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Amygdala activation to masked happy facial expressions

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Abstract

The amygdala has a key role in automatic non-conscious processing of emotions. Highly salient emotional stimuli elicit amygdala activity, and happy faces are among the most rapidly perceived facial expressions. In backward masking paradigms, an image is presented briefly and then masked by another stimulus. However, reports of amygdala responses to masked happy faces have been mixed. In the present study, we used functional magnetic resonance imaging (fMRI) to examine amygdala activation to masked happy, sad, and neutral facial expressions. Masked happy faces elicited greater amygdala activation bilaterally as compared to masked sad faces. Our findings indicate that the amygdala is highly responsive to non-consciously perceived happy facial expressions. (*JINS*, 2010, *16*, 1–5)

Keywords: Functional magnetic resonance imaging, Subliminal, Non-conscious, Unconscious, Happy, Sad, Emotion

INTRODUCTION

Facial expressions are a crucial component of social interactions and emotional experience (Snyder & Cantor, 1980). Affective facial processing is mediated by a distributed neural system that consists of multiple bilateral regions (Haxby, Hoffman, & Gobbini, 2000). In particular, the amygdala plays a central role in processing the social relevance of information gleaned from faces (Costafreda, Brammer, David, & Fu, 2008). Happy faces are highly salient and among the most easily perceived facial expressions (Bruce & Young, 1986). However, early reports suggested that there were few neural responses to happy facial expressions (Whalen, Rauch, Etcoff, NcInerney, Lee, & Jenike, 1998; Kestler-West et al., 2001), but a recent meta-analysis of functional neuroimaging studies has revealed significant amygdala activation to happy faces (Costafreda et al., 2008).

Emotional appraisals occur explicitly as well as *implicitly* at a non-conscious level which may precede explicit, cognitive assessments (Sergent, Ohta, MacDonald, & Zuck, 1994). Using the method of backward masking (Esteves & Ohman, 1993), stimuli are presented outside of conscious awareness. Functional neuroimaging studies have identified the amygdala as a key structure in automatic nonconscious processing of affective facial expressions (Costafreda et al., 2008). Yet, when the presentation has been non-conscious, the data have been mixed (Whalen et al., 1998; Killgore & Yurgelun-Todd, 2004). Whalen et al. (1998) reported a relative reduction in amygdala activation to masked happy faces, but the task was a series of happy faces alternating with fearful faces within the same trial, which may have reduced the salience of the happy faces. In contrast, Killgore and Yurgelun-Todd (2004) presented masked happy and sad faces in separate trials and observed increased amygdala activation to happy faces. However, the facial stimuli were presented alongside simple cross-hair stimuli which may have inflated the effect of the affective faces.

In the present study, we sought to examine amygdala activation to non-conscious presentation of happy faces. The stimuli were masked happy and sad faces presented in separate trials. Within the trials, we also included masked neutral faces as a control condition for the affective facial expressions. We expected significant amygdala activation to masked happy faces.

MATERIALS AND METHODS

Participants were 10 healthy right-handed adults (7 male), mean age 25.2 years [standard deviation (*SD*) 3.2 years],

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without a personal history of a psychiatric disorder or in first-degree relatives or a neurological illness according to a structured clinical interview. The study was approved by the Research Ethical Committee of the Institute of Psychiatry and the South London and Maudsley NHS Trust Ethics (Research) Committee, and all participants provided written informed consent

In an event-related, functional magnetic resonance imaging (fMRI) design, masked happy and masked sad faces were presented in separate trials. In each condition, the first stimulus (non-conscious stimulus) was presented for 33 ms, immediately followed by the second stimulus (mask) for 200 ms. Mean intertrial interval (ITI) was 5 s, jittered across conditions. The conditions were: happy/neutral (H/N), sad/neutral (S/N), neutral/neutral (N/N), and cross-hair/cross-hair (X/X), containing 28 stimuli for each condition with an equal number of male and female faces (14 of each) (Figure 1) (Lundqvist, Flykt, & Öhman, 1998). The face stimuli were all of the same emotional intensity of expression. The masked happy faces trial consisted of 28 H/N, 28 N/N, and 28 X/X stimuli, and the masked sad faces trial was 28 S/N, 28 N/N, and 28 X/X stimuli. The happy and sad trials were identical in design except for the primary emotion (i.e., happiness or sadness). The order of presentation of the happy and sad trials was counterbalanced between subjects.

