

# Gender differences in subliminal affective face priming: A high-density ERP study

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## Research article

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## Abstract

**Background** Subliminal affective priming effects (SAPEs) refer to the phenomenon by which the presentation of an affective prime stimulus influences the subsequent affective evaluation of a target stimulus. Previous studies have shown that behavioural performance is affected more by unconsciously processed stimuli than by consciously processed stimuli. However, the impact of SAPEs on the face-specific N170 component is unclear. In the current study, we investigated how subliminal processing of fearful faces affected the N170 for subsequent supraliminal target faces using event-related potentials (ERPs).

**Methods** We used event-related potentials (ERPs) to study how SAPEs for fearful faces affect the N170 for subsequent supraliminal target faces. Japanese adults (n=51, 24 females) participated in this study. Subliminal prime faces (neutral or fearful) were presented for 17 ms, followed by a backward mask for 283 ms and target faces for 800 ms (neutral, emotionally ambiguous-fearful, or fearful). ERPs (128-ch) were recorded while participants judged the expression of target faces as neutral or fearful.

**Results** Behavioral data revealed that participants judged target faces as more fearful in the fearful face prime condition compared with the neutral prime condition, regardless of emotional expression. Interestingly, we found gender-related differences in N170 amplitude; only female participants exhibited enhanced N170 amplitude for neutral faces primed by fearful faces. Therefore, a noticeable gender difference exists in the neural processing of subliminally perceived facial emotions.

**Conclusions** Our ERP results suggest the existence of a gender difference in target-face processing preceded by subliminally presented face stimuli in the right occipito-temporal regions.

## Background

Facial information conveys the emotional status of others, making it possible to infer others' intentions and appropriately alter subsequent social cognition and behaviour. Previous studies have suggested that specific neural substrates in the extrastriate cortex are involved in face processing [1–5]. The inferior occipital gyrus (occipital face area, OFA) is thought to be involved in the initial processing and encoding of face parts [6], while the fusiform face area (FFA) mediates the analysis of holistic face structure [7], and the superior temporal sulcus (STS) is involved in the analysis of eye gaze, facial expression, and identity [8]. Electrophysiological studies using event-related potentials (ERPs) have indicated that early face processing occurs approximately 100–200 ms post-stimulus onset, reflected by the P1 [9] and N170 ERP components in occipito-temporal regions [1,2,10]. N170 is reported to function as a face specific component, exhibiting maximal negative peak amplitude over the right occipito-temporal regions, possibly corresponding to the FFA or STS [11,12]. Accumulating evidence has also suggested that N170 is sensitive to emotional expression [13–15]. Thus, the early processing represented by N170 is thought to be responsible for the detailed face processing that underlies the differentiation of basic emotions.

The social significance of emotional information is often assumed to be processed automatically. Although most studies define happiness, sadness, anger, disgust, surprise, and fear as basic emotions, whether a set of basic emotions actually exists remains controversial [16]. However, based on the evolutionary viewpoint, stimuli that indicate an imminent threat, such as fearful facial expressions, should be rapidly processed in order to evoke extremely rapid social reactions [17]. For instance, activity in the amygdala is known to reflect various categories of emotion such that responses to fearful faces or bodily expressions are especially rapid and more intense when subjects unattended or unconsciously perceived fearful stimulus [18–21]. Lesion studies have demonstrated that unconsciously perceived fearful face stimuli produce an amygdala response in the damaged hemifield, including the right fusiform cortex [22,23]. Similarly, our previous study [24] reported that subliminally presented face stimuli elicited an occipital P1 (approximately 120 ms) component, and also demonstrated a trend towards larger P1 amplitude in the subliminal fearful face condition. Several studies have claimed

that such unconscious fear processing depends on a subcortical route that typically bypasses the visual cortex. Instead, the amygdala receives threat-related visual information via the superior colliculus and pulvinar thalamus [19,21,25]. Thus, the subcortical pathway to the amygdala, probably including a magnocellular channel, is thought to be specialized for processing the low spatial frequency information of fearful images [26]. With this type of subcortical route, such information would be perceived without consciousness. Together, these findings suggest that unconsciously perceived affective (particularly threat-predicting) information may modulate the amygdala-visual cortex network by regulating subsequent volitional behaviour.

Amygdala modulation of occipital regions is thought to underlie subliminal affective priming, the phenomenon by which subliminal presentations of affective prime stimuli shift subsequent affective evaluation of supraliminal target stimuli [27,28]. Such affective evaluation suggests the existence of a fast-acting affective information-processing system, in accord with the finding that people are able to form a preference among several items before they are able to explain the reasons for that preference [27,28]. Recent neurophysiological and neuroimaging studies have used the backward masking paradigm to reveal the neural substrates underlying the processing of subliminal affective objects. Several ERP studies have suggested that ERP components, such as N2/P3 recorded from a midline site, are enhanced more by subliminally presented fearful faces than by supraliminally presented fearful faces [14,29–33]. Furthermore, Pegna et al. [34] reported that subliminally presented fearful faces elicited larger N170 amplitudes than did supraliminally presented fearful faces. These findings indicate a double dissociation for subliminal versus supraliminal processing of fearful faces [32], raising the possibility that subliminally presented fearful face stimuli may affect the perception of subsequently presented supraliminal face stimuli via subcortical visual pathways. However, subliminal affective priming effects (SAPes) on subsequent supraliminally presented target-face stimuli remain unclear because of the superimposition of ERP responses for the prime and target faces. In previous behavioural studies, affective priming effects have been obtained with a relatively short (300 ms) prime-target interval (stimulus onset asynchrony; SOA) [35]. Therefore, the current study sought to investigate neural activity accompanying SAPes using high-density electroencephalogram (EEG) with the subliminal priming paradigm adapted from Fazio et al. [35]. We hypothesized that subliminally presented fearful face stimuli would negatively shift the subsequent emotional judgment of the target face. Accordingly, we predicted that face-related ERP components (P1, N170, P2) would be more prominent in the fearful face priming condition than in the neutral priming condition. Interestingly, previous behavioural evidence has suggested a gender difference in affective priming, with female participants tending to respond to positive affective information and male participants exhibiting strong priming effects in emotionally negative conditions [36,37]. Therefore, we predicted that the results would reveal gender-related ERP differences in SAPes for fearful faces.

## Methods

### Participants

Fifty-one healthy Japanese adults (20–32 years old; mean age:  $23.1 \pm 3.1$  years; 24 females) participated in this study. All participants were right-handed and all had normal or corrected-to-normal vision. None had a history of neurological or psychiatric disorders. All participants provided written informed consent. The experimental procedure was approved by the Ethics Committee at the Graduate School of Medical Science, Kyushu University (24078), and methods were carried out in accordance with the relevant guidelines. This research was conducted in accordance with the Helsinki Declaration as revised 1989.

### Visual stimuli

Prime-face stimuli consisted of grayscale photographs of four Japanese actors (two males and two females) exhibiting fearful or neutral expressions, resulting in a total of eight different prime-face stimuli. Target-face stimuli included fearful,

ambiguous-fearful, and neutral expressions displayed by four Japanese actors (two males and two females), who were not the actors used for the prime-face stimuli. We used Morpher 3.1 freeware to generate a series of target-face stimuli with ambiguous-fearful emotional facial expressions. Ambiguous-fearful stimuli were created by morphing together faces with fearful and neutral expressions. We generated three types of ambiguous-face stimuli that differed in their fearful to neutral face ratios (25%, 50%, and 75% fearful faces). Thus, a total of 12 different facial stimuli were included in the ambiguous-fearful subset of target-face stimuli (i.e., 3 graded intensity levels  $\times$  4 identities, see Fig. 1A). The original facial images were taken from the ATR face database (ATR Promotions, Inc., Kyoto, Japan) (for detailed descriptions of the face database, see section Supplementary Information SI.1). All face stimuli were converted to grayscale and cropped into an oval shape to exclude all hair and non-facial contours. Scrambled mask stimuli were generated using a MATLAB R2013a (The MathWorks Inc., Natick, MA, USA) script. All images were equated for luminance ( $15 \text{ cd/m}^2$ ) and contrast (80%). All stimuli were presented on a 19-inch CRT monitor (refresh rate: 60 Hz) at a viewing distance of 114 cm, and subtended a visual angle of  $5.3^\circ \times 8^\circ$  ( $283 \times 413$  pixels) (See Fig. 1A).

