Published in final edited form as:

Brain Cogn. 2012 October ; 80(1): 15–22. doi:10.1016/j.bandc.2012.04.005.

The amygdala is involved in affective priming effect for fearful faces

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Abstract

The object of this study was to investigate whether the amygdala is involved in affective priming effect after stimuli are encoded unconsciously and consciously. During the encoding phase, each masked face (fearful or neutral) was presented to participants six times for 17 ms each, using a backward masking paradigm. During the retrieval phase, participants made a fearful/neutral judgment for each face. Half of the faces had the same valence as that seen during encoding (congruent condition) and the other half did not (incongruent condition). Participants were divided into unaware and aware groups based on their subjective and objective awareness assessments. The fMRI results showed that during encoding, the amygdala elicited stronger activation for fearful faces than neutral faces but differed in the hemisphere according to the awareness level. During retrieval, the amygdala showed a significant repetition priming effect, with the congruent faces producing less activation than the incongruent faces, especially for fearful faces. These data suggest that the amygdala is important in unconscious retrieving of memories for emotional faces whether they are encoded consciously or unconsciously.

Keywords

amygdala; unconscious; emotional memory; affective priming

1. Introduction

In recent years, studies have shown that subsequent memory processes for emotional stimuli could be enhanced even when they are processed unconsciously (e.g., Ghuman & Bar, 2008; Ruys & Stapel, 2008; Sweeny et al., 2009; Thomas & LaBar, 2005; Yang et al., 2011). In an experimental environment, priming paradigms (e.g., subliminal affective priming, lexical decision) are usually adopted to explore the effect of prior unconscious experience on subsequent behavioral performance. For example, after participants were unconsciously presented happy faces, they rated target neutral faces more positively and recognized them better 24 hours later (Sweeny et al., 2009). Our recent study (Yang et al., 2011) also showed

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that after participants were presented with masked faces six times, they judged fearful faces more quickly minutes later when the primes were fearful than when they were neutral.

Converging evidence from neuroimaging studies and those with patients suffering brain lesions has shown that the amygdala, together with subcortical regions, plays an important role in processing emotional stimuli unconsciously (for reviews, see Phelps & LeDoux, 2005; Tsuchiya & Adolphs, 2007). Moreover, the amygdala activity during encoding is related to subsequent memory processes (e.g., Dannlowski et al., 2007; Nomura et al., 2004; Suslow et al., 2006). For example, in the study by Nomura et al. (2004), participants were presented with masked angry or neutral primes for 35 ms. Although the authors did not find significant behavioral priming effect, the degree of amygdala activity was positively correlated with recognition performance of angry targets. Similarly, in the study by Dannlowski et al. (2007), participants were asked to rate preference for a neutral target face that followed a 33-ms exposure to a priming face (angry, sad, happy or neutral). The affective priming effect was not significant either, but the amygdala activity was correlated with negative bias scores (i.e., subjects rated neutral faces more negatively). On the other hand, these studies only scan the encoding phase and examined the correlation between subsequent behavioral performance and the amygdala activity during encoding. It is unclear whether the amygdala is involved in affective priming effect during retrieval phase. The priming effect is manifested as decreased brain activation for repeated rather than for new stimuli in perceptual regions and prefrontal cortices (Schacter et al., 2004; Grill-Spector et al., 2006). Thus it is necessary to explore whether the repetition-induced suppression of brain activation is manifested when repeated emotional faces are compared with new items.

The objective of this study was to explore whether the amygdala is involved in affective priming effect after fearful faces are unconsciously encoded. Participants were presented with a masked face (fearful or neutral) six times for a duration of 17 ms each exposure during encoding. Five minutes later, they were asked to make an emotional judgment for faces of congruent (fear–fear, neutral–neutral faces), incongruent (fear–neutral, neutral–fear faces) and new faces (Yang et al., 2011). Both the encoding and retrieval phases were scanned using functional magnetic resonance imaging (fMRI). To ensure that participants processed the faces unconsciously, a backward masking paradigm was used and further, participants were divided into unaware and aware groups based on subjective and objective assessments.

