Differentially tuned responses to restricted versus prolonged awareness of threat: A preliminary fMRI investigation

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A B S T R A C T

Responses to threat occur via two known independent processing routes. We propose that early, reflexive processing is predominantly tuned to the detection of congruent combinations of facial cues that signal threat, whereas later, reflective processing is predominantly tuned to incongruent combinations of threat. To test this prediction, we examined responses to threat–gaze expression pairs (anger versus fear expression by direct versus averted gaze). We report on two functional magnetic resonance imaging (fMRI) studies, one employing prolonged presentations (2 s) of threat–gaze pairs to allow for reflective processing (Study 1), and one employing severely restricted (33 ms), backward masked presentations of threat–gaze pairs to isolate reflexive neural responding (Study 2). Our findings offer initial support for the conclusion that early, reflexive responses to threat are predominantly tuned to congruent threat–gaze pairings, whereas later reflective responses are predominantly tuned to ambiguous threat–gaze pairings. These findings highlight a distinct dual function in threat perception.

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1. Introduction

Initial processing of threat is thought to involve feed-forward inputs to low-level visual areas including the striate cortex (SC_x), fusiform gyrus (FG), and temporal sulci/gyri (Adolphs, 2002a, 2002b), as well as direct, subcortical projections from the thalamus to the amygdala, posterior cingulate cortex (PCC), and orbitofrontal cortex (OFC; Palermo & Rhodes, 2007; Robinson & Petersen, 1992; Vuilleumier, 2002). Such responses are believed to underlie early threat detection and the initialization of adaptive behavioral responding (LeDoux, 1995, 1998; Vuilleumier, 2002). Later conscious-level processing of threat is thought to involve highly interconnected structures including the amygdala, OFC, FG, temporal sulci/gyri, and SC_x, regions thought to be involved in more elaborate cognitive and perceptual processing (Adolphs, 2002a, 2002b; Davis, 1992; LeDoux, 1995, 1998). As such, threat perception appears to unfold along at least two parallel pathways (Adolphs, 2002a, 2002b; Davis, 1992; LeDoux, 1998). Indeed, evidence suggests these processing routes are doubly dissociable, both temporally and structurally (Halgren & Marinkovic, 1995; LeDoux, 1995; Liddell, Williams, Rathjen, Shevrin, & Gordon, 2004; Morris,

DeGelder, Weiskrantz, & Dolan, 2001; Morris, Öhman, & Dolan, 1998, 1999; Vuilleumier, 2002; Williams et al., 2004). Whether they are differentially responsive to various combinations of threat cues, however, has not been previously established.

2. The role of eye gaze in threat perception

Using speeded reaction time tasks and self-reported emotion perception, Adams and Kleck (2003, 2005) found that direct gaze facilitated processing speed and increased the perceived intensity of facially communicated approach-oriented emotions (e.g., anger), whereas averted gaze facilitated facially communicated avoidance-oriented emotions (e.g., fear; see also Adams, Gordon, Baird, Ambady, & Kleck, 2003; Fox, Mathews, Calder, & Yiend, 2007; Graham & LaBar, 2007; Hadjikhani, Hoge, Snyder, & de Gelder, 2008; Hess, Adams, & Kleck, 2007; N'Diaye, Sander, & Vuilleumier, 2009; Rigato, Farroni, & Johnson, 2010; Sander, Grandjean, Kaiser, Wehrle, & Scherer, 2007; Sato, Yoshikawa, Kochiyama, & Matsumura, 2004).

Prior to these recent investigations, studies examining neural responses to anger and fear tended to utilize facial expressions posed only with direct gaze. These studies revealed greater amygdala recruitment to fear than anger faces (Whalen et al., 2001), a puzzling finding when considering that anger is arguably the clearer threat signaling both the source and target of imminent

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aggression. In an attempt to address this issue, Whalen and colleagues hypothesized that amygdala activation may be directly proportional to the amount of ambiguity that surrounds the source of a perceived threat (Whalen, 1998). Adams et al. (2003) corroborated this threat-ambiguity hypothesis by crossing eye gaze with threat displays, replicating the pattern of greater amygdala activation to fear than to anger expressions for direct gaze faces only, and finding the reverse pattern for averted gaze faces.

