Long-term Academic Stress Enhances Early Processing of Facial Expressions

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Abstract

Exposure to long-term stress can lead to a variety of emotional and behavioral problems. Although widely investigated, the neural basis of how long-term stress impacts emotional processing in humans remains largely elusive. Using event-related brain potentials (ERPs), we investigated the effects of long-term stress on the neural dynamics of emotionally facial expression processing. Thirty-nine male college students undergoing preparation for a major examination and twenty-one matched controls performed a gender discrimination task for faces displaying angry, happy, and neutral expressions. The results of the Perceived Stress Scale showed that participants in the stress group perceived higher levels of long-term stress relative to the control group. ERP analyses revealed differential effects of long-term stress on two early stages of facial expression processing: 1) long-term stress generally augmented posterior P1 amplitudes to facial stimuli irrespective of expression valence, suggesting that stress can increase sensitization to visual inputs in general, and 2) long-term stress selectively augmented fronto-central P2 amplitudes for angry but not for neutral or positive facial expressions, suggesting that stress may lead to increased attentional prioritization to processing negative emotional stimuli. Together, our findings suggest that long-term stress has profound impacts on the early stages of facial expression processing, with an increase at the very early stage of general information inputs and a subsequent attentional bias toward processing emotionally negative stimuli.

Keywords: long-term stress, event-related potentials, facial expression, P1, P2

1. Introduction

Studies in animals and humans have shown that long-term exposure to stress has a variety of consequences on cognition, emotion, and behavior (Lederbogen et al., 2011; Lupien, McEwen, Gunnar, & Heim, 2009; Schwabe, Dalm, Schachinger, & Oitzl, 2008). More specifically, participants suffering from long-term stress typically showed cognitive deficits (e.g., Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Liston, McEwen, & Casey, 2009; Wu et al., 2014) and augmented emotional responses (Lupien et al., 2009).

Behavioral research reported that a high level of long-term stress was associated with greater affective reactivity (van Eck, Nicolson, & Berkhof, 1998). It was also reported that individuals who are exposed to long-term stress perceived more negative emotion, such as anxiety and depression (Jun & Choi, 2015; Spada, Nikčević, Moneta, & Wells, 2008), and exhibit more negative emotional behavior, such as anger/hostility and aggressive behavior (Haller & Kruk, 2006; Sprague, Verona, Kalkhoff, & Kilmer, 2011). These findings are particularly relevant for patients with disorders associated with long-term stress, such as major depression and anxiety disorder (McEwen, 2004; McWilliams, Cox, & Enns, 2003; Rimmele & Lobmaier, 2012). Recently, some studies explored the underlying neurocognitive mechanism of these behavioral alterations under long-term stress (Golkar et al., 2014; Lederbogen et al., 2011). Functional magnetic resonance imaging (fMRI) results showed that a period of exposure to a stressful social environment (i.e., city living) was associated with increased amygdala activity (Lederbogen et al., 2011). In addition, long-term stress exposure impaired the connectivity between amygdala and anterior cingulate cortex (ACC), which correlated with the ability to down-regulate negative emotion (Golkar et al., 2014). There is, however, little knowledge about the dynamic processes when these alterations occur.

Event-related brain potentials (ERPs), with their higher temporal resolution, can be used to distinguish the neural sub-processes involved in behavior and have been utilized to investigate dynamic information processing (Hillyard & Kutas, 1983). By using ERPs, researchers are able to reveal the distinct effects of stress on the different processing phases. For example, one study found that early stages of visual processing were enhanced and late stages were attenuated when participants were under acute stress, suggesting that acute stress exerts dissociable effects on different stages of information processing (Shackman, Maxwell, McMenamin, Greischar, & Davidson, 2011).

Facial expressions are often used in ERP studies as they are one of the most important emotional signals for humans (Eimer & Holmes, 2007). Previous researches have proposed the models of face processing (e.g., Bruce and Young, 1986; Haxby et al., 2000) as well as the related ERP components (Calder, Rhodes, Johnson, & Haxby, 2011 for a review). Several components, including occipital-temporal P1 and N170, fronto-central P2, and central-parietal P3 are involved in the different stages of face processing (Eimer, Holmes, & McGlone, 2003; Luo, Luo, Feng, He, & Wang, 2010). The effect of emotional facial expressions begins as early as the P1 component, which reflect coarse perceptual discrimination of faces (Eimer et al., 2003; Vuilleumier and Pourtois, 2007). N170 reflects the structural encoding of facial features and configurations (Itier & Taylor, 2004; see Eimer, 2011 for a review). Enhanced processing as indicated by N170 effects were found for emotional expressions (Blau, Maurer, Tottenham, & McCandliss, 2007; Rellecke, Sommer, & Schacht, 2012), though there are still debates about the emotional modulation on N170 (e.g., Eimer, 2002). P2 is an early fronto-centrally distributed positivity which peaks approximately 180 ms after stimulus onset (see Eimer & Holmes, 2007 for a review). It is sensitive to the affective content of visual stimuli, reflecting rapid detection of