Subjects were unaware of the backward masking nature of the study and were informed only that they would see a series of briefly presented photographs of faces. Debriefing following the scans, subjects did not indicate any awareness of the masked facial stimuli. For each face condition, subjects were asked to indicate the sex of the face (male or female) by lateral movement of a joystick; no hand movement was required in response to the cross-hair conditions. All of the facial masks were of a neutral expression, and participants were asked to determine the sex of the presented face as our meta-analysis of neuroimaging studies revealed that implicit affective processing generally elicits greater amygdala activation in comparison to explicit affective judgments (Costafreda et al., 2009) and high accuracy of sex identification has been found in previous studies (Fu et al., 2004, 2007, 2008). Response latency and accuracy of the gender decision were recorded.

Single-shot, gradient echo planar imaging was used to acquire 380 T2*-weighted image volumes on a 1.5 Tesla GE Excite MRI scanner (General Electric, Milwaukee, WI, USA) at the Institute of Psychiatry, Maudsley NHS Trust, UK. For each volume, 16 non-contiguous axial planes parallel to the inter-commissural plane were collected with the following parameters: TR = 2000 ms, TE = 40 ms, slice thickness = 7 mm, slice skip = 0.7 mm, FOV = 24 cm, image acquisition matrix 64×64, yielding an in-plane spatial resolution of 3.75×3.75 mm^2. Four additional acquisitions were also made at the beginning of each run to set the longitudinal magnetization into steady state. To facilitate co-registration of the fMRI data in standard space, a 43-slice, high-resolution inversion recovery echo planar image was acquired of the whole brain, parallel to the same inter-commissural landmark: TR = 3000 ms, TE = 40 ms, slice thickness = 3 mm, slice skip 0.3 mm, NEX = 8, image acquisition matrix 128×128, in-plane spatial resolution of 1.875×1.875 mm^2.

The fMRI data analysis (XBAM, http://brainmap.it) began with image correction for subject motion and smoothed with a Gaussian filter (FWHM 8.8 mm). Neural responses to the experimental paradigm were detected by fitting a linear model in which each component of the design was convolved separately with two gamma variate functions (peak responses at 4 s and 8 s). A goodness-of-fit statistic was computed, consisting of the ratio of the sum of squares of deviations from the mean image intensity due to the model (over the whole time series) to the sum of squares of deviations due to the residuals (SSQ). Each affective condition (H/N, S/N, N/N) was contrasted to the

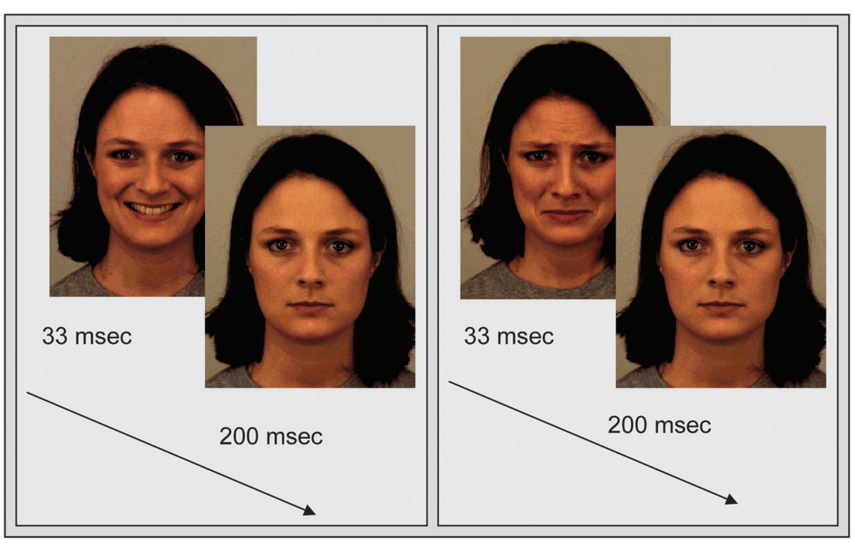

Fig. 1. Each facial stimulus condition consisted of two rapidly presented stimuli: the first face depicting either a sad or a happy expression which was presented for 33 milliseconds (ms) followed by a second ''mask'' face of the same person expressing a neutral expression which was presented for 200 ms; thus, the happy or sad expression was "masked" by the neutral expression. There was an additional control condition of neutral faces masked by neutral expressions of the same person.

cross-hair control condition (X/X). In the happy faces trial, the contrasts were: (H/N – X/X) and (N/N – X/X); and similarly, in the sad faces trial, the contrasts were: (S/N – X/X) and (N/N – X/X). Differences between the happy and sad trials were essentially a factorial design: [(H/N – N/N) – (S/N – N/N)] and [(S/N – N/N) - (H/N – N/N)], in which each affective (H/N, S/N, or N/N) condition had been contrasted to the cross-hair (X/X) control condition.

