

Amygdala responses to averted vs direct gaze fear vary as a function of presentation speed

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We examined whether amygdala responses to rapidly presented fear expressions are preferentially tuned to averted vs direct gaze fear and conversely whether responses to more sustained presentations are preferentially tuned to direct vs averted gaze fear. We conducted three functional magnetic resonance imaging (fMRI) studies to test these predictions including: Study 1: a block design employing sustained presentations (1 s) of averted vs direct gaze fear expressions taken from the Pictures of Facial Affect; Study 2: a block design employing rapid presentations (300 ms) of these same stimuli and Study 3: a direct replication of these studies in the context of a single experiment using stimuli selected from the NimStim Emotional Face Stimuli. Together, these studies provide evidence consistent with an early, reflexive amygdala response tuned to clear threat and a later reflective response tuned to ambiguous threat.

Keywords: threat perception; amygdala; fMRI; eye gaze; fear expression

INTRODUCTION

When examining socio-emotional perception, particularly at the neural level, it is necessary to consider both reflexive and reflective processes (cf. Lieberman *et al.*, 2002; Lieberman 2003; Cunningham and Zelazo, 2007). This is particularly true of threat perception, which is known to involve both early and late processing streams (Davis, 1992; LeDoux, 1998; Adolphs, 2002a, 2002b), which appear to be dissociable both temporally and structurally (Halgren and Marinkovic, 1995; LeDoux, 1995; Morris *et al.*, 1998, 1999, 2001; Vuilleumier, 2002; Liddell *et al.*, 2004; Williams *et al.*, 2004). Herein we propose that these two processing routes are not only neurally distinct, but are functionally tuned to different aspects of the threatening stimulus. Specifically, we propose that reflexive vs reflective processes may be differentially tuned to clear vs ambiguous combinations of threat cues, respectively.

Most previous studies examining neural responses to anger and fear have tended to utilize only faces displaying direct gaze, with an underlying assumption that gaze direction should not meaningfully influence the perception of such basic threat signals as anger and fear. However, anger and fear signal different types of threat for which eye gaze direction is arguably differentially informative (Adams *et al.*, 2010a;

Adams and Nelson, 2011). Anger signals a threat from the person making the expression. When coupled with direct eye gaze, therefore, observers gain information regarding both the source of threat and that the threat is directed at them. When coupled with averted gaze, however, the anger signal is a more ambiguous message of imminent danger to the observer. Fear, on the other hand, signals that an expressor perceives an external threat in the environment. Thus, when coupled with direct gaze, there is ambiguity regarding the source of threat. In this case, averted gaze offers information regarding where that threat might be located. Notably, averted gaze and fear expressions also both signal avoidance, whereas direct gaze and anger both signal approach. Thus, direct-gaze anger and averted-gaze fear, when combined, convey congruent signals. Consistent with both of these interpretations, averted-gaze fear and direct-gaze anger have been found to be perceived as more intense and recognized more quickly and accurately than direct gaze fear and averted-gaze anger (Adams and Kleck, 2003, 2005; Adams *et al.*, 2006; Adams and Franklin, 2009; Sander *et al.*, 2007; Benton, 2010). A growing number of studies utilizing a variety of techniques now offer growing support for the important role of gaze direction in emotion processing, particularly in threat perception (e.g. Sato *et al.*, 2004; Fox *et al.*, 2007; Graham and LaBar, 2007; Hess *et al.*, 2007; Hadjikhani *et al.*, 2008; N'Diaye *et al.*, 2009; Ewbank *et al.*, 2010; Rigato *et al.*, 2010).

When initially examining the role of eye gaze in neural responses to threat displays, we found greater amygdala activation when participants viewed ambiguous threat-gaze

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pairs (direct fear, averted anger) *vs* clear threat-gaze pairs (averted fear, direct anger; Adams *et al.*, 2003). At the time, this finding seemed to resolve a puzzling issue in the literature: early neuroimaging papers reported robust and consistent amygdala responses to fear faces, but to a lesser extent angry faces (e.g. Whalen *et al.*, 2001). Yet, anger—at least when coupled with direct gaze—arguably signals a clear and immediate threat to the observer, as it indicates both the source and target of imminent aggression. Our findings, therefore, corroborated a working hypothesis at the time that amygdala responses increase proportionally with the degree of ambiguity surrounding the source of threat (see Whalen, 1998).

