

Processing of Fearful Faces Exhibits Characteristics of Subcortical Functions

Kairui Yu¹, Junzhen Guo¹, Zhenjie Xu¹, Feiyang Shi¹, Xiaoqian Yu¹, Fang Fang^{2, 3, 4}, and Yingying Wang¹

¹Department of Psychology and Behavioral Sciences, Zhejiang University

²School of Psychological and Cognitive Sciences and Beijing Key Laboratory of Behavior and Mental Health, Peking University

³IDG/McGovern Institute for Brain Research, Peking University

⁴Peking-Tsinghua Center for Life Sciences, Peking University

A subcortical pathway is thought to have evolved to facilitate fear information transmission, but direct evidence for its existence in humans is lacking. In recent years, rapid, preattentive, and preconscious fear processing has been demonstrated, providing indirect support for the existence of the subcortical pathway by challenging the necessity of canonical cortical pathways in fear processing. However, direct support also requires evidence for the involvement of subcortical regions in fear processing. To address this issue, here we investigate whether fear processing reflects the characteristics of the subcortical structures in the hypothesized subcortical pathway. Using a monocular/dichoptic paradigm, Experiment 1 demonstrated a same-eye advantage for fearful but not neutral face processing, suggesting that fear processing relied on monocular neurons existing mainly in the subcortex. Experiments 2 and 3 further showed insensitivity to short-wavelength stimuli and a nasal-temporal hemifield asymmetry in fear processing, both of which were functional characteristics of the superior colliculus, a key hub of the subcortical pathway. Furthermore, all three experiments revealed a low spatial frequency selectivity of fear processing, consistent with magnocellular input via subcortical neurons. These results suggest a selective involvement of subcortical structures in fear processing, which, together with the indirect evidence for automatic fear processing, provides a more complete picture of the existence of a subcortical pathway for fear processing in humans.

Public Significance Statement

achieve rapid and automatic processing of threat information (LeDoux, 1996; Öhman & Mineka, 2001; Pessoa & Adolphs, 2010). However, due to the deep location and rapid response of the subcortical structures, which pose methodological challenges for functional magnetic resonance imaging, magnetoencephalography, and electroencephalogram (EEG) techniques, evidence for such a subcortical pathway to the amygdala in humans has mainly been inferred indirectly from neuroimaging and behavioral findings. For instance, compared to neutral information, threat information, such as fearful faces, snakes, and electric shock signals, can be detected rapidly (Bannerman et al., 2010; Méndez-Bértolo et al., 2016; Wang et al., 2023), preattentively (Luo et al., 2010; Morris et al., 1999; Vuilleumier et al., 2001), and preconsciously (Gomes et al., 2018; Morris et al., 1998, 1999; Whalen et al., 1998), suggesting its independence from the cortical pathways. However, an alternative to the subcortical pathway model suggests that some shortcut cortical pathways could achieve such threat processing equally well (Pessoa & Adolphs, 2010), casting further doubt on the existence of the subcortical pathway.

To validate the subcortical pathway hypothesis, indirect evidence has been gathered from an array of different studies using different approaches. To date, support for the subcortical pathway has come from three main sources. First, neuroimaging studies have shown enhanced responses to fear information in the amygdala in the absence of conscious awareness, either due to unconscious manipulations of backward masking (Morris et al., 1998; Wang et al., 2023; Whalen et al., 1998; L. M. Williams et al., 2006) or interocular suppression (Pasley et al., 2004; M. A. Williams et al., 2004) or due to

convergently hinting that subcortical regions are involved in threat processing. These results, together with the existing evidence for rapid, preconscious fear processing in the amygdala and the structural basis for the subcortical pathway, suggest the existence of a subcortical pathway to the human amygdala for threat processing.

Experiment 1: Same-Eye Advantage for LSF Fear Perception

Experiment 1 aimed to explore whether the processing of fearful faces relies on subcortical structures. Using a monocular/dichoptic paradigm (Gabay, Burlingham, & Behrmann, 2014; Gabay, Nestor, et al., 2014), we presented two consecutive faces, a priming face and a probe face, monocularly to the same eye or dichoptically to different eyes. Because the subcortex contains only monocular neurons, it should be invisible to priming faces presented to the different eye. Therefore, if fear processing takes place in the subcortex, the priming effect in the subcortex should be observed in the same-eye condition rather than in the different-eye condition. That is, a same-eye facilitation effect is expected for fear processing. Furthermore, because the subcortical pathway is thought to carry only crude visual input to the amygdala through magnocellular neurons (Vuilleumier et al., 2003), the same-eye facilitation should be selective to the LSF component.

Method

Transparency and Openness

All data of all experiments are publicly available via Open Science Framework and are accessible at <https://osf.io/dnx5m/>. The experiments reported here were not preregistered and the data were collected in 2022–2023.

Participants

Participants were 30 adults (21 participants reported their gender as female and nine as male, 19–27 years, $M_{\text{age}} = 22.1$ years, $SD = 5.6$) recruited from Zhejiang University. The sample size was similar to conventional sample sizes in prior experiments using the same paradigm (Collins et al., 2017; Gabay, Burlingham, & Behrmann, 2014; Zhao et al., 2023). All participants had normal or corrected-to-normal vision and were naive to the purpose of the experiments. They provided written informed consent and received monetary compensation for participation. The experimental procedure was approved by the Human Subject Review Committee of Zhejiang University.

Apparatus and Software

The stimuli were presented on a 21-in. cathode-ray tube screen with a refresh rate of 75 Hz and a resolution of $1,024 \times 768$ pixels. Participants viewed the screen with both eyes through a stereoscope so that the left and right halves of the screen were projected to the left and right eyes, respectively. A cardboard divider was attached to the chin rest to block the participants' direct view of the screen. A white rectangular frame ($5^\circ \times 6.3^\circ$) was presented at the center of each half of the screen to minimize eye movements. Participants adjusted the stereoscope to fuse the perception of two eyes before starting the experiment. The MATLAB (The MathWorks) and Psychtoolbox-3 toolbox were used to display the visual stimuli.

Materials

Stimuli were faces of 48 actors (24 females) posing fearful and neutral expressions. The faces were obtained from two databases: Radboud Faces Database (<https://www.socsci.ru.nl:8180/RaFD2/RaFD>) and NimStim Set of Facial Expressions (<https://danlab.psychology.columbia.edu/content/nimstim-set-facial-expressions>). Images were processed following the procedure by McFadyen et al. (2017). All images were gray-scaled, equalized in mean luminance, reshaped into the same size ($5^\circ \times 6.3^\circ$), and cropped to exclude most hair and background. In addition to the original (broad spatial frequency [BSF]) face images, faces containing low- or high-spatial-frequency information were created by filtering the BSF faces using a low-pass cutoff of <6 cycles per image (LSF) and a high-pass cutoff of >24 cycles per image (high spatial frequency [HSF]; Wang et al., 2023), respectively. Images were drawn randomly from the stimuli set for each condition for each participant.

