Specifically, we tested the null hypothesis (H0) of no differences in ERPs amplitudes between odors. We assessed whether differences among conditions were significant with a montecarlo-permutation procedure with n=10000 permutations, followed by cluster correction procedure to handle multiple hypothesis testing [26]. Post-hoc analyses were carried out by means of paired t-test with Bonferroni correction.

III. RESULTS

A. SAM rating

The ANOVAs on valence and arousal ratings did not show any significant effect of odor factor (all p-values>0.20). In Table I, we report the mean and the standard deviation of the ratings of valence and arousal for each odor condition. The null hypothesis of equal effect sizes among stimuli could not be rejected, indicating that subjects rated all stimuli equal in terms of valence and arousal.

TABLE I
RATINGS OF SELF-REPORTED VALENCE AND AROUSAL FOR EACH ODOR CONDITION: MEAN (SD).

	ODOR		
	CA	**h-HBO**	**f-HBO**
Valence	5.05 (0.42)	4.49 (3.29)	5.01 (0.45)
Arousal	3.15 (1.30)	3.29 (1.25)	3.21 (1.23)

B. ERP analysis

Out of all subjects recruited for the study two were removed because of irreducible artefacts in the EEG signals. For the remaining subjects, an average of 38 artefact-free epochs per condition was obtained after visual inspection. The results of the ANOVAs revealed significant differences among the three odor-related potentials only for C3 as shown in Fig. 2. Particularly, significant differences among odors were found in the late components of the ERP (LPP) for ROI centered on C3. This cluster highlighted a consistent long-lasting effect of h-HBO and f-HBO, with respect to CA, on ERP waveforms. More specifically, we observed that ERP amplitudes were higher during the presentation of HBOs, compared to CA. Interestingly, these differences occurred in a physiologically plausible time-range, by starting around 1500ms and protracted until the end of the epoch (i.e., late LPP according to [27]). Within this time-range, post-hoc analyses highlighted that differences in ERP amplitude were more widespread for the CA vs. f-HBO contrast, than for the CA vs. h-HBO one.

We did not observe any relevant significant difference among odors for the other considered ROIs, nor for the early components of the ERP (i.e., P100, N170, VPP), which are thus not reported.

IV. DISCUSSION

In this preliminary study, we investigated the modulatory effects of happiness and fear body odors on neutral face processing through ERP analysis. To reduce the number of multiple comparisons, based on the literature [22], [23], [24], we focused on a set of ROIs to evaluate the influence of happiness and fear body odors on well-established ERP components of face processing, i.e.: P100, N170, VPP, LPP, as well as on behavioral measures of valence and arousal obtained with the SAM. Although preliminary, our results highlighted that emotional body odors of happiness and fear induce changes in LPP amplitude compared to clean air.

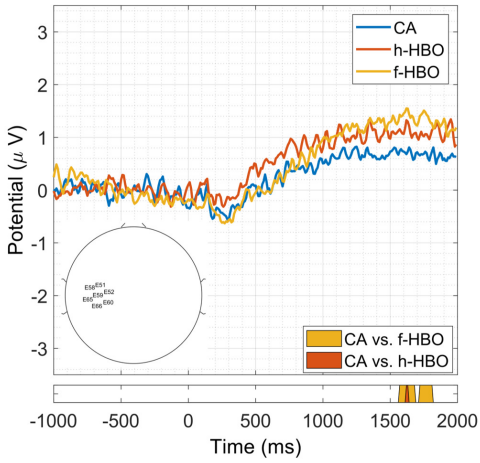

Fig. 2. ERP plots for C3 ROIs. (top) ERP time courses for CA, h-HBO and f-HBO. (bottom) Time-ranges for which the results of the 1x3 ANOVA were significant along with Post-hoc outcomes ($p < 0.05$).

The statistical analysis of arousal and valence ratings of the neutral faces did not highlight any significant difference among odor contexts. As expected, the faces were rated to be neutral and low arousing, and this is regardless the odor condition. Nevertheless, we must also consider that, although previous studies showed that neutral faces were rated as more pleasant/unpleasant during pleasant/unpleasant odor contexts [28], other studies did not find any significant effect of odor context on neutral face ratings [29], [30]. Yet, the nature of the HBOs used in this study (i.e., h-HBO and f-HBO), may behave differently with respect to common odors.

Group analysis on ERP components highlighted significant differences in the LPP component at C3 cluster for different odors. More specifically, we observed that ERP amplitude was higher for the happiness and fear body odors, compared to the clean air. This result is in line with the notion that positive and negative emotional stimuli trigger a higher amplitude of LPP at central and parietal sites, compared to neutral stimuli. Accordingly, our results may suggest that the emotional body odors can modulate the cortical activity to some extent comparable to other emotional stimuli widely used in the literature, such as visual or acoustic stimuli. Of note, differences between HBOs and CA were more consistent and widespread for the f-HBO, compared to h-HBO. In this view, this result offer interesting insights for further investigation on the central physiological response to HBOs characterizing different emotional states.

Interestingly, we observed these changes in LPP amplitude after $1500ms$ from stimulus onset. Previous findings found that HBOs affect the early LPP [9], but limiting the analysis to time windows less than 1 s. However, emotionally salient stimuli of different nature can induce notable differences also in the late LPP [7]. Such prolonged increase in the late positive potential was suggested to reflect a sustained

engagement with emotionally significant content [31], [32], as well as a repeated activation of cortico-limbic appetitive and defensive systems throughout the duration of stimulus presentation [7]. In this view, we can hypothesize that the same mechanism could be involved in the processing of ambiguous stimuli (i.e., neutral faces) when simultaneous HBOs modulate their brain processing,

Overall, this suggests that cortical positivity may be a more sensitive measure than subjective ratings in discriminating human body odors. Indeed, this measure may indicate unaware attentional and motivational processes that are not perceivable with subjective reports of emotional experience.

The exploratory nature of our work led to a high number of statistical tests performed for comparing conditions. Accordingly, we believe that confirmatory analysis on a bigger sample size will be needed. Nevertheless, the observed trends in late LPP are consistent with previous literature in terms of time-range, spatial location and emotional nature of stimuli [7]. In this view, our study found interesting results in the effects of fear and happiness HBO on neutral face processing in healthy subjects.

Given the ability of body odors to convey emotional information, and their ability to modulate cortical activity, future studies should explore body odors processing in affective disorders characterized by social impairments, such as depression and social anxiety, increasing our understanding of these complex disorders. Additionally, it would be of particular interest evaluating how brain connectivity involved in olfactory processing [33], [34] is affected by HBOs.

In conclusion, although preliminary, our results suggest that emotional HBOs of fear and happiness possibly modulate the late components of the LPP. Here, we analyzed a restricted number of ROIs because of limited statistical power due to the relatively limited number of subjects. However, we believe that further investigation will be needed by increasing the number of subjects participating in the study, allowing for more detailed analysis of changes in ERP components, as well as for confirmatory results on the neural correlates of happiness and fear body odor processing.

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