Since this initial fMRI study, a new even more puzzling issue has emerged. Three subsequent neuroimaging studies (Sato *et al.*, 2004; Hadjikhani *et al.*, 2008; N'Diaye *et al.*, 2009) have been published that—although confirming the important role of gaze in threat perception at the neural level—revealed the opposite pattern of effects as our own (i.e. greater amygdala responses to congruent *vs* ambiguous threat-gaze pairs). Recently, important moderators of the gaze by threat display interaction have been explored, including individual differences in trait and state anxiety levels (Fox *et al.*, 2007; Ewbank *et al.*, 2010), shifts in progesterone levels along the menstrual cycle (Conway *et al.*, 2007) and the relative discriminability of gaze and expression at the stimulus level (Graham and LaBar, 2007). None of these, however, can account for the current discrepancy across these studies, leaving this issue unresolved.

One aspect of threat perception yet to be examined that may help address this new puzzle is processing speed. When scrutinizing previous studies for similarities and differences, it became evident that the presentation parameters employed ('rapid'—300 ms presentation: Hadjikhani *et al.*, 2008; 'dynamic'—rapidly unfolding from neutral to expressive: N'Diaye *et al.*, 2009; and 'peripheral'—presented to the left or right of fixation: Sato *et al.*, 2004), all favor visual input via the magnocellular pathway, which is implicated in initial, rapid orienting responses to threat (Vuilleumier *et al.*, 2003). Our original study, on the other hand, used prolonged (2 s), focally presented, static threat faces, which represent presentation parameters favoring additional visual input via the parvocellular pathway. Another recent study using prolonged (1 s), focally presented, static images yielded similar amygdala effects to our own—greater amygdala responses to incongruent *vs* congruent pairings of gaze when coupled with two approach-oriented emotional displays, averted *vs* direct happy and angry expressions (Straube *et al.*, 2009). These findings suggest the possibility that threat processing along parallel visual pathways may account for the differences reported in the literature, thereby implicating a dual process.

Dual process models have played a critical role in helping shape our understanding of a broad range of social processes and are of particular importance to consider at the neural level (Lieberman *et al.*, 2002; Satpute and Lieberman, 2006).

Specifically, these models focus on the duality of 'reflexive' *vs* 'reflective' responding, for which distinct neural underpinnings are known to exist. Reflexive pathways are implicated in automatic responding, whereas reflective pathways act in a more controlled, top-down manner to guide, control, respond to and fine tune information processing. Distinct neurocognitive pathways such as these exist arguably so that reflexive and reflective processes can operate in a parallel, coordinated manner for maximally efficient responding.

The dual process distinction has a direct parallel in the threat perception literature as well, the putative 'low road' *vs* 'high road'. Low road responses are thought to involve only crude information about the environment provided predominantly by magnocellular inputs and unfold in a quick and efficient manner, allowing for survival-enhancing behaviors to be enacted before threat strikes. High road responses, on the other hand, are thought to build upon a slower and more detailed account of the environment, thereby allowing for modulation of initial low road perceptions and behaviors (LeDoux, 1998; Palermo and Rhodes, 2007). Little is known about how these systems interact, however, particularly as to whether they exhibit differential responses to combinations of threatening cues, such as eye gaze and expression. Given the existing evidence for distinct reflexive *vs* reflective neural responses to threat, these observations give rise to a straightforward prediction in the context of the current work: presentation conditions favoring relatively rapid *vs* more sustained threat processing will give rise to differentially increased amygdala responses to averted *vs* direct gaze fear, respectively. Addressing this hypothesis is the focus of our current series of studies.

Current study

We designed a series of studies that vary the presentation speed of fear displays, from relatively sustained to very rapid, to examine the proposed temporal dynamics of compound threat cue processing. These studies rule out other obvious potential differences in methodology that could otherwise account for such differences across studies, including features of the stimuli (e.g. total number, prototypicality) and differences in design (i.e. block *vs* event-related). Importantly, Hadjikhani *et al.* (2008) utilized 300 ms stimulus presentations (i.e. 300 ms fear expression, 1200 ms fixation). To create an identical design varying only in stimulus presentation duration, keeping all other aspects of the paradigm equivalent, we employed both 300 ms presentations with 1200 fixation for our rapid presentation condition, a direct replication of Hadjikhani *et al.* (2008) and a 1 s stimulus presentation with 500 ms fixation for our sustained duration comparison condition. Critically, initial reflexive attention has previously been found to be triggered as early as 50–100 ms, becoming fully engaged ~300 ms, whereas intentional responding is thought to occur as early as 500–700 ms (e.g. Posner, 1980; Driver *et al.*, 1999; Cooper

and Langton, 2006; Jones *et al.*, 2010), making these stimulus durations well-suited to our goal of targeting obligatory *vs* intentional threat responses.