Procedure

Each trial started with the appearance of a white fixation cross at the center of the rectangular frame in each visual field for 500 ms. Two faces, one priming and one probe face, were then presented sequentially on the screen, each for 200 ms and interleaved by a 500-ms fixation interval (Figure 1). The two consecutive faces were presented to the same eye in the same-eye condition and to different eyes in the

$t(29) = 3.95, p < .001, \text{Cohen's } d = 0.72$. No significant same-eye advantage was observed for the BSF, $M_{\text{difference}} = 0, 95\% \text{ CI } [-2.72, 2.72], t(29) = 0, p = 1.000, \text{Cohen's } d = 0$, or HSF, $M_{\text{difference}} = 0.44, 95\% \text{ CI } [-2.34, 3.23], t(29) = 0.33, p = .747, \text{Cohen's } d =$

Figure 2
Percentage of Correct Emotion Discrimination for Fearful and Neutral Faces



Note. (A) Same-eye presentation induced a facilitation effect on LSF but not HSF or BSF fear discrimination than different-eye presentation. (B) No same-eye versus different-eye presentation difference was observed for the neutral face independent of the SF information it contained. Error bars represent SEMs across participants. LSF = low spatial frequency; HSF = high spatial frequency; BSF = broad spatial frequency; SF = spatial frequency; SEM = standard error of the mean. See the online article for the color version of this figure. *** $p < .001$ (significant differences between the monocular and dichoptic presentation conditions).

participants reported their gender as female and nine as male, 18–28 years, $M_{\text{age}} = 21.4$ years, $SD = 6.0$ adults from Zhejiang University. All participants had normal or corrected-to-normal vision, were not color blind, and were naive to the purpose of the experiments. They provided written informed consent and received monetary compensation for participation. The experimental procedure was approved by the Human Subject Review Committee of Zhejiang University.

Materials

The same apparatus was used as in Experiment 1. In both experiments, LSF face images were used as probes while LSF gratings were used as priming stimuli. The face images in Experiment 2a were the LSF face images from Experiment 1. Sixty LSF face images were drawn randomly from the stimuli set for each condition for each participant, resulting in a total of 480 images being used. To prevent cultural influences on emotion recognition caused by unfamiliarity with and misrecognition of facial expressions from other cultures (Wang et al., 2019), Experiment 2b used face images from the Chinese Facial Affective Picture System (Gong et al., 2011). The LSF face images were created using the same criteria as Experiment 1. In each trial, a face image from a certain condition was randomly selected from the 25 LSF face images for each participant. As the total number of face images was roughly half that used in Experiments 1 and 2a, we reduced the number of trials to 240 in Experiment 2b.

Experiments 2a and 2b used the same priming grating stimuli. The priming grating stimuli had a cutoff of 5 cycles per image. There were two types of grating stimuli: gray (colorimetric values: $x = 0.30$, $y = 0.30$) and chromatic purple/blue ($x = 0.183/0.270$, $y = 0.087/0.087$; Tamietto et al., 2009). To ensure that the SF information was

determined solely by the color differences in the gratings, the gratings were carefully matched for their physical attributes; that is, all stimuli had the same mean luminance (10.8 cd/m^2) and were of the same size (8°). The gratings were oriented 45° clockwise or counterclockwise relative to the vertical orientationAQ11.

Procedure

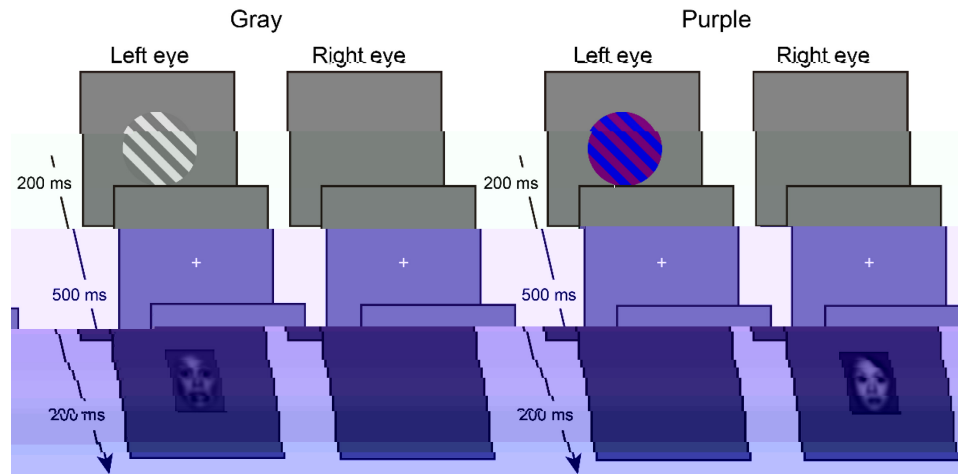
The procedure, which was identical in Experiments 2a and 2b, was similar to that in Experiment 1 except that gray or purple/blue gratings were used as the priming stimuli (Figure 3). Specifically, a gray or purple/blue LSF grating was presented for 200 ms to a random eye. After a 500-ms interval, an LSF fearful or neutral face was presented to the same (monocular presentation) or different (dichoptic presentation) eye than the LSF grating for 200 ms. Participants were asked to judge whether the probe face showed a fearful or neutral expression and did not need to respond to the gratings. The response was required to be made within 3,000 ms. Participants completed 480 trials in six blocks (80 trials/block). The order of trials from different conditions was randomized within each block. Perceptual fusion from the two eyes was checked at the beginning of each block.

Results

Experiment 2a

Given that the monocular advantage was only observed for the LSF fearful faces in Experiment 1, we predicted that the subcortical characteristics would be present in the fearful but not in the neutral condition. Therefore, for Experiment 2a, we performed a 2 (priming condition: gray and purple) \times 2 (eye source: same and different) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA

Figure 3
The Experimental Procedure in Experiments 2a and 2b



Note. A gray (left) or purple/blue (right) grating with LSF information was presented to one eye through a stereoscope, followed by an LSF face image presented to the same (left) or different (right) eye. Participants judged the emotion the probe face expressed. From the *NimStim Set of Facial Expressions* (<https://danlab.psychology.columbia.edu/content/nimstim-set-facial-expressions>). LSF = low spatial frequency; ms = milliseconds. See the online article for the color version of this figure.

