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Second-hand stress: inhalation of stress sweat enhances neural response to neutral faces

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This study investigated whether human chemosensory-stress cues affect neural activity related to the evaluation of emotional stimuli. Chemosensory stimuli were obtained from the sweat of 64 male donors during both stress (first-time skydive) and control (exercise) conditions, indistinguishable by odor. We then recorded event-related potentials (ERPs) from an unrelated group of 14 participants while they viewed faces morphed with neutral-to-angry expressions and inhaled nebulized stress and exercise sweat in counter-balanced blocks, blind to condition. Results for the control condition ERPs were consistent with previous findings: the late positive potential (LPP; 400–600 ms post stimulus) in response to faces was larger for threatening than both neutral and ambiguous faces. In contrast, the stress condition was associated with a heightened LPP across all facial expressions; relative to control, the LPP was increased for both ambiguous and neutral faces in the stress condition. These results suggest that stress sweat may impact electrocortical activity associated with attention to salient environmental cues, potentially increasing attentiveness to otherwise inconspicuous stimuli.

Keywords: emotion; attention; event-related potential; late positive potential; pheromones

INTRODUCTION

The existence of alarm pheromones—chemosensory stress cues communicated between members of the same species—is well established in non-human mammals; when animals inhale odors secreted by acutely stressed conspecifics, they express neurobiological and behavioral changes consistent with heightened threat assessment (Fanselow, 1985; Zalaquett and Thiessen, 1991; Dielenberg and McGregor, 2001; Dielenberg *et al.*, 2001; Kikusui *et al.*, 2001). In our fMRI experiment and its subsequent replication, we showed that humans also activate the amygdala during inhalation of sweat taken from an independent sample of emotionally stressed individuals, with exercise sweat as a control (Mujica-Parodi *et al.*, 2009). Importantly, participants were unable to perceptually differentiate between the sweat odors, suggesting that the amygdala response was specific to emotional, rather than olfactory, discrimination. Psychophysiological and behavioral research have additionally demonstrated that inhalation of human stress sweat augments the defensive startle reflex (Prehn *et al.*, 2006; Pause *et al.*, 2009) as well as enhancing perception and discrimination of fearful (Zhou and Chen, 2009) and angry (Mujica-Parodi *et al.*, 2009) faces.

Based on this work, we tested the novel hypothesis that chemosensory stress cues may enhance neural activity that indexes perceptual salience and sustained attention. In particular, we focused on the late positive potential (LPP) component of the event-related potential (ERP). The LPP is observed as a sustained parietally maximal positivity that begins ~200 ms following stimulus presentation (Sutton *et al.*, 1965; Foti *et al.*, 2009). The LPP is larger (i.e. more positive) for emotional than neutral stimuli, and this effect is evident throughout the duration of picture presentation (Schupp *et al.*, 2004; Leppanen *et al.*, 2007; Holmes *et al.*, 2008; Foti *et al.*, 2009; Hajcak *et al.*, 2009; MacNamara and Hajcak, 2009). In a series of studies, we have shown that the magnitude of the LPP is sensitive to stimulus meaning and salience: when pictures are preceded by negative compared to neutral descriptions, the LPP is increased (Foti and Hajcak, 2008; Macnamara *et al.*, 2009). Further, directing attention to more or less arousing aspects within unpleasant pictures dynamically modulates the amplitude of the LPP (Dunning and Hajcak, 2009; Hajcak *et al.*, 2009). Based on these and other similar data, we have argued that the LPP indexes sustained attention based on the appraised salience of visual stimuli (Hajcak *et al.*, 2010). We hypothesized that stress sweat might generalize threat appraisal from angry faces (i.e. overt threat) to ambiguous and neutral faces (i.e. potential threat)—and that the increased salience of neutral

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and ambiguous faces under stress sweat conditions would be reflected in an increased LPP.

MATERIALS AND METHODS

Participants

Olfactory stimuli were obtained from the sweat of 64 male 'Donors' (ages 18–50 years, $\mu = 25$, s.d. = 9). All Donors participated in two conditions: a 'stress' condition, which consisted of a first-time tandem skydive (15 min rise to altitude, jump at 4 km/13 000 ft., with 1 min freefall and 4 min under the parachute before landing) and an 'exercise' condition conducted for the same length of time (20 min running on a treadmill at maximum comfort level) and at the same time of day. Previous analyses of cortisol and self-reported state anxiety confirmed that the experimental paradigm reliably induced an intense state of emotional stress and that exercise provided an acceptable control (Mujica-Parodi *et al.*, 2009). We tested the effects of the Donor sweat samples on a non-overlapping group of 14 'Detector' participants [males ($N = 6$): ages 20–22 years, $\mu = 21$, s.d. = 1; females ($N = 8$): ages 20–35 years, $\mu = 24$, s.d. = 5]. Detectors inhaled the stress and exercise sweat, blind to condition, while they viewed a range of stimuli during which we recorded EEG.