2. Method

2.1. Participants

Twenty-seven (22.45 ± 1.78 years old, 14 male) right-handed, healthy native Chinese-speaking students at Peking University took part in this experiment. They had normal or corrected-to-normal vision and no history of neurological trauma or psychiatric disorders. All participants were compensated for their participation and given informed consent in accordance with the procedures and protocols approved by the Human Participants Review Committee of Peking University.

2.2. Materials

Sixty faces photographs from 30 persons (15 male and 15 female) showing either fearful or neutral expressions were used. All photos were selected from the NimStim Emotional Face Stimuli set (Tottenham et al., 2009). Half of the faces were western people, and half eastern people. Each face was categorized as fearful or neutral based on its valence and arousal scores, determined by a group of 37 raters at Peking University using the nine-point scale. The fearful faces were more unpleasant ($3.01 \pm .30$ vs. $4.49 \pm .39$, $t(59) = 25.12$, $p < 0.001$)

and more arousing (6.23 ± 0.28 vs. 4.20 ± 0.26 , $t(59) = 45.25$, $p < 0.001$) than the neutral faces. Phase-scrambled images for all the faces were created to serve as masks and control stimuli, each preserving the color and spatial frequency of the original picture without depicting the face form.

The faces were first divided into three sets (20 faces from 10 persons in each). During encoding, faces in two sets were randomly presented as fearful or neutral. During retrieval, faces in each of the two sets were further randomly divided into two subsets to be presented as fearful or neutral. The other set was only presented during retrieval as a new condition, with half being fearful and half being neutral. Therefore, there were six conditions referring the relations between encoding and retrieval: fearful–fearful (FF), fearful–neutral (FN), neutral–fearful (NF), neutral–neutral (NN), new fearful (F) and new neutral (N) faces. The prime and target faces were the same in the fearful–fearful and neutral–neutral conditions, so they were referred to as a congruent condition. The neutral–fearful and fearful–neutral conditions were referred to as an incongruent condition. Altogether, there were 20 affectively congruent facial stimuli (10 fearful–fearful, 10 neutral–neutral) and 20 affectively incongruent (10 fearful–neutral, 10 neutral–fearful). The stimuli were counterbalanced so that each face had an equal chance to be presented in each condition for both encoding and retrieval phases.

2.3. Procedure

The experiment included four phases: unconscious encoding of faces, a distraction task, a subsequent priming task (retrieval) and post-awareness assessments (Fig. 1, Yang et al., 2011). Participants performed the tasks in the scanner for the first three phases but outside of the scanner for the last phase. During the unconscious encoding phase, a block design was used to present faces in four runs. In each run, there were four blocks, with stimulus blocks and scrambled blocks interleaved. Altogether, 16 blocks, including eight stimulus blocks (four fearful and four neutral) and eight scrambled blocks were pseudorandomly assigned into four runs. In each block, each of the five faces or scrambled pictures was presented for 17 ms (refresh rate of the projector as 60 Hz) and masked for 483 ms six times. The mask for a given face was created using the same face as for the prime in that trial. The mask for a given scrambled picture was the same as the scrambled picture. Then, to ensure that the participants attended to the screen, a horizontal or vertical bar was shown and participants had to judge its orientation as quickly and accurately as possible within 1.5 s. Each block lasted 37.5 s. Because a 12 s fixation was inserted in the first run, the total scanning time lasted 11.6 min. The order of runs was balanced across participants.

During the distraction task, participants were asked to subtract 7 from 1000 continuously for five minutes. Then during the priming task, an event-related design was used to present faces. The 40 prime faces and 20 new faces (10 fearful and 10 neutral) were pseudorandomly assigned to two runs so that no more than three faces that had the same valence dimension were presented consecutively. For each trial, each face was presented on the center of the screen for 2 s, with an interstimulus interval of 10 s. Of the 40 prime faces, half had the same facial expressions as those used in the subliminal encoding (congruent condition) and the other half had new expressions (incongruent condition). The participants were asked to decide whether each face was fearful or neutral as quickly and accurately as possible. Each run lasted 6 min. The order of runs and the button pressing activities were counterbalanced across participants. Participants had an opportunity to practice before the formal test.