## Rating of ambiguous-fearful target-face stimuli

Participants sat in a dimly lit, sound-attenuated, and electrically shielded room. The experiment was programmed using Presentation software (version 16.3, Neurobehavioral Systems, 2016; <http://www.neurobs.com/>) to display the prime and target-face stimuli.

Before the experiment, we used the three morphed ambiguous-fearful face categories (25% fearful, 50% fearful, and 75% fearful) (Fig. 1A) to determine the ambiguous-fearful target face for each participant. A face was defined as ambiguous-fearful for a participant if they judged it to be fearful about 50% of the time (chance level). At the beginning of each test, a scrambled mask stimulus with a red fixation cross appeared in the middle of the screen for 1000 ms. Following a scrambled mask, one of the test faces (neutral, one of three fearful morph levels, or fearful) was displayed on the screen for 1000 ms and then replaced by a scrambled mask. Each of the five face categories was presented 48 times in pseudo-random order. Participants were instructed to judge whether the target face was "neutral" or "fearful" by clicking the left or right mouse button with their right hands. Response buttons were counterbalanced across participants. We defined the ambiguous fearful target face category as stimuli for which the fearful response rate (RR) of each participant was approximately 50%. After determining the ambiguous-fearful faces for each participant, the stimuli that were ambiguous-fearful for all participants were used as the ambiguous-fearful face stimuli for the ERP experiment.

## Affective subliminal priming task

We adopted the backward masking paradigm from Mitsudo et al. [24]. Each trial began with mask stimuli and a red fixation cross lasting 1000 ms. A prime-face stimulus was displayed for 17 ms, immediately followed by the mask stimulus for 283 ms (SOA was 300 ms) and then a target-face stimulus for 800 ms (see Fig. 1B). Participants were instructed to judge whether the target face was fearful or neutral as quickly as possible by pressing the left or right mouse button with their right index finger. The response button assignment was counterbalanced across participants. After the experiments, participants were asked if they had noticed the prime faces. Because Mitsudo et al. [24] reported that threshold durations for subliminal stimuli ranged from 20 to 50 ms, our choice of a 17-ms duration was sufficient for avoiding conscious perception of the prime stimuli.

Each prime-face stimulus subset (neutral or fearful) included four stimuli (two male actors and two female actors) and each target-face stimulus subset (neutral, ambiguous-fearful, or fearful) also comprised four identities. Six different trial types were formed from these prime and target pictures (i.e., neutral prime/neutral target, neutral prime/ambiguous-fearful target, neutral prime/fearful target, fearful prime/neutral target, fearful prime/ambiguous-fearful target, and fearful prime/fearful target). Each trial type was presented 144 times in a pseudorandom order. Thus, the total number of trials in

the experiment was 864 (6 trial types  $\times$  144 times). RRs and reaction times (RTs) were calculated from the behavioural data for each participant.

## ERP recordings

ERP data were recorded while participants performed the affective subliminal priming task. Continuous ERPs were recorded using a high-density 128-channel system (NetAmps 200, Electrical Geodesics Inc., Eugene, OR, USA), and data were on-line bandpass filtered from 0.01 to 100 Hz and sampled at 1000 Hz. ERP data were recorded continuously with the vertex electrode (Cz) as a reference and offline re-referenced to the average reference. We processed ERP data offline using Net Station 4.2 software (Electrical Geodesics Inc., Eugene, OR, USA) with a 0.5–30 Hz bandpass filter, and segmented data from 100 ms before prime-face onset to 700 ms after target-face onset, corrected to a 100 ms pre-stimulus baseline. EEG epochs containing artefacts (EEG voltage exceeding 100  $\mu$ V or eye movements in excess of 55  $\mu$ V) were automatically rejected from further analysis.

## Data analysis

We determined the time windows for ERP components based on butterfly plots and a microstate analysis [38]. First, we drew the butterfly plots by superimposing all 128 channels for all conditions and sought out salient ERP components by visual inspection (Fig. 2).

Next, we applied the microstate analysis to determine significant ERP components and their associated time windows. The aim of a microstate-segment analysis is to provide information about the brain activity associated with the sequence of discrete (and non-periodic) information-processing operations evoked by a stimulus or task [38]. Microstate segment analyses were carried out using Microstate Analysis Toolbox (MST) [39] from the EEGLAB toolbox[40]. All ERP data from the 6 conditions (male or female  $\times$  6 stimuli) were added together and grand-averaged. Then, they were segmented into 3 and 20 microstate classes using a K-means algorithm with 50 random initializations for clustering. Finally, we selected microstates using three criteria; global explained variance (GEV), cross-validation (CV), and Krzanowski-Lai (K-L) criterion. In our data, the suitable number of segments for explaining the all epochs was 18. Accordingly, we fit the microstate prototypes back to all grand-averaged data. This procedure enabled us to label the EEG epochs with the microstate prototypes.

## Statistical analysis

For SAPEs, RRs, and RTs were analysed with a  $2 \times 3 \times 2$  repeated-measures analysis of variance (ANOVA) with prime-face category (neutral or fearful) and target-face category (neutral, ambiguous-fearful, or fearful) as within-participant factors, and gender (female or male) as the between-participant factor. ERP amplitudes and latencies were analysed using a  $2 \times 3 \times 2$  repeated-measures ANOVA with prime-face category (neutral or fearful), target-face category (neutral, ambiguous-fearful, or fearful), and laterality (left or right region of interests (ROI)) as within-participant factors. The results of the microstate analysis suggested significant gender-based differences in the time windows for each ERP component, especially N170. Thus, ANOVAs were performed separately by gender. All analyses were conducted with SPSS (version 22; IBM Corp, USA). All significant *p*-values were corrected by Greenhouse-Geisser correction, and Bonferroni correction was used for multiple comparisons.

Multiple regression analyses were also conducted to evaluate the correlation between behavioural and ERP results. We evaluated the correlation between within-participant factors (prime face and target face categories), behavioural data, and amplitudes and latencies of each ERP component. Thus, dummy variables were used to indicate intra-participant factors. Dummy variables for prime- and target-face categories were determined by the emotional intensity of the face categories

(prime face: neutral = 1, fearful = 2, target face: neutral face = 1, ambiguous-fearful face = 2, fearful face = 3). Multiple regression analysis was performed separately for gender and laterality.

## Results

### Behavioural results

Data from five participants were discarded because they contained inappropriate behavioural responses (i.e., mean RR or mean RT for the target-face stimuli exceeded more than three times the inter-quartile range). Thus, the final analysis included data from 46 participants. After the experiment, all participants reported that they were completely unaware that prime-face stimuli had been used. Behavioural results indicated that mean RR for ambiguous-fearful target faces was  $33.8\% \pm 3.1\%$ .

Table 1 summarizes the results of the three-way ANOVA (prime  $\times$  target  $\times$  gender) for RRs and RTs. We found a significant main effect of Prime Face ( $F(1, 44) = 4.456, p = 0.041, \eta^2 = 0.09$ ). RRs for the fearful prime condition were significantly higher than those for the neutral prime condition (fearful prime condition vs. neutral prime condition =  $44.06 \pm 1.48\%$  vs.  $43.29 \pm 1.39\%$ ,  $p = 0.032$ ) (Fig. 3A). We also observed a significant main effect of Target Face ( $F(1.28, 56.60) = 762.79, p < 0.001, \eta^2 = 0.95$ ). Post hoc analysis revealed significantly higher RRs for fearful target-face stimuli than for neutral and ambiguous-fearful target-face stimuli (fearful target-face vs. neutral target-face =  $90.55 \pm 1.16\%$  vs.  $4.35 \pm 0.60\%$ ,  $p < 0.001$ , fearful target-face vs. ambiguous-fearful target-face =  $90.55 \pm 1.16\%$  vs.  $33.77 \pm 3.04\%$ ,  $p < 0.001$ ). RRs for ambiguous-fearful target-face stimuli were also significantly higher than those for neutral target-face stimuli (ambiguous-fearful target-face vs. neutral target-face =  $33.77 \pm 3.04\%$  vs.  $4.35 \pm 0.60\%$ ,  $p < 0.001$ ) (Fig. 3B). We did not find any significant main effect of Gender or any significant interactions between Prime Face, Target Face, or Gender.