We ran three fMRI studies varying the presentation speed (1 s *vs* 300 ms) of direct *vs* averted gaze fear faces. Study 1 (1 s) and Study 2 (300 ms) utilized stimuli selected from the highly standardized and widely validated Pictures of Facial Affect (Ekman and Friesen, 1976), previously used by Adams *et al.* (2003). Study 3 then directly replicated these findings in the context of a single experiment with stimuli selected from the NimStim Emotional Face Stimuli database (Tottenham *et al.*, 2009), previously used by Hadjikhani *et al.* (2008). Thus, the current series of experiments aimed to reconcile differences across these previous studies.

Study 1: amygdala responses to 1 s presentations of direct *vs* averted fear faces

Methods

Participants. Fifteen participants (nine female, six male) completed this study. Fourteen of the participants were Caucasian and one male was African American. Participants were all right-handed, with normal or corrected to normal vision, between 18 and 30 years, with no reported history of neurological impairments or disorders.

Stimuli. Eight separate models (four female, four male) displaying fear expressions were selected from the Pictures of Facial Affect (Ekman and Friesen, 1976). To reduce potential habituation effects, we also included neutral displays of these same exemplar faces morphed at 20% with their corresponding happy expressions.

Design and procedure. Participants passively viewed blocks of fear faces and 20% joy faces with fixation trials interspersed to help decrease habituation. Participants completed two functional runs. Each run consisted of an initial 20 s resting fixation, followed by 24 blocks, including six direct fear (DF), six averted fear (AF), three direct neutral (DN), three averted neutral (AN) and six additional fixation blocks (+). Each block lasted 16 s, including 12 trials consisting of a face displayed for 1 s, followed by a 500 ms fixation. For each of two runs, blocks consisted of the following run order: (+, DF, DN, DF, +, AF, NA, AF, +, AF, DN, DF, +, AF, DN, DF, +, AF, DN, DN, + DF, AN, AF, +) with the reverse order used in the second run. Run orders were counterbalanced across subjects.

Data acquisition and analysis

Data was acquired using an Intera 3.0 Tesla Philips scanner with custom built 3-axis balanced-torque head gradient coil and end capped birdcage RF coils. A T1-weighted three-dimensional image was acquired for anatomical structure (TR = 8.05 ms, TE = 3.7 ms, flip angle = 8°, FOV = 256 × 150 mm³, acquisition matrix = 256 × 256 × 150 and a SENSE factor = 2). SENSE factor refers to sensitivity

encoding used to reduce overall scan time using multiple image coils with parallel scan times, thereby preserving spatial resolution. It is represented as a factor of *k*-space samples that have been reduced. Echo planar imaging (EPI) was used for functional data acquisition (TR = 3 s, TE = 30 ms, flip angle = 90°, voxel size = 3 × 3 × 3 mm, 48 interleaved slices, 134 volumes, thickness = 3 mm, no gap and SENSE factor = 2). The first 6 volumes collected preceded the task run and were dropped from analysis due to EPI saturation effects. Preprocessing was conducted in SPM5. Images were realigned using a least squares approach and a 6-parameter rigid body spatial transformation to the first functional image, coregistered using the structural and the functional data in 3D using rigid body transformations. The coregistered anatomical images were segmented and normalized to the MNI space using these segmentation parameters, which formed the basis of spatial normalization of the functional images. To allow for inter-subject averaging, the functional images were smoothed with 8 mm FWHM isotropic Gaussian smoothing kernel.

Statistical analysis was performed using a mass-univariate GLM approach. Stimulus conditions were modeled as delayed boxcar functions convolved with a standard HRF model. We included six regressors to account for head motion (*x*, *y* and *z* plane and roll, pitch and yaw). These were included in the first-level analysis along with our trial conditions. Low-frequency signal components were eliminated with a standard SPM5 high-pass filter of 1/128 Hz. Subject-specific contrasts were estimated using a fixed-effects model. For group analysis, these estimates were entered in 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 direct gaze minus averted gaze fear and vice versa (height: $P < 0.005$, uncorrected, extent: 10 voxels). Notably, Lieberman and Cunningham (2009) recently argued that this threshold is optimal to balance between Type 1 and Type 2 errors. 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/micro.html>). Coordinates are reported in MNI space.

Results

As predicted, responses to direct minus averted gaze fear presentations revealed greater left amygdala response—thereby replicating Adams *et al.* (2003); (see Figure 1). No amygdala responses were apparent when comparing averted minus direct fear, even at greatly reduced thresholds (for full list of activations, refer to Supplementary Table S1).

Study 2: amygdala responses to 300 ms presentations of direct and averted fear

Methods

Participants. Eighteen Caucasian participants (nine female, nine male) were recruited for financial compensation.