During the post-awareness assessment outside of the scanner, participants were first asked whether they saw anything between the fixation and the scrambled mask. They then completed a detection task to assess their objective awareness. Same as that during the

encoding phase, after each of the 40 masked faces was presented (refresh rate of the monitor as 60 Hz) for six times as 17 ms each, participants were asked to judge whether the facial expression was fearful or neutral as quickly and accurately as possible, and make confidence rating ranged from 1 to 6. The faces were presented pseudorandomly so that no more than three faces that had the same valence dimension were presented consecutively. The button press was counterbalanced across participants.

2.4. fMRI acquisition

The fMRI data were collected on a Siemens 3 Tesla scanner (Magnetom Trio; Siemens). Stimuli were presented through a back projector (refresh rate 60 Hz). Subjects viewed the stimuli through a mirror attached to the head coil. Because the faces were presented very quickly for the encoding phase, the block design was adopted to increase the detection power of the fMRI acquisition for the masked faces. Because participants were asked to make emotional judgment for the retrieval phase, the event-related design was more appropriate. The anatomical data were acquired using a high-resolution SPGR sequence (TR = 845 ms, FOV = 22 cm, matrix = 256×256 , resolution = $1 \times 1 \times 1.3 \text{ mm}^3$) after the retrieval phase. The whole brain functional data were acquired using a gradient echo, echo-planar imaging (EPI) sequence (interleaved scanned, TR = 3 sec, TE = 39 ms, flip angle = 90° , FOV = 22 cm, matrix = 96×96 , slice = 33, resolution = $2.3 \times 2.3 \times 3 \text{ mm}^3$).

2.5. Data analysis

The standard d' measures and the area under the ROC curve (A') were computed for each participant (Pessoa et al., 2005). Participants were considered as being unaware when they claimed that they did not perceive any face or facial parts during encoding (subjective criterion) or when their detection task performance was at chance level (i.e., d' or A' values were not significantly different from chance level, objective criterion) (Yang et al., 2011). One participant's data was excluded because of excessive head motion. Among the remaining participants, 13 were aware of faces during encoding and the other 13 were not. Thus the aware group was included as a between-subject factor to enter into behavioral and fMRI data analysis. Accuracy and reaction times (RT) of the affective priming task were recorded for each participant.

The fMRI data were analyzed using the AFNI software package (Cox, 1996). The first three EPI volumes in each run were discarded because of equilibrium magnetization issue. The remaining images were corrected for slice timing offset and aligned to the EPI volume acquired most closely in time to the anatomical scan. Data were smoothed spatially using a 3 mm full width at half maximum Gaussian kernel and then scaled by the mean of the time series at each voxel. The EPI data from different runs were concatenated before individual subject analyses. For the encoding phase, the response to each condition compared with the scrambled baseline was estimated in regression analysis. The model included two regressors of interest (fearful and neutral) and six regressors of noninterest (motion parameters). For the retrieval phase, the response to each condition compared with the fixation baseline was estimated. The model included six regressors of interest (fearful–fearful, neutral–fearful, neutral–neutral, fearful–neutral, new fearful and new neutral), each of which was created through the convolution with an impulse response of gamma-variate function and six regressors of noninterest (motion parameters). Anatomical and statistical volumes were then transformed into standard stereotaxic space of the Talairach and Tournoux atlas (Talairach & Tournoux, 1988).