salient facial emotions and the mobilization of attentional resources (Bertsch, Bohnke, Kruk, Richter, & Naumann, 2011; Eimer & Holmes, 2007); P3 reflects a higher-level cognitive process, for example, distinguishing the emotion of facial expression (Campanella, Quinet, Bruyer, Crommelinck, & Guerit, 2002; Luo et al., 2010). Previous results have shown the increased P3 following the emotional compared to neutral faces (see Hajcak, MacNamara, & Olvet, 2010 for a review).

Several ERP studies have reported that stress or alterations of stress hormones alter neural activities to facial expressions (Bertsch et al., 2011; van Marle, Hermans, Qin, & Fernández, 2009; van Peer, Spinhoven, van Dijk, & Roelofs, 2009). For example, participants in the higher stressful provoked condition showed increased occipital P1 amplitudes compared to less provoked participants for all facial expressions (Bertsch et al., 2011). Also, larger right N170 amplitudes to angry faces were reported when participants were under the social evaluative stress of public speaking (Wieser, Pauli, Reicherts, & Muhlberger, 2010). As to the stress hormones, the oral administration of cortisol decreased the amplitude of early frontocentral P2 for faces, particularly for negative expressions (e.g., angry faces) in both healthy participants (Bertsch et al., 2011) and in patients with social anxiety disorder (van Peer et al., 2009). These results indicated that stress may enhance the early stages of face processing by increasing the attentional prioritization to the salient stimuli, especially to the negative emotional stimuli. Similar results were found in individuals with disorders associated with long-term stress. For example, anxious individuals showed higher amplitudes of P1 to faces regardless of expression, indicating the hypervigilance to the social salient stimuli (Peschard, Philippot, Joassin, & Rossignol, 2013). Besides, an early attentional bias for angry faces as indexed by P2 amplitudes were found in individuals with social anxiety disorder. But some researchers did not find effects on these early

components (Weinberg & Hajcak, 2010). These findings were either induced by a short period of stress, or within a population with stress-related disorder, and cannot be generalized to the healthy population. Moreover, given that acute and long-term stress impact body and brain differently (McEwen, 2004), the neural basis of how long-term stress impacts on emotional expression processing still remains largely elusive.

Therefore, we recruited healthy participants to investigate the mechanism of emotional information processing under stress. The present study aims to investigate the effects of long-term stress on neural dynamics for processing emotionally salient stimuli (i.e., facial expressions). The participants in the long-term stress group were college students who were undergoing a period of psychological stress for around six months as they prepared for a major academic examination. Previous research in humans has utilized long-term preparation for academic examination as a naturalistic stressor (Gonzalez-Cabrera, Fernandez-Prada, Iribar-Ibabe, & Peinado, 2014; Liston et al., 2009). In this study, participants in the stress group prepared for the National Postgraduate Entrance Exam (NPEE), which is one of the most important and highly competitive exams within the national educational system (Duan et al., 2013). Normally, students begin to effortfully prepare for about half a year before the exam. The acceptance rate into a graduate program following the exam has been less than 33% over the last ten years (Sohu.com, 2014). Questionnaires on the duration of preparation for the exam and perceived stress level were collected to assess the long-term stress.

Since previous studies have demonstrated that long-term exposure to stress was associated with greater reaction to emotional stimuli (Golkar et al., 2014; Lederbogen et al., 2011; Sprague et al., 2011), we predict that long-term stress would augment sensitivity to processing of emotional expressions. As some results showed that stress may enhance the early attentional

distribution to the salient stimuli, which is indexed by greater amplitudes of P1, N170 and P2 to facial expressions (Bertsch et al., 2011; van Peer et al., 2009; Wieser et al., 2010), we are expected enhanced amplitudes of early components to facial expressions for stress group compared with non-stress controls. Specially, we predicted increased P1, N170 and P2 to negative expressions for stress group compared with the control group. Besides, we will also examine whether the late ERPs to the facial expressions are affected by long-term stress.