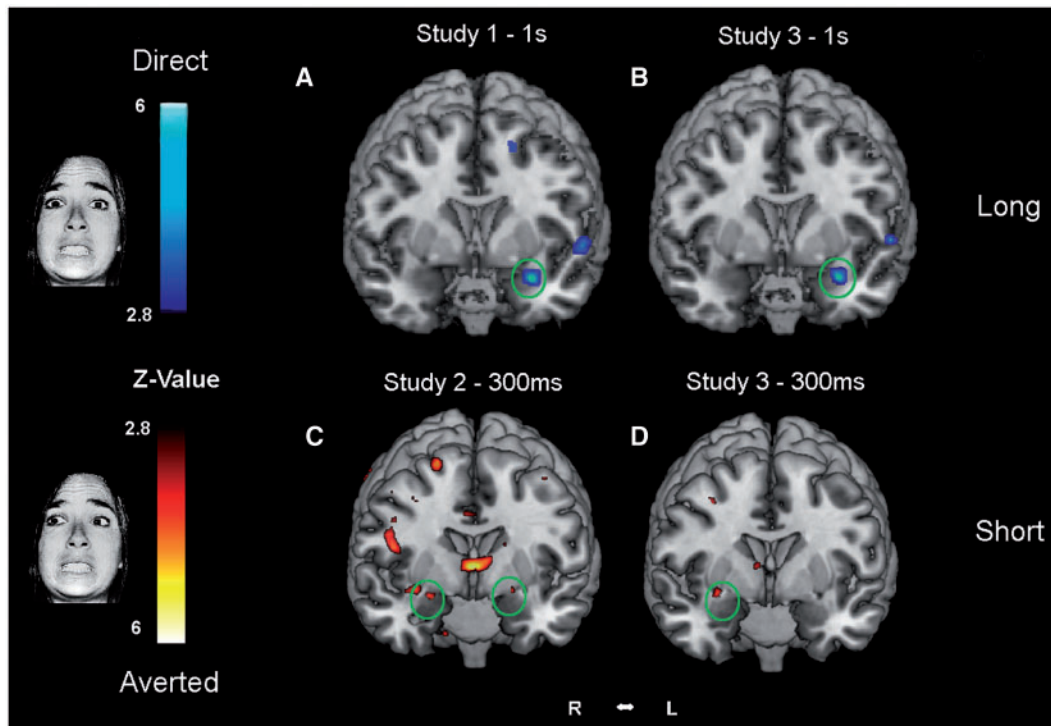


Fig. 1 Activation maps correspond to whole-brain analyses for Study 1 (A) and Study 2 (B). Activations for Study 3 (C and D) correspond to whole-brain analyses, but are restricted to the ROI masks determined by Studies 1 and 2. Activations in Study 3 represent points of direct overlap with Study 1 for the 1 s presentation condition (C) and with Study 2 for the 300 ms presentation condition (D) for the contrasts averted minus direct fear (displayed in the bottom panel) and direct minus averted fear (displayed in the top panel). Significant left amygdala activation (circled in green) was found for direct minus averted fear in both Studies 1 and 3 (1 s presentation condition) and significant right dorsal amygdala/SI (circled in green) was apparent to averted minus direct fear in both Studies 2 and 3 (300 ms presentation condition).

Participants were all right-handed, in the age group of 18–30 years, with normal or corrected to normal vision and reported having no history of neurological impairments or disorders. Two participants (both male) showed significant signal drop out in the amygdala and thus were excluded from the analyses.

Stimuli. The same fear expressions used in Study 1 were used here. In order to keep this design identical to that used by Hadjikhani *et al.* (2008), the neutral baseline and fixation blocks were dropped.

Design and procedure. Participants completed a passive viewing task using the same procedure employed by Hadjikhani *et al.* (2008), which involved an ABA design with alternating blocks of averted and direct gaze fear. Participants viewed eight blocks of fear expressions. Blocks lasted 24 s and included 16 trials each. Each trial consisted of a fearful face displayed for 300 ms followed by a 1200 ms fixation.

Data acquisition and analysis

fMRI scanning employed a 3-T Siemens Tim Trio scanner with a standard 12-channel headcoil for data acquisition. Scanning consisted of two experimental functional runs

and a T1-weighted anatomical scan collected for coregistration and normalization. High-resolution T1-weighted were collected using MP-RAGE (128 sagittal slices 1.33 mm thick, 256×256 matrix). Functional scans used EPI involving 192 dynamic whole-brain T2*-weighted images per run collected in an oblique axial orientation (TR = 2 s, TE = 30 ms, flip angle = 90° , voxel size = $3.125 \times 3.125 \times 5$ mm, 32 interleaved slices, 192 volumes, thickness = 5 mm, gap = 1 mm). Foam padding around the head was used to minimize head movement. Images were viewed using a back-projection system. Data were preprocessed and analyzed in the same way as reported in Study 1. Notably, these data were drawn from a larger study examining cross-cultural influences of gaze and fear processing (Adams *et al.*, 2010b). Given evidence for cultural effects in that study, the current analysis focuses only on US Caucasian participants viewing US Caucasian fear expressions. The contrasts comparing direct and averted gaze fear reported here were not previously published.

