Fear is Fast in Phobics: The Time Course of Amygdala Activation in Response to Fear-relevant Stimuli

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Evolutionary fitness dictates that threat must be detected quickly (*1, 2*). Efficient identification of sources of potential harm requires an ability to detect threat-related stimuli based on relatively simple stimulus features and at any position in the perceptual field, thus resulting in a system which functions in a relatively rapid, automatic fashion with minimal processing of incoming sensory information (*1, 2*). A growing corpus of data indicate that the amygdala is involved in fear processing and that moreover, it likely holds the key to rapid detection of potentially threatening stimuli (*2, 3*). An independent line of psychophysiological research has found that specific phobics respond rapidly to phobia-relevant stimuli (*4, 5*). One possible mechanism underlying this accelerated response is exacerbation of the already rapid response of the amygdala to fear-relevant cues. To better understand the disjunction between normal and pathological fear, and the neural substrates of this distinction, we examined the chronometry of the amygdalar response in phobic fear, particularly the rapidity of its onset.

Extensive data implicates the amygdala in negative affect, especially fear (*3*). Lesions of the amygdala block fear conditioning in numerous sensory modalities (*6*). In human neuroimaging studies increased amygdala activation has been found during viewing of negatively-valenced pictures (*7, 8*) and fear faces (*9, 10*) even when presented preattentively (*11*). Importantly, Breiter and colleagues (*9*) found that the amygdala response to fearful faces habituated rapidly. In addition, fMRI studies have shown that amygdala activation habituates over repeated conditioning trials or presentations of affective stimuli (*12, 13, 14*). Furthermore, Phelps and colleagues (*15*) found greater amygdala activation to a threat condition only when the first half of each of the trials was included in the analysis. Thus, not only does amygdala activation habituate across repeated trials, but attenuation of signal from the amygdala occurs within individual trials as well. These data suggest that the amygdala may play an immediate, but short-lived role in the unfolding reaction to a negative affective stimulus. In contrast to these data demonstrating amygdala involvement in fear-related processes, several ^{15}O positron emission tomography (PET) studies of specific animal phobia have not found amygdala activation in response to phobia-relevant cues (*16, 17*). Given the mounting evidence indicating amygdalar habituation, it is likely that the temporal resolution of PET is not sufficient to capture these relatively fleeting patterns of activation.

Independent research has suggested that phobic fear is characterized by an abnormally early onset of the fear response. Globisch and colleagues (*4*) found potentiation of the startle eyeblink response to phobic compared to neutral and pleasant stimuli among snake and spider phobics. Furthermore, this potentiation was evident as early as 300 ms following the onset of the picture, earlier than potentiation is typically found in response to non-phobic, aversive stimuli (*18*), suggesting that phobic fear is associated with a fast onset.

In the current study we used an event-related fMRI paradigm to assess the magnitude and timing of the amygdalar response to fear-relevant compared to neutral and unpleasant nonphobic pictorial stimuli among female spider phobics (N = 13) and controls (N = 14). To enable fine-grained temporal sampling of activation in the amygdala, five coronal slices centered on the amygdala were collected at an effective time resolution of 300 ms. To extract magnitude and time to onset and peak of amygdala activation, a gamma variate function was fit to the resulting BOLD (blood-oxygen level dependent) responses. We demonstrate that in response to phobia-relevant stimuli phobics exhibit more rapid onset of amygdala activation than controls. We also show that among the phobics amygdala activation in response to spider pictures is more rapid than responses to negative or neutral pictures.

Method

Participants

Female undergraduates scoring greater than 20 (94^{th} percentile in the current sample) on the Spider Phobia Questionnaire (*19;* SPQ) were classified as phobics, and women scoring a 0 or 1 were classified as nonphobic controls. From this pool, thirty right-handed women (15 phobics, 15 controls) screened with the Structured Clinical Interview for DSM-IV Axis I Disorders, Non-patient version (*20*) met criteria for participation in the study including, presence or absence of spider phobia and absence of a history of depression, psychosis or other anxiety disorders. Two phobics and one control were dropped due to movement artifact yielding a final sample of 27 participants (13 phobics: mean age = 18.46, 14 controls: mean age = 19.21). The two groups did not differ on age ($p > .50$).

Materials and Design

Affective pictures were presented over the course of 3 functional scans. Given evidence of amygdala habituation over time, only data from the first scan are presented here. During the first scan 20 spider, 20 negative, and 20 neutral pictures were presented via Silent Vision fiber optic goggles (Avotec, Inc., Jensen Beach, FL). Negative and neutral pictures were selected from the International Affective Picture System (*21*). Spider pictures were selected from various websites. Pictures were presented in a pseudorandom order for 300 ms followed by a 15000 ms ITI.