on the emotion recognition accuracy of the probe faces. The three-way interaction effect was significant (Figure 4A), $F(1, 29) = 12.18, p = .002, \eta_p^2 = .30$, suggesting different results patterns in the fearful and neutral face condition. Further analysis showed that the priming condition by eye source interaction effect was significant in the fearful face condition, $F(1, 29) = 16.13, p < .001, \eta_p^2 = .36$. Simple effect analyses showed a same-eye advantage for LSF fearful faces primed by a gray LSF grating. Specifically, when the gray LSF grating was presented to the same eye, the recognition of LSF fearful faces was increased significantly when compared to the grating being presented to the different eye, $M_{\text{difference}} = 4.02, 95\% \text{ CI } [2.06, 5.98], t(29) = 4.20, p < .001, \text{Cohen's } d = 0.77$. No significant same-eye versus different-eye difference was observed in the purple/blue grating priming condition, $M_{\text{difference}} = -2.24, 95\% \text{ CI } [-4.81, 0.33], t(29) = -1.78, p = .086, \text{Cohen's } d = 0.33$. In contrast to the fearful face condition, the priming condition by eye source interaction effect was not significant for the neutral face condition, $F(1, 29) = 0.14, p = .712, \eta_p^2 = .01$. No significant same-eye advantage was observed under either gray, $M_{\text{difference}} = -2.05, 95\% \text{ CI } [-4.23, 0.00], t(29) = -1.93, p = .063, \text{Cohen's } d = 0.35$, or purple/blue, $M_{\text{difference}} = -1.53, 95\% \text{ CI } [-3.59, 0.54], t(29) = -1.51, p = .141, \text{Cohen's } d = 0.28$, LSF grating priming. Therefore, the same-eye facilitation effect was abolished when S-cones, which exist in the LGN but not in the SC, were stimulated. This result suggests that the SC underlies the same-eye advantage of LSF fear processing.

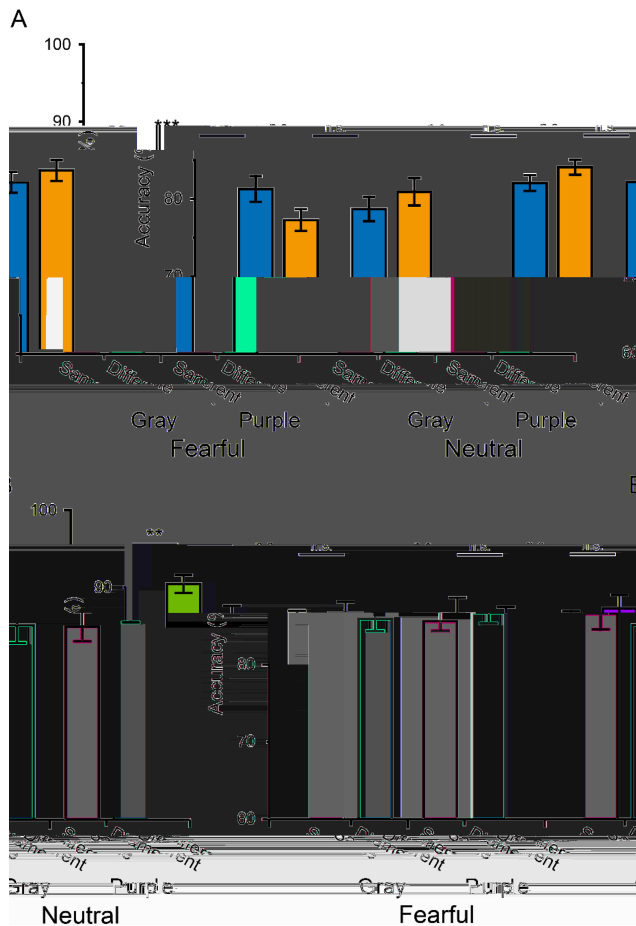
To rule out the speed-accuracy trade-off, we performed a 2 (priming condition: gray and purple) \times 2 (eye source: same and different) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA on the RT. No significant three-way interaction effect was observed, $F(1, 29) = 1.20, p = .282, \eta_p^2 = .04$, and no significant priming condition by eye source interaction effect was observed for RT in either emotion condition, fearful, $F(1, 29) = 1.01, p = .324, \eta_p^2 = .03$; neutral, $F(1, 29) =$

$0.11, p = .740, \eta_p^2 = .004$. The main effect of eye source was not significant, fearful, $F(1, 29) = 0.11, p = .738, \eta_p^2 = .003$; neutral, $F(1, 29) = 2.01, p = .167, \eta_p^2 = .06$, either. Simple effects analyses further showed no significant same- versus different-eye RT difference under the gray, fearful, $M_{\text{difference}} = -0.01, 95\% \text{ CI } [-0.03, 0.01], t(29) = -0.85, p = .403, \text{Cohen's } d = 0.15$; neutral, $M_{\text{difference}} = -0.01, 95\% \text{ CI } [-0.03, 0.01], t(29) = -0.93, p = .358, \text{Cohen's } d = 0.17$, or purple/blue, fearful, $M_{\text{difference}} = 0.003, 95\% \text{ CI } [-0.02, 0.02], t(29) = 0.29, p = .774, \text{Cohen's } d = 0.05$; neutral, $M_{\text{difference}} = -0.01, 95\% \text{ CI } [-0.03, 0.01], t(29) = -1.34, p = .191, \text{Cohen's } d = 0.24$, LSF grating priming.

Experiment 2b

The same analyses were performed as in Experiment 2a. First, we performed a 2 (priming condition: gray and purple) \times 2 (eye source: same and different) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA on the emotion recognition accuracy of the probe faces. The three-way interaction effect was not significant (Figure 4B), $F(1, 29) = 0.32, p = .576, \eta_p^2 = .01$. Nevertheless, we went on to investigate whether the priming effects differed for different eye sources in the fearful and neutral emotion condition, respectively. We performed a 2 (priming condition: gray and purple) \times 2 (eye source: same and different) on the fearful face condition and found a significant priming condition by eye source interaction effect, $F(1, 29) = 5.95, p = .021, \eta_p^2 = .17$, suggesting that the same-eye advantage was different in the two priming conditions. Simple effect analyses further showed that, when the gray LSF grating was presented to the same eye, the recognition of LSF fearful faces was increased significantly when compared to the grating being presented to the different eye, $M_{\text{difference}} = 4.47, 95\% \text{ CI } [1.38, 7.54], t(29) = 2.96, p = .006, \text{Cohen's } d = 0.54$. No significant same-eye versus different-eye difference was observed in the purple/blue grating priming condition, $M_{\text{difference}} = -1.10, 95\% \text{ CI } [-2.20, 0.00], t(29) = -1.10, p = .274, \text{Cohen's } d = 0.18$.

Figure 4
Emotion Recognition Accuracy Under Different Priming Conditions



Note. Emotion recognition results of Experiments 2a (A) and 2b (B). In both experiments, gray rather than purple LSF grating induced a facilitation effect on LSF fear recognition when the grating and the face was presented to the same eye than to different eyes. No same-eye versus different-eye facilitation effect was observed for neutral faces independent of whether the priming grating was gray or purple. Error bars represent SEMs across participants. LSF = low spatial frequency; n.s. = nonsignificant; SEM = standard error of the mean. See the online article for the color version of this figure.