Results

As predicted, neural responses to averted minus direct gaze fear revealed significant amygdala activation, specifically in bilateral dorsal amygdalae/substantia innominata (SI). No amygdala response was apparent in the reverse contrast,

again even at greatly reduced thresholds (for full list of activations, refer to Supplementary Table S2).

Study 3: replication and extension of Studies 1 and 2 utilizing a different stimulus set

Methods

Participants. About 29 (15 female, 14 male) Caucasian participants were selected in the age ranging between 18 and 35. Participants had normal or corrected to normal vision and were free of neurological impairment. About 15 participants (7 female, 8 male) were randomly assigned to the 1 s presentation condition and 14 participants (8 female, 6 male) to the 300 ms presentation condition. Two participants (male) were subsequently dropped from analyses for excessive movement, both from the 1 s condition.

Stimuli. Stimuli used here were the same as those used by Hadjikhani *et al.* (2008), selected from the NimStim Emotional Face Stimuli database (Tottenham *et al.*, 2009) and included eight models displaying fear expressions, four female and four male.

Design and procedure. Participants passively viewed fearful faces in an ABA block design alternating between averted and direct gaze, with a total of 16 blocks of fearful faces. Each block consisted of 16 stimulus presentations. For the sustained presentation condition, stimuli were presented for 1 s followed by a 500 ms fixation. For the rapid presentation condition, stimuli were presented for 300 ms followed by a 1200 ms fixation between each stimulus. Otherwise, trial length between the two conditions was identical.

Data acquisition and analysis

We collected and preprocessed fMRI data as described in Study 1. An initial whole-brain analysis was conducted using the same thresholds utilized in Studies 1 and 2, (height: $P < 0.005$, uncorrected, extent: 10 voxels). In order to examine a precise replication of Studies 1 and 2, we also applied inclusionary region of interest (ROI) masks derived from each of these studies for direct comparison with the current presentation conditions. Thus, an ROI mask from Study 1 was used to examine corresponding neural responses for the 1 s presentation condition and an ROI mask from Study 2 was used to examine the 300 ms presentation condition. ROI masks were thresholded at $P < 0.05$, uncorrected, a commonly used threshold when employing an inclusive mask in this manner (e.g. Peigneux *et al.*, 2004; Coull *et al.*, 2011). Significant clusters were then based on a contrast of direct gaze minus averted gaze fear and vice versa when applying these masks (height: $P < 0.005$, uncorrected, extent: 10 voxels).

In this way, we were able to examine activations specifically overlapping with those found from in Studies 1 and 2 to employ a more stringent assessment of the reliability and consistency of effects across studies. Finally, we ran ROI

analyses pulling beta weights from an 8 mm sphere centered over the peaks of the amygdalae activation reported in Studies 1 and 2 (i.e. left amygdala in Study 1, bilateral dorsal amygdala/SI in Study 2), to directly compare activation across presentation duration for the comparison of direct vs averted gaze.

Results

For sustained presentations (1 s), left amygdala responses to direct minus averted gaze fear directly replicated those found in Study 1. Likewise, for rapid presentations (300 ms), neural responses to averted minus direct gaze fear displays replicated those found in the right dorsal amygdala/SI found in Study 2, but not for left dorsal amygdala/SI (Figure 1). Using activations pulled based on our three ROIs derived from Studies 1 and 2, we next computed a 2 (presentation duration: 300 ms, 1 s) \times 3 (ROI: left amygdala—Study 1, left and right dorsal amygdala/SI—from Study 2) mixed factorial (between-within) ANOVA. This analysis revealed a main effect of presentation duration, $F(1, 25) = 9.47$, $P < 0.005$, partial $\eta^2 = 0.275$, such that amygdala responses to fast presentations were greater for averted than for direct gaze fear, whereas amygdala responses to slow presentations were greater for direct than for averted gaze fear (Figure 2). Although there was not an interaction between type of ROI and presentation duration, for the sake of completeness, we also ran single sample *t*-tests for each ROI for each presentation duration separately. The only regions reaching significance based on these analyses were left amygdala in the sustained presentation condition, $t(12) = 2.52$, $P < 0.05$, such that activation was greater for direct minus averted gaze ($\beta = 0.087$, s.e. = 0.035) and right dorsal amygdala/SI in the rapid presentation condition, $t(13) = -2.23$, $P < 0.05$, such that activation was greater for averted minus direct gaze ($\beta = -0.051$, s.e. = 0.025; all other P 's > 0.2), thereby directly replicating the pattern found in Studies 1 and 2 for these ROIs. Overlapping activations when employing the whole-brain inclusionary masks derived from Studies 1 and 2 were also evident in several additional regions of interest (Table 1).