Data were permuted by a wavelet-based method (Bullmore et al., 2001), which permitted a data-driven calculation of the null distribution of SSQ under the assumption of no experimentally-determined response. As we expected activation in the amygdala, we examined *a priori* regions of interest (ROI) (Lancaster et al., 2000) in the right and left amygdala.

We also conducted an exploratory whole-brain analysis, in which contrasts were performed by fitting the data at each intracerebral voxel by linear modeling of the contrast of interest by minimizing the sum of absolute deviations. To increase sensitivity and reduce confounds due to multiple comparisons, the analysis was extended from the voxel to cluster level (Bullmore, Suckling, Overmeyer, Rabe-Hesketh, Taylor, & Brammer, 1999). Observed and randomized SSQ data for each individual were normalized into standard space (Talairach & Tournoux, 1988), and maps of activated regions computed using the contrast median (Brammer et al., 1997). The null distribution was computed by permuting data between groups, and the model was refitted at each voxel.

RESULTS

The mean response latency was: 951 ms (*SD* 186 ms) for masked sad faces, 1008 ms (193 ms) for masked happy faces, and 833 ms (177 ms) masked neutral faces. There were no significant differences in response latency ($F = 3.82$; $df = 27$; $p = .53$) or accuracy of the gender decision ($\chi^2 = 0.003$; $df = 1$; $p = .67$) between the facial expressions (happy, sad, neutral).

In the ROI analysis, greater bilateral activation to masked happy faces relative to masked sad faces was observed in the right ($t = 2.70$; $df = 9$; $p = .02$) and left amygdala ($t = 3.54$; $df = 9$; $p = .006$) (Figure 2). This was not attributable to differences in activation in the neutral contrasts as there were no significant differences in the neutral contrasts within the masked happy and masked sad faces trials in the left or right amygdala [i.e., (N/N – X/X) in sad trial *vs.* (N/N – X/X) in happy trial: right amygdala, $t = -0.79$; $df = 0$; $p = 0.44$; and left amygdala, $t = -0.89$; $df = 9$; $p = .40$]. Moreover, masked happy faces showed a significantly greater activation than masked neutral faces in the in left amygdala (two-tailed t test: $t = 3.36$; $df = 9$; $p = .008$) but not in right amygdala ($t = 1.74$; $df = 9$; $p = .115$), while masked sad faces had a trend toward reduced activation relative to masked neutral faces in the left ($t = -2.21$; $df = 9$; $p = .054$) and right ($t = -2.00$; $df = 9$; $p = .076$) amygdala.

In the whole-brain analysis, masked happy faces relative to masked sad faces showed greater activation in the right amygdala (Talairach coordinates: x = 27, y = −5, z = −22; volume: 24 voxels, $p = .02$ uncorrected) and in a region which encompassed the left amygdala, extending into the hippocampus and having a peak in the left superior temporal gyrus (Brodmann's area 38) (x = −26, y = 10, z = −26; volume: 95 voxels, $p = .003$ corrected). No regions showed greater activation to masked sad faces as compared to masked happy faces, and there were no significant differences in the neutral contrasts within the happy and sad trials.