2. Method

2.1 Participants

Sixty-three healthy male undergraduate students with normal or corrected-to-normal vision took part in the study. Forty-two of them (stress group) had spent about six months of preparation for the NPEE. The other 21 students were from the same college, matched in terms of age and grade, but did not participate in any academic examinations or interviews within one month before or after the experiment. Only male students participated in this study to avoid any confounding sex influence on stress effects (Backovic, Zivojinovic, Maksimovic, & Maksimovic, 2012; Baeken et al., 2012; Weekes et al., 2008). Inclusion criteria were no history of serious diseases, such as psychiatric or neurological disorders or any other major chronic physiological disorder; no chronic use of any neurological, psychiatric, or endocrine medicine; no current diseases; and no medication use within two days of participation in the study or irregular life style. Overnight shift workers or participants with irregular circadian rhythm, excessive alcohol users (more than two alcoholic drinks daily), or nicotine users (more than five cigarettes a day) were excluded. All participants were assessed with the Life Events Scale (LES; Nakamoto & Mori, 2008; Willemsen et al., 2010) to exclude other major life stressors of the past and within one month in the future.

Three participants were excluded from the data analysis because of excessive ocular and muscle artifacts (over 50% trials were removed due to artifacts). Finally, 39 participants in the stress group and 21 participants in the control group remained. The stress group and control group were matched with respect to age ($M \pm SD$: stress group 22.1 ± 1.0 years vs. control group 22.1 ± 1.0 years). All participants provided informed consent and received monetary compensation for participation. The experiment was approved by the Ethics Committee of Human Experimentation at the Institute of Psychology, Chinese Academy of Sciences.

2.2 Stimuli

The stimuli consisted of photographs of six actors (three female), which were taken from Asian faces in of the NimStim set of facial expressions (Herman, Flak, & Jankord, 2008; one female and one male), the Japanese and Caucasian Facial Expressions of Emotion (JACFEE) (Biehl et al., 1997; one male), and the Chinese Facial Expressions of Emotion (Wang & Markham, 1999; two female and one male). Each actor displayed a happy, neutral, and angry expression (see Figure 1). A total of 18 photographs were used in the experiment. All the faces were removed hair, clothing, background and the other external features. Stimuli were equated in terms of size, gray scale parameters, luminance, contrast and central alignment of the face within the image. The size of the faces were 220×267 pixel. Faces were equated for mean pixel luminance using the adjustments for brightness and contrast functions in Photoshop 8.0. All facial stimuli were presented were shown on a black background and subtended a viewing angle of $7.7 \times 6.3^\circ$.

The proportion of correct judgments for happy faces ($M = 92.8\%$, $SD = 0.024$) was higher than for angry faces ($M = 78.3\%$, $SD = 0.096$). There was no difference in correct judgments between the neutral ($M = 83.2\%$, $SD = 0.028$) and the other two emotional faces, which was

consistent with previous studies, confirming that happy expressions have higher recognition rates than negative expressions (Herman et al., 2008). The rated intensity of neutral faces ($M = 4.95$, $SD = 0.42$) was lower than of the other two emotional faces ($M = 6.91, 6.02$, $SD = 1.18, 0.80$, for angry and happy, respectively). There was no difference between the intensity ratings of angry and happy faces.

Figure 1 here

2.3 Procedure

The participants completed questionnaires and were then prepared for the EEG recordings by being fit with an electrode cap. Before the present task, participants completed an auditory classical S1-S2 task, which lasted 8 to 10 minutes. After that, participants rested for about 5 minutes and then began with the gender discrimination task while the EEG data were collected.

The gender discrimination task was adapted from a previous study (Sprengelmeyer & Jentsch, 2006). Participants were seated in a quiet and normally lit room with their eyes approximately 70 cm from a computer monitor. The whole experiment was divided in two blocks and each block consisted of 144 experimental trials. There was a short break between the two blocks. For each facial emotion, there were 48 trials in one block. All face stimuli were presented individually and in random order. In each trial, a 2×2 cm white fixation cross appeared at the center of the screen for a random interval of 1200–1600 ms. After the fixation, a facial expression was presented until the participants responded. The maximum response time was 2000 ms, after which or the participants' response the next trial began. Participants were required to respond to the gender of the facial stimuli in each trial as fast and accurately as

possible. Half of the participants were instructed to press the left key when male faces appeared and the right key when female faces appeared, the other half received the reverse instructions. Participants pressed the left and right key by index fingers of their left and right hands, respectively.