** $p < .01$. *** $p < .001$ (significant differences between the monocular and dichoptic priming conditions).

$[-4.54, 2.33]$, $t(29) = -0.66$, $p = .518$, Cohen's $d = 0.12$. In contrast to the fearful face condition, the priming condition by eye source interaction effect was not significant for the neutral face condition, $F(1, 29) = 2.70$, $p = .111$, $\eta_p^2 = .09$. No significant same-eye advantage was observed under either gray, $M_{\text{difference}} = 1.48$, 95% CI $[-1.85, 4.81]$, $t(29) = 0.91$, $p = .371$, Cohen's $d = 0.17$, or purple/blue, $M_{\text{difference}} = -2.30$, 95% CI $[-4.83, 0.24]$, $t(29) = -1.85$, $p = .075$, Cohen's $d = 0.34$, LSF grating priming. Therefore, our results consistently show that the same-eye facilitation effect was abolished when S-cones, which exist in the LGN but not in the SC, were stimulated.

Finally, we performed a 2 (priming condition: gray and purple) \times 2 (eye source: same and different) \times 2 (emotion: fearful vs. neutral)

repeated measures ANOVA on the RT. No significant three-way interaction effect was observed, $F(1, 29) = 1.72$, $p = .200$, $\eta_p^2 = .06$. No significant priming condition by eye source interaction effect was observed for RT in either emotion condition, fearful, $F(1, 29) = 0.003$, $p = .960$, $\eta_p^2 = .00$; neutral, $F(1, 29) = 2.25$, $p = .145$, $\eta_p^2 = .07$. However, we still explored the same-eye effects in each priming and emotion condition. Simple effects analyses showed significant same- versus different-eye RT differences under the gray LSF grating priming for both emotions, fearful, $M_{\text{difference}} = -0.02$, 95% CI $[-0.04, -0.003]$, $t(29) = -2.44$, $p = .021$, Cohen's $d = 0.44$; neutral, $M_{\text{difference}} = -0.02$, 95% CI $[-0.05, -0.001]$, $t(29) = -2.14$, $p = .041$, Cohen's $d = 0.39$, and under the purple/blue LSF grating priming for fearful, $M_{\text{difference}} = -0.02$, 95% CI $[-0.04, -0.003]$, $t(29) = -2.38$, $p = .024$, Cohen's $d = 0.43$, but not neutral, $M_{\text{difference}} = -0.002$, 95% CI $[-0.03, 0.02]$, $t(29) = -0.21$, $p = .837$, Cohen's $d = 0.04$, emotion. Therefore, the same-eye facilitation effect in the gray grating priming condition was unlikely due to speed-accuracy trade-off.

Experiment 3: Nasal–Temporal Asymmetry for Saccades to LSF Fear

To strengthen the evidence for the involvement of the SC in fear processing, Experiment 3 further explored another characteristic of the SC, namely the nasal–temporal asymmetry of fear processing. Since more fibers are projected to the SC from the nasal hemiretina than from the temporal hemiretina (Perry & Cowey, 1985; Pollack & Hickey, 1979), the SC is expected to be more sensitive to fear information presented to the temporal than to the nasal hemifield. Furthermore, given that the SC is involved in oculomotor behavior (Bannerman et al., 2010; Cynader & Berman, 1972; Koller & Rafal, 2019), this function is likely to be reflected directly in the saccadic response. Therefore, in Experiment 3, we used eye-tracking to investigate the saccadic response to fearful faces that were presented in either the temporal or the nasal hemifield. Two independent experiments were performed to ensure the reliability of the results.

Participants

The same sample size determination and participant recruitment criteria were used as in Experiment 2. Thirty adults from Zhejiang University took part in Experiments 3a (20 participants reported their gender as female and 10 as male, 19–31 years, $M_{\text{age}} = 23.1$ years, $SD = 7.3$) and 3b (23 participants reported their gender as female and seven as male, 19–29 years, $M_{\text{age}} = 22.2$ years, $SD = 7.1$), respectively.

Materials

Stimuli in Experiment 3a were LSF and HSF face images ($10^\circ \times 12.6^\circ$) from Experiment 1. The stimuli in Experiment 3b were LSF and HSF fearful and neutral face images from the Chinese Facial Affective Picture System (Gong et al., 2011). Images of 25 identities were obtained for each condition. The same criteria as in Experiment 1 were used to generate the LSF and HSF face images. Images were drawn randomly from the stimuli set for each condition for each participant.

Eye-Tracking Apparatus

An Eyelink1000 eye-tracking device continuously recorded eye position at a sampling rate of 500 Hz. Eyelink1000 analysis software was used to measure saccade latency and velocity. The same processing criteria were utilized in both experiments. Saccades were detected with a velocity of 30 degrees/second and acceleration of 8,000 degrees/second². Trials with saccades faster than 50 ms were excluded from the analysis.

Procedure

The experiment comprised two eye-tracking sessions carried out under monocular eye viewing using an eye patch. One session was completed with left eye viewing and the other with right eye viewing. Sessions were counterbalanced to control for order effects. Participants were instructed to place their head and chin on a chin rest and to look straight at the center of the computer screen (Figure 5).

The procedure was identical in Experiments 3a and 3b. A white fixation was constantly presented at the center of the screen. A trial started when the eye position was maintained within 2° from the central fixation for 1,000 ms. After a 200-ms gap, two faces, one neutral and one fearful, were presented on the left and right sides of the central fixation, with an eccentricity of 12°. Depending on which eye was used, the left and right image could correspond to either the temporal or nasal hemifield. The pairs of neutral and fearful faces stayed for 100 ms on the screen in three possible random stimulus onset asynchronies: (a) fearful preceding neutral face onset by 50 ms, (b) neutral preceding fearful face onset by 50 ms, and (c) simultaneous onset. The small interval between the first and last presented faces and the presence of the simultaneous onset trials increased the difficulty of the task and were designed to encourage automatic saccadic responses driven by the stimulus. Participants were unknown about the simultaneous onset condition and were required to make a saccade to the location where the first presented face was shown. Participants completed a total of 600 trials in six blocks. Faces with different spatial frequency information were presented in separate blocks. The order of trials from different conditions was randomized within each block.