The three-way ANOVA (prime  $\times$  target  $\times$  gender) for RTs revealed a significant main effect of Target Face ( $F(1.41, 62.16) = 11.11, p < 0.001, \eta^2 = 0.20$ ) (Table 1). Post hoc analysis revealed that RTs for the ambiguous-fearful target faces were significantly higher than those for neutral faces (ambiguous-fearful target-face vs. neutral target-face =  $539.34 \pm 14.98$  ms vs.  $514.40 \pm 13.22$  ms,  $p < 0.001$ ) (Fig. 3C). No other main effects or interactions were significant.

## ERP results

### Microstate analysis

Figure 2 shows butterfly plots for each group. We found three major components within an approximately 400–550 ms time window (100–250 ms after target face onset) for male and female data sets. Interestingly, we observed temporal differences among these components between female and male groups. The microstate analysis (Fig. 4, Supplementary Information Fig. S1) revealed three microstates between 418–659 ms (118–359 ms after target face onset) for each prime  $\times$  target condition. Microstate topography suggested that each microstate responded strongly among regions near the occipito-temporal electrodes. The first microstate overlapped between 418–447 (114–147) ms in females and between 419–447 (119–147) ms in males. The second microstate overlapped between 473–503 (173–203) ms in females and at 444–488 (144–188) ms in males. The third microstate overlapped between 511–545 (211–245) ms in females and between 515–559 (215–259) ms in males. We used these time windows to determine the conventional ERP components; P1, N170, and P2. Peak amplitudes and latencies for the ERP components were calculated at clusters of electrodes, corresponding to specific ROIs. ROIs for each component were defined as the electrode that recorded the maximum amplitude and its three spatially adjacent electrodes. P1 was measured around electrodes at O1 and O2. N170 was measured around electrodes at T5 and T6. P2 was measured around electrodes at T5 and T6 (for detailed descriptions of ROI definitions, see section SI.2, Supplementary Fig. S2).

## P1 component

The results of the three-way ANOVA (prime × target × laterality) are shown in Table 2. We found a significant main effect of Target Face on P1 amplitude (Males:  $F(2, 46) = 8.31, p < 0.001, \eta^2 = 0.27$ , Females:  $F(2, 42) = 8.79, p < 0.001, \eta^2 = 0.30$ ), suggesting that P1 amplitude for fearful target faces is significantly greater than that for neutral and ambiguous fearful target faces in both genders (Male: fearful vs. neutral vs. ambiguous-fearful target face =  $5.03 \pm 0.35 \mu\text{V}$  vs.  $4.80 \pm 0.33 \mu\text{V}$  vs.  $4.75 \pm 0.35 \mu\text{V}$ ,  $0.001 < p < 0.023$ , Female: fearful vs. neutral vs. ambiguous-fearful target face =  $4.35 \pm 0.30 \mu\text{V}$  vs.  $4.06 \pm 0.31 \mu\text{V}$  vs.  $4.09 \pm 0.308 \mu\text{V}$ ,  $0.003 < p < 0.008$ ). We also found a significant main effect of Laterality in males ( $F(1, 23) = 11.36, p < 0.001, \eta^2 = 0.33$ ), with P1 amplitude being significantly larger in the right occipital ROI than in the left ROI (Right vs. Left =  $5.17 \pm 0.38 \mu\text{V}$  vs.  $4.54 \pm 0.34 \mu\text{V}$ ,  $p = 0.003$ ) (for the time-course representation of the ERPs, see Supplementary Fig. S3).

A three-way ANOVA (prime × target × laterality) of P1 latencies revealed a significant main effect of Laterality in females ( $F(1, 21) = 5.27, p = 0.03, \eta^2 = 0.032$ ) (Table 2) such that P1 latency in the right ROI was significantly shorter than in the left ROI (Right vs. Left =  $424.48 \pm 1.18 \text{ ms}$  vs.  $426.0 \pm 1.34 \text{ ms}$ ,  $p = 0.032$ ). We found no significant main effects for the prime conditions.

## N170 component

Although no significant difference in prime conditions or target-face categories was evident in the grand averaged waveforms (or the time course representation of the ERPs, see Supplementary Fig. S3), a definite gender difference in SAPEs was found in the grand averaged waveforms and scalp topography in the right ROI (Fig. 5). Although visual inspection indicated that N170 amplitudes for neutral target-face stimuli were larger in the fearful prime condition than in the neutral prime condition for female participants, no such differences were evident for male participants (Fig. 5B, 5D). Three-way ANOVA (prime × target × laterality) calculated for N170 amplitude revealed significant interactions in each group (females: Prime Face × Target Face × Laterality ( $F(2, 42) = 4.88, p = 0.017, \eta^2 = 0.19$ ); males: Prime Face × Laterality ( $F(1, 23) = 12.29, p = 0.002, \eta^2 = 0.35$ )) (Table 2). Further statistical analysis revealed gender differences in SAPEs. N170 amplitude for neutral target faces in the fearful prime-face condition was significantly larger than that in the neutral prime-face condition, but only in the right ROI of the female participants ( $-1.63 \pm 0.20 \mu\text{V}$  vs.  $-1.42 \pm 0.19 \mu\text{V}$ ,  $p = 0.013$ ) (Fig. 5B, D and 6B). In contrast, no significant SAPEs were found in the male participants. We only found that N170 amplitude in the right ROI during the fearful priming condition was significantly larger than that in the left ROI ( $-2.98 \pm 0.32 \mu\text{V}$  vs.  $-2.86 \pm 0.32 \mu\text{V}$ ,  $p = 0.046$ ) (Fig. 6A). We did not observe any main effects of prime condition, target face, or laterality.

Three-way ANOVA (prime × target × laterality) calculated for N170 latency revealed a significant interaction between Prime Face and Laterality in female participants ( $F(1, 21) = 5.04, p = 0.036, \eta^2 = 0.19$ ) (Table 2). Further analysis revealed that N170 latency in the left ROI was significantly shorter in the fearful prime condition than in the neutral prime condition ( $477.82 \pm 1.50 \text{ ms}$  vs.  $479.68 \pm 1.62 \text{ ms}$ ,  $p = 0.036$ ). No main effects or interactions were significant for N170 latency in male participants (Table 2).

## P2 component

A three-way ANOVA (prime × target × laterality) calculated for P2 amplitude revealed a significant main effect of Target Face for each gender (Males:  $F(1.50, 34.41) = 14.53, p < 0.001, \eta^2 = 0.39$ , Females:  $F(1.58, 33.26) = 4.15, p = 0.023, \eta^2 = 0.17$ ) (Table 2). A direct comparison between target-face categories in male participants revealed that P2 amplitudes were higher for neutral target faces than for ambiguous-fearful ( $1.92 \pm 0.27 \mu\text{V}$  vs.  $1.74 \pm 0.26 \mu\text{V}$ ,  $p < 0.001$ ) and fearful target faces ( $1.92 \pm 0.27 \mu\text{V}$  vs.  $1.59 \pm 0.26 \mu\text{V}$ ,  $p < 0.001$ ). In contrast, we found no significant differences among the target face categories in female participants. ANOVA also revealed a significant interaction of Target Face × Laterality in females ( $F(2,$

42) = 4.10,  $p = 0.024$ ,  $\eta^2 = 0.16$ ), and Prime Face $\times$  Laterality in males ( $F(1, 23) = 6.92$ ,  $p = 0.015$ ,  $\eta^2 = 0.23$ ) (Table 2). P2 amplitude in the female right ROI was significantly larger for ambiguous-fearful target faces than for fearful target faces ( $1.96 \pm 0.24 \mu\text{V}$  vs.  $1.75 \pm 0.22 \mu\text{V}$ ,  $p = 0.028$ ). In contrast, P2 amplitude in the right male ROI was significantly larger in the fearful prime condition than in the neutral prime condition ( $1.68 \pm 0.28 \mu\text{V}$  vs.  $1.54 \pm 0.30 \mu\text{V}$ ,  $p < 0.05$ ) (for a time-course representation of the ERPs, see Supplementary Fig. S3). We found no main effects or interactions for P2 latency in either group (Table 2).

## Correlations between each ERP component and intra-participant factors in behavioural data

Unlike the N170, the P1 and P2 components did not show significant relationships with intra-participant factors. N170 amplitude in the right hemisphere was only positively correlated with RT in the male participants ( $r = 0.38$ ,  $p < 0.001$ ). N170 latency in both hemispheres was also positively correlated with RT in males (left:  $r = 0.55$ ,  $p < 0.001$ , right:  $r = 0.50$ ,  $p < 0.001$ ). In females however, we did not find any significant correlations between N170 amplitude or latency and within-participant factors (Table 3).