2.4 Questionnaires

The Perceived Stress Scale (PSS 10-item version; Cohen, 1988; Z. Wang et al., 2011) was used to assess the long-term stress level. The scale measures how often participants feel that life has been overwhelming, uncontrollable, and unpredictable over the last month (Cohen, 1988; Leung, Lam, & Chan, 2010), and has been used frequently as an index for the perception of long-term stress (Liston et al., 2009; Tomiyama, Dallman, & Epel, 2011). The 10-items version (PSS-10) is a short version of the original (PSS-14), and has demonstrated adequate reliability and validity (Cohen, 1988; Wang et al., 2011). It consists of six negative and four positive items. Each item is scored from 0 (*never*) to 4 (*very often*), and higher scores indicate higher levels of long-term stress. Considering the potential influence of personality (De Pascalis, Strippoli, Riccardi, & Vergari, 2004), the Big Five Personality Scale was used (Donnellan, Oswald, Baird, & Lucas, 2006; Zhang, Shi, Zhao, & Wang, 2012) to ensure that the observed group differences were not due to pre-existing personality trait factors.

2.5 ERP Recordings

The EEG was recorded from 64 Ag/Ag-Cl-electrodes mounted in an elastic cap (Neuroscan Inc., USA). Electrodes were referenced on-line to the left mastoid and off-line algebraic re-reference to the average of left and right mastoids. The ground electrode was placed on the forehead, between the frontal midline electrode sites Fz and FPz. The vertical electrooculogram (VEOG) was recorded from a pair of electrodes that were placed above and

below the left eye. The horizontal electrooculogram (HEOG) was recorded by another pair placed at 1 cm from the outer canthi of each eye. Electrode impedance was kept below 5 k Ω . Signals were amplified by a Neuroscan SynAmps² amplifier (Neuroscan Inc., USA) with a 0.05–100 Hz bandpass filter and the sampling rate was 1000 Hz.

The EEG data were filtered with a 30 Hz lowpass filter and epoched into bins of 1000 ms (including 200 ms pre-stimulus as baseline). The EEG signal was corrected by removing ocular artifacts with the Neuroscan software (Semlitsch, Anderer, Schuster, & Presslich, 1986). Trials were excluded from the analysis with an artifact detection criterion of ± 100 μ V.

2.6 Data Analysis

Independent samples t-tests were used to compare the differences between the stress and control group on scores from questionnaires. Some participants' data from the questionnaires have previously been reported (Duan et al., 2013; Wu et al., 2014), but those prior reports did not include behavioral and ERP data in the present gender discrimination task. Repeated measures analyses of variance (ANOVAs) were used to investigate effects of Group (stress vs. control group), Emotion (angry, happy, and neutral) and Facial gender (female, male) on reaction times (RTs) and accuracy of the gender discrimination task. All trials with incorrect responses and trials with RTs slower than 2000 ms or faster than 100 ms were excluded from behavioral and ERP analyses.

The P1, P2 and P3 components were measured and analyzed. For P1, we obtained peak amplitude and latency, as the peak of this early visual component is well-defined and occurs in a very short time-window (Handy, 2005). The peak amplitude and latency of P1 were measured 85-155 ms after stimulus onset. The peaks of P1 were usually measured at occipital sites. But the maximum of P1 peaks may vary across studies and individuals (Luck, 2014). For example, most

of previous studies found that the P1 elicited by facial stimuli was maximal at O1 and O2 sites, but some of others found that at more lateral parietal-occipital electrodes (e.g., PO5/PO6 in Jemel, Pisani, Calabria, Crommelinck, & Bruyer, 2003; Pourtois, Grandjean, Sander, & Vuilleumier, 2004). In this study, bilateral parietal-occipital sites PO5 (left) and PO6 (right) were selected to measure P1 where the maximum amplitude was observed. For the components N170, P2 and P3, the mean amplitudes were analyzed (Luck & Kappenman, 2011). Bilateral parietal-occipital sites P7 (left) and P8 (right) were selected to measure N170; midline frontal-central sites (Fz, FCz, and Cz), and midline central-parietal sites (Cz, CPz, and Pz) were selected to measure P2 and P3, respectively, where the maximum amplitudes were observed for each component (also used in previous studies, e.g., Bertsch et al., 2011; Campanella, et al., 2002; Huang & Luo, 2006). The time windows were 150-190 ms for N170, 160–200 ms for P2 and 400–600 ms for P3, during which the maximum amplitudes were observed on the grand average ERPs (also used in previous studies, e.g., Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Huang & Luo, 2006).