3. Reflexive- versus reflective-level processing

Based on early animal models LeDoux (1998) proposed two distinct pathways for the processing of threatening stimuli, both involving the amygdala. He referred to a "low road" (i.e., fast processing route), a direct subcortical pathway from the thalamus to the amygdala. This route is thought to be involved in the detection of highly salient threat cues eliciting automatic responding to potential danger. He also referred to a "high road" (i.e., slower processing route), which involves subcortical and cortical networks underlying conscious evaluation of the stimulus and its contextual cues. This route underlies a more deliberate cognitive and behavioral response to threat. Although these early studies examined audition in rats, current models of face perception indicate a similar dual pathway in the human visual system (e.g., Vuilleumier, Armony, Driver, & Dolan, 2003). Given that these pathways represent distinct neural routes, it stands to reason that they may also be functionally distinct, i.e., tuned to different processing demands. Yet, evidence for such a functional distinction remains limited. The amygdala is involved in the early detection of highly salient threat cues (Adolphs & Tranel, 2000; LeDoux, 1998). It is also active in (1) deciphering ambiguity such as in high-level decision making (Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005), (2) responses to ambiguous visual stimuli such as complex clips from surrealist films (Hamann, Ely, Hoffman, & Kilts, 2002), and (3) responses to ambiguity surrounding threat (Adams et al., 2003).

It has been further speculated that although initially more attention grabbing, clear threat cues likely give way to attentional disengagement sooner than ambiguous threat cues given their aversive nature (see Mogg, Garner, & Bradley, 2007). This perspective assumes two mechanisms at play, an attention shift and an attention maintenance response (see also Serences et al., 2005). Such differences in attention might also explain differences in amygdala responses to threat-related ambiguity versus clarity. Current findings in the literature hint at such a dual function. For instance, Adams et al.'s (2003) evidence for heightened amygdala response to ambiguous versus clear threat cues employed extremely sustained presentations of threat stimuli (2 s), allowing for prolonged attention maintenance. Recently, however, studies employing more rapid presentations (300 ms) have revealed the opposite pattern, i.e., greater amygdala responses to congruent versus ambiguous threat–gaze pairings (Hadjikhani et al., 2008).

4. The current work

We predicted that under conditions of sustained processing allowing for reflective evaluation, neural responses would be preferentially tuned to incongruent threat–gaze cues (averted anger and direct fear), whereas under conditions of severely restricted awareness threat responses would be preferentially tuned to congruent threat–gaze cues (direct anger and averted fear). To examine this question, we used fMRI to explore neural responses to clear versus ambiguous threat/gaze displays under conditions of sustained awareness (Study 1; 2 s) and severely restricted awareness (Study 2; 33 ms, backward masked).

5. Study 1: threat responses under prolonged presentations durations

5.1. Method

5.1.1. Participants

Thirteen healthy, right handed Caucasian males under 30 years of age with normal or corrected vision, and no history of neurological impairment or disorders participated in this study. Three participants were removed from subsequent analysis, one due to failure to respond to the gender discrimination task, and two for technical problems with MRI acquisition and data reconstruction.

5.1.2. Stimuli

Facial displays of anger and fear were selected from four sets of photographs: the Pictures of Facial Affect (Ekman & Friesen, 1976), a set developed by Kirouac and Doré (1984), the Montreal Set of Facial Displays of Emotion (Beaupré & Hess, 2005), and a set developed by Adams and Kleck (2001). These were the same stimuli previously found to yield gaze by emotion interaction effects in a speeded reaction time task (Adams & Kleck, 2003) and that were used in our previous fMRI experiment showing gaze by emotion interactions at the neural level (Adams et al., 2003). In order to reduce potential habituation effects, we included trials of neutral facial displays blended slightly with happy expressions (20%; see also Phillips et al., 1997). All photographs were presented as grayscale images approximately 3.5×3.5 in. Gaze direction was manipulated using Adobe Photoshop™ (see Fig. 1).

5.1.3. Design and procedure

Participants were presented with 360 pictorial trials in two runs across six conditions: 3 (Emotion displayed: angry, fear, neutral [blended with 20% joy]) × 2 (Gaze direction: direct, averted). Each experimental trial lasted 2500 ms and consisted of a 500 ms central fixation followed by a face presented for 2000 ms during which participants performed a gender discrimination task by pressing a right or left button, to insure they were alert and attending to the faces. These trials were randomly interspersed with 172 (2500 ms) central fixation (+) trials across two runs.

