

Note

A left amygdala mediated network for rapid orienting to masked fearful faces

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ABSTRACT

A rapid response to environmental threat is highly adaptive and fearful facial expressions serve as important threat cues. The biological significance of these threat cues is demonstrated by neuroimaging findings of amygdala responses to backward masked fearful faces. Additionally, behavioral dot-probe studies reveal that backward masked fearful faces modulate spatial attention. However, little is known about the behavioral impact of the amygdala sensitivity to masked fearful faces. Using a dot-probe task with event-related functional magnetic resonance imaging (fMRI), we provide the first evidence that the amygdala is involved in orienting to backward masked fearful faces. Furthermore, this spatial attention-related amygdala response was correlated with activity in the anterior cingulate, superior temporal sulcus, and lingual gyrus.

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Fearful faces are significant biological indicators of potential threat that are detected and processed in the emotionally responsive amygdala (Adolphs, Tranel, Damasio, & Damasio, 1994). Emotional processing theories claim the amygdala automatically mediates an orienting response to both detailed and crude threat signals (LeDoux, 1998; Ohman & Mineka, 2001). Consistent with this theoretical stance, behavioral effects in dot-probe studies indicate that unmasked (Armony & Dolan, 2002; Cooper & Langton, 2006; Pourtois, Grandjean, Sander, & Vuilleumier, 2004) and masked (Carlson & Reinke, 2008; Fox, 2002; Mogg & Bradley, 1999; Mogg & Bradley, 2002) threatening faces modulate spatial attention. Additionally, backward masking neuroimaging studies report increased amygdala activity for threat-related relative to neutral faces (Liddell et al., 2005; Morris, Ohman, & Dolan, 1998; Whalen et al., 1998). Neuroimaging dot-probe studies suggest unmasked fearful faces facilitate visual processing (Pourtois et al., 2004; Pourtois, Schwartz, Seghier, Lazeyras, & Vuilleumier, 2006) and human lesion research indicates the amygdala mediates fear-related enhancements in occipital cortex (Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004).

The directing or orienting of spatial attention to crude/masked threat signals is an important aspect of the fear response; however,

the neural characteristics of this behavior have yet to be assessed. Previous fMRI fearful face dot-probe studies (e.g., Pourtois et al., 2006) have not compared directed vs. undirected spatial attention and have only assessed detailed/unmasked fearful faces. Here, we tested the hypothesis that the amygdala directs spatial attention to backward masked fearful faces through a network of brain structures that include the emotion- and attention-related anterior cingulate cortex (ACC; Armony & Dolan, 2002; Bush, Luu, & Posner, 2000).

1. Methods

Twelve (seven male and five female) right handed individuals between the ages of 18 and 35 participated in the study. Potential subjects were screened for prescription and recreational drug usage, neurological and psychological histories, and for metal that could not be removed from their body. Monetary compensation was provided to subjects for participating. Subjects gave informed consent and were treated in accordance to the guidelines of the Institutional Review Board of Southern Illinois University Carbondale.

A dot-probe task (MacLeod & Mathews, 1988) was performed while brain activity was examined with event-related fMRI. Four (two male and two female) gray scale facial identities of fearful and neutral expressions (Gur et al., 2002) were used for the initial faces. A fifth neutral female face from this database was used as the mask. Stimuli were presented using the IFIS system on an MRI-compatible LCD-screen mounted to the head coil with a field of view of 7.5°. Each trial began with a 1000 ms fixation cue. Next, two faces were simultaneously presented (33 ms) to the left and right of fixation (outer edges of the faces were separated by 15° of visual angle) and immediately masked by neutral faces (100 ms). Masks were offset by approximately 1° of visual angle on the vertical Y-axis to reduce apparent motion (Liddell et al., 2005). Horizontal shifts were not used (to reduce biasing participants' attention). Masks were followed by a left visual field (LVF) or right visual field (RVF) target dot (750 ms) and a jittered (500–2000 ms) intertrial interval (Supplementary Fig. 1).

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With an fMRI-compatible response pad, subjects used their right index finger for LVF targets and right middle finger for RVF targets. The LCD-screen and response pad were controlled via a fiber optic cable by a control-room PC equipped with E-Prime 1.1 (Psychology Software Tools, Pittsburgh, PA).

