



The amygdala is involved in affective priming effect for fearful faces

J. Yang^{a,*}, Z. Cao^{a,1}, X. Xu^{a,1}, G. Chen^b

^aDepartment of Psychology, Peking University, Beijing 100871, China

^bScientific and Statistical Computing Core, NIMH/NIH/DHHS, 9000 Rockville Pike, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:

Accepted 15 April 2012

Available online 23 May 2012

Keywords:

Amygdala
Unconscious
Emotional memory
Affective priming

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.

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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, 2006; Ruys & Stapel, 2008; Sweeny, Grabowecky, Suzuki, & Paller, 2009; Thomas & LaBar, 2005; Yang, Xu, Du, Shi, & Fang, 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 h later (Sweeny et al., 2009). Our recent study (Yang et al., 2011) also showed 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 (Grill-Spector, Henson, & Martin, 2006; Schacter, Dobbins, & Schnyer, 2004). 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

* Corresponding author. Fax: +86 10 62761081.

E-mail address: yangjj@pku.edu.cn (J. Yang).

¹ These authors contributed equally to the work.

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 face 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 (10 fearful–fearful, 10 neutral–neutral) and 20 affectively incongruent (10 fearful–neutral, 10 neutral–fearful) facial stimuli. 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 5 min. 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 during 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 during 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 s, 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, Japee, & Ungerleider, 2005). Participants were considered as being unaware when they claimed that they did not perceive any face or facial parts during

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