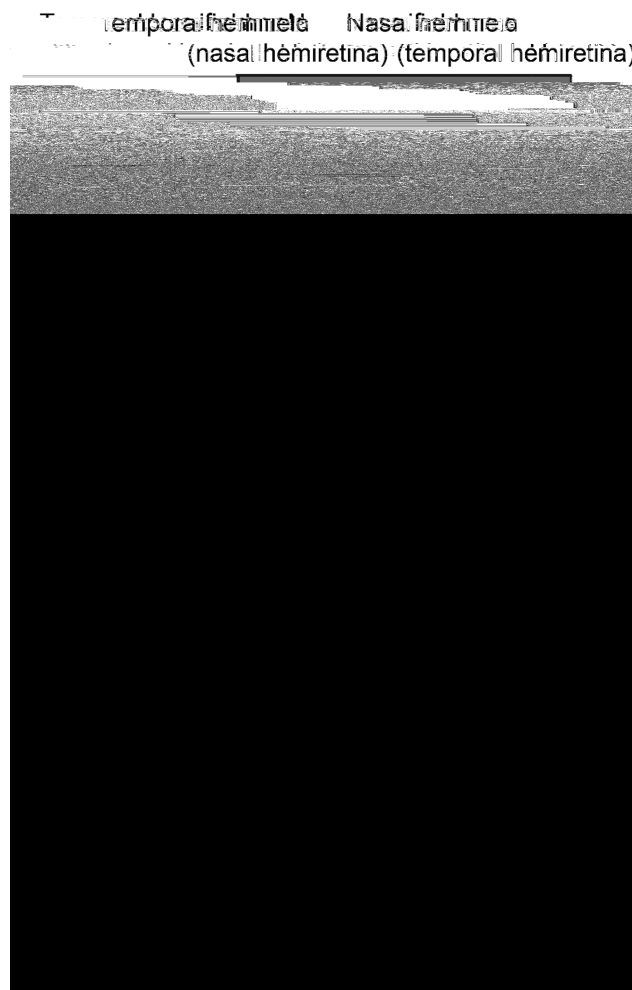
Results

Experiment 3a

We first calculated the percentage of correct saccade response in each condition. We found that the saccade accuracy was not significantly different from the chance level of 50% in the fearful-first (LSF temporal, $p = .233$; LSF nasal, $p = .736$; HSF temporal, $p = .290$; HSF nasal, $p = .169$) or the neutral-first (LSF temporal, $p = 1.000$; LSF nasal, $p = .619$; HSF temporal, $p = .736$; HSF nasal, $p = .390$) condition. We then performed a 2 (hemifield: temporal and nasal) \times 2 (SF: LSF and HSF) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA on the saccade accuracy. The three-way interaction effect was not significant (Figure 6A), $F(1, 29) = 0.70$, $p = .408$, $\eta_p^2 = .02$. Repeated measures ANOVA did not show a significant interaction effect between SF (LSF and HSF) and hemifield (temporal and nasal) on the saccade accuracy for the fearful-first, $F(1, 29) = 0.51$, $p = .479$, $\eta_p^2 = .02$, or neutral-first, $F(1, 29) = 0.67$, $p = .422$, $\eta_p^2 = .02$, condition, either. Furthermore, no

Figure 5

A Schematic Depiction of the Experimental Procedure and Monocular Viewing Setting in Experiments 3a and 3b

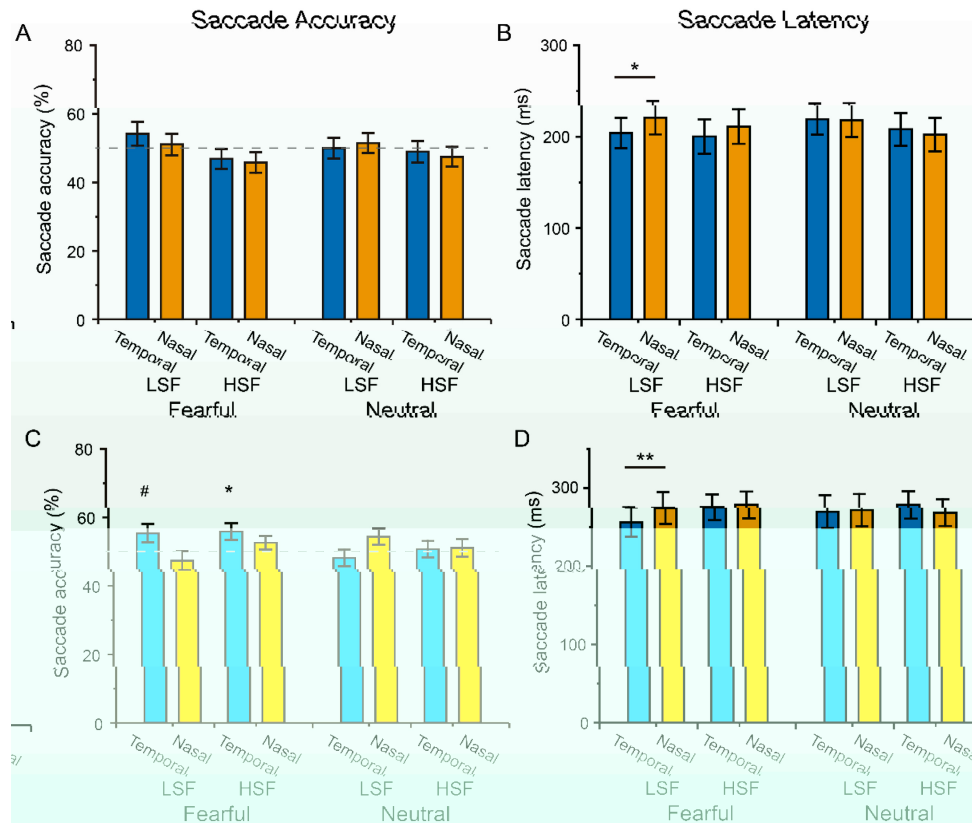


Note. (Top) Two faces, one fearful and one neutral, were presented sequentially to one eye (the left eye in the graphic illustration). Participants were asked to make a saccade to the first presented face. (Bottom) Due to the monocular viewing setting, when viewing with the left eye, the face at the left and right from the central fixation was presented to the temporal and nasal hemifield, respectively. From the *NimStim Set of Facial Expressions* (<https://danlab.psychology.columbia.edu/content/nimstim-set-facial-expressions>).

significant nasal–temporal asymmetry effects ($ps > .24$) were observed. These results suggest that participants were not explicitly aware of which face was presented first during the experiment, suggesting that the saccadic response was mainly stimulus-driven.

Next, we explored whether the fearful face presented to the temporal hemifield would facilitate the saccade response by reducing the saccade latency. We first performed a 2 (hemifield: temporal and nasal) \times 2 (SF: LSF and HSF) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA on the saccade latency. The three-way interaction effect was not significant (Figure 6B), $F(1, 29) = 0.001$, $p = .972$, $\eta_p^2 = .00$. Next, we performed a 2 (SF: LSF and HSF) \times 2 (hemifield: temporal and nasal) repeated measures ANOVA on the saccade latency to the fearful-first condition. The interaction effect

Figure 6
Accuracy and Latency of Saccades to Faces Presented to the Temporal and Nasal Hemifields



Note. (A, C) The saccade accuracy of each condition in Experiments 3a (A) and 3b (C). The saccade accuracy of each condition was mostly at the chance level. The saccade accuracy did not differ between the temporal and nasal conditions, showing no nasal-temporal asymmetry effects in either LSF or HSF condition for either fearful or neutral faces. (B, D) The saccade latency of each condition in Experiments 3a (B) and 3b (D). The saccade latency to LSF fearful faces presented to the temporal hemifield was significantly shorter than that to the nasal hemifield. The saccade latency to neutral faces was not modulated by the spatial frequency or hemifield of the presented faces. Error bars represent SEMs across participants. LSF = low spatial frequency; HSF = high spatial frequency; SEM = standard error of the mean. See the online article for the color version of this figure.