## Discussion

In the current study, we explored the neural correlates of SAPEs in terms of behavioural performance and high-density ERPs. We compared ERPs in response to three types of target-face stimuli (neutral, ambiguous-fearful, and fearful) after participants were primed by neutral and fearful face stimuli that were presented for only 17 ms, and were thus subliminal. The results revealed that the rates of fearful judgments for all target faces preceded by a fearful prime-face stimulus were significantly greater than those preceded by a neutral prime-face stimulus. We also found that SAPEs for the face-specific N170 component were only evident in female participants (see Fig. 5). Interestingly, multiple regression analysis revealed that N170 amplitude and latency were significantly correlated with RTs in males but not females. These results suggest that the N170 component primarily reflects the neural basis of the SAPE, and its impact on behavioural performance may differ between genders. Several previous studies have reported right-lateralized N170/M170-components in the occipito-temporal area in male participants, but not in female participants [41,42]. Although Choi et al. [43], reported gender differences in N170 emotional sensitivity, there have been no studies demonstrating a gender difference of SAPEs in ERPs, including the face-specific N170 component.

Our behavioural data revealed that emotional judgments of target faces were affected by subliminally presented fearful face stimuli. Although affective priming typically leads to shorter reaction times when judging targets [27,28], we did not observe such an effect. Rather, we observed longer RTs for the ambiguous-fearful target faces than for the neutral or fearful target faces. We are not certain that this finding reflects the difficulty of the emotional judgment or a negative effect of priming. Interestingly, N170 amplitude and latency positively correlated with male-participant RTs. Thus, our behavioural results indicate that subliminal perception of fearful faces biased facial judgment toward fearful. Positive correlations between RT and N170 latencies in males also suggest that early neural processing of target faces might affect how fast they can be judged.

Previous studies have demonstrated that emotional judgments of neutral objects are affected by briefly presented emotional prime faces [28,44]. In a positive (happy) prime condition, participants evaluated neutral objects more positively than they did in a neutral prime condition. Similarly, participants evaluated neutral objects more negatively in a negative (angry, fearful, or disgusted) prime condition than they did in a neutral condition. This effect was prominent in the prime-target valence-congruent condition. Furthermore, other studies have reported gender differences in the affective priming effect. Gohier et al. [37] reported greater sensitivity to negative emotional prime-face stimuli in females than in males, whereas Donges et al. [36] demonstrated stronger positive priming in females than in males. In accord with previous

studies, our behavioural data revealed a negative priming effect of fearful faces, in which RRs for target faces primed by fearful faces were significantly higher than those primed by neutral faces. Although this result demonstrated the SAPE in the fearful prime-face condition regardless of target facial expression, we found no gender differences in any priming condition. This discrepancy between our behavioural results and those from other studies may be related to the longer durations of prime stimuli that were used in previous studies (33 ms or 250 ms), given that some participants in previous studies exhibited extremely short subthresholds (< 33 ms) for visual stimuli [24,45].

Although our results revealed no significant main effect of laterality for the N170, we found a gender-related difference in the neural processing of target-face stimuli. Specifically, we observed larger N170 amplitudes in the right hemisphere of females in response to neutral target-face stimuli primed by fearful face stimuli compared to those primed by neutral face stimuli. In contrast, larger N170 amplitudes were found in the right ROI of males than in the left ROI when primed by fearful face stimuli. The N170 component is generated in the FFA, while the STS is considered to be specialized for visual processing and encoding of high level features such as structural information contained within faces [1,10,46,47]. Although some researchers [30,48] have claimed that the N170 is relatively insensitive to emotion, several studies have observed the sensitivity of this component to emotional facial expressions [13,15,49]. Based on these controversial findings, many previous studies have examined the emotion-based modulation of the N170 component by looking for effects related to the emotional valence of prime stimuli. ERP studies using the affective priming paradigm have reported enhanced N170 amplitude for target stimuli when the emotional valence of prime visual stimuli or scenes is congruent with that of target stimuli [50–52]. Although negative evidence for affective priming effects on the N170 component has been reported [53,54], one explanation for this discrepancy between the current findings and previous studies could be the difference in SOAs. The reports of SAPEs on the subsequent supraliminally presented target face that have been demonstrated by ERP studies using the backward masking paradigm [14,55] are currently inconclusive because of the overlapped ERP responses for prime and target faces. In the present study, we chose an SOA of 300 ms because it has been used in several previous studies [35,50,56] and has been shown to be effective for facilitating the affective priming effect. Moreover, Itier et al. [46] demonstrated a repetition effect on N170 amplitude in which repetition of the same face identity suppressed the N170 response, leading to increased N170 amplitude in prime-target incongruent conditions (e.g., different expressions of faces with the same identity or different identities). These findings further suggest that automatic rapid encoding of emotional information included in prime stimuli is implemented in the brain, and that such information is integrated with the emotional content of subsequent target stimuli by the FFA, STS, or OFA. From this perspective, the N170 modulation observed in female participants could be considered a prime-target incongruent repetition effect. However, it remains unclear why this effect was not observed in male participants, and why other prime-target incongruent pairs did not show repetition effects.

Because the presentation duration in the current study was extremely short (17 ms), it also remains unclear whether subliminal emotional properties were processed in the occipito-temporal face-sensitive areas. We assume that the gender differences we observed in the N170 can be partially explained by the functional connectivity between the FFA and amygdala [57,58]. Evolutionarily, unconscious processing of threat-related stimuli should be beneficial because it allows extremely rapid motor responses to the stimuli [17]. The amygdala is involved in pre-attentive, rapid processing of threat-related stimuli, whereby it receives subcortical visual information via the superior colliculus and pulvinar thalamus [19], and anatomical evidence for subcortical emotional pathway connections has also been shown in nonhuman primates and humans [21]. The sensitivity of the amygdala to emotional content has generally been investigated using the phenomenon by which subliminally presented emotional visual stimuli enhance amygdala activation [59–61]. A previous study reported that the amygdala appears to be involved in automatically elicited negative evaluative shifts in response to negative face priming [62]. Because the modulation of the visual cortex by emotional face stimuli is dependent on the amygdala [63,64], it is possible that modulation of N170 amplitude reflects the contribution of amygdala output to the visual cortex [63]. Dima et al. [65] suggested that negative emotional information is conveyed to the ventral prefrontal cortex (VPFC) via multiple pathways, including the inferior occipital gyrus, FFA, and the amygdala, and is processed among these pathways in

parallel. In addition, functional magnetic resonance imaging (fMRI) studies have reported gender differences in the processing of emotional properties in which females exhibited enhanced activity in emotion-specific areas such as the amygdala, the orbitofrontal cortex, and the anterior cingulate cortex in response to negative stimuli. In males, cognition and cognitive control areas such as the prefrontal and superior parietal regions were recruited to process negative content, while the amygdala showed attenuated activation [66,67]. Taken together, we speculate that in females, subliminally-presented fearful face stimuli in the fearful prime-neutral target condition activate emotion-specific areas including the amygdala. These areas then stimulate the FFA and process neutral target faces as emotionally negative (enhanced N170 amplitude). In males however, subliminally perceived fearful-face information is conveyed to the VPFC via the amygdala and subsequent target faces are cognitively processed.

Although previous studies reported prime-target emotional incongruency effects on P1 [54,68], the present study failed to demonstrate SAPEs in the P1 and P2 components, despite the emotional sensitivity shown by these components. In previous studies, P1 amplitude was reported to be enhanced by fearful faces significantly more than by happy or neutral faces, while the frontal N1, as the counterpart to the P1, was modulated by passive viewing of emotional expressions [69,70]. Similarly, previous studies have reported larger P2 amplitudes for face stimuli with configuration modifications than for faces with feature modifications or the original faces [71]. Thus, P1 is thought to exhibit rapid responses to threat-related stimuli, while P2 reflects the initial stages of facial expression cognition, regardless of preceding emotional stimuli.