Fig. 1. Example stimuli showing one exemplar face in each of the 2 (expression: anger, fear) by 2 (gaze: direct, averted) treatment conditions.

5.1.4. Data acquisition and analysis

Imaging was performed on a GE Medical Systems 1.5 Tesla Sigma scanner with a standard quadrature head coil. Visual images were back-projected onto a screen by an LED projector and participants viewed the images through a mirror on the head coil. Multiple structural images were acquired using a spoiled-gradient (SPGR) technique and T1-weighted images (TR = 7.7 ms, TE = 3 ms, thickness = 1.2 mm). Functional images used a gradient echo-planar image (EPI) sequence using blood-oxygen level-dependent (BOLD) contrast (TR = 2.5 s, TE = 35 ms, flip = 90°). Each of two functional runs consisted of 266 interleaved whole-brain acquisitions (25 noncontiguous axial slices, 4.5 mm thickness, 1 mm gap). Functional volumes were corrected for differences in slice timing, realigned, corrected for movement-related artifacts, coregistered with each participant's anatomical data, normalized to the Montreal Neurological Institute (MNI) template, and spatially smoothed using a 6-mm gaussian kernel in SPM99 (Wellcome Department of Cognitive Neurology).

Subject-specific contrasts were estimated using a fixed-effects model. These contrast images were used to obtain subject-specific estimates for each effect. For group analysis, these estimates were then entered into a second-level analysis treating participants as a random effect, using one-sample t-tests at each voxel. Clusters were localized based on the contrast of ambiguous (averted-gaze anger/direct-gaze fear) minus clear (direct-gaze anger/averted-gaze fear) threat and vice versa (height: $p < .005$, uncorrected, extent: 10 voxels). Lieberman and Cunningham (2009) recently recommended that $p < .005$, extent = 10 voxels is an optimal threshold to balance between Type 1 and Type 2 errors. Critically, when examining amygdala responses as an ROI, Monte Carlo simulations have previously determined that a threshold of $p < .01$, extent = 5 voxels corresponds to $p < .05$, small-volume correction for anatomically defined bilateral amygdala (see Kim, Somerville, Johnstone, Alexander, & Whalen, 2003). The threshold we employ for whole-brain analysis here is more stringent, and thus we interpret our a priori predictions for the primary ROI, the amygdala, in this study as corresponding to $p < .05$ small volume correction. The ROI approach described above was also used to pull clusters in order to examine direct comparisons between our treatment conditions to explicate the nature of the interaction. In our tables of activations, we also indicate when regions survive a more stringent threshold of $p < .001$, extent = 10 voxels. For illustration purposes all group contrast images were overlaid onto a representative T1 template anatomical image using MRIcron (http://www.sph.sc.edu/comd/rorden/mricro.html). Coordinates are reported in MNI space.

In order to examine differences in global activation in the brain as well, we performed a number of chi-square analyses on overall number of voxels activated (see also Canli, Desmond, Zhao, Glover, & Gabrieli, 1998). To compute the chi-square analysis comparing brain activation to ambiguous minus clear versus clear minus ambiguous contrasts, we determined the total number of voxels reaching threshold (i.e., $p < .005$, uncorrected) for each condition. We also computed chi-square analyses to examine possible laterality differences by combining the total number of voxels that reached threshold for each treatment condition and comparing across hemispheres.

5.2. Results

5.2.1. Clear minus ambiguous threat

Most notable in this analysis were clusters of activation in motor-planning areas, including the left and right premotor cortex (PMC), and right supplementary motor area (SMA). In addition, portions of the left inferior frontal gyrus, right superior temporal sulcus (STS), and right cingulate gyrus were significantly more

Table 1

Regions of increased activation associated showing greater activation to clear minus ambiguous and to ambiguous minus clear threat/gaze pairings in Study 2 (height: $p < .005$, uncorrected; extent: 10 voxels). Regions reported posterior to anterior.