Three-way ANOVAs on the P1 and N170 components were conducted with Emotion (angry, happy, neutral) and Hemisphere (left, right) as within-subject factors, and Group (stress vs. control) as a between-subjects factor. Two three-way ANOVAs on the P2 component were conducted with Emotion (angry, happy, neutral), Group (stress vs. control), and Electrode position. In the ANOVA on the P2 component, the Electrode position was anterior (Fz), anterior-middle (FCz) and middle (Cz); in the ANOVA on the P3 component, the Electrode position was middle (Cz), middle- posterior (CPz) and posterior (Pz). P values were corrected by Greenhouse-Geisser adjustment. When the ANOVA revealed significant main effects of Emotion, post hoc analyses of Bonferroni were used to examine the significance levels.

Whenever the interaction with Group factor was significant, post-hoc tests between the two groups for each Emotion or Site/Hemisphere were calculated with paired t-tests (two tailed). Measures of effect size are reported using eta square (partial η^2).

3. Results

3.1 Questionnaires

The average durations of the preparation for the examination were reported in Table 1 ($n = 36$ for the data of preparing duration, with data from 3 students missing). The stress group reported a significantly higher stress level than the control group on the PSS ($t(58) = 2.463$, $p = .020$). There were no differences on the Big Five personality traits between the two groups ($ps > .10$).

3.2 Behavioral Results

The ANOVAs revealed no differences between groups, neither for accuracy (main effect of Group $F_{(1,58)} = 0.041$, $p = .841$) nor for RTs (main effect of Group $F_{(1,58)} = 0.037$, $p = .848$). Neither the main effects of Emotion (accuracy: $F_{(1.659, 96.2)} = 0.334$, $p = .676$; RTs: $F_{(2,116)} = 2.172$, $p = .119$) nor the interactions of Group $\times$ Emotion (accuracy: $F_{(1.659, 96.2)} = 0.365$, $p = .655$; RT: $F_{(2,116)} = 1.224$, $p = .298$) reached the level of significance. There was a significant main effect of Facial gender for RTs ($F_{(1,58)} = 6.917$, $p = .011$) and significant interactions of Emotion $\times$ Facial gender for RTs ($F_{(2,116)} = 38.358$, $p < .001$) and for accuracy ($F_{(2,116)} = 25.385$, $p < .001$). Participants responded faster to the female faces compared with male faces. Post hoc tests revealed that as to neutral faces, participants responded faster and more accurate to female faces than male faces ($ps < .001$); As to happy faces, participants responded faster but less accurate to the female faces than male faces ($ps < .05$); As to angry faces, participants responded slower and

less accurate to female faces than male faces ($ps < .01$). However, the interactions with the Group factor failed to reach significance (accuracy: Facial gender $\times$ Group $F_{(1,58)} = 0.552, p = .460$; Emotion $\times$ Facial gender $\times$ Group $F_{(2,116)} = 0.214, p = .801$; RT: Facial gender $\times$ Group $F_{(1,58)} = 0.173, p = .679$; Emotion $\times$ Facial gender $\times$ Group $F_{(2,116)} = 2.462, p = .090$).

Table 1 here

3.3 ERP Results

P1. The ANOVAs showed a significant main effect of Group on peak amplitude ($F_{(1,58)} = 4.414, p = .040$, partial $\eta^2 = .071$, Figure 2). The faces elicited a more positive P1 in the stress group compared to the control group. Neither the main effect of Emotion ($F_{(2,116)} = 0.963, p = .385$) nor the interaction between Group and Emotion ($F_{(2,116)} = 0.359, p = .699$) reached the level of significance. None of the other interactions reached the level of significance for the P1 peak amplitude (Group $\times$ Hemisphere $F_{(1,58)} = 0.830, p = .366$; Emotion $\times$ Hemisphere $F_{(2,116)} = 0.969, p = .383$; Emotion $\times$ Group $\times$ Hemisphere $F_{(2,116)} = 0.462, p = .631$) No effects reached the level of significance for the P1 peak latency (Group $F_{(1,58)} = 0.023, p = .879$; Emotion $F_{(2,116)} = 0.954, p = .388$; Group $\times$ Emotion $F_{(2,116)} = 0.824, p = .441$; Group $\times$ Hemisphere $F_{(1,58)} = 0.691, p = .409$; Emotion $\times$ Hemisphere $F_{(1.785, 103.529)} = 0.534, p = .568$; Emotion $\times$ Group $\times$ Hemisphere $F_{(1.785, 103.529)} = 0.065, p = .921$).