For the group analysis, voxel-wise mixed-effects analysis of variance (ANOVA) was performed on the beta weights from the individual analysis. For the encoding data, the ANOVA included 'subject' as a random factor, 'facial expression' (fearful, neutral) and

'group' (unaware, aware) as fixed factors. For the retrieval data, the ANOVA included 'facial target' (fearful, neutral), 'condition' (congruent, incongruent, new) and 'group' (unaware, aware) as fixed factors and 'subject' as a random effect. Because repeated stimuli usually elicit less activation than new stimuli because of repetition suppression (Schacter et al., 2004), the priming effect was defined as significantly decreased activation for congruent vs. incongruent conditions and for old vs. new faces. Correspondingly, the priming effect for the fearful faces was taken as the signal change between fearful–fearful and neutral–fearful faces and the priming effect for the neutral faces was taken as the signal change between neutral–neutral and fearful–neutral faces. The activation (main effects and contrasts) reported here was at a voxel-wise significance level of $p < 0.02$ (two-tailed). Monte Carlo simulations were used to correct for multiple comparisons and to determine the minimum cluster size at a significance level of 0.05 (volume = 319 mm³). Small volume correction (SVC) was applied for the anatomically constrained amygdala ($p < 0.05$, corrected, volume = 75 mm³).

3. Results

3.1. Behavioral results

The analyses of d' and A' confirmed that the two groups of participants had different awareness levels (0.09 ± 0.13 vs. 0.49 ± 0.50 , $F(1,25) = 10.27$ for d' ; 0.54 ± 0.05 vs. 0.67 ± 0.14 , $F(1,25) = 11.94$ for A' , both $p < 0.003$). For the aware participants ($N=13$), 11 of them met subjective criterion because they perceived parts of faces (especially eyes), and 7 of them met objective criterion. There were 5 of the aware participants met both subjective and objective criteria. The aware participants had above chance levels of d' and A' ($p < 0.001$), but the unaware participants ($N=13$) had a chance level in detecting the faces ($p > 0.1$). No significant effects were found in the accuracy of the bar judgment between the two groups during encoding (0.97 vs. 0.97 , $p > 0.9$).

Participants were successful in making emotional judgment during retrieval ($Mean \pm SD$, $98\% \pm 3\%$). The ANOVA analysis included two within-subject factors: target (fearful, neutral) and prime (fearful, neutral) and a between-subject factor (aware, unaware). The results showed a significant target effect for RTs ($F(1,24) = 7.41$, $p < 0.01$; partial $\eta^2 = 0.24$), suggesting that the fearful faces were judged more quickly than the neutral faces. Other effects and interactions were not significant ($p > 0.1$). Specifically, there were no significant group effects for both RTs ($F(1,24) = 0.38$, $p > 0.54$; partial $\eta^2 = 0.02$) and accuracy ($F(1,24) = 0.03$, $p > 0.87$; partial $\eta^2 = 0.01$), and no significant interactions related to awareness ($p > 0.2$).

3.2. Encoding phase

The voxel-based ANOVA revealed a significant effect of awareness in many regions (Table 1). There was stronger activation in the amygdala ($-18, -8, -19$, $t(25) = 4.42$) for the unaware group and stronger activation in the prefrontal cortex ($-34, 18, 29$, $t(25) = 5.56$), occipital region ($-32, -69, 19$, $t(25) = 4.16$) and the fusiform gyrus ($-24, -39, -24$, $t(25) = 4.99$) for the aware group. There was a significant interaction between emotion and awareness in the right amygdala ($21, -6, -15$, $F(1,24) = 14.40$), left medial frontal cortex ($-2, 25, -4$, $F(1,24) = 14.60$) and subcortical regions ($24, -21, 5$, $F(1,24) = 17.73$, 76 mm³).

Consistent with previous studies, for unaware participants, fearful faces (vs. neutral) produced stronger activation in the right amygdala ($20, -5, -8$, $t(12) = 6.39$) and the right pulvinar ($6, -11, 6$, $t(12) = 4.96$) (Fig. 2). In addition, their activation was significantly correlated ($r = 0.55$, $p < 0.05$). Fearful faces also elicited stronger activation than neutral

faces in the superior temporal sulcus (STS), occipital regions and the anterior temporal cortex, but their cluster sizes were small and did not survive the cluster-level correction. In contrast, for aware participants, fearful faces elicited stronger activation in the left amygdala ($-23, -3, -15, t(12) = 6.39, 28 \text{ mm}^3$), right fusiform gyrus ($38, -38, -20, t(12) = 4.48, 64 \text{ mm}^3$) and the left STS ($-60, -45, 8, t(12) = 4.44$).