DISCUSSION

Amygdala responses have been documented in early, presumably reflexive, detection of highly salient threat cues (LeDoux, 1998; Morris *et al.*, 1998; Adolphs and Tranel, 2000). Research has also implicated the amygdala in later, top-down modulated, processing of ambiguity, such as in high-level decision making (Hsu *et al.*, 2005), responses to complex visual stimuli (Hamann *et al.*, 2002) and ambiguity surrounding threat (Adams *et al.*, 2003). The current results are consistent with these findings, suggesting that amygdala responses to averted vs direct gaze fear expressions vary depending on whether presented rapidly and thus presumably engaging reflexive responses, vs more slowly, presumably allowing for more reflective responses to the threat displays.

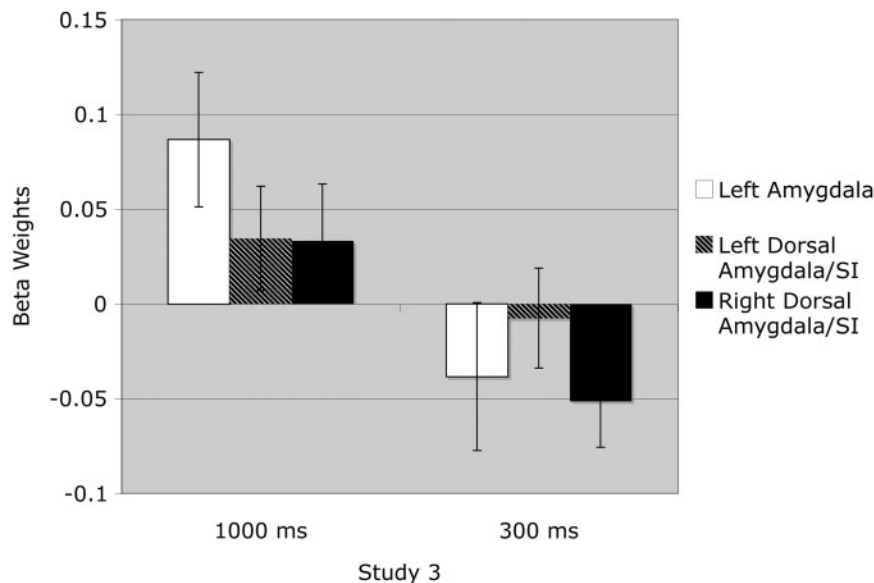


Fig. 2 Bars represent the mean beta weights of 8 mm clusters centered over the peaks of the three amygdala ROIs derived from Studies 1 and 2 (i.e. left amygdala in Study 1, bilateral dorsal amygdala/SI in Study 2).

Specifically, Study 1 revealed greater left amygdala activation to direct *vs* averted fear using 1 s presentation durations, replicating Adams *et al.*'s (2003) original pattern of findings. Using these same stimuli, we also replicated the reverse pattern of activation in Study 2 using 300 ms stimulus durations (see Hadjikhani *et al.*, 2008). Specifically, we found greater response to averted *vs* direct fear in bilateral dorsal amygdalae/SI, which has been previously implicated in threat vigilance and action preparedness (see Whalen *et al.*, 2001; Kim *et al.*, 2003). Study 3, employing identical trial timing parameters as Studies 1 and 2 with a unique stimulus set, resulted in highly reliable and consistent replications of these effects in both the left amygdala response during sustained presentations and the right dorsal amygdala/SI response during rapid presentations. Critically, direct comparisons of these ROIs showed a significant flip in amygdala sensitivity to direct *vs* averted gaze fear, displays as a function of presentation duration. These findings, therefore, offer both a direct replication of Studies 1 and 2, while at the same time providing direct evidence consistent with an early response system tuned to clear threat and a later response system tuned to threat-related ambiguity.