Data Acquistion

MR data were acquired using a GE EchoSpeed 1.5 Tesla scanner (Waukesha, WI) equipped with high-speed, whole body gradients and a standard clinical whole-head transmit-receive quadrature birdcage headcoil. A T2* weighted gradient-echo echo-planar (EPI) pulse sequence was used to collect 5 coronal slices centered on the amygdala (slice thickness: 5mm, 1 mm interslice gap, TE/TR = 50/600 ms, FV = 24 x 24 cm, a = 65º, matrix = 64 x 64. 1540 images per scan). By using an ITI not evenly divisible by the TR (acquisition time for one full set of 5 coronal slices), we were able to achieve a time resolution of 300 ms or half of the acquisition TR. Structural images were acquired using an axial 3D SPGR (TE/TR = 8/20ms, FOV = 24 x 24 cm, a = 35º, NEX = 1, 256 x 256, 124 slices, slice thickness = 1.0-1.2 mm). Subjects were fit with a bite bar to minimize head movements.

Following the three functional scans subjects were removed from the scanner and rated a subset (10 each of unpleasant, neutral, and spider images) of the pictures on valence and arousal. Each picture was presented for 3 s followed by two 9-point Likert scale ratings, one for valence and one for arousal. Due to technical problems rating data for 2 control subjects were lost.

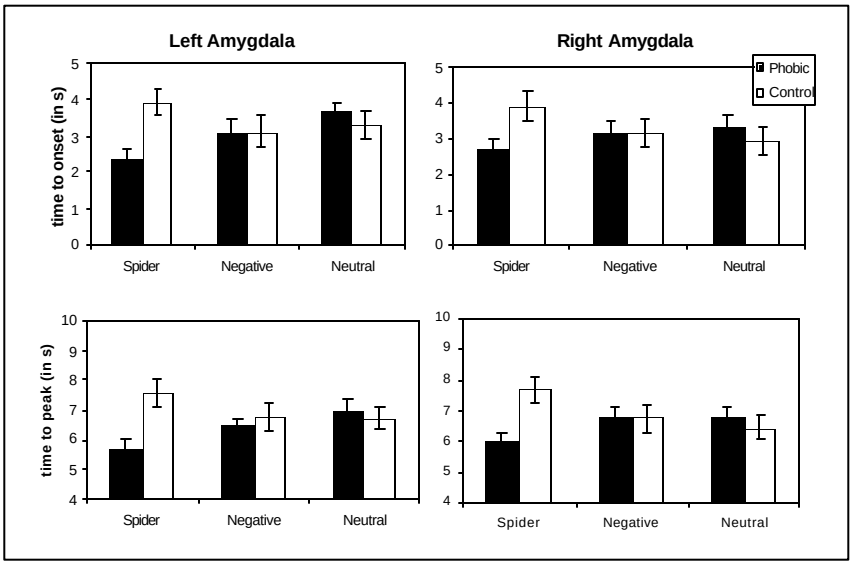
Data reduction and analysis

Using the AFNI software suite (*22*) data processing included offline image reconstruction, spatial smoothing with a Hamming window (FWHM = 1 voxel), motion correction, removal of skull and ghost artifacts, and introduction of bandpass temporal Fourier filter to remove high and low frequency (bandpass = .21-.4 Hz). Within run trials were aggregated such that the 20 trials per picture type (spider, negative, neutral) were averaged to create a 50-point (15 s) time series with 300 ms resolution for each condition. A gamma variate function was fit to the functional time series data to model the hemodynamic response and assess both magnitude (percent signal change from baseline) and timing (time to onset and time to peak in seconds) of activation.

Given that our predictions concerned amygdala activation, the primary analysis was performed using voxels identified to be in the amygdala for each individual subject. A region of interest (ROI) including all functional voxels containing amygdalar tissue (as identified in the SPGR anatomies) was drawn on EPI images for each slice for each subject. The results of the gamma variate fit, including percent signal change, time to onset, and time to peak response were extracted for ROI voxels. For each hemisphere averages of the gamma variate parameters across all voxels in the amygdala ROIs were calculated. A Group (Phobic, Control) X Picture Condition (Spider, Negative, Neutral) X Hemisphere (Left, Right) ANOVA was computed separately for each gamma variate parameter.