3.3. Retrieval phase

The aware and unaware groups did not differ in the amygdala activation during retrieval, so the results from the two groups were reported together. There was stronger activation for fearful faces (vs. neutral faces) in large regions of the temporal region, frontal cortices, cingulate cortex and the amygdala. Specifically, the new fearful faces elicited stronger activation in the amygdala than the new neutral faces (left, $-20, -4, -15, t(25) = 3.41$; right, $29, -4, -19, t(25) = 4.63$). The region in the amygdala was larger (left, 324 mm^3 ; right, 294 mm^3) than that measured during encoding (left, 28 mm^3) for the aware participants, suggesting that awareness level was not the same under the two manipulations.

For the priming effect, the congruent faces elicited weaker activation than the incongruent faces in the right amygdala (Fig. 3, left), the anterior and orbital frontal cortices, the cingulate gyrus, the middle temporal gyrus and the subcortical regions (Table 2). The congruent effect in the amygdala was mainly caused by the priming effect for fearful faces. As illustrated in Figure 4, the fearful–fearful faces (FF) elicited weaker activation than did the neutral–fearful faces (NF) in the amygdala ($t(25) = 4.01, 91 \text{ mm}^3$), although participants judged both of them as fearful faces. In contrast, neutral–neutral (NN) faces elicited *stronger* activation than the fearful–neutral (FN) faces in the amygdala ($t(25) = 3.16, 30 \text{ mm}^3$) ($p < 0.01$, uncorrected). To clarify the extent to which the amygdala was involved in the priming effect for fearful faces, we determined the cluster in the amygdala as a region of interest from the congruent effect, and extracted percent signal changes from each condition. The results showed a significant interaction between prime and target ($F(1,24) = 14.5, p < 0.001$; partial $\eta^2 = .38$), showing that the priming effect for fearful faces (fearful–fearful vs. neutral–fearful, $p < 0.001$) larger than that for neutral faces (neutral–neutral vs. fearful–neutral, $p < 0.05$) (Fig. 3, right). There was no significant effect for the aware and unaware group ($F(1,24) = 0.03, p > 0.86$; partial $\eta^2 = .001$). These results suggest that the amygdala is involved in affective priming, especially for fearful faces.

4. Discussion

The objective of this study was to explore whether the amygdala was involved in affective priming effect during memory retrieval. There were two main findings. First, the amygdala showed a significant repetition-priming effect, with the congruent faces eliciting decreased activation when compared with the incongruent faces. Moreover, the priming effect in the amygdala was more outstanding for fearful faces than for neutral faces. Second, the amygdala activation was different for unaware and aware participants during encoding, but similar for the two groups during retrieval. Our study suggested that activation of the amygdala is important for affective priming effect during the retrieval phase.

4.1. The amygdala and affective priming for fearful faces

The novel finding of our study was that the amygdala was involved in a significant priming effect for congruent (vs. incongruent) faces, especially for fearful faces. The fearful–fearful and neutral–neutral faces elicited decreased activation in the amygdala than those of fearful–neutral and neutral–fearful faces. Typically, a priming effect refers to changes in RT or response accuracy for repeated items in comparison with new items (Tulving & Schacter, 1990). These behavioral changes link to reduced neural activity (i.e., repetition suppression)

and to decreased brain activation for repeated rather than for new stimuli in perceptual regions and the prefrontal cortices (Schacter et al., 2004; Grill-Spector et al., 2006). However, when stimuli are unfamiliar, repeated stimuli lead to increased, rather than decreased, activation in related regions (Henson et al., 2000). Therefore, our results suggest that congruent faces is encoded successfully and thus need less brain activation to be involved during retrieval. More importantly, although both fearful and neutral faces elicited decreased activation in the amygdala for congruent vs. incongruent condition, the priming effect was larger for fearful than for neutral faces. Combined with previous findings on significant correlations between unconscious encoding and subsequent memory performance (e.g., Dannlowski et al., 2007; Suslow et al., 2006), These data provided evidence that the amygdala is not only important in the unconscious processing of fearful faces, but it is also involved in the subsequent implicit memory for fearful faces.