$p < .06$. * $p < .05$. ** $p < .01$ (significant differences between the monocular and dichoptic priming conditions).

was not significant, $F(1, 29) = 0.19$, $p = .670$, $\eta_p^2 = .01$, either. Nevertheless, with the a priori hypothesis that the fearful face presented to the temporal hemifield would facilitate the saccade response, we still performed simple effects analyses and indeed observed a faster saccade latency for LSF fearful faces presented to the temporal relative to nasal hemifield, $M_{\text{difference}} = -16.1$, $t(29) = -2.32$, $p = .014$ one-tailed, Cohen's $d = 0.42$. No significant difference in saccade latency was observed for HSF fearful faces presented in the temporal and nasal hemifields, $M_{\text{difference}} = -10.6$, $t(29) = -0.93$, $p = .180$ one-tailed, Cohen's $d = 0.17$. In contrast to the fearful face condition, no significant interaction or simple effects were observed to either LSF or HSF neutral faces ($ps > .05$). Therefore, a temporal hemifield advantage was observed for LSF fear as revealed by faster saccade latency.

Experiment 3b

The same analyses were performed as Experiment 3a. First, we calculated the percentage of correct saccade response in each condition.

A trend of above-chance level saccade accuracy was found only when the LSF (Figure 6C), $M_{\text{difference}} = 5.37$, $t(29) = 1.98$, $p = .057$, Cohen's $d = 0.36$, and HSF, $M_{\text{difference}} = 5.87$, $t(29) = 2.42$, $p = .022$, Cohen's $d = 0.44$, fearful faces were presented to the temporal hemifield. However, repeated measures ANOVA did not show a significant three-way interaction effect between 2 (hemifield: temporal and nasal) and 2 (SF: LSF and HSF) and 2 (emotion: fearful vs. neutral), $F(1, 29) = 1.66$, $p = .208$, $\eta_p^2 = .05$, or a significant two-way interaction effect between SF (LSF and HSF) and hemifield (temporal and nasal) on the saccade accuracy for either the fearful-, $F(1, 29) = 0.92$, $p = .344$, $\eta_p^2 = .03$, or neutral-first, $F(1, 29) = 1.42$, $p = .244$, $\eta_p^2 = .05$, condition. No significant nasal-temporal asymmetry effects ($ps > .09$) were observed, either. Therefore, no nasal-temporal asymmetry effects were observed in the saccade accuracy.

Next, we went on testing whether the fearful face presented to the temporal hemifield would facilitate the saccade response. We performed a 2 (hemifield: temporal and nasal) \times 2 (SF: LSF and HSF) \times 2 (emotion: fearful vs. neutral) repeated measures ANOVA on the

saccade latency. The three-way interaction effect was not significant (Figure 6D), $F(1, 29) = 0.07$, $p = .786$, $\eta_p^2 = .003$. Next, we performed a 2 (SF: LSF and HSF) \times 2 (hemifield: temporal and nasal) repeated measures ANOVA on the saccade latency to the fearful-first condition. We observed a marginally significant interaction effect, $F(1, 29) = 3.96$, $p = .056$, $\eta_p^2 = .12$. Consistent with our a priori hypothesis, simple effects analyses showed a faster saccade latency for LSF fearful faces presented to the temporal relative to nasal hemifield, $M_{\text{difference}} = -18.01$, $t(29) = -2.71$, $p = .006$ one-tailed, Cohen's $d = 0.49$. No significant difference in saccade latency was observed for HSF fearful faces presented in the temporal and nasal hemifields, $M_{\text{difference}} = -3.08$, $t(29) = -0.53$, $p = .299$ one-tailed, Cohen's $d = 0.10$. In contrast to the fearful face condition, no significant interaction or simple effects were observed to either LSF or HSF neutral faces ($ps > .05$). Therefore, a temporal hemifield advantage was observed specifically for LSF fear (Figure 6).

Discussion

The present study tested the subcortical pathway hypothesis for fear processing. We speculated that if fear-related information is transmitted to the amygdala via subcortical structures, the perception of fearful faces would exhibit characteristics indicative of subcortical functions. Across five experiments, we observed that the detection of fearful faces displayed features aligning with the characteristics of subcortical structures implicated in the subcortical pathway hypothesis. Specifically, the perception of fearful faces demonstrated a same-eye advantage (Experiment 1), suggesting processing by monocular neurons. Priming with short-wavelength gratings abolished the same-eye advantage (Experiments 2a and 2b), consistent with the known insensitivity of the SC to short-wavelength light. Furthermore, a nasal-temporal asymmetry manifested in the saccadic response to fearful faces (Experiments 3a and 3b), mirroring the nasal-temporal anatomical asymmetry of fibers projecting to the SC. Finally, these observed features were selectively present for LSF but not HSF fearful faces (Experiments 1–3), as predicted by magnocellular inputs to the amygdala. Taken together, the confluence of these four features point to information transmission via the subcortex, the SC in particular, thereby supporting the existence of a subcortical pathway in fear processing.

Our experiments provide four lines of evidence for the involvement of subcortical brain structures in fearful face processing. First, all three experiments, especially Experiments 1 and 3, showed

properties of subcortical brain regions, the SC in particular, suggest that even if multiple-wave pathways underlie the transmission of threat information to the amygdala, the multiple-wave pathways still receive information from the subcortical pathway. Therefore, we suggest that our results are more consistent with the subcortical explanation than with the multiple-wave explanation. Overall, our finding of subcortical features in fear processing complements the existing evidence and strengthens the case for fear processing by the subcortical pathway.

Controversial evidence exists for the subcortical pathway hypothesis. For instance, regarding the reflection of subcortical characteristics, previous studies have shown that the amygdala relies equally on the LSF and HSF information in emotion processing, reflecting its dependence on multiple sources from retinal and cortical input (McFadyen et al., 2017; Tamietto & Morrone, 2016). Behaviorally, a preference for HSF information in fearful face recognition has been observed (Smith & Schyns, 2009), along with facilitated recognition of HSF fearful faces (Stein et al., 2014). However, without identifying the precise temporal information of these processes, it is always possible that the processing is influenced by the feedback signals from the cortical pathway. Therefore, a comprehensive investigation combining the recording of the temporal information and the examination of the subcortical involvement is needed. Notably, studies such as Méndez-Bértolo et al. (2016) and Wang et al. (2023), utilizing direct intracranial EEG recordings from the human amygdala, have shown LSF specificity in rapid fear-selective responses, thereby excluding the contribution of cortical feedback information. Another plausible explanation of the controversial evidence posits that both the cortical and the subcortical pathways are involved in fearful emotion processing. Even with the subcortical pathway engaged, the cortical pathway may still play a critical role. As accumulating evidence underscores the existence of the subcortical pathway, understanding the interactions between the cortical and the subcortical pathways emerges as a pivotal research focus.