Anatomical location	MNI coordinates			t Value
	x	y	z	
Clear minus ambiguous (2 s)				
L. Superior temporal sulcus	−62	−38	6	8.32[*]
R. Cingulate gyrus	12	−10	50	5.34
L. Premotor cortex	−32	−2	62	8.47[*]
L. Inferior frontal cortex	−60	2	22	4.66
R. Anterior superior temporal sulcus	58	4	−20	4.32
R. Supplementary motor cortex	6	6	56	6.13[*]
R. Premotor cortex	46	6	44	5.48[*]
L. Hippocampus	−20	26	16	6.88[*]
R. Ventromedial prefrontal cortex	10	28	−30	4.69
Ambiguous minus clear (2 s)				
L. Striate cortex	−8	−86	−2	7.92[*]
R. Striate cortex	10	−82	−6	5.46[*]
L. Fusiform gyrus	−44	−62	−20	5.55[*]
L. Cingulate gyrus/precuneus	−2	−54	30	6.89[*]
R. Parahippocampal gyrus	30	−52	−8	4.57
R. Intraparietal sulcus	58	−42	64	5.00
L. Intraparietal sulcus	−48	−38	72	4.77
L. STS/temporoparietal junction	−62	−32	32	6.94[*]
L. Inferior temporal gyrus	−52	−28	−28	5.42[*]
R. Hippocampus	30	−24	0	5.73[*]
L. Amygdala	−20	−6	−22	4.08
L. Anterior superior temporal sulcus	−14	14	−40	7.45[*]
R. Dorsolateral prefrontal cortex	24	46	54	4.95
L. Dorsolateral prefrontal cortex	−26	48	56	6.77[*]
L. Orbitofrontal cortex	−14	48	−10	6.01[*]
R. Orbitofrontal cortex	16	50	−8	4.99

[*] Signifies cluster remains significant at $p < .001$, uncorrected; extent: 10 voxels.

responsive to clear versus ambiguous threat (see Table 1, top panel).

5.2.2. Ambiguous minus clear threat

As predicted, the left amygdala showed greater activation to ambiguous versus clear threat displays (see top panel, Fig. 2), thereby replicating Adams et al. (2003). In order to clarify the nature of this interaction, we pulled an ROI cluster and examined direct comparisons between anger and fear. Following the pattern found in our previous work (Adams et al., 2003), and in Whalen et al. (2001), amygdala responses to direct gaze faces were greater for fear than anger expressions, $t(9) = −2.67$, $p < .05$, whereas amygdala responses to averted gaze faces were greater for anger than fear $t(9) = 2.35$, $p < .05$.

In addition, significant effects were found in visual areas including the left and right striate cortex (SC_x), left STS, left inferior temporal gyrus (ITG), and left and right fusiform gyrus (FG). Other areas important for emotion processing were also found including left orbitofrontal cortex (OFC), and the left posterior cingulate (see Table 1, bottom panel).

5.2.3. Chi-square

Overall, there were significantly more voxels active in the ambiguous minus clear threat–gaze contrast (voxels = 2138) than in the clear minus ambiguous threat–gaze contrast (voxels = 1246), $\chi^2(1, N = 3384) = 235.1$, $p < .0001$. In addition, significantly more voxels were activated overall in the left hemisphere (voxels = 1988) than the right (voxels = 1396), $\chi^2(1, N = 3384) = 103.57$, $p < .0001$.[1]

These findings offer evidence consistent with the conclusion that neural responses to sustained presentations of threat are more engaged by ambiguous versus clear threat cues. Regions implicated in the processing of threat displays were preferentially responsive

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[1] The effects of the chi-square analysis hold for reduced thresholds as well.
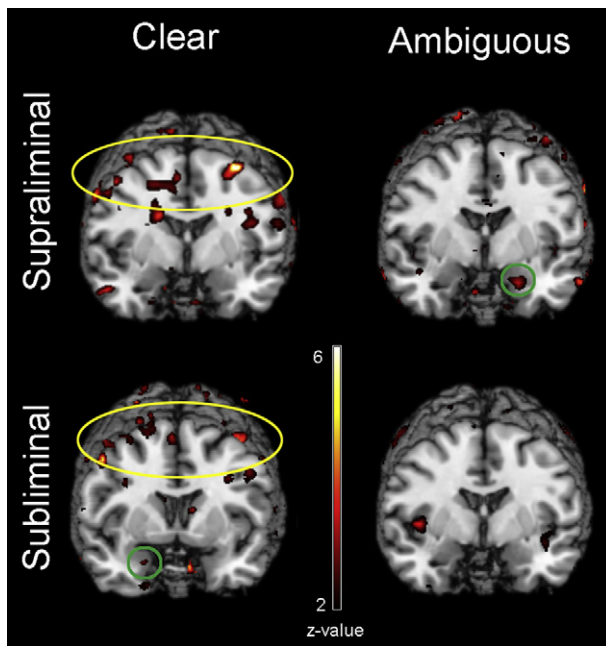
Fig. 2. Activation maps corresponding to Study 1 (top) and Study 2 (bottom) for clear minus ambiguous (left), and ambiguous minus clear threat (right). Activation maps show significant amygdala activation to ambiguous minus clear threat for Study 1, and a trend to clear minus ambiguous threat for Study 2 (circled in green). Premotor and supplementary motor cortices to clear minus ambiguous threat are apparent in both studies (circled in yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to ambiguous versus clear threat, and there were overall more voxels reaching our set threshold for the ambiguous versus clear threat–gaze pairings.