There are two possible mechanisms to account for the role of the amygdala in the priming effect for fearful faces. One is that fearful faces attract more attention mediated by the amygdala (Phelps & LeDoux, 2005; Tsuchiya & Adolphs, 2007), which is related to enhanced memory for emotional stimuli (Hamann, 2001; Talmi et al., 2008). Emotional stimuli increase attentional vigilance because detecting biological relevance is important for human survival. Thus, fearful faces are processed more quickly during encoding and are more available for subsequent processing (Suslow et al., 2006). The other possibility is that unconsciously processed fearful faces enhance memory consolidation. It is known that the amygdala interacts with the MTL (for reviews, see LaBar & Cabeza, 2006; McGaugh, 2000; Phelps, 2006) and the prefrontal cortex (McGaugh, 1996) to modulate consolidation for explicit emotional memories. Considering that there was no significant difference between aware and unaware participants for the priming effect, it is possible that similar mechanisms mediate retrieval after unconscious encoding. The amygdala can lower neural thresholds in sensory systems and increase neurotransmitter thresholds (e.g., cholinergic, dopaminergic and noradrenergic neurotransmitters), which in turn enhance priming effect for emotional faces (LaBar & Cabeza, 2006; McGaugh, 2000). Thus, the longer the retention, the more memory retrieval might be enhanced for emotional faces.

4.2. Consciousness and priming effect

In studying subliminal affective priming, it is crucial to ensure that subjects process stimuli unconsciously because affective priming effect could be contaminated by a conscious component (Fazio & Olsson, 2003; Milders et al., 2008; Pessoa et al., 2005; LM Williams et al., 2004). In our study, half of the participants were aware of the presence of faces after a duration of 17 ms presented *six times*. By using both objective and subjective criteria, aware and unaware participants were separated optimally. Consistent with previous studies (for reviews, see Phelps & LeDoux, 2005; Tsuchiya & Adolphs, 2007), we found that the amygdala and the pulvinar were involved in unconsciously encoding of fearful faces (vs. neutral faces), and their activation was significantly correlated. It should be noted that there was no significant difference between the two groups during retrieval in both behavior and fMRI signal change, suggesting that they rely on similar neural mechanisms for the priming effect.

Our results also showed that the consciousness level modulated laterality of the amygdala activity. During encoding, the amygdala was activated on the left among the aware participants and on the right among the unaware participants. During retrieval, the right amygdala was associated with a significant priming effect for congruent faces. This was consistent with the view that the right amygdala is associated with unconscious processing of facial expression and the left amygdala is involved in awareness or semantic processing of the stimuli (e.g., Hariri et al., 2004; Morris et al., 1998).

We manipulated unconscious processing by dividing participants into aware and unaware groups, which was different from some previous studies that manipulated presentation times to differentiate awareness level (e.g., Carlsson et al., 2004; Williams et al., 2006). For example, by presenting the faces for 17 ms (followed by a 150 ms mask) subjects were unable to detect the presence of face stimuli, but by presenting the faces for 500 ms they could discriminate fearful and neutral faces (Williams et al., 2006). We believe that longer presentation times lead to different brain activation patterns, because there were different activity patterns observed when subjects viewed the faces for 17 ms (during encoding) or for 2 s (during retrieval). When compared fearful faces with neutral faces, there was small amount of activation in visual regions when the faces were presented for 17 ms even when participants were aware of the presentation. However, strong activation was seen in the amygdala and visual regions (STS, fusiform gyrus) when the faces were presented for 2 s. Further studies on implicit emotional processing should consider these differences and adopt appropriate presentation methods to distinguish aware from unaware groups.