Our study has some methodological limitations. First, it lacked a control condition. We might have overlooked other SAPEs (both behavioural and electrophysiological) because we did not compare the prime condition with a primeless condition. Second, we only studied the emotion of fear. Are SAPEs solely evident in the fearful face-priming condition? We are therefore cautious about how to interpret the SAPEs we observed. In the future, we need to use both subliminal priming and primeless conditions and include other prime and target faces (i.e., happy, sad, angry, and disgusted faces). Third, although the intensity levels for male and female face images were almost equal (see SI.2), the number of face stimuli used in our study was small. Because the ATR face database contains 4 female and 6 male actors, and because we wanted the number of male/female faces to be equal, we were limited to 4 female and 4 male face images. Furthermore, we did not consider individual face actors as fixed factors. Kret et al.[72] reported that males showed higher brain activity in several regions of interest, including FFA and STS, following fearful and angry male bodily expressions. Further analysis will be necessary to determine the effect of 'face actor' on SAPEs.

Finally, a current study revealed that SAPEs were more pronounced in the fearful prime-neutral target condition in females. However, we failed to find a significant interaction between prime and target conditions in males, even using the ambiguous-fearful target face, which we expected to show salient SAPEs. Further study will be necessary to consider these problems.

## Conclusions

In the current study, female participants exhibited increased N170 amplitude in neutral prime trials with fearful target faces, whereas male participants exhibited increased N170 amplitude in the right ROI in all fearful prime conditions. Significant positive correlations between ERPs and behavioural performance were observed only in male participants. Our ERP results suggest the existence of a gender difference in target-face processing preceded by subliminally presented face stimuli in the right occipito-temporal regions.

## Abbreviations

SAPEs: subliminal affective priming effects; ERPs: event-related potentials; OFA: occipital face area; FFA: fusiform face area; STS: the superior temporal sulcus; SOA: stimulus onset asynchrony; EEG: electroencephalogram; SI: supplementary information; RR: response rate; RT: reaction time; MST: microstate analysis toolbox; GEV: global explained variance; CV:

cross-validation; K-L: Krzanowski-Lai; ANOVA: analysis of variance; ROI: region of interests; VPFC: ventral prefrontal cortex; fMRI: functional magnetic resonance imaging.

## **Declarations**

### **Ethics approval and consent to participants**

This study was carried according to the Declaration of Helsinki and the Guideline for Good Clinical Practice. The experimental procedure was approved by the Ethics Committee at the Graduate School of Medical Science, Kyushu University (24078). All participants signed an informed consent.

### **Consent to publish**

Not applicable

### **Availability of data and materials**

The dataset used or analyzed during the current study are available from the corresponding author on reasonable request.

### **Competing Interests**

The authors declare no competing interests.

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### **Author Contributions**

MT, TM, EY, NT, and ST conceived and designed the experiments. KO, EY, and HN contributed experimental programs and analysis tools. MT performed the experiments and analysed the data. MT, TM, KO, EY, NT, and ST wrote the paper. The study was supervised by TM and ST.

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## Tables

Table 1. Three-way repeated measures ANOVA results for the behavioural data

Factor	RR				RT			
	df	F	$\eta^2$	P	df	F	$\eta^2$	P
Prime face	1, 44	4.456	0.09	<b>0.04</b>	1, 44	0.070	0.00	0.07
Target face	1.28, 56.60	762.79	0.95	<b>p &lt;0.001</b>	1.41, 62.16	11.11	0.20	<b>p &lt;0.001</b>
Gender	1, 44	1.4		0.24	1, 44	0		0.96
Prime face × Target face	1.70, 74.99	0.59	0.01	0.53	2, 88	0.27	0.01	0.76
Prime face × Gender	1, 44	12.43	0.04	0.20	1, 44	0.52	0.01	0.48
Target face × Gender	1.28, 56.60	2.44	0.05	0.09	1.41, 62.16	1.26	0.03	0.28
Prime face × Target face × Gender	1.70, 74.99	0.08	0.00	0.89	2, 88	0.06	0.00	0.95

Prime faces comprised fearful and neutral faces displayed by four Japanese actors (two males and two females). Target faces comprised fearful, ambiguous-fearful, and neutral faces displayed by four Japanese actors (two males and two females), who were not the same actors used for the Prime faces.

Abbreviations: RR, the fearful response rate; RT, reaction time.

Bold values indicate  $p < 0.05$  in this table and subsequent tables.

Table 2. Four-way repeated measures ANOVA results for the P1, N170 and P2 components

## Males

Factor	Amplitude				Latency			
	df	<i>F</i>	$\eta^2$	<i>P</i>	df	<i>F</i>	$\eta^2$	<i>P</i>
P1								
Prime face	1,23	0.02	0.00	0.89	1,23	0.58	0.03	0.45
Target face	<b>2,46</b>	<b>8.31</b>	<b>0.27</b>	<b>0.00</b>	2,46	0.62	0.03	0.54
Laterality	<b>1,23</b>	<b>11.36</b>	<b>0.33</b>	<b>0.00</b>	1,23	2.65	0.10	0.12
N170								
Prime face	1,23	0.17	0.01	0.69	1,23	1.73	0.07	0.20
Target face	1.52, 34.93	0.59	0.03	0.56	2,46	0.12	0.01	0.88
Laterality	1,23	0.42	0.03	0.42	1,23	0.00	0.00	0.96
Prime face × Laterality	<b>1,23</b>	<b>12.29</b>	<b>0.35</b>	<b>0.00</b>				
P2								
Prime face	1,23	2.76	0.11	0.11	1,23	0.73	0.03	0.40
Target face	<b>1.50, 34.41</b>	<b>14.53</b>	<b>0.39</b>	<b>0.00</b>	2,46	0.24	0.01	0.79
Laterality	1,21	2.78	0.11	0.11	1,23	1.02	0.04	0.32
Prime face × Laterality	<b>1,23</b>	<b>6.92</b>	<b>0.23</b>	<b>0.02</b>				

## Females

Factor	Amplitude				Latency			
	df	<i>F</i>	$\eta^2$	<i>P</i>	df	<i>F</i>	$\eta^2$	<i>P</i>
P1								
Prime face	1,21	1.23	0.06	0.28	1,21	0.19	0.01	0.67
Target face	<b>2,42</b>	<b>8.79</b>	<b>0.30</b>	<b>0.00</b>	2,42	0.51	0.02	0.60
Laterality	1,21	2.35	0.10	0.14	<b>1,21</b>	<b>5.27</b>	<b>0.20</b>	<b>0.03</b>
N170								
Prime face	1,21	0.10	0.01	0.76	1,21	3.02	0.13	0.10
Target face	2,42	0.39	0.02	0.68	2,42	0.17	0.01	0.84
Laterality	1,21	0.19	0.01	0.67	1,21	2.25	0.10	0.15
Prime face × Laterality					<b>1,21</b>	<b>5.04</b>	<b>0.19</b>	<b>0.04</b>
Prime face × Laterality	<b>2,42</b>	<b>4.88</b>	<b>0.19</b>	<b>0.01</b>				

Target face ×									
Laterality									
P2									
Prime face	1,23	1.26	0.06	0.27	1,21	0.01	0.00	0.94	
Target face	<b>1.58, 33.26</b>	<b>4.15</b>	<b>0.17</b>	<b>0.02</b>	2,42	2.36	0.10	0.11	
Laterality	1,21	3.67	0.15	0.07	1,21	0.58	0.03	0.45	
Target face ×	<b>2,42</b>	<b>4.10</b>	<b>0.16</b>	<b>0.02</b>					
Laterality									

Table 3. Relationships between intra-subject factors (prime face category and target face category) and ERP components