This study was approved by the Institutional Review Board of State University of New York at Stony Brook; all participants provided informed consent.

Stimuli

Sweat samples were obtained from Donors using previously described (Mujica-Parodi *et al.*, 2009) procedures, which were designed to prevent bacterial growth ensuring samples were odor-free, as well as to preserve both hydrophilic and hydrophobic components. A previous double-blind forced-choice odor-discrimination experiment, using the same stimuli presented for this study, determined that subjects rated test and control sample odors as 'neutral' and were unable to perceptually distinguish them (Mujica-Parodi *et al.*, 2009). The same analysis for the subset of subjects who participated in this study confirmed that these subjects were unable to discriminate between the stress and exercise sweat [one-sample *t*-test: $t(13) = -0.62$, $P = 0.547$] and rated them equally on a Likert scale for strength [ANOVA: $F(1, 13) = 1.36$, $P = 0.27$] and valence [ANOVA: $F(1, 13) = 0.51$, $P = 0.487$]. Sweat samples were extracted, diluted with water and presented to participants via a proprietary nebulizing olfactometer optimized for semi-volatiles. Sweat samples were presented only during inhalation, which was controlled using auditory cues to pace breathing. These included two tones (2500 ms each) of varying pitch and equal volume. Visual stimuli were the Pictures of Facial Affect (Paul Ekman Inc., Oakland, CA, USA). We produced nine levels (10–90%) of morph (MorphMan 3.0, STOIK Imaging, Moscow Russia) equally distributed between the Neutral (0%) and Angry (100%) poles. Three male faces (E.M., J.J., P.E.) were selected based on a non-olfactory pilot experiment ($N = 12$) as they were the most consistent in producing classic psychometric curves.

Procedure

Each trial of the task consisted of a 500-ms rest period followed by a cued 2500-ms inhalation, a 200-ms presentation of a face and a cued 2500-ms exhalation. Participants were asked to attend to a fixation-cross presented at the center of the screen in the periods between faces. The task consisted of four runs with seven trials per morph level (i.e. 63 trials per run). Each run started with six random presentations at 0 and 100% levels; these were not included in the analyses, but functioned to 'pin' the Neutral and Angry endpoints. We then followed with 63 presentations of the 10–90% morphed faces lasting a total of 6.5 min. The morph levels were selected randomly (1/9), as was the particular face (1/3). Researchers monitored participants from outside the room via both respiration data and video camera in order to ensure respiratory compliance. The total experiment lasted for 30 min, with 2-min breaks between runs, counterbalanced for order.

ERPs

We recorded continuous EEG using the ActiveTwo BioSemi system (BioSemi, Amsterdam, The Netherlands). Recordings were taken from 64 scalp electrodes based on the 10/20 system, as well as from two electrodes that were placed on the left and right mastoids. The electrooculogram generated from blinks and eye movement was recorded from four electrodes: two ~1 cm above and below the participant's right eye, one ~1 cm to the left of the left eye and one ~1 cm to the right of the right eye. The ground during acquisition was formed by the Common Mode Sense active electrode and the Driven Right Leg passive electrode.

All the bioelectric signals were digitized on a laboratory microcomputer using ActiView software (BioSemi, Amsterdam, The Netherlands) and were analyzed off-line using Brain Vision Analyzer (Brain Products, Germany). EEG was sampled at 512 Hz. Off-line, all data were re-referenced to the numeric mean of the mastoids and band-pass filtered between 0.1 and 30 Hz; the EEG was corrected for blinks and eye movements using the method developed by Gratton *et al.* (1983). In addition, a semi-automated procedure was used to identify and reject physiological artifacts according to the following criteria: a voltage step of >50.0 μV between sample points, a voltage difference of >300.0 μV within a trial and a maximum voltage difference of <0.50 μV within 100-ms intervals. Remaining artifacts were detected through visual inspection.