4.3. Other regions

In addition to the amygdala, other regions were involved in encoding and retrieving memories of fearful faces unconsciously. During encoding, we found small activation in the STS and lingual gyrus for the fearful vs. neutral faces, but their activation levels were not correlated with that of the amygdala. There have been inconsistent findings on whether the fusiform gyrus, the STS/STG are activated when emotional faces are unconsciously processed (Pasley et al., 2004; MA Williams et al., 2004; c.f., Jiang & He, 2006; Kouider et al., 2007). Some indicated that the visual cortices are not preferentially activated for emotional faces (e.g., Pasley et al., 2004; MA Williams et al., 2004). Others found that the cortical regions are selectively activated for unconscious processing of emotional stimuli (e.g., Jiang & He, 2006). Our results showing activation in the STS seem to support the view that some visual regions are activated under the unconscious condition. However, because of insignificant correlation between the STS and the amygdala, further investigation is needed to clarify the mechanism of visual activation under unconscious conditions.

During retrieval, the ventral prefrontal cortex, fusiform gyrus and medial temporal cortex showed decreased activation for congruent vs. incongruent faces. Consistent with our findings, Kouider et al. (2007) also found that the long-lag priming effect is associated with decreased activity in the ventral frontal cortex and with increased activity in the parietal and frontal regions. These regions might interact with each other, thereby modulating memory storage for fearful faces (Nomura et al., 2004).

4.4. Limitations and future directions

Unlike our previous results (Yang et al., 2011), the behavioral priming effect in this study was not significant. This mainly arose from different experimental details for the fMRI scanning. Instead of being disappeared once the participants responded, the stimuli were presented for 2 s during retrieval in the scanner. Instead of using duration of 12 ms, the presentation time could be 17 ms due to refresh rate of the projector in the scanner. These manipulation changes might have diminished quicker responses to the fearful–fearful faces and increase the probability to aware the stimuli. Further studies could also increase sample sizes and assess awareness in the scanner to better define aware and unaware participants and increase the detection power. In addition, our study did not distinguish the effects of valence and arousal on the affective priming effect, because fearful faces differed from neutral faces in both dimensions. Previous studies did not find significant activation of the amygdala in the priming of positive faces, although this priming effect seems to be more robust in behavior (Dannlowski et al., 2007; Sweeny et al., 2009). Therefore, valence might not be the critical factor to modulate affective priming effect, and arousal may play an

important role (Thomas & LaBar 2005). Further studies that include different emotional faces, such as happy, angry and sad faces, could help elucidate whether arousal or valence plays a role in affective priming effect.

4.5. Conclusions

We found that the amygdala was involved in unconscious encoding of fearful faces, and subsequent affective priming effect, especially for fearful faces. These findings highlight the role of the amygdala in cognitive processes and help us understand the neural mechanisms of how our memory is modulated by emotional experiences, even when it is acquired unconsciously.

Highlights

- A long-term priming effect was tested for unconsciously encoded faces.
- The amygdala showed decreased activation for congruent vs. incongruent faces.
- The priming effect in the amygdala is more obvious for fearful faces than for neutral faces.

Acknowledgments

This research was supported by grants from the Global Research Initiative Program, National Institutes of Health, USA (R01TW007897, J. Yang, 2008), and the National Science Foundation of China (30870769, J. Yang, 2009). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Fig. 1. Experimental procedures. During encoding, the masked faces were presented six times and participants were asked to judge the orientation of the bar. During retrieval, participants performed an emotional judgment task.

Fig. 2.

Brain activation during encoding. For the unaware participants, fearful faces elicited stronger activation in the right amygdala and the right pulvinar. For the aware participants, fearful faces elicited stronger activation in the superior temporal sulcus and the amygdala (uncorrected). The left hemisphere is displayed on the left.

Congruent <
incongruent

Fig. 3.

Amygdala activation during retrieval. The congruent faces elicited decreased activation in the right amygdala when compared with the incongruent faces. The left hemisphere is displayed on the left.

Fig. 4. Brain activation during retrieval. The fearful–fearful (FF) faces elicited less activation in the right amygdala than did neutral–fearful (NF) faces and new faces. The left hemisphere is displayed on the left. Note that the activation in the amygdale for NN vs. FN was small and did not survive the correction.