Directed spatial attention trials consisted of one fearful and one neutral initial face with the fearful face occurring equally in either the LVF or RVF. These trials were half congruent (target dot on the same side as the fearful face) and half incongruent. Undirected attention trials consisted of either both fearful (FF) or both neutral (NN) 33 ms faces. Whereas directed attention trials are thought to contain a shift in spatial attention to the location of the fearful face, undirected trials are independent of an attentional bias to one face over the other. This difference in attentional bias enabled us to assess amygdala activity for *directed vs. undirected* spatial attention. Differential amygdala activity in the cued LVF (or RVF) vs. NN contrast should reveal activity associated both with fearful face processing and spatial attention elicited by masked fearful faces. On the other hand, differential activity in the LVF (or RVF) vs. FF+NN contrast should reveal amygdala processing strictly associated with masked fearful face-elicited spatial attention and not fearful face processing per se as the total ratio of fearful and neutral faces (1:1) is held constant in this contrast.

A 1.5T Phillips whole body scanner equipped with a head coil was used to acquire T2* weighted scans with an EPI sequence sensitive to BOLD signal using the following parameters: TR=2500 ms, TE=50 ms, flip angle=90°, matrix dimensions=64 × 64, slices=26, slice thickness=5.5 mm, gap=0. Anatomical T1-weighted structural scans were also acquired. Standard preprocessing procedures were performed in SPM5, including: image realignment corrections for head movements, slice timing corrections, normalization to standard 2 mm × 2 mm × 2 mm Montreal Neurological Institute space, and spatial smoothing with a Gaussian full-width-at-half-maximum 10 mm filter. First level single subject statistical parameter maps were created for each condition using the general linear model in SPM5. A full factorial second-level model was created with six levels (LVF: congruent and incongruent, RVF: congruent and incongruent, neutral-neutral, and fearful-fearful).

2. Results

To examine the efficacy of the masking procedure, a separate sample (see [Supplementary Methods](#)) of subjects attempted to correctly identify each trial type (LVF, RVF, FF, and NN). This control task was identical to the dot-probe task with the exception that after the initial face-mask pairing, subjects attempted to identify the trial type. Fourteen out of 15 subjects failed to identify trial types above chance ([Supplementary Table 1](#)). While the majority of control subjects failed to detect the nature of the masked faces, we do not wish to claim that facial processing was subliminal for all fMRI subjects. Nonetheless, these backward masking procedures appear to be successful at restricting the processing of the initial (masked) faces.

For reaction times (RTs), a cued visual field (left, right fearful face) by target congruency (congruent, incongruent) repeated-measures analysis of variance revealed a significant two-way interaction ($F_{1,11} = 5.68, P = 0.036$): congruent trials were faster than incongruent trials in the LVF ($P = 0.006$) but did not differ in the RVF ($P = 0.589$, [Supplementary Fig. 2](#)). This interaction is consistent with previous research (Fox, 2002; Mogg & Bradley, 1999; Mogg & Bradley, 2002) and suggests that in the current experiment LVF, but not RVF, fearful faces captured spatial attention.

Bilateral amygdala region of interest analyses were performed in SPM5 using Masks for Regions of Interest Analysis (Walter et al., 2003) with a search volume corrected α of 0.05. The FF vs. NN comparison resulted in increased left amygdala activity ($P = 0.005$, [Fig. 1a](#)), which confirms previous findings (Liddell et al., 2005; Morris et al., 1998; Pasley, Mayes, & Schultz, 2004; Whalen et al., 1998; Williams et al., 2006; Williams, Morris, McGlone, Abbott, & Mattingley, 2004) that suggest the amygdala is sensitive to crude threatening faces. However, the laterality of this amygdala response has been inconsistent (*left*: Pasley et al., 2004, *right*: Morris et al., 1998, and *bilateral*: Liddell et al., 2005; Whalen et al., 1998; Williams et al., 2004, 2006). This lateralized amygdala activity is further explored in the discussion.

To test the hypothesis that the amygdala mediates the modulation of spatial attention to masked fearful faces we separately compared the cued LVF and cued RVF to the NN and FF+NN undirected attention conditions. As presented in [Table 1](#), the results revealed increased left amygdala activity in the LVF vs. NN ($P = 0.003$, [Fig. 1b](#)) and LVF vs. FF+NN ($P = 0.019$, [Fig. 1c](#)) attention contrasts; however masked RVF fearful faces did not enhance amygdala activity. While previous research (Carlson & Reinke, 2008) suggests that perceptual inconsistencies between the initial faces and masks do not facilitate reaction times, it is unclear how these inconsistencies might effect BOLD. However, the tight coupling between behavioral and BOLD effects (both differences in LVF, but not RVF) suggests the left amygdala is associated with enhancements in spatial attention to masked fearful faces.