6. Study 2: neural responses to threat under severely restricted awareness

6.1. Method

6.1.1. Participants

Twelve healthy Caucasian males under the age of 30 participated in this study, all right handed, with normal or corrected vision, and no history of neurological impairment or disorders. Two participants were removed from the subsequent analysis for excessive head movement during scanning, leaving ten participants.

6.1.2. Stimuli

Faces were selected from the Ekman and Friesen (1976) Pictures of Facial Affect set. Eight identities (four males, four females) were selected. These faces displayed anger and fear expressions.[2] All photographs were presented as grayscale images approximately 3.5×3.5 in. Gaze direction was manipulated using Adobe Photoshop™, insuring that the facial muscle patterning associated with emotional expression remained identical across treatment conditions, differing only in gaze direction.

6.1.3. Design and procedure

Scanning involved alternating 31.5 s epochs of backward masked fearful direct- and averted gaze fearful and angry target

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[2] These stimuli were validated using a speeded emotional sort task used by Adams and Kleck (2003). Using this task, the predicted interaction was found, $F(1, 38) = 5.818$, $p < .05$, where direct anger and averted fear were faster decoded than averted anger and direct fear. There were no main effects for either emotion or gaze.

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displays. Target faces were presented for 33 ms, after which a different neutral exemplar face (the mask) with parallel gaze direction was presented for 150 ms. Participants passively viewed these stimulus trials. Eight exemplar faces were presented with both anger and fear expressions for a total of 56 masked stimuli per condition. Rest periods consisted of a 28 s epoch presenting low-level fixation (+). Each run included eight sets of stimulus presentations and four rests in a block design fashion lasting approximately 6 min, 4 s. During each of three runs, participants viewed the following orders of stimulus blocks: Order 1: +,DA,AA,+, DF,AF,+,AA,DA,+, AF,DF,+; Order 2: +,DA,AA,+,AF,DF,+,DF,AF,+, AA,DA,+; Order 3: +,DF,AF,+,DA,AA,+, AF,DF,+,AA,DA,+ (A = averted gaze, D = direct gaze by A = anger expression, F = fear expression, + = fixation). The sequence of runs was counterbalanced across participants. A post-scan manipulation check using a subset of these stimuli was performed on nine of the participants, revealing no differences in recognition accuracy of the rapidly presented target expressions for any of participants tested (Mean = .517, SD = .11, where chance = .50), confirming that awareness was severely restricted.

6.1.4. Data acquisition and analysis

Imaging was performed on a GE Medical Systems 1.5 Tesla Sigma scanner with a standard quadrature head coil as in Study 1. Visual images were back-projected onto a screen by an LED projector and participants viewed the images through a mirror on the head coil. Structural images were acquired as described in Study 1. Functional images were acquired with a gradient EPI sequence using BOLD contrast (TR = 2800 ms, TE = 70 ms, flip = 90°). During each of three functional runs there were 126 interleaved whole-brain acquisitions (25 noncontiguous slices, 4.5 mm thickness, 1 mm gap). The design matrix was organized in a block design fashion.