Table 1

Brain activation during encoding

F > N, unaware						
Left/Right	Area	Region	t-value	X	Y	Z
L	Frontal	Anterior frontal cortex	5.69	-19	22	-2
R	Frontal	Medial frontal cortex	4.06	1	21	-6
R	Temporal	Anterior temporal gyrus	5.97	25	11	-31
L	Temporal	Anterior temporal gyrus	5.40	-34	-1	-25
R	Subcortical	Pulvinar	5.13	24	-24	10
R	Subcortical	Amygdala	6.39	20	-5	-8
F > N, aware						
Left/Right	Area	Region	t-value	X	Y	Z
L	Frontal	Middle frontal gyrus	6.48	-28	1	28
R	Frontal	Inferior frontal gyrus	4.46	39	23	4
L	Frontal	Cingulate	3.88	-4	-36	42
L	Parietal	Precuneus	5.24	-22	-73	25
R	Temporal	Anterior temporal	5.48	43	1	-14
L	Temporal	STS	4.44	-60	-45	8
R	Temporal	Temporal	5.95	45	-26	13

Table 2

Brain activation during retrieval

Congruent vs. Incongruent						
Left/Right	Area	Region	X	Y	Z	t-value p value
R	Frontal	Anterior frontal	41	21	27	-3.90 $p < 0.001$
L	Frontal	Anterior frontal	-17	52	9	-3.82 $p < 0.001$
R	Frontal	Orbital frontal	31	29	-6	-4.82 $p < 0.001$
L	Frontal	Anterior frontal	-27	37	37	-5.12 $p < 0.001$
R	Frontal	Anterior frontal	15	40	12	-4.35 $p < 0.001$
R	Frontal	Anterior cingulate	14	26	26	-4.98 $p < 0.001$
R	Temporal	Fusiform gyrus	28	-55	-10	-6.74 $p < 0.001$
L	Temporal	Fusiform gyrus	-35	-51	-5	-5.46 $p < 0.001$
R	Temporal	MTL	58	-18	-11	-4.60 $p < 0.001$
L	Temporal	MTG	-55	-36	1	-5.38 $p < 0.001$
R	Temporal	MTG	57	-40	-8	-4.81 $p < 0.001$
R	Subcortical	Subcortical	22	-13	10	-4.69 $p < 0.001$
R	Subcortical	Amygdala	27	0	-14	-4.36 $p < 0.001$
FF vs. NF						
Left/Right	Area	Region	X	Y	Z	t-value p value
L	Frontal	Anterior frontal	-20	35	39	-4.99 $p < 0.001$
L	Frontal	Cingulate	-10	47	17	-4.32 $p < 0.001$
R	Frontal	Cingulate	15	41	7	-4.53 $p < 0.001$
L	Frontal	Anterior frontal	-16	48	7	-5.65 $p < 0.001$
R	Temporal	Fusiform gyrus	18	-82	-4	-5.95 $p < 0.001$
L	Temporal	MTG/occipital	-55	-36	-2	-4.92 $p < 0.001$
R	Temporal	MTG	47	-32	12	-4.99 $p < 0.001$
R	Subcortical	Subcortical	24	1	15	-5.21 $p < 0.001$
R	Subcortical	Insula	47	-8	-4	-5.51 $p < 0.001$
R	Subcortical	Amygdala	32	4	-18	-4.01 $p < 0.001$
NN vs. FN						
Left/Right	Area	Region	X	Y	Z	t-value p value

Congruent vs. Incongruent								
	Left/Right	Area	Region	X	Y	Z	t-value	p value
	R	Frontal	Inferior frontal	36	3	29	-4.19	$p < 0.001$
	L	Frontal	Inferior frontal	-43	-1	30	-4.51	$p < 0.001$
	L	Temporal	Fusiform	-34	-52	-4	-4.96	$p < 0.001$
	R	Temporal	Fusiform/occipital	38	-68	-8	-3.82	$p < 0.001$
	R	Subcortical	Hippocampus	37	-13	-17	-5.83	$p < 0.001$
	R	Frontal	Inferior frontal	47	19	6	3.85	$p < 0.001$
	L	Frontal	Anterior frontal	-20	44	29	5.24	$p < 0.001$