Left amygdala activity (active cluster from the LVF vs. FF+NN contrast) was included as a covariate in a whole brain analysis of LVF trials to assess a potential correlated network involved in directing attention to masked fearful faces. The left amygdala significantly ($P's < 0.05_{FWE}$) covaried with the left ACC (IACC), right lingual gyrus (rLG) and superior temporal sulcus (rSTS) in addition to other limbic and frontal areas (see [Table 1](#)). Differences in correlations were assessed by using the voxel of interest box in SPM5 to extract data from the most active voxel in the rLG, IACC, and rSTS. Follow-up Fisher's z -tests indicated that activity in the left amygdala was significantly more correlated with the IACC ($z = 2.16, P = 0.016$), rLG ($z = 2.31, P = 0.011$), and rSTS ($z = 2.18, P = 0.014$) in the LVF condition (IACC $r = 0.83$, rLG $r = 0.88$, rSTS $r = 0.85$) compared to the undirected condition (IACC $r = 0.16$, rLG $r = 0.17$, rSTS $r = .18$, [Supplementary Fig. 3](#)). A behavioral LVF "attention index" (LVF congruent–incongruent RTs) was computed for each subject and was included as an additional covariate (with left amygdala activity) in the whole brain analysis of LVF trials. For LVF trials, a whole brain conjunction analysis (Nichols, Brett, Andersson, Wager, & Poline, 2005) of correlated left amygdala activity and correlated LVF attention index activity revealed that within the previously identified attention network, that the IACC ($P < 0.001_{uncorrected}$) and rLG ($P = 0.006_{uncorrected}$) covaried with both measures (see [Table 1](#) for additional areas of covariation).

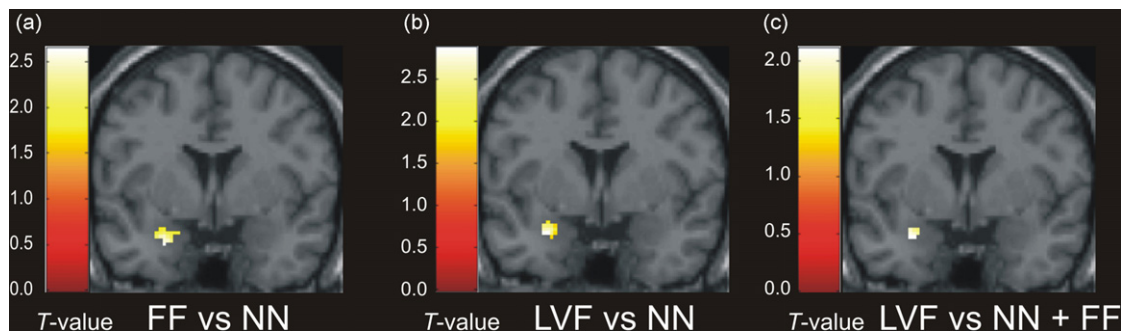


Fig. 1. Left amygdala ($y = 2$) responsivity to (a) masked fearful faces, (b) LVF fearful face and attention-related processing, and (c) LVF fearful face-elicited spatial attention. Activation displayed at $P = 0.05$, adjusted for search volume.

Table 1
Masked fearful face-related spatial attention activity.