For each functional run, data were preprocessed as described in Study 1 using SPM99 software. The different conditions (AD, AA, FD, FA) were then modeled as boxcar functions, convolved with a hemodynamic response function. To identify regions revealing significant BOLD changes to clear versus ambiguous threat, statistics were computed on a voxel-by-voxel basis using GLM. These individual results were then submitted to a second-level random-effects group analysis to create mean t-images. Clusters were localized based on the contrast of clear (direct-gaze anger/averted-gaze fear) minus ambiguous (averted-gaze anger/direct-gaze fear) threat and vice versa (height: $p < .005$, uncorrected; extent threshold: 10 voxels). Primary analyses involved direct contrasts of clear minus ambiguous threat and vice versa. For the sake of completeness, we employed one additional contrast at a reduced threshold (height: $p < .02$, uncorrected; extent threshold: 5 voxels) to explore trend-level amygdala activation for clear minus ambiguous threat. We did this given a priori evidence for right amgydala responsivity to subliminal presentations of threat (see Morris et al., 1998). Because Monte Carlo simulations have previously determined that a threshold of $p < .01$, extent = 5 voxels corresponds to $p < .05$, small volume correction for anatomically defined bilateral amygdala, we felt this would serve an adequate reduced threshold for exploring unilateral amygdala response. Coordinates are reported in MNI space.

6.2. Results

6.2.1. Clear minus ambiguous threat

Most notable were activations in the sensory cortices including right SC_x, right STS, left and right ITG, and right FG (see Table 2, top panel). Other areas of interest included the left OFC, right DLPFC and VMPFC, right PCC and anterior cingulate, and right thalamus. The predicted interaction was also found in the right SMA, and the left PMC, consistent with the notion that this processing stream

Table 2
Regions of increased activation associated showing greater activation to clear minus ambiguous and to ambiguous minus clear threat/gaze pairings in Study 1 (height: $p < .005$, uncorrected; extent: 10 voxels). Regions reported posterior to anterior.

Anatomical location	MNI coordinates			*t* Value
	x	*y*	*z*	
Clear minus ambiguous (subliminal)				
R. Striate cortex	24	−96	−2	5.34
L. Inferior temporal cortex	−44	−78	−4	4.6
R. Fusiform gyrus	24	−40	−16	6.91*
R. Posterior cingulate	18	−38	14	5.47*
L. Inferior temporal cortex	−56	−28	−24	4.52
L. Superior temporal gyrus	−46	−26	14	6.57*
L. Premotor cortex	−38	−8	58	4.83
R. Amygdala	26	−2	−22	3.02+
R. Supplementary motor cortex	10	12	58	4.42
R. Ventromedial prefrontal cortex	14	22	−14	5.89*
R. Dorsolateral prefrontal cortex	50	34	40	8.14*
L. Orbitofrontal cortex	−12	36	−16	5.34
L. Dorsolateral prefrontal cortex	−48	36	32	4.11
L. Orbitofrontal cortex	−8	42	−20	4.24
Ambiguous minus clear (subliminal)				
L. Intraparietal sulcus	−54	−34	48	5.18
Superior colliculus	0	−32	−4	5.04
R. Insula	44	−14	2	5.39*
R. Insula	44	−2	2	7.35*
R. Medial orbitofrontal cortex	2	10	−24	4.72
R. Anterior superior temporal sulcus	50	22	−26	5.93*
L. Orbitofrontal cortex	−32	36	−16	5.88*
L. Dorsolateral prefrontal cortex	−56	42	10	4.59

* Signifies cluster remains significant at $p < .001$, uncorrected; threshold extent: 10 voxels.

+ Reduced threshold, $p < .02$, uncorrected; threshold extent: 5 voxels. Peak voxel, $p < .007$.

is involved in the initialization of an early response system (see Fig. 2). However, neither right nor left amygdala activation was evident at our set threshold. Given its importance to the threat perception network outlined herein, we therefore examined trend-level activation using an ROI analysis with a reduced threshold ($p < .02$, uncorrected) in order to explore this response. This analysis revealed a cluster of right amygdala activation with greater response to clear versus ambiguous threat–gaze pairings as predicted (peak voxel, $p = .007$).

6.2.2. Ambiguous minus clear threat

This contrast yielded significant clusters in the left intraparietal sulcus, right insula, right cerebellum, left OFC and DLPFC, and the right anterior STG (see Table 2, bottom panel). No amygdala activation was evident even at reduced thresholds.