A pattern of amygdala activation that emerged across Studies 1 and 2 that was replicated in Study 3, might suggest evidence for a laterality effect. Specifically, reflective threat perception was found to be more left lateralized (Studies 1 and 3–1 s condition) whereas reflexive processing was found to be more right lateralized (Studies 2 and 3–300 ms condition). This finding is consistent with work showing more right-lateralized amygdala activation to subliminal threat *vs* more left-lateralized amygdala activation to supraliminal threat (see Morris *et al.*, 1998). These findings are also

consistent with the notion of a dual process threat response where threat cues are processed along dissociable pathways. However, we urge strong caution when interpreting the pattern of laterality in the current studies. Laterality effects for amygdala responses to rapidly presented threat remain mixed (e.g. Hadjikhani *et al.*, 2008; Carlson *et al.*, 2009). Further, a direct comparison of activations across our three ROIs in Study 3 yielded no statistical evidence for laterality, even though when tested independently, the activations reaching threshold across the timing conditions did seem consistent with a laterality account. In general, patterns of lateralized amygdala responses are often based on activations that do or do not reach threshold. Future work examining direct statistical comparisons of left *vs* right amygdala activation will be necessary to clarify the role that laterality may play in reflexive *vs* reflective threat responses.

Given that the current set of studies examined differential responses only to fear expressions, future research will also be necessary to determine the extent to which these findings generalize to other clear *vs* ambiguous threat-gaze pairs, such as those involving anger and disgust expressions. The current findings also should not be taken to imply that the same timing parameters will apply to all expressions, paradigms, or individuals. Overall intensity of expression and salience of gaze as visual cues, as well as the relative discriminability of these cues, can influence how readily threat is detected and even whether gaze is integrated in the threat response at all (see Graham and LaBar, 2007). Differences in amygdala responses have also been found when viewing fear faces from different cultural groups (Chiao *et al.*, 2008; Adams *et al.*, 2010c). Amygdala responses have further been found to be sensitive to a variety of individual differences including

Table 1 ROI masks derived from Studies 1 and 2 to show overlapping activation in Study 3 (height: $p < .005$, uncorrected; extent: 10 voxels)

Anatomical location	MNI coordinates				
	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> -value	Extent
1 s presentation					
Direct minus averted					
L. cuneus	-18	-66	20	3.8	27
L. cerebellum	-8	-46	-8	3.87	19
R. cerebellum	12	-38	-8	4.39	119
R. posterior cingulate	10	-28	44	4.12	41
L. posterior cingulate	-18	-26	36	7.67	220
L. superior temporal sulcus	-52	-26	0	5.38	173
R. superior temporal sulcus	58	-26	10	3.84	12
R. midbrain	2	-16	-8	4.01	11
R. claustrum	34	-12	-6	4.25	29
L. amygdala	-28	8	-26	3.95	7 ^a
R. posterior orbitofrontal cortex	18	14	-16	3.57	16
Averted minus direct					
N/A					
300 ms Presentation					
Direct minus averted					
N/A					
Averted minus direct					
L. cerebellum	-20	-66	-44	6.42	283
R. middle temporal gyrus	42	-64	14	3.59	20
L. temporo-parietal junction	-46	-40	26	4.57	60
L. postcentral gyrus	-16	-38	58	4.13	36
R. cingulate	20	-32	60	3.49	11
Midbrain	0	-30	-18	3.97	28
L. thalamus	-12	-26	-4	6.01	226
R. temporo-parietal junction	44	-26	20	4.22	40
R. thalamus	6	-20	-2	3.4	15
L. superior temporal gyrus	-60	-14	-2	3.7	25
L. premotor cortex	-30	-12	40	4.26	83
L. cingulate	-8	-10	52	4.25	55
R. supplementary motor area	30	-4	46	3.8	44
R. dorsal amygdala/Sl	30	0	-8	4.03	81
R. caudate	6	2	8	3.7	21

Study 1 was used to derive a mask to examine the 1 s condition and Study 2 for the 300 ms condition. Regions are reported posterior to anterior.

^aMonte Carlo simulations have previously determined that a threshold of $P < 0.01$, extent = 5 voxels corresponds to $P < 0.05$, small-volume correction for bilateral amygdalae (see Kim *et al.*, 2003). When following this approach, $P < 0.01$, the cluster extent here = 18 voxels.

temperament (Schwartz *et al.*, 2003), state and trait anxiety (Etkin *et al.*, 2004), social phobia (Stein *et al.*, 2002), sex differences (McClure *et al.*, 2004; Phan *et al.*, 2006) and genetic variation (e.g. Bertolino *et al.*, 2005; Canli *et al.*, 2005). Of particular relevance to the current findings, the tendency toward attentional biases to fearful expressions are positively correlated with both state and trait anxiety (Mathews and MacLeod, 1985; MacLeod *et al.*, 1986; Mogg *et al.*, 2000). Individuals high in trait anxiety (Mathews *et al.*, 2003; Putman *et al.*, 2006; Fox *et al.*, 2007) and trait fearfulness (Tipples, 2006) also show enhanced attentional shifts in the direction cued by fearful gaze. Even more specifically, recent work has demonstrated that gaze by emotion (anger and fear)

interactions in amygdala responses are modulated by state anxiety (Ewbank *et al.*, 2010). Given that high anxious individuals have particular problems reflectively disengaging from threatening stimuli (Bishop *et al.*, 2007), future work of this kind may offer critical insight into the reflexive/reflective nature of such responses.