ERPs were constructed for each of the nine morph levels (10–90% anger), which were also averaged for neutral (10–30% anger), ambiguous (40–60% anger) and angry (70–90% anger) faces. For each ERP average, the 200-ms window prior to picture onset served as the baseline. The

LPP was scored as the average activity at POz, where it was numerically maximal, and was evaluated in both an early (250–400 ms) and late (400–600 ms) window. In addition to the LPP, the earlier N1 (i.e. 140–160 ms) and P2 (i.e. 190–220 ms) were also analyzed at FCz and Cz, respectively, where they were numerically maximal. To assess the general role of the stress sweat on the amplitude of the N1, P2 and the early/late portions of the LPP, as well as potential interactions with morph-level, we performed a Repeated-Measures ANOVA, with within-subject factors of 'stress *vs* exercise' and 'morph-level' (10–90%). To further interpret significant ANOVA results, we compared paired *t*-tests for each of the averaged 'neutral', 'ambiguous' and 'angry' levels, comparing between 'stress' and 'exercise' conditions. All subjects had between 12 and 14 trials per each of the nine morph levels, with >99% of instances having 13 or 14 trials per morph level. Each ERP average (which was combined from three morph levels to make 'neutral', 'ambiguous' and 'angry' conditions) contained between 39–42 trials. No subjects or faces were lost due to EEG artifact, nor was the number of trials different between the two conditions [$F(1, 13) < 0.001$, $P = 1.00$].

RESULTS AND DISCUSSION

Compared to exercise, the stress condition was associated with increased LPPs overall [early portions of the LPP: $F(1, 13) = 8.02$, $P = 0.014$; late portion of the LPP: $F(1, 13) = 5.38$, $P = 0.037$; Figure 1A]. Interactions between arousal and morph level were also significant for the late, but not early portion of the LPP [early portions of the LPP: $F(8, 6) = 0.64$, $P = 0.731$, late portion of the LPP: $F(8, 6) = 6.24$, $P = 0.019$). Although normative LPP modulation by morph level was found for the exercise condition in the later LPP window [$F(8, 104) = 3.25$, $P = 0.002$], it was notably absent for the stress condition [$F(8, 104) = 0.79$, $P = 0.611$]. Thus, more angry faces were associated with a larger LPP only in the exercise (i.e. control) condition. LPP amplitude in the later window for each averaged level is presented in Figure 1A. Paired *t*-tests for each averaged (i.e. neutral, ambiguous, angry) level (Figure 1B) revealed that the later portion of the LPP was increased in response to the stress sweat for the neutral stimuli [$t(13) = 2.53$, $P = 0.025$], with trend-level increases for ambiguous [$t(13) = 2.01$, $P = 0.066$] stimuli. No differences in condition were observed for overtly threatening stimuli [$t(13) = -0.79$, $P = 0.44$]. The increased LPP in response to neutral faces during stress compared to exercise sweat is depicted in Figure 2: the average activity from 400 to 600 ms was more positive over occipital/parietal sites (Figure 2A), and is evident as an increased positivity at POz (Figure 2B). Investigating the earlier N1 and P2 ERP components confirmed that the stress sweat effects were specific to the LPP: results for the N1 and P2 components were negative for all of the contrasts described above.

Overall, ERP results suggest that stress sweat does not impact ERPs that have been linked to selective attention (i.e. the N1 and P2; Luck and Kappenman, 2011). However, stress sweat enhanced both the early and late portions of the LPP elicited by all stimuli—suggesting that alarm pheromones may increase the salience of biologically relevant faces. Moreover, stress sweat led to a specific enhancement of the late portion of the LPP in response to non-threatening faces. Thus, stress sweat increased processing of all faces beginning ~250 ms after stimulus presentation. This effect was larger for neutral and ambiguous faces in the later window because both stress and control sweat produced the normative increase in the later LPP in response to angry faces.

Results for the exercise condition were consistent with previous results on emotional processing, in which aversive stimuli are associated with an increased LPP (Schupp *et al.*, 2004; Leppanen *et al.*, 2007; Holmes *et al.*, 2008; Foti *et al.*, 2009; Hajcak *et al.*, 2009; MacNamara and Hajcak, 2009). Ambiguous faces did not elicit an increased LPP in the