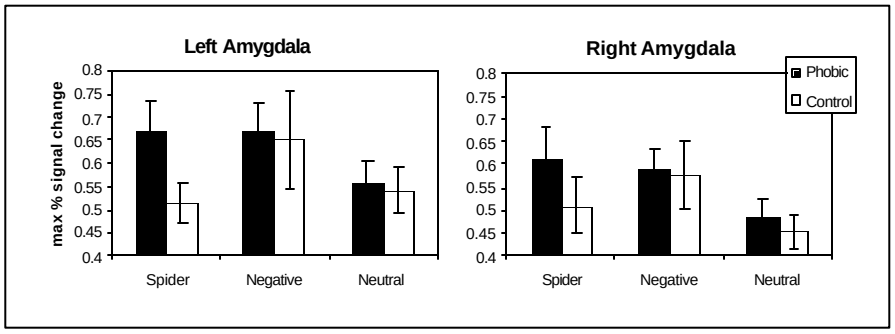
Results

Time to onset & peak of amygdala activation. The time to onset and time to peak of amygdalar responses to phobogenic stimuli consistently differentiated between the two groups, as well as between picture valences among the phobics (Fig. 1, right). There was a significant group by picture condition interaction for both time to onset, $F(2,50) = 4.9$, $p < .01$, and time to peak of response, $F(2,50) = 5.74$, $p = .006$. Compared to nonphobics, phobics had faster onset, $t = 3.28$, $p < .003$, and time to peak, $t = 3.02$, $p < .006$, of response to spider stimuli in the left amygdala. In addition to the Group X Picture Condition interaction and faster activation to spider stimuli for phobics compared to nonphobics, phobics also had significantly faster time to onset in the left

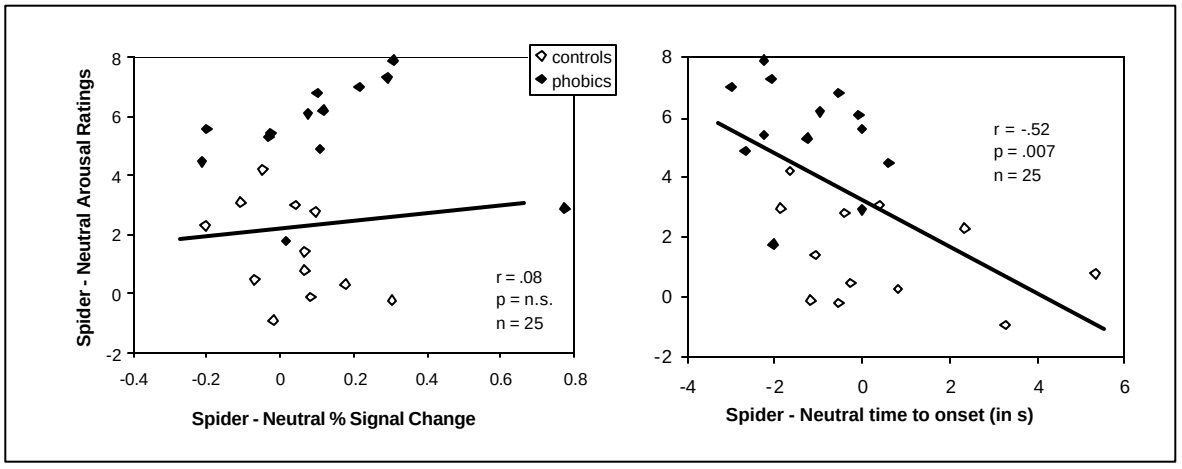


amygdala for spiders compared to neutral, $t(11) = 3.88$, $p = .002$, and negative, $t(11) = 2.65$, p = .02, pictures. Analogous *t* tests revealed faster time to peak for spiders compared to both neutral (left: $t(11) = 3.44$, $p < .005$) and negative (left: $t(11) = 2.60$, $p = .02$; right: $t(11) = 3.12$, $p = .009$) stimuli among phobics.

Magnitude of amygdala activation. Analysis of mean percent signal change for a region of interest (ROI) containing voxels in the amygdala yielded a significant main effect for picture condition, with larger responses to spider and negative compared with neutral pictures (Fig. 2, right). There was no interaction between group and picture condition. There was a significant main effect for Picture Condition, $F(2,50) = 4.42$, $p = .02$, such that responses to neutral pictures were smaller than those to negative, $t(25) = 3.46$, $p = .002$, and to a lesser extent spider, $t(25) = 1.89$, $p < .07$, pictures. Planned contrasts comparing percent signal change in response to spiders versus neutral pictures among the phobics revealed marginally greater activation in response to spiders in the right, $t(11) = 2.05$, $p < .06$, but not left amygdala ($p > .10$). Thus, differences in magnitude of amygdala activation are attributable primarily to the valence of the stimuli, not group membership. There was also a main effect for Hemisphere, $F(1,25) = 4.76$, $p = .04$, indicating greater magnitude of response in the left compared to right amygdala, $t(25) = 2.21$, $p < .04$.