Figure 2 here

N170. The results showed a significant interaction of Emotion $\times$ Hemisphere ($F_{(2,116)} = 3.956, p = 0.023$, partial $\eta^2 = 0.064$) on the N170 mean amplitudes. Post hoc contrasts revealed that the angry faces elicited a less negative N170 than the neutral faces at right hemisphere ($p = 0.042$). However, neither the main effects of Emotion and Group nor the interaction between them reach significance (Group $F_{(1,58)} = 0.061, p = .805$; Emotion $F_{(2,116)} = 2.195, p = .116$; Group $\times$ Emotion $F_{(2,116)} = 0.835, p = .437$). No other effects reached the level of significance (Group $\times$ Hemisphere $F_{(1,58)} = 0.015, p = .903$; Emotion $\times$ Group $\times$ Hemisphere $F_{(2,116)} = 2.781, p = .066$).

P2. The ANOVAs showed a marginally significant main effect of Group ($F_{(1,58)} = 3.588, p = .063$, partial $\eta^2 = .058$) and a significant main effect of Emotion ($F_{(2,116)} = 10.926, p < .001$, partial $\eta^2 = .159$). Neutral faces elicited lower positivity than angry faces ($p < .001$) and happy faces ($p < .05$). The interaction between Emotion and Site was significant ($F_{(4,232)} = 3.071, p = .031$, partial $\eta^2 = .050$). Post hoc tests revealed that neutral faces evoked lower positivity than angry or happy faces over the FCz and Cz electrode sites ($ps < .017$); angry faces evoked larger positivity than to neutral faces over the FCz electrode sites ($p = .001$).

Most importantly, a significant interaction between Group and Emotion was found ($F_{(2,116)} = 3.132, p = .047$, partial $\eta^2 = .051$, see Figure 3). Post hoc tests showed that the stress group displayed larger P2 amplitudes compared with the control group for angry faces ($p = .021$); the P2 amplitudes to happy or neutral faces did not differ significantly between the two groups ($ps > 0.1$, see Figure 4). The emotion effects for each group were also assessed. For the stress group, angry faces evoked greater P2 amplitudes than happy faces ($p = .029$) and neutral faces (p

$< .001$); happy faces evoked greater P2 amplitudes than neutral faces ($p = .001$). For the control group, the differences between the three emotions did not reach the significance ($ps > .10$).

Neither the interaction of Group $\times$ Site ($F_{(2,116)} = 0.307, p = .607$) nor Emotion $\times$ Group $\times$ Site ($F_{(4,232)} = 0.324, p = .815$) was significant.

Figure 3 here

Figure 4 here

P3. The results showed a significant main effect of Emotion ($F_{(2,116)} = 4.127, p = .019$, partial $\eta^2 = .066$) and an interaction between Emotion and Site ($F_{(3.221, 186.83)} = 9.219, p < .001$, partial $\eta^2 = .137$). Angry faces elicited greater positivity than happy faces ($p < .01$). Post hoc contrasts found that angry faces evoked larger positivity than to happy ($p = .034$) and neutral ($p = .023$) faces over the Cz electrode sites; angry faces evoked larger positivity than to happy faces ($p = .017$) over the CPz electrode sites; and happy faces evoked lower positivity than to angry ($p = .004$) and neutral faces ($p = .045$) over the Pz electrode sites. Neither the main effect of Group ($F_{(1,58)} = 0.010, p = .922$) nor the interaction between Group and Emotion ($F_{(2,116)} = 1.517, p = .224$) was significant. No other effects reached the level of significance (Group $\times$ Site $F_{(2,116)} = 0.266, p = .637$; Emotion $\times$ Group $\times$ Site $F_{(3.221, 186.83)} = 0.995, p = .400$).

Table 2 here

4. Discussion

The present study explored the effect of long-term stress on the processing of emotionally salient stimuli, that is, facial expressions in humans. The PSS results showed that the students in the stress group perceived a significantly higher level of long-term stress than the controls. By using ERPs, we found that long-term stress had differential effects on two early stages of facial expression processing: 1) Long-term stress augmented posterior P1 amplitudes to facial stimuli irrespective of expression valence, and 2) long-term stress selectively augmented fronto-central P2 amplitudes for angry but not for neutral or positive expressions.