## Males

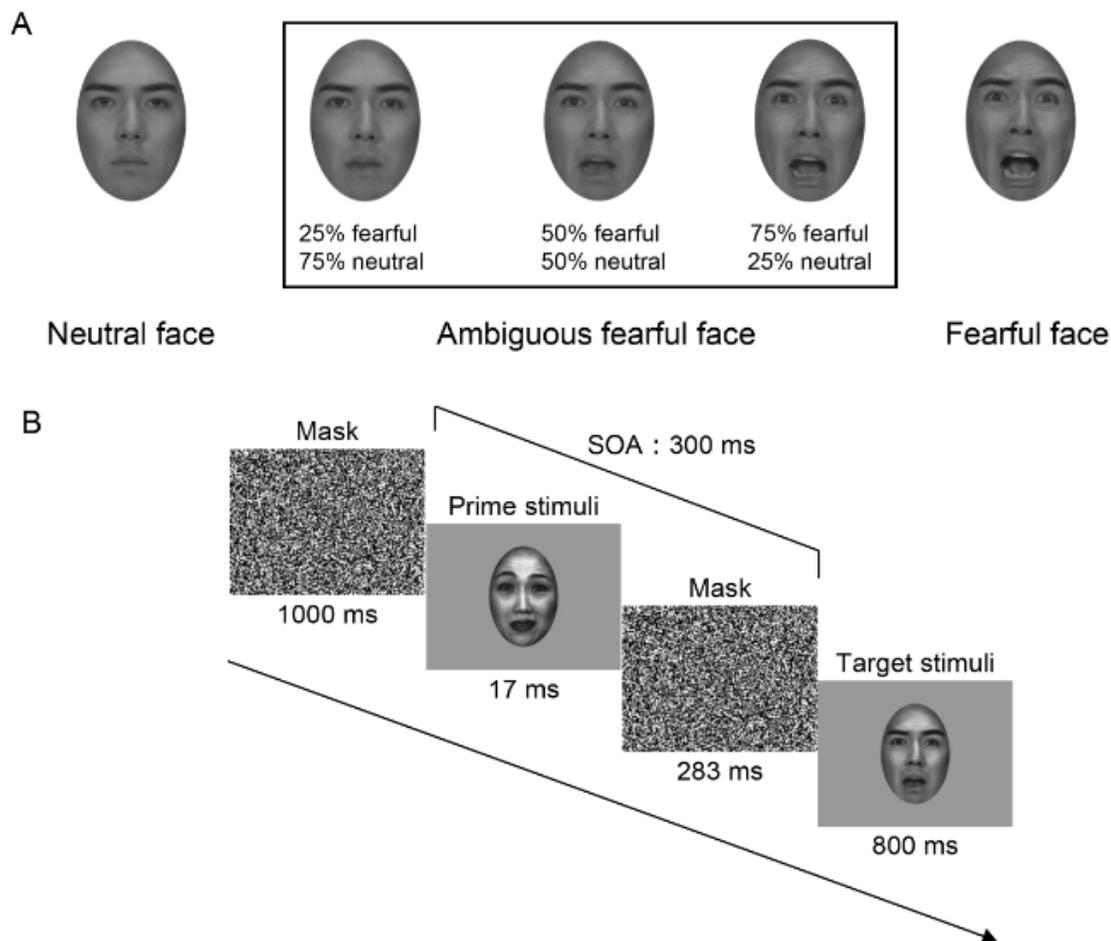
	Left				Right			
	$R^\dagger$	$p$	$R^{\ddagger}$	$p$	$r$	$p$	$R^2$	$p$
N170 amplitudes								
RR	0.02	n.s.			0.01	n.s.		
RT	-0.04	n.s.			0.38	< 0.001	0.15	< 0.001**
Prime	-0.03	n.s.			0.04	n.s.		
Target	0.03	n.s.			0.02	n.s.		
	Left				Right			
	$r$	$p$	$R^2$	$p$	$r$	$p$	$R^2$	$p$
N170 latencies								
RR	0.00	n.s.			0.03	n.s.		
RT	0.55	< 0.001	0.30	< 0.001**	0.50	< 0.001	0.25	< 0.001**
Prime	-0.02	n.s.			-0.02	n.s.		
Target	0.02	n.s.			0.03	n.s.		

## Females

	Left				Right			
	$r$	$p$	$R^2$	$p$	$r$	$p$	$R^2$	$p$
N170 amplitudes								
RR	-0.02	n.s.			0.01	n.s.		
RT	-0.02	n.s.			-0.03	n.s.		
Prime	0.02	n.s.			-0.01	n.s.		
Target	0.01	n.s.			-0.01	n.s.		
	Left				Right			
	$r$	$p$	$R^2$	$p$	$r$	$p$	$R^2$	$p$
N170 latencies								
RR	0.05	n.s.			0.00	n.s.		
RT	0.08	n.s.			0.00	n.s.		
Prime	-0.06	n.s.			-0.01	n.s.		
Target	-0.01	n.s.			0.02	n.s.		

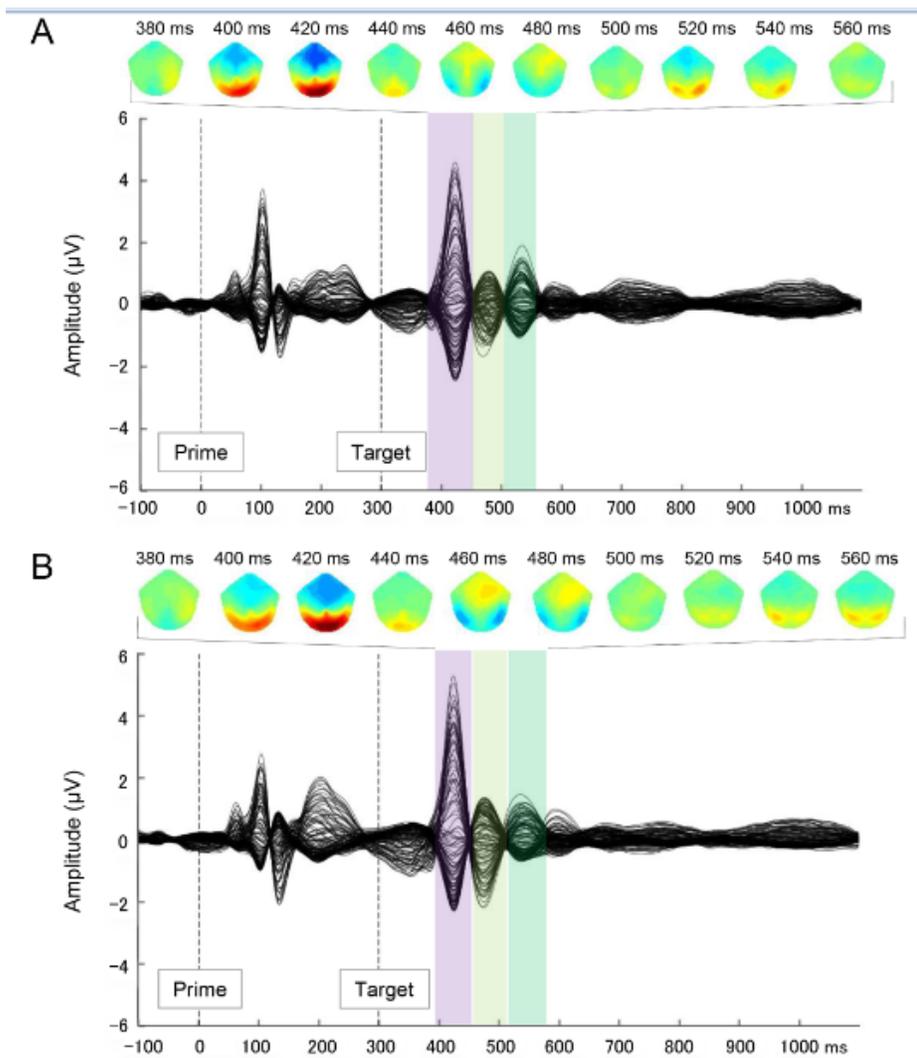
\* $p < 0.05$ , \*\* $p < 0.01$ , † Pearson's correlation, ‡ Multiple correlation from stepwise regression analysis