Experiment 2 indicated the involvement of the SC in fear processing, yet it could not conclusively rule out the contribution of earlier substrates in the visual pathway, such as the retina. Consequently, Experiment 3 further investigated the SC's involvement using a different paradigm and a different property. Specifically, Experiment 3 employed the nasal-temporal asymmetry to as a marker for SC functions, as demonstrated by a preference for saccadic orientation toward the temporal hemifield (Bertini et al., 2008; Dodds et al., 2002; Koller et al., 2019; Sumner et al., 2002; Teller et al., 1993; Tomalski & Johnson, 2012) and heightened SC activity in response to visual stimuli in the temporal hemifield (Sylvester et al., 2007). However, conflicting evidence exists, with studies reporting a lack of nasal-temporal asymmetry (Honda, 2002; Walker et al., 2000). Additionally, Bompas et al. (2008) demonstrated a nasal-temporal asymmetry for S-cone stimuli in humans, suggesting that this nasal-temporal asymmetry is not solely dependent on the SC. Given these contradictory findings, Experiment 3 incorporated special manipulations to increase the involvement of the SC. First, considering that the SC plays a major role in oculomotor behavior, we measured participants' saccadic orienting response rather than the manual key response to the first presented face. It has been shown that the SC is selectively activated by visually guided reaching behavior (Himmelbach et al., 2013; Stuphorn et al., 2000; Werner et al., 1997) and that saccades rely more on the magnocellular pathway than manual responses (Bompas & Sumner, 2009; Zhu et al.,

2021). Second, given that the subcortical pathway is suggested to serve automatic fear processing, it can be recruited automatically without the need of explicit knowledge (Gomes et al., 2018; Morris et al., 1998, 1999; Wang et al., 2023; Whalen et al., 1998). For this goal, the interval between the two presentations were kept small in the two experiments and one third of the trials were simultaneous presentation trials that were blind to the participants. As revealed by the saccade accuracy data, participants were mostly unaware of which face was presented first at the conscious level. This manipulation ensured that participants' responses were stimulus-driven rather than the result of high-level decision making (Bannerman et al., 2010). Nevertheless, comprehensive evidence from various perspectives is necessary to thoroughly investigate the association between nasal-temporal symmetry and the SC.

The potential involvement of subcortical structures in fear processing raises questions about the role of these structures in emotion processing. For example, the specificity of LSF processing may indicate sensitivity to coarse-scale information within the visual input, and the nasal-temporal asymmetry could suggest faster threat processing in the temporal hemifield. Nevertheless, fear processing showing characteristics of subcortical processing does not mean that these characteristics all have functional significance for fear processing in a natural environment. Realistically, individuals seldom experience dichoptic or monocular views of their surroundings. A subsequent question is the practical applications of subcortical fear processing. Given that threat-related information is a major source for affective disorders such as phobia, anxiety, and posttraumatic stress disorders (Chavanne & Robinson, 2021; Garfinkel et al., 2014), direct or indirect modulations on these subcortical structures could be a plausible way for mitigating excessive affective responses in psychiatric conditions (Battaglia et al., 2018, 2023).

In summary, the present study investigated the subcortical pathway hypothesis for threat processing by examining the involvement of subcortical structures during fearful face processing. By showing four distinctive functional features of subcortical brain regions, including LSF selectivity, same-eye advantage, S-cone insensitivity, and nasal-temporal asymmetry, we suggest that the fearful face information is likely to be mediated by the subcortical structures. Combined with the existing evidence of rapid, preattentive, and pre-conscious processing in the amygdala, the current evidence supports fear information transmission to the amygdala through a subcortical pathway in the human brain.

Constraints on Generality

The present study suffers from some limitations which constrain the generality of the observed subcortical characteristics in emotion processing. One limitation is that it only examined emotion processing with face stimuli. To date, consistent evidence for rapid and subcortical fear processing has mainly been obtained with face stimuli (e.g., Méndez-Bértolo et al., 2016). However, for a comprehensive understanding of adaptive functioning, it is imperative to extend investigations to other threatening stimuli, such as snakes and frightening scenes, which may exhibit similar characteristics (Carretié et al., 2022; Öhman & Mineka, 2001). Moreover, our exploration was confined to the processing of fearful emotion. While previous evidence has refuted subcortical processing of happy emotion (e.g., Méndez-Bértolo et al., 2016; Morris et al., 2001), limited attention has been devoted to exploring other emotional dimensions, such

as valence, arousal, and dominance. A thorough investigation involving diverse stimuli and measurement approaches is essential to decipher the driving factors behind the observed effects. Without answering the above questions, it is still immature to conclude the function and mechanism of the subcortical pathway. Furthermore, from an ecological perspective, the subcortical processing hypothesis emphasizes speed over accuracy. However, in the present study, subcortical characteristics were primarily reflected in recognition accuracy (Experiments 1 and 2). Although it is anticipated that the subcortical characteristics should manifest in recognition speed, future studies are required to investigate this hypothesis. Finally, it remains elusive regarding the influence of cultural factors on subcortical fear processing. Despite the subcortical pathway hypothesis emphasizing the processing of coarse, LSF information (LeDoux, 1996; Vuilleumier et al., 2003), it does not dismiss cultural influences, as the influence of cultural factors on face and emotion recognition has been demonstrated across different information scales (Chen et al., 2018; Jack et al., 2009, 2012). By comparing recognition outcomes using faces from the Chinese (e.g., Experiment 2b) and Western (e.g., Experiment 2a) cultures, we observed heightened recognition accuracies for fearful faces ($ps < .02$) and a more pronounced facilitation in LSF fear recognition (see Figure 4B). Notably, the same-eye advantage in the luminance-defined priming condition persisted in both experiments, suggesting that subcortical processing may be, at least in part, independent of cultural influences. However, a nuanced investigation into the specific impact of cultural factors on subcortical processing of threat information is essential, necessitating future research endeavors.