The stress group showed larger amplitudes of the early posterior P1, and this effect was distributed across all three expressions. The parieto-occipital P1 is thought to reflect processing of low-level features of visual stimuli (Hinwood et al., 2011; Linkenkaer-Hansen et al., 1998). This early stage of processing is fast, automatic, and coarse (Hinwood et al., 2011). The enhanced P1 in our study indicated that exposure to long-term stress enhanced the processing of visual inputs irrespective of the stimulus valence. This finding is consistent with previous ERP studies, which found enhanced P1 amplitudes under the laboratory-induced stress (Bertsch et al., 2011). But the present results did not replicate the emotional effect which has been reported in the study with acute stress (Wieser, et al., 2010). Therefore, the P1 effect in the present study

may indicate a general hypervigilance to visual input. From an evolutionary perspective, it is wise to stay alert to environmental stimuli especially in stress situations (Rimmele & Lobmaier, 2012). Previous studies have found that stress potentiates early stages of visual processing (Schwabe & Wolf, 2010; Shackman et al., 2011). Our findings suggest that individuals who were under long-term stress heightened their vigilance to visual inputs.

Furthermore, we found a larger fronto-central P2 for angry faces in the stress group compared to the control group. Numerous ERPs studies have reported the 'negative bias', i.e., greater responses to negative stimuli, such as angry or fearful faces compared with neutral or positive stimuli (Bar-Haim et al., 2007; Putwain et al., 2011; Stefanics, Csukly, Komlosi, Czobor, & Czigler, 2012). The latencies and resources of ERP components that index the negative bias varied across experimental paradigms. The present result showed an enhanced P2 elicited by emotional faces compared with neutral faces. It is consistent with previous research, in which enhanced fronto-central P2 amplitudes were found for the emotional faces compared to the neutral faces (Ashley, Vuilleumier, & Swick, 2004; Bertsch, Bohnke, Kruk, Richter, & Naumann, 2011), as well as the emotional words and pictures (Kissler, Assadollahi, & Herbert, 2006; Weinberg & Hajcak, 2010), implicating this early fronto-central positivity indexed an initial, rapid detection and attentional prioritization of salient emotional stimuli (see Eimer & Holmes, 2007 for a review). Note that the P2 component was distributed at fronto-central sites in our study. The results were inconsistent with some previous studies that measured the occipito-temporal P2 component (e.g., Faerber, Kaufmann, & Schweinberger, 2015; Kolassa et al., 2009; Stahl, Wiese, & Schweinberger, 2008; Stahl, Wiese, & Schweinberger, 2010). In the present study, the tendency for a larger P2 in the stress group suggests that individuals under long-term stress allocate more attention to the processing of angry expressions in this early

phase.

Moreover, the difference between the two groups occurred only for angry faces. Enhanced P2 amplitudes to emotional expressions were considered to represent activity within the neural network involved in the early detection of emotions, which includes limbic structures as well as interconnected neocortical regions (Eimer & Holmes, 2007). Therefore, the enhancement of P2 to angry faces in the stress group suggests that long-term stress sensitizes the organism for rapid detection of facial expression and prioritizes attention to negative emotional stimuli. This finding provides an explanation why individuals suffering from long-term stress exhibit more negative emotional behavior (Haller et al., 2006; Sprague et al., 2011). On the one hand, it is an adaptive behavior to rapidly detect social threat signals in stress situations. On the other hand, maintaining such high sensitivity long term might play a causative role in stress-related disorders such as anxiety or depression (Davidson, Pizzagalli, Nitschke, & Putnam, 2002; Etkin & Wager, 2007).

The understanding of neuroendocrine mechanisms underlying the increased P2 towards angry faces under long-term stress is limited. By means of source localization of ERP, previous study found that P2 was localized in the orbitofrontal cortex (OFC; Rigoni, Polezzi, Rumiati, Guarino, & Sartori, 2010). Intracranial recordings of ERPs to facial expressions also identified the OFC as a potential source of P2 (Kawasaki et al., 2001). The OFC is not only part of the neural network underlying emotional processing but also a target region of stress cortisol (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). Functional disturbances of the OFC have been observed in post-traumatic stress disorder (Driessen et al., 2004). Thus, the increased P2 in our study could reflect an enhancement of OFC activity to negative emotional information under long-term stress.