# Figures



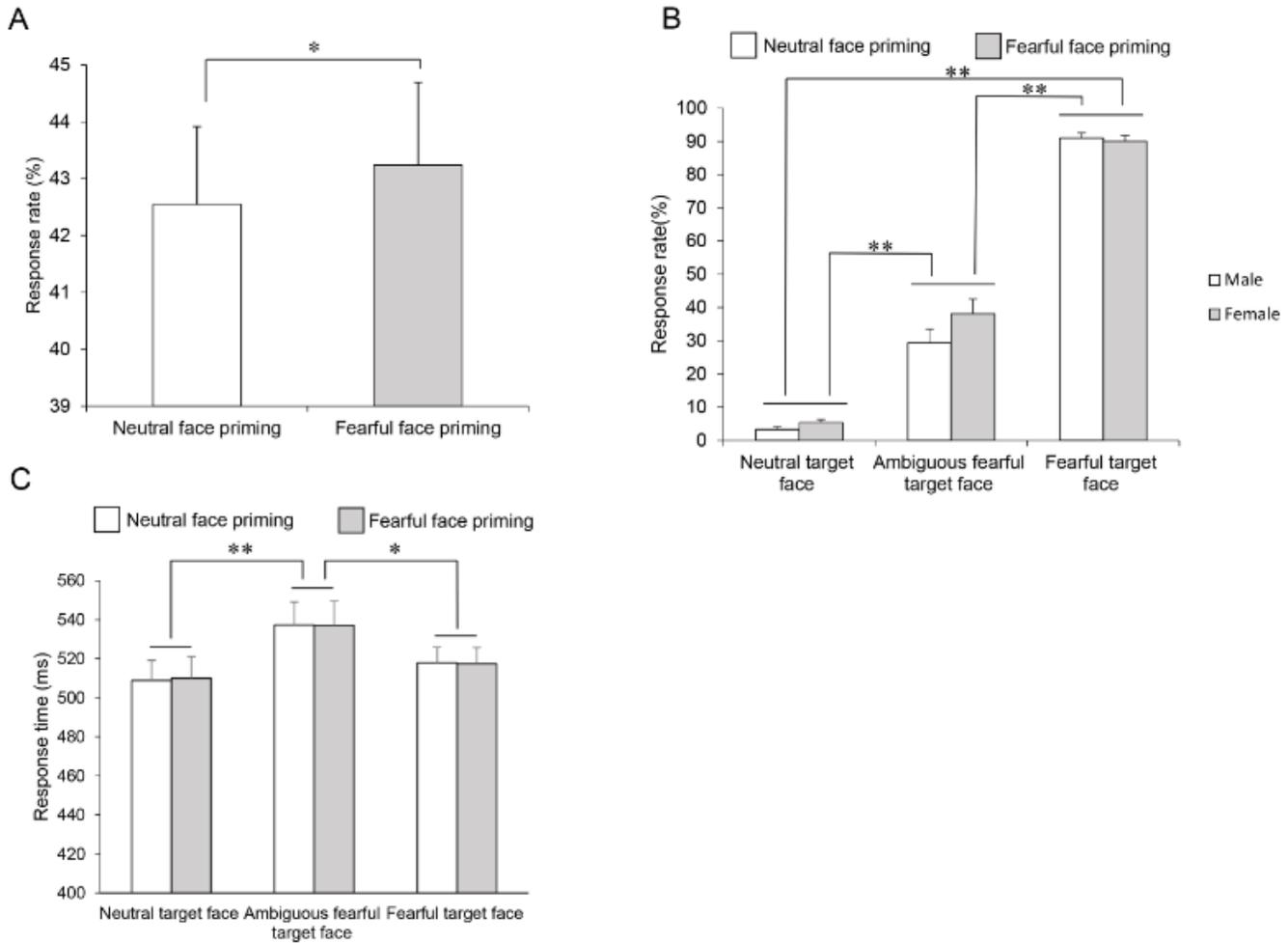
**Figure 1**

Behavioural results for response rates (RRs) and reaction times (RTs). RRs for the fearful face-priming condition were significantly higher than those for the neutral face-priming condition (A). RRs for fearful target-face stimuli were significantly higher than those for neutral and ambiguous-fearful target faces (B). RRs for ambiguous-fearful target faces were also significantly higher than those for neutral target faces. Gender differences were not significant. Reaction times (RTs) for the ambiguous-fearful target-face stimuli were significantly higher than those for neutral and fearful target faces (C). Error bars represent the SEM. \* $p < 0.05$ , \*\* $p < 0.01$



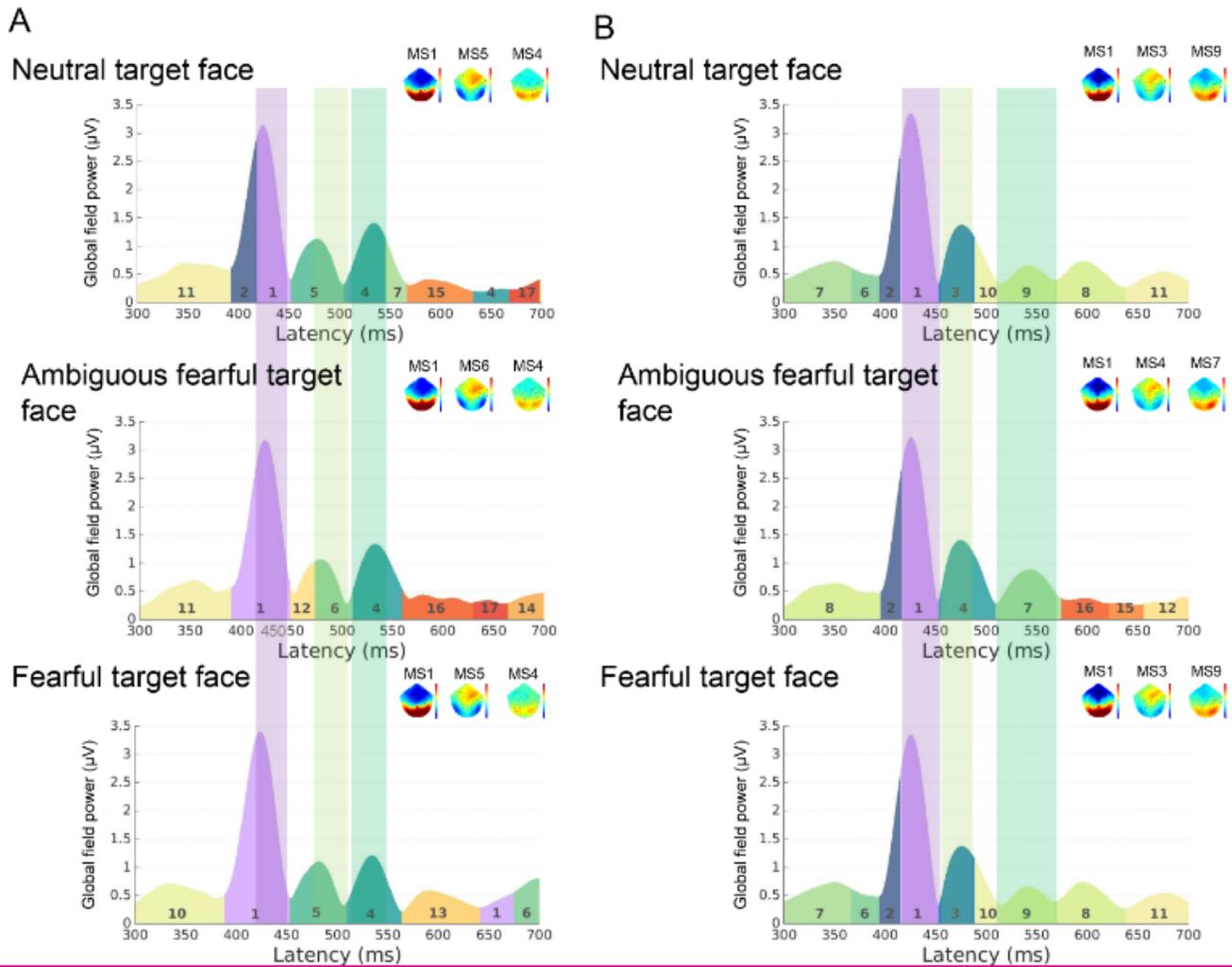
**Figure 2**

Group-averaged ERPs during the subliminal priming task, with waveforms from the 128 electrodes superimposed. All ERP data for the 6 conditions (female (A) or male (B)  $\times$  6 stimuli) were summed and grand-averaged. The ERP responses to the subliminally primed faces and target faces are clearly delineated. Note that three microstates are evident and shaded in purple, light green, and green. These microstates were further analysed.