6.2.3. Chi-square analysis

Across the whole brain, there were significantly more voxels reaching threshold to the clear minus ambiguous condition (voxels = 502) than the ambiguous minus clear condition (voxels = 380), χ^2 (1, $n = 882$) = 16.88, $p < .001$. There were also significantly more active voxels in the right hemisphere (voxels = 525) than the left hemisphere (voxels = 324), χ^2 (1, $n = 849$) = 47.58, $p < .001$.[3]

Overall these findings are consistent with the conclusion that neural responses to rapidly presented threat are more engaged by clear versus ambiguous threat–gaze pairs. Regions known to be implicated in the processing of threat displays were preferentially responsive to clear versus ambiguous threat, and there were overall more voxels reaching threshold for clear versus ambiguous threat–gaze pairings.

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[3] One cluster with a peak centered midline between the hemispheres was excluded.

7. General discussion

The two studies reported above offer initial evidence for differential attunements of reflexive versus reflective processing to clear versus ambiguous threat, respectively.

Critically, Study 1 revealed the predicted pattern of effects, such that sustained threat presentations yielded overall greater neural responsivity to ambiguous versus congruent threat–gaze pairings, a pattern apparent for many of the predicted regions of interest including left amygdala, thereby replicating Adams et al. (2003). These findings are consistent with the self-regulation hypothesis of attention to threat (Mogg et al., 2007; Serences et al., 2005), which predicts that conscious processing of threat will yield greater attentional disengagement from clear threat cues while attentional maintenance will continue for ambiguous-threat cues that require greater processing demands. Overall, more voxels reached threshold for incongruent versus congruent threat–gaze pairs, and more voxels also reached threshold in the left versus right hemisphere. Together these findings suggest that sustained presentations, which allow for more reflective processing, yield responses preferentially tuned to threat-related ambiguity, supporting theoretical assumptions that this level of processing is geared toward clarifying the nature of ambiguity surrounding threat.

Study 2 revealed that areas related to visual and socioemotional processing were predominantly responsive to congruent threat–gaze pairings under conditions of severely restricted awareness (33 ms, backward masked). Many of the predicted regions of interest were found to be significantly more activated to clear than to ambiguous threat. Areas related to motor-planning were also activated in this condition. In addition, overall activation (i.e., total number of voxels reaching threshold) was greater for congruent versus incongruent threat–gaze pairs, and more voxels were found to reach threshold in the right versus left hemisphere. When employing a reduced threshold to explore the right, unilateral amgydala response, this yielded greater activation to clear versus ambiguous threat as well.

The lack of a more robust amygdala response to clear versus ambiguous threat in Study 2 may indicate that integration of subtle visual cues such as eye gaze in the amygdala is more difficult at such brief exposures. Early processing of threat, bypassing visual cortices, and proceeding along a subcortical route with direct projections from the retina to superior colliculus, to the pulvinar nucleus of the thalamus and then onto the amygdala (Morris et al., 1999; Palermo & Rhodes, 2007) is thought to be attuned to only low spatial frequencies, which confer global configural information but not fine-tuned detail (Vuilleumier et al., 2003). Thus, although initial orienting responses occur to subliminally presented threat faces (Mogg & Bradley, 1999; Morris et al., 1999), the information received may simply be too coarse for gaze direction to exert substantial influence on this effect.

In order for gaze to interact with expression under conditions of such restricted awareness, a requisite level of visuoperceptual clarity would be necessary (see Adolphs, 2002a, 2002b). That said, different amygdala responses have been found to subliminally presented anger displays when posed on two different models, where one face was aversively conditioned with a noise burst and another not (Morris et al., 1998). This finding is compelling in the current context as it suggests a fairly high level of visuoperceptual clarity necessary to decipher subtle identity cues on otherwise highly similar, prototypical anger displays. Similar effects were found in patients with a cortically blind field (Morris et al., 2001). Given these previous findings, and the known role of the amygdala in early threat detection, we explored amygdala activation using a reduced threshold ($p < .02$, uncorrected, extent = 5 voxels) in order to better inform future research efforts in this area. At this more liberal threshold, we found more responsivity to clear

than ambiguous threat in the right amygdala, as predicted (see Morris et al., 1998). Given the important role of the amygdala in threat detection and in regulating cognitive, behavioral, and perceptual responses to threat, and trend-level responses that follow our predictions, as well as the pattern of other key ROIs, future examination of the role of the amygdala in such reflexive responses to compound threat cues is clearly warranted.