Analysis and region	Hemisphere	MNI coordinates			Voxels	Maximally activated voxel	
		x	y	z		t-value	p-value
Amygdala ROI analyses							
FF vs. NN	L	−26	0	−24	71*	2.64	0.005
LVF vs. NN	L	−30	0	−22	57*	2.87	0.003
LVF vs. NN + FF	L	−30	0	−22	17*	2.11	0.019
Whole brain left amygdala covariation (LVF trials)							
Anterior cingulate	L	−4	46	−4	329***	6.85	<0.05 _{FWE}
Lingual gyrus	R	10	−52	4	142***	8.80	<0.05 _{FWE}
Superior temporal sulcus	R	50	−32	−4	82***	7.72	<0.05 _{FWE}
Parahippocampal gyrus/hippocampus	L	−22	−18	−16	112***	7.74	<0.05 _{FWE}
Cingulate	L	−4	24	30	327***	6.78	<0.05 _{FWE}
Inferior frontal gyrus	L	−38	28	6	29***	6.76	<0.05 _{FWE}
Whole brain conjunction of left amygdala and attention index covariation (LVF trials)							
Anterior cingulate	L	−8	48	−8	52**	5.97	<0.001
Lingual gyrus	R	10	−54	2	4**	3.18	<0.006
Cingulate	L	−6	26	32	43**	8.05	<0.001
Anterior temporal pole	L	−34	8	−18	123**	5.73	<0.001
Parahippocampal gyrus/hippocampus	L	−22	−30	−14	26**	5.23	<0.001
Parahippocampal gyrus	R	26	−48	8	33**	4.97	<0.001
Brain stem	R	4	−20	−14	6**	4.40	0.001

* $p < 0.05_{\text{svc}}$.

** $p < 0.01_{\text{uncorrected}}$ (conjunction).

*** $p < 0.005_{\text{FDR}}$.

3. Discussion

Consistent with previous research (Carlson & Reinke, 2008; Fox, 2002; Mogg & Bradley, 1999; Mogg & Bradley, 2002), our behavioral results indicate that backward masked LVF fearful faces enhanced spatial attention. In line with these behavioral results, we found left amygdala activity for LVF fearful faces. On the other hand, RVF fearful faces did not enhance RTs nor elicit amygdala activity. Collectively, these results suggest the left amygdala mediates the facilitation of spatial attention to masked fearful faces. Additionally, left amygdala activity was found to be positively correlated with IACC, rSTS, and rLG activity when visuospatial attention was directed to backward masked LVF fearful faces, but not when attention was undirected. Finally, the results revealed that the IACC and rLG were associated both with subjects' attention-related left amygdala activity and behavioral attention index.

The observed ipsilateral amygdala processing of masked fearful faces contradicts the contralateral processing typically observed in the visual system. However, a review of the neuroimaging literature (Baas, Aleman, & Kahn, 2004) indicates a greater reported incidence of left, compared to right, amygdala activity. Indeed, we found left, but not right, amygdala activity in the FF vs. NN and LVF attention contrasts. Evidence from retrograde tracing studies in rats indicates that the amygdala receives bilateral input from visual nuclei in the brainstem, which may provide a neuroanatomical subcortical substrate for ipsilateral visual processing (Usunoff, Itzev, Rolfs, Schmitt, & Wree, 2006). The aforementioned amygdala characteristics may explain our left lateralized effects.

We provide the first evidence that the left amygdala mediates enhancements of spatial attention to masked fearful faces through a network including the IACC, rSTS, and rLG. Based on previous findings, we speculate that the amygdala detects fearful (or emotional) stimuli relayed from subcortical visual nuclei (Liddell et al., 2005; Morris, DeGelder, Weiskrantz, & Dolan, 2001; Pasley et al., 2004; Usunoff et al., 2006) and interacts with the emotion- and attention-related ACC (Armony & Dolan, 2002; Bush et al., 2000) to modulate contralateral visual processing (rLG and rSTS) through amygdala dependent feedback (Vuilleumier et al., 2004). The STS processes changeable aspects of face perception, such as eye gaze direction and emotional expression (Haxby, Hoffman, & Gobbini,

2000) and has been found to be correlated with the amygdala during crude fearful face processing (Jiang & He, 2006). Our results add to findings of unmasked fearful face-elicited attentional enhancements in visual cortex (Pourtois et al., 2004, 2006) and suggest this enhancement may be mediated by the amygdala. The amygdala and ACC may be involved in orienting spatial attention to crude threat signals, whereas enhancements in contralateral visual processing may reflect the site of attentional capture. While we did not assess the neural structures involved in the disengagement of spatial attention, previous research (with unmasked faces) points to intraparietal and orbitofrontal areas (Armony & Dolan, 2002; Pourtois et al., 2006). In sum, the threat- or emotion-initiated left amygdala, IACC and rLG network may reflect a rapid and automatic modulation of the "spotlight of attention" (Posner, 1980), which produces an adaptive facilitation in behavioral responses to stimuli within this retinotopically distinct visual location.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropsychologia.2009.01.026.

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