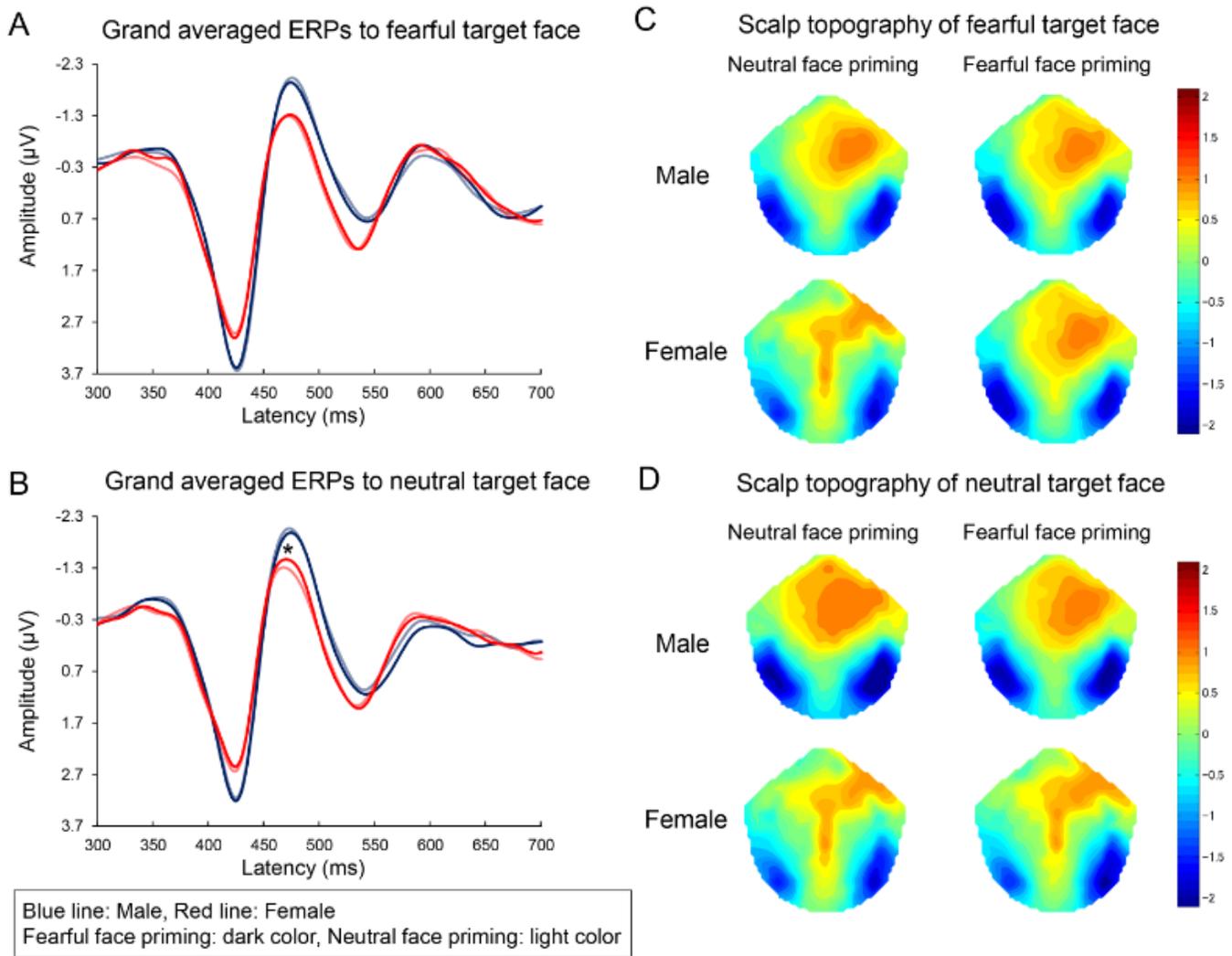
**Figure 3**

Microstate analysis outlining periods of topographic differences in the grand mean ERPs for females (A) and males (B). Fearful faces were used as the subliminal prime. Scalp topographies show microstate maps obtained from the cross-validation procedure. The microstate maps are displayed in sequence of occurrence from left to right. Insets show the different patterns for each condition. Red and blue indicate positive and negative potential values, respectively. The onset of microstate 6 (MS6) for the ambiguous-fearful target face, which corresponded to the N170 time window in females, was later than MS3 and MS4, which corresponded to N170 in males.



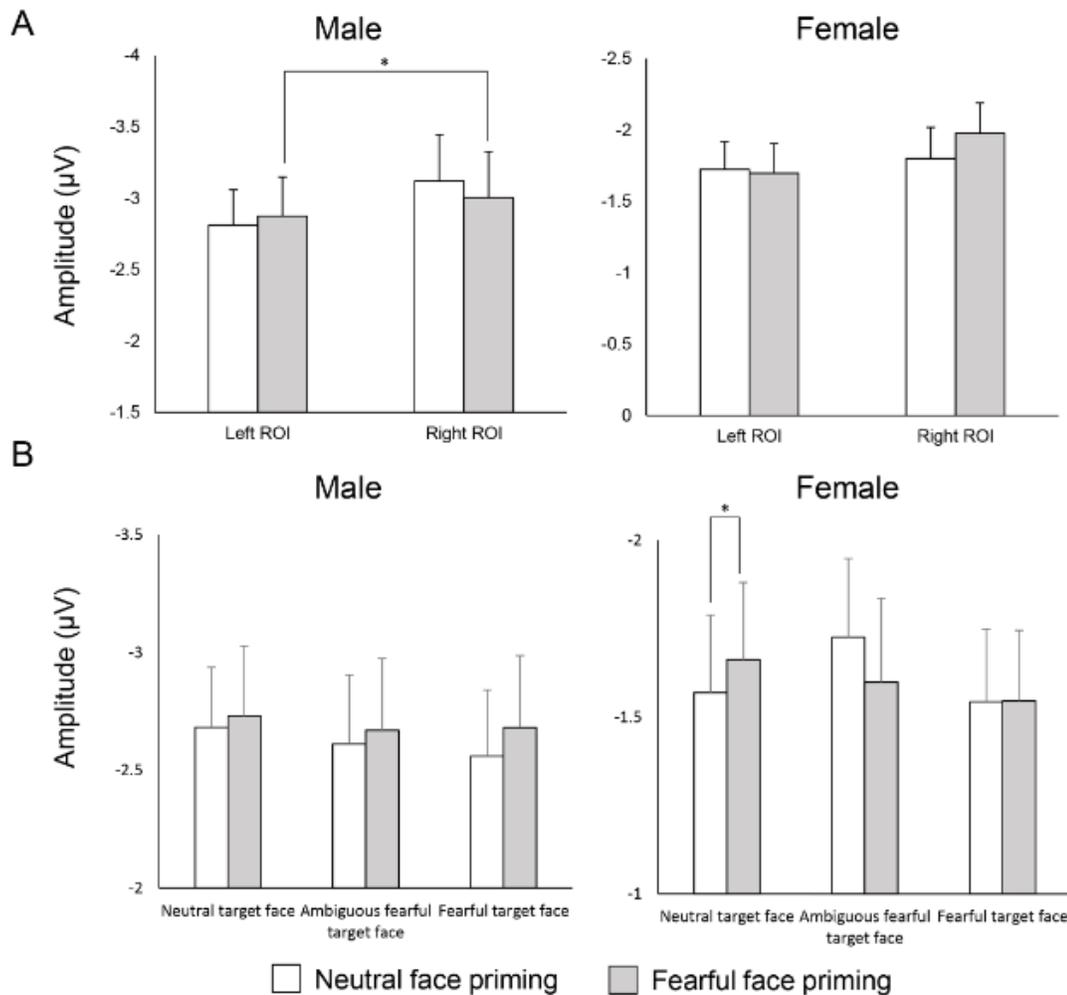
**Figure 4**

Grand averaged waveforms for the N170 in the right ROI of male and female participants and their scalp topographies. N170 amplitudes in female participants differed from those in males for both primed conditions (A, B) as did the scalp topography (C, D). In female participants, N170 amplitudes for the fearful prime condition were slightly larger than those for the neutral prime condition. For graphical presentation, ERP waveforms are shown 300 ms after the prime-stimuli onsets. The y-axis ranges from  $-2.3$  to  $3.7$   $\mu\text{V}$  for better viewing of the N170 waveforms.



**Figure 5**

N170 amplitudes in the right ROIs of male participants were significantly larger in the fearful face-prime condition than in the neutral face-prime condition for target faces primed by fearful face stimuli (A). N170 amplitudes in the right ROIs of female participants were significantly larger for neutral target faces in the fearful prime-face condition than for neutral targets in the neutral prime-face condition (B).



**Figure 6**

(A) Examples of the face stimuli used in the current experiment. Ambiguous fearful face stimuli were created by morphing neutral and fearful faces together, while keeping the same identity. Three types of 25% step-morphed faces were used to define emotionally ambiguous fearful faces for each participant. (B) The experimental procedures. Each trial began with mask stimuli and a red fixation cross lasting for 1000 ms. A prime-face stimulus (neutral or fearful) was presented for 17 ms and immediately followed by the mask stimulus for 283 ms (SOA was 300 ms). A target face stimulus (neutral, ambiguous-fearful, or fearful) then appeared for 800 ms. Participants were instructed to judge whether the target face was fearful or neutral as quickly as possible by pressing the left or right mouse button

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.pdf](#)