Unlike the enhancements during early stages, we did not observe a stress effect on the N170 and P3 component. Previous studies indicated that the modulation of anxious on the N170 required participants to explicitly process emotion (Mühlberger et al., 2009). In the present study, the task was to discriminate the gender rather than the emotion of faces. Attending to emotion or not may result in the inconsistent results of N170 effect. Besides, the late component P3 reflects conscious evaluation and selection, and can be modulated by the task demand, too. The task or goal-relevance was also an important factor modulating the effects of stress on information processing (van Peer, Spinhoven, & Roelofs, 2010). So the results might be different if the emotion of faces was task-relevant, for example, in an emotion discrimination or evaluation task (e.g., Weymar et al, 2012).

There are some limitations that should be addressed. First, only male undergraduates were recruited in our study. Thus, the reported results cannot be generalized to the female population. Second, data were collected only during the examination preparation period. A longitudinal study is needed to explore how stress impacts emotional processing over time. Third, there might be other factors mediating the effects of stress on emotional processing, for example, whether the emotion is task or goal-relevant (e.g. emotion categorization or evaluation task), or whether coping strategies are available to deal with the stress. Further research should consider these related factors to explore the role of stress on emotional processes.

In conclusion, our results suggest that long-term stress related to academic examination amplified neural dynamics for processing of facial expressions and had distinct effects at different processing stages. Long-term stress generally increased sensitization to visual inputs as revealed by enhanced P1 amplitudes, as well as increased attentional prioritization to negative emotional stimuli as indexed by P2 amplitudes. Such alterations might increase survival

probability but also become a risk factor for developing psychiatric disorders such as affective disorder.

Author Note

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Table 1. Descriptive statistics of questionnaires and behavioral data by group: mean $\pm$ SD.

	Stress group	Control group
PSS 10 *	17.5 $\pm$ 2.6	14.8 $\pm$ 4.6
Review duration (in months)	5.5 $\pm$ 2.9	
Review intensity (hours/day)	9.5 $\pm$ 1.5	
Reaction Time (ms)	544 $\pm$ 75	548 $\pm$ 60
Accuracy (%)	93.7 $\pm$ 4.2	93.9 $\pm$ 4.2

Note. PSS 10 represents the Perceived Stress Scale 10-item version.

* indicates significant ($p < .05$) difference between the stress and control group.

Table 2. Means (and standard errors) of amplitudes (in uV) and latencies (in ms) on the selected electrode sites.

	P1		N170	P2	P3
	Peak latencies	Peak amplitudes	Mean amplitudes	Mean amplitudes	Mean amplitudes
<i>Stress</i>					
Angry faces	119.9 (1.5)	3.71 (0.32)	-2.51(0.38)	7.68 (0.58)	10.96 (0.59)
Neutral faces	119.2 (1.4)	3.74 (0.33)	-2.74(0.38)	6.97 (0.58)	10.00 (0.58)
Happy faces	118.2 (1.5)	3.68 (0.33)	-2.73(0.37)	6.37 (0.56)	10.31 (0.60)
<i>Control</i>					
Angry faces	118.2 (2.1)	2.56 (0.43)	-2.43(0.52)	5.35 (0.79)	10.46 (0.81)
Neutral faces	120.0 (2.0)	2.72 (0.44)	-2.42(0.52)	5.38 (0.79)	10.23 (0.79)
Happy faces	118.1(2.0)	2.48 (0.45)	-2.66(0.51)	4.91 (0.76)	10.29 (0.81)

Note. The amplitudes and latencies were from selected electrode sites where the maximum amplitudes were observed: PO5/6 for P1; P7/8 for N170; Fz, FCz, and Cz for P2; Cz, CPz, and Pz for P3.

Figure 1. Schematic illustration of two consecutive experimental trials and the stimulus examples.

Figure 2. Upper panel: Grand averaged ERPs in the stress and control group at parieto-occipital electrode sites (PO5/6). The red and green dash lines indicate peaks of the P1 for stress and control group, separately. Lower panel: Comparison of the scalp distributions of the P1 peak between the stress and control group.

Figure 3. Grand averaged ERPs to three facial expressions in stress and control group at Fz, FCz and Cz electrode sites. Shaded areas indicate significant Group effect (stress vs. control) during P2 (160-200ms) intervals ($p < 0.05$).

Figure 4. Left panel: difference waves (stress minus control group) for angry, neutral and happy facial expressions at Fz, FCz and Cz electrode sites. Right panel: topographic maps showing scalp distributions of the difference waves of P2 components (160–200 ms) for angry, neutral and happy facial expressions.