References

- Bannerman, R. L., Milders, M., & Sahraie, A. (2010). Attentional bias to brief threat-related faces revealed by saccadic eye movements. *Emotion, 10*(5), 733–738. <https://doi.org/10.1037/a0019354>
- Battaglia, S., Garofalo, S., & Di Pellegrino, G. (2018). Context-dependent extinction of threat memories: Influences of healthy aging. *Scientific Reports, 8*(1), Article 12592. <https://doi.org/10.1038/s41598-018-31000-9>
- Battaglia, S., Nazzi, C., & Thayer, J. F. (2023). Heart's tale of trauma: Fear-conditioned heart rate changes in post-traumatic stress disorder. *Acta Psychiatrica Scandinavica, 148*(5), 463–466. <https://doi.org/10.1111/acps.13602>
- Bertini, C., Leo, F., & Ládavas, E. (2008). Temporo-nasal asymmetry in multisensory integration mediated by the Superior Colliculus. *Brain Research, 1242*, 37–44. <https://doi.org/10.1016/j.brainres.2008.03.087>
- Bompas, A., Sterling, T., Rafal, R. D., & Sumner, P. (2008). Naso-temporal asymmetry for signals invisible to the retinotectal pathway. *Journal of Neurophysiology, 100*(1), 412–421. <https://doi.org/10.1152/jn.90312.2008>
- Bompas, A., & Sumner, P. (2009). Oculomotor distraction by signals invisible to the retinotectal and magnocellular pathways. *Journal of Neurophysiology, 102*(4), 2387–2395. <https://doi.org/10.1152/jn.00359.2009>
- Carretié, L., Fernández-Folgueiras, U., Álvarez, F., Cipriani, G. A., Tapia, M., & Kessel, D. (2022). Fast unconscious processing of emotional stimuli in early stages of the visual cortex. *Cerebral Cortex, 32*(19), 4331–4344. <https://doi.org/10.1093/cercor/bhab486>
- Chavanne, A. V., & Robinson, O. J. (2021). The overlapping neurobiology of induced and pathological anxiety: A meta-analysis of functional neural activation. *American Journal of Psychiatry, 178*(2), 156–164. <https://doi.org/10.1176/appi.ajp.2020.19111153>
- Chen, C., Crivelli, C., Garrod, O. G. B., Schyns, P. G., Fernández-Dols, J.-M., & Jack, R. E. (2018). Distinct facial expressions represent pain and pleasure across cultures. *Proceedings of the National Academy of Sciences of the United States of America, 115*(43), E10013–E10021. <https://doi.org/10.1073/pnas.1807862115>
- Collins, E., Park, J., & Behrmann, M. (2017). Numerosity representation is encoded in human subcortex. *Proceedings of the National Academy of Sciences of the United States of America, 114*(14), E2806–E2815. <https://doi.org/10.1073/pnas.1613982114>
- Cynader, M., & Berman, N. (1972). Receptive-field organization of monkey superior colliculus. *Journal of Neurophysiology, 35*(2), 187–201. <https://doi.org/10.1152/jn.1972.35.2.187>
- Dodds, C., Machado, L., Rafal, R., & Ro, T. (2002). A temporal/nasal asymmetry for blindsight in a localisation task: Evidence for extrageniculate mediation. *Neuroreport, 13*(5), 655–658. <https://doi.org/10.1097/00001756-200204160-00024>
- Gabay, S., Burlingham, C., & Behrmann, M. (2014). The nature of face representations in subcortical regions. *Neuropsychologia, 59*, 35–46. <https://doi.org/10.1016/j.neuropsychologia.2014.04.010>
- Gabay, S., Nestor, A., Dundas, E., & Behrmann, M. (2014). Monocular advantage for face perception implicates subcortical mechanisms in adult humans. *Journal of Cognitive Neuroscience, 26*(5), 927–937. https://doi.org/10.1162/jocn_a_00528
- Garfinkel, S. N., Abelson, J. L., King, A. P., Sripada, R. K., Wang, X., Gaines, L. M., & Liberzon, I. (2014). Impaired contextual modulation of memories in PTSD: An fMRI and psychophysiological study of extinction retention and fear renewal. *The Journal of Neuroscience, 34*(40), 13435–13443. <https://doi.org/10.1523/JNEUROSCI.4287-13.2014>
- Garvert, M. M., Friston, K. J., Dolan, R. J., & Garrido, M. I. (2014). Subcortical amygdala pathways enable rapid face processing. *NeuroImage, 102*, 309–316. <https://doi.org/10.1016/j.neuroimage.2014.07.047>
- Gomes, N., Soares, S. C., Silva, S., & Silva, C. F. (2018). Mind the snake: Fear detection relies on low spatial frequencies. *Emotion, 18*(6), 886–895. <https://doi.org/10.1037/emo0000391>
- Gong, X., Huang, Y., Wang, Y., & Luo, Y. (2011). Revision of the Chinese Facial Affective Picture System. *Chinese Mental Health Journal, 25*(1), 40–46. <https://doi.org/10.3969/j.issn.1000-6729.2011.01.011>
- Himmelbach, M., Linzenbold, W., & Ilg, U. J. (2013). Dissociation of reach-related and visual signals in the human superior colliculus. *NeuroImage, 82*, 61–67. <https://doi.org/10.1016/j.neuroimage.2013.05.101>
- Honda, H. (2002). Idiosyncratic left–right asymmetries of saccadic latencies: Examination in a gap paradigm. *Vision Research, 42*(11), 1437–1445. [https://doi.org/10.1016/S0042-6989\(02\)00050-0](https://doi.org/10.1016/S0042-6989(02)00050-0)
- Horton, J. C., Dagi, L. R., McCrane, E. P., & de Monasterio, F. M. (1990). Arrangement of ocular dominance columns in human visual cortex. *Archives of Ophthalmology, 108*(7), 1025–1031. <https://doi.org/10.1001/archophth.1990.01070090127054>
- Inagaki, M., Inoue, K., Tanabe, S., Kimura, K., Takada, M., & Fujita, I. (2023). Rapid processing of threatening faces in the amygdala of nonhuman primates: Subcortical inputs and dual roles. *Cerebral Cortex, 33*(3), 895–915. <https://doi.org/10.1093/cercor/bhac109>
- Jack, R. E., Blais, C., Scheepers, C., Schyns, P. G., & Caldara, R. (2009). Cultural confusions show that facial expressions are not universal. *Current Biology, 19*(18), 1543–1548. <https://doi.org/10.1016/j.cub.2009.07.051>
- Jack, R. E., Garrod, O. G. B., Yu, H., Caldara, R., & Schyns, P. G. (2012). Facial expressions of emotion are not culturally universal. *Proceedings of the National Academy of Sciences of the United States of America, 109*(19), 7241–7244. <https://doi.org/10.1073/pnas.1200155109>
- Koller, K., & Rafal, R. D. (2019). Saccade latency bias toward temporal hemifield: Evidence for role of retinotectal tract in mediating reflexive saccades. *Neuropsychologia, 128*, 276–281. <https://doi.org/10.1016/j.neuropsychologia.2018.01.028>
- Koller, K., Rafal, R. D., Platt, A., & Mitchell, N. D. (2019). Orienting toward threat: Contributions of a subcortical pathway transmitting retinal afferents to the amygdala via the superior colliculus and pulvinar. *Neuropsychologia, 128*, 78–86. <https://doi.org/10.1016/j.neuropsychologia.2018.01.027>

- LeDoux, J. E. (1996). *The emotional brain: The mysterious underpinnings of emotional life*. Simon & Schuster.
- Luo, Q., Holroyd, T., Majestic, C., Cheng, X., Schechter, J., & Blair, R. J. (2010). Emotional automaticity is a matter of timing. *Journal of Neuroscience*, *30*(17), 5825–5829. <https://doi.org/10.1523/JNEUROSCI.BC-5668-09.2010>
- Marrocco, R. T., & Li, R. H. (1977). Monkey superior colliculus: Properties of single cells and their afferent inputs. *Journal of Neurophysiology*, *40*(4), 844–860. <https://doi.org/10.1152/jn.1977.40.4.844>
- Marzi, C. A., Mancini, F., Metitieri, T., & Savazzi, S. (2009). Blindsight following visual cortex deafferentation disappears with purple and red stimuli: A case study.