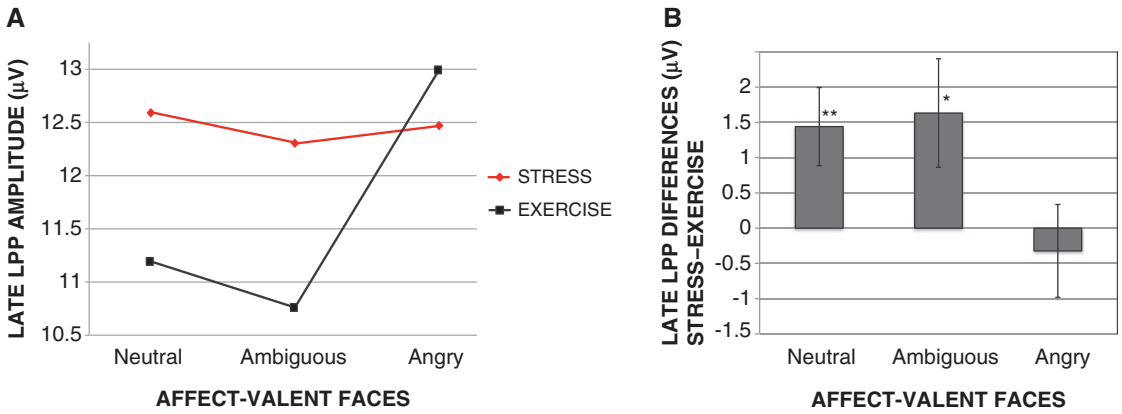

Fig. 1 Stimulus-locked ERP amplitudes in the later LPP window, collected as participants viewed affect-valent faces while inhaling the stress and exercise sweat from a non-overlapping group of male donors (**A**), and differences between stress and exercise conditions for each average (i.e., neutral, ambiguous, angry) (**B**). Participants showed overall heightened LPP during inhalation of stress sweat (repeated-measures ANOVA, early portion of LPP: $P = 0.014$, late portion of LPP: $P = 0.037$), with the later LPP response showing particular increases to otherwise neutral stimuli (* = $p < .10$; ** = $p < .05$).

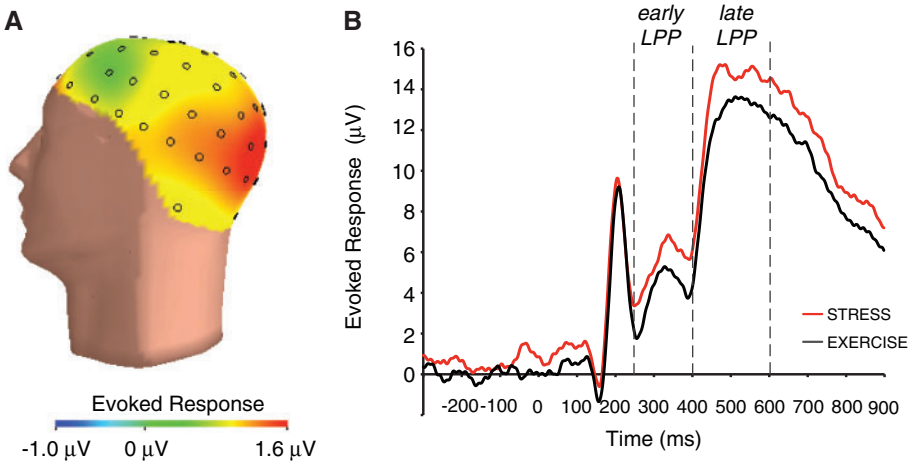

Fig. 2 Activation map (**A**) and waveform at POz (**B**) show stress, as compared to exercise, sweat increases activation of visual cortical processing in the time-range of the LPP (400–600 ms) after viewing affect-neutral (10–30% angry) faces.

exercise condition; to our knowledge, no previous studies have examined whether the LPP is larger for ambiguous facial expressions of anger, although there is evidence suggesting that only maximally angry faces potentiate the defensive startle reflex (Dunning *et al.*, 2010). In contrast, the stress condition was associated with heightened LPP regardless of emotional expression; differences between fear and exercise sweat were seen most prominently in response to neutral facial stimuli which, under normal conditions, would not elicit an increased LPP.

Our results suggest that chemosensory stress cues may impact electrocortical activity that indexes sustained attention to salient environmental stimuli, supporting previous studies of human stress sweat showing increased defensive startle (Prehn *et al.*, 2006; Pause *et al.*, 2009), amygdala activation (Mujica-Parodi *et al.*, 2009), cognitive performance (Chen *et al.*, 2006), and evaluation of ambiguously affective stimuli (Mujica-Parodi, *et al.*, 2009; Zhou and Chen, 2009). The pattern of observed LPP results shows that stress sweat had a specific effect on neutral and ambiguous facial stimuli—there was no evidence for a preferential impact of fear sweat in response to maximally angry faces. One possible interpretation of these data is that chemosensory stress cues may act as a low-resolution alert. By increasing overall vigilance, the stress cues may enhance sensory processing of subtle environmental features that might otherwise be overlooked (in our study, by augmenting visual processing of inconspicuous visual stimuli), thereby increasing receptivity to more detailed information in the assessment of potential threat.

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