There are also two routes implicated in attention to facial stimuli worth considering in the present context (Palermo & Rhodes, 2007). First is a top-down affective attentional route, driven primarily by the VMPFC. Second is a cognitive top-down route driven by the DLPFC, which is involved in task set demands and working memory. Evidence for activation in these and associated attentional networks was apparent in our two studies. Study 1 showed greater bilateral intraparietal sucli activation to ambiguous versus clear threat, as well as greater VMPFC to clear versus ambiguous threat cues. Study 2 revealed greater extrastriate and fusiform responses to clear versus ambiguous threat, and greater intraparietal and DLPFC activation to ambiguous versus clear threat conditions. Activity in the visual orienting attentional network likely accounts for the pattern of significant activations found for many of the primary regions of interest (e.g., right ACC, thalamus; Posner & Petersen, 1990). These findings suggest that early attentional weighting to threat is predominantly tuned to congruent cues consistent with this level of processing being preferentially geared toward detecting highly salient threat cues. In order to make sense of the role of attentional networks in threat perception, a critical next step will be to more precisely model such spatiotemporal dynamics, which will necessitate multi-modal data collection, such as combined EEG and fMRI (see Debener, Ullsperger, Siegel, & Engel, 2006) allowing simultaneous examination of both the spatial and temporal distribution of neural responses to clear versus ambiguous threat cue combinations.

Interestingly, some evidence of STS/G activation was apparent for all contrasts reported in Studies 1 and 2, though in nonoverlapping areas. Given the known role of the STS as an advanced stage of visual integration of facial information (Perrett & Mistlin, 1990), it is likely that this region plays an important role in pooling various permutations of threat–gaze information at different stages along the processing stream. In addition, both fMRI studies revealed more activation in motor planning areas including the SMA and premotor cortex to the presentation of clear versus ambiguous threat–gaze pairings. This finding suggests that motor planning is reflexively triggered and remains engaged even during more reflective processing of congruent threat–gaze pairings.

Across the two studies there was also some evidence for laterality effects, with relatively more left hemispheric involvement in reflective threat responses, and more right hemispheric involvement in reflexive threat responses. These findings bolster the conclusion that such processes are dissociable, but we report them with caution. Although they are consistent with some work demonstrating subliminal responses to threatening stimuli appearing to recruit more right amygdala responses, whereas supraliminal responses recruit more left amygdala (Morris et al., 1998), there are notable exceptions (e.g., Carlson, Reinke, & Habib, 2009) suggesting that at this point any firm conclusions for differential functioning based on hemispheric laterality is likely to be oversimplified. Future research efforts are necessary to employ stronger tests of laterality before such conclusions can be drawn.

An important limitation of the current work is the use of different experimental paradigms across the studies, which did not allow for direct comparison. Study 1 employed an event-related design and had participants engage in a gender discrimination task. Study 2 employed a block design and passive viewing. We chose to the current approach in order to directly replicate our previous findings in Study 1, and to employ a well-established par-

adigm for tapping into rapid, backward masked threat responses in Study 2. As an initial inquiry into this issue, we felt it important to use previously validated paradigms before varying these parameters to allow for more direct comparison (i.e., within the context of a single study). We also felt that first we needed to demonstrate a direct replication of our previous effects, given recent evidence for opposite effects using more rapid presentation parameters (i.e., Hadjikhani et al., 2008). There is important consistency between these studies to highlight as well, in that they were run on the same scanner drawing from the same participant population, within the same timeframe, making conceptual comparisons more meaningful. Clearly, future research examining these effects will benefit from varying only the timing parameters within the context of a single study to allow for direct comparisons.

In sum, by varying gaze direction, and thereby threat clarity, the current work offers evidence for a sustained attunement to threat-related ambiguity that is differentiated from an earlier more reflexive attunement to clear threat–gaze cues. Theoretically such attunements would support an early response system that not only detects threat and sets in motion an adaptive behavioral response to it, but a parallel response system that acts to either confirm and perpetuate the response already set in motion, or disconfirm and inhibit inappropriate responses before fully engaged. The interplay of reflexive and conscious processing streams in threat perception is not well understood beyond that they appear to have distinct neural underpinnings, and operate in parallel. Our work suggests that this independence allows them to benefit from different attunements to the threatening stimulus thereby responding to different processing demands, at least in the specific case of threat perception.

Acknowledgments

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