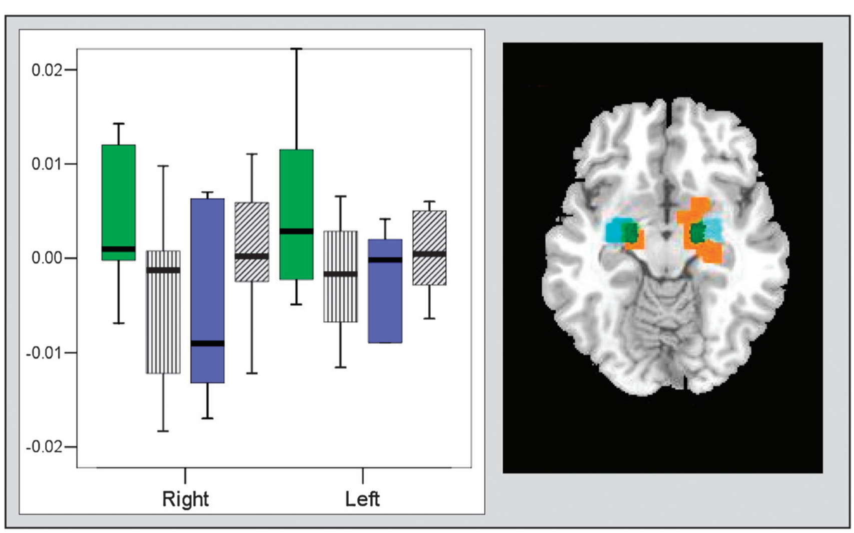

Fig. 2. Transverse section of brain showing greater bilateral amygdala activity in response to masked happy faces relative to masked sad faces at Talairach coordinate $z = -12$, presented in radiological convention. Results from the region of interest (ROI) analysis are colored in blue, results from the whole-brain analysis are colored in orange, and the overlapping regions are colored in green. The box graph shows the right and left amygdala responses as measured by ROI to masked happy (green color) and masked sad (blue color) faces. The ROI amygdala responses to neutral faces (gray vertical lines) in the masked happy trials and to neutral faces (gray diagonal lines) in the masked sad trials are also presented. The y-axis represents the sum of squares quotient (SSQ) for each contrast.

DISCUSSION

Non-conscious presentation of happy facial expressions was associated with bilateral amygdala activation. The amygdala is engaged by strong emotional stimuli (Costafreda et al., 2008), and the present study supports the high salience of happy faces even when observed below the threshold of conscious awareness.

The emotional facial expressions were masked by neutral faces, and the effects of the masked happy and sad faces were measured in comparison with masked neutral faces. High amygdala responsivity to masked happy faces relative to masked sad faces supports theories of the arousal dimension of affect in which sadness is associated with less expressed arousal than fear, anger, or happiness (Watson & Tellegen, 1985). Moreover, neutral facial expressions are themselves implicitly evaluated along valence judgments, such as trustworthiness and attractiveness, which modulates amygdala activity (Todorov & Engell, 2008). The lack of a significant effect of masked sad faces on amygdala activation relative to neutral faces may reflect a comparable salience of neutral and sad expressions to healthy individuals.

It has been purported that the initial detection of a stimulus, which may be automatic, is mediated by the right amygdala through a direct subcortical thalamic pathway while the more evaluative response occurs through an indirect cortical route to the left amygdala (LeDoux, Sakaguchi, & Reis, 1984). In a meta-analysis of amygdala responsivity, Costafreda et al. (2008) noted greater activation of the right amygdala to masked presentations of affective facial expressions. However, the present study, as in Killgore and Yurgelun-Todd (2004), found increased bilateral amygdala activation to masked happy faces relative to masked sad faces. Right lateralization of amygdala activation is a relative response because both amygdalae typically show strong engagement, which has often been greater in the right (Costafreda et al., 2008).

Activation of adjacent regions extending into the parahippocampal region and temporal cortex were also observed with masked happy faces. Recruitment of a network of regions is required for implicit and explicit affective judgments of facial expressions (Habel et al., 2007; Todorov & Engell, 2008). The present study suggests that processing of masked happy faces engages more extensive regions of the network, including the superior temporal cortex which is responsive to facial movements (Haxby et al., 2000).

Limitations of the present study include the small sample size which was sufficient for investigation of the amygdala as an *a priori* hypothesis. Although the whole-brain analysis further substantiated our findings, a larger sample may have provided greater power to examine effects in the entire network involved in affective facial processing (Haxby et al., 2000; Todorov & Engell, 2008). As well, we did not observe a significant difference in responses latency between the masked happy, sad, and neutral facial conditions, although there appears to have been a marginal effect. The finding is likely due to a combination of the small sample size and the inter-subject variability often found in response latency data. Indeed, behavioral (non-imaging) studies of masked emotional primes typically use a much larger sample size to detect emotional effects (Van den Bussche, Van Den Noortgate, & Reynvoet, 2009). Future studies should include a more sensitive quantitative measure of arousal, such as galvanic skin responses (GSR), which have been used in previous research to demonstrate that perceptual (Kotzé & Möller, 1990) and cognitive (Wagar & Dixon, 2006) processes can occur outside of conscious awareness. Recent research has also linked GSR to amygdala responsivity (Laine, Spitler, Mosher, & Gothard, 2009), thus suggesting that detectable increases in autonomic responses would occur during amygdalar responses to masked emotional stimuli.

In summary, the amygdala demonstrated significant activation to masked presentation of happy facial expressions. These findings support a role for the amygdala in processing non-consciously perceived affective stimuli and suggest a stronger bilateral response to happy faces.

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AUTHOR QUERIES

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