Research into the interplay of reflexive and reflective threat responses remains nascent. One possibility is that reflexive and reflective processing streams have distinct neural underpinnings, allowing them to operate in parallel (e.g. Satpute and Lieberman, 2006). In the current context, such processing streams could work in tandem to produce the most efficient and adaptive threat response, allowing for rapid threat detection followed by later confirmation or disconfirmation of the threat before a behavioral response is fully engaged. Another possible mechanism has been proposed in the 'iterative reprocessing' model (Cunningham and Zelazo, 2007; see also Pessoa and Adolphs, 2010). This model posits that, rather than employing separate processing streams per se, the brain proceeds from reflexive to reflective processing via successive processing cycles, engaging higher-level cortical regions as the processing proceeds from reflexive to reflective. The initial few cycles may engage the amygdala and early-response cortical threat detection regions, such as the orbitofrontal cortex (OFC) (Thorpe *et al.*, 1983; Tomita *et al.*, 1999). As the stimulus is being evaluated through successive iterations, each cycle may incorporate more detailed information about the stimulus, engaging additional prefrontal regions (see Cunningham *et al.*, 2004). Both the 'dual process' and 'iterative reprocessing' models predict greater activation in the lateral prefrontal cortex (LPFC), rostral anterior cingulate cortex (rACC) and medial PFC (mPFC) during more reflective processing. Our work does not address which of these approaches can best explain our findings. Closer examination of spatiotemporal dynamics and effective connectivity among these regions while performing tasks similar to ours will be very helpful in clarifying a general understanding of the mechanisms underlying reflexive and reflective responses to compound threat cues.

Another possibility is that congruent threat cues not only capture attention more readily, but due to their aversive nature, may lead to earlier attentional disengagement as compared to ambiguous threat cues (see also Mogg *et al.*, 2007). Indirect support for this explanation is exemplified in a study conducted by Cooper and Langton (2006), using a dot probe attention task, in which they found that when presented for 100 ms, angry faces captured attention more readily than happy faces, whereas by 500 ms happy faces appeared to hold attention more than anger. The regulation explanation for these findings assumes a temporal stage model of emotion processing, with an early orienting and a later attentional maintenance response (e.g. Halgren and Marinkovic, 1995; see also Serences *et al.*, 2005). Supporting such a temporal model, an ERP study compared subliminal vs supraliminal responses to fear faces, finding temporally

and cortically dissociable activations associated with each that were argued to directly correspond to dual process models of threat detection (Liddell *et al.*, 2004).

Methodologically, the current work holds implications for future fMRI studies of this kind by underscoring the importance of considering temporal dynamics in neural responses to threat and perhaps other socio-emotional cues. As previous research has documented that threat responses are both temporally and spatially distributed, it is critical to consider how brain responses vary across the temporal aspect of the perceptual stream. A single ‘snapshot’ of activation at a particular point along that stream will miss such variation and can thus result in seemingly discrepant, even opposing findings across studies, as was the case here. The current work offers one example of how ostensibly minor differences in presentation parameters can give rise to major discrepancies in responses acquired. Focal *vs* peripheral, large *vs* small and dynamic *vs* static presentations may also yield differential neural responses to threat given that these can differentially favor visual input via magnocellular and parvocellular pathways. This observation further underscores the importance of considering more broadly how presentation methods systematically influence neural responses in neuroimaging paradigms of this type.

Conceptually, this work has implications for our growing understanding of threat perception more generally. To date the interplay of reflexive and reflective processing streams in threat perception remains not well understood. What is known is that they appear to have distinct neural underpinnings, which may allow them to operate in an interactive parallel manner (see also Townsend and Wenger, 2004). Clearly, closer examination of spatiotemporal dynamics will be essential in clarifying our understanding of the threat response. Attention to similar issues within other research domains is also likely to prove fruitful, given the prevalence of dual modes of processing across a wide variety of socio-emotional, cognitive and perceptual phenomena (e.g. attention, memory, appraisal, categorization).

SUPPLEMENTARY DATA

Supplementary data are available at SCAN online.

Conflict of Interest

None declared.

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