Correlations with self-report. Moreover, timing of activation was also predictive of self-reported arousal in response to the pictures, whereas magnitude of activation was not (Fig. 4 below). Hierarchical regressions indicated that while magnitude of activation (percent signal change) did not account for significant variance in arousal ratings, faster time to onset and time to peak were both associated with higher arousal ratings for spider compared to neutral pictures. Entered as the first step in the regression, left amygdala spider - neutral time to onset predicted 27.3% of the variance in arousal ratings, $F(1,23) = 8.63$, $p < .007$, with left amygdala percent signal change adding 0.1% of variance ($p > .80$). Reversing the order of entry, spider - neutral percent signal change predicted a nonsignificant 0.6% of variance in arousal ratings ($p > .70$), with time to onset contributing an additional 26.8% of variance, $F(2,22) = 8.13$, $p < .009$. Similarly, for time to peak when entered as the first step in the regression, left hemisphere time to peak predicted 36.0% of the variance in spider - neutral arousal ratings, $F(1,23) = 12.93$, $p < .002$, with percent signal change adding 0.5% of the variance ($p > .65$). When entered first, percent signal change predicted an almost identical 0.6% of the variance in arousal difference scores, $F(1,23) = 0.14$, p >.70, with the bulk of the variance being predicted by time to peak (35.9%), $F(2,22) = 12.43$, $p < .002$). Similar results were present for the right amygdala, with percent signal change accounting for 2.6% of the variance when entered as the first step, $F(1,23) = 0.62$, $p > .40$), time to peak adding 21.5%, $F(2,22) = 6.22$, $p < .02$, and in a separate regression time to onset adding a marginally significant 14.3%, $F(2,22) = 3.78$, $p < .06$. No significant effects were found for time to onset, time to peak, or percent signal change in predicting negative–neutral activation, suggesting that variations in the time course of amygdala activation are consequential for reactivity to phobic stimuli specifically. As is evident in the scatterplot the phobic subjects rated spider pictures as more arousing than the controls, $t(23) = 9.79$, $p < .001$. Thus, timing of activation is associated with subjects' affective response to phobia-relevant stimuli, but magnitude of activation is unrelated.



Discussion

These data suggest that phobic fear is associated with a rapid response to phobia-relevant stimuli in the amygdala. The lack of robust effects for magnitude of amygdala activation seen here is consistent with several earlier reports failing to find increased amygdala activation in specific phobia (*16, 17*). In contrast, faster time to onset and time to peak of amygdala activation to spiders consistently discriminated between groups and conditions. Moreover, while time to onset and time to peak predicted ratings of arousal in response to the phobia-relevant pictures, magnitude of activation did not.

Evolutionary accounts of the instantiation of fear systems in the brain have emphasized early and reliable recognition of threatening stimuli as a crucial key to survival (*2*). In keeping with this notion, individuals with specific phobia exhibit an exaggerated preattentive bias for identifying threatening animals embedded in a complex visual display (*5*), a rapid onset of the fear-potentiated blink response to phobogenic pictures (*4*), and elevated skin conductance responses to phobia-relevant stimuli that are masked, and thus not consciously perceived (*24*). Phobic fear responses are thought to operate in a relatively crude fashion with an emphasis on minimizing false negatives at the risk of an increased number of false positives (*1, 2*). Indeed, previous investigators have suggested that phobics fail to circumvent "false" fear reactions that may normally be circumvented via more extensive cortical processing (*25*). Öhman and Soares (*24*) further suggested that rapid activation of the amygdala in particular facilitates responses to masked phobia-relevant stimuli, perhaps via the "quick and dirty transmission route" described by LeDoux (*3*). The amygdala can be rapidly activated by incompletely processed stimuli via a direct pathway from thalamic nuclei with minimal input from higher cortical regions (*26, 27*). While this circuitry may be adequate only for processing very crude environmental stimuli, the existence of this pathway underscores the possibility that the amygdala is involved in rapid processing of emotional stimuli.

Supporting the claim that rapid processing can occur via the direct thalamo-amygdalar pathway, auditory fear conditioning induces plasticity in amygdala neurons (*28, 29*), and this plasticity is evident earlier than that seen in auditory cortex, suggesting that the early plasticity in amygdala neurons results from direct thalamo-amygdala projections (*29*). In humans, covariation between the amygdala, the pulvinar nucleus of the thalamus, and the superior colliculus has been found in response to masked conditioned faces (*30*). Furthermore, a normal pattern of posterior thalamic-amygdala covariation is evident in a patient with an extensive lesion to left primary visual cortex, even when stimuli are presented to his blind hemifield and thus not consciously detectable (*31*). Given the absence of input from primary visual cortex, responses to the stimuli presented in the blind hemifield likely resulted from the pathway from the retina to the amygdala via the superior colliculus and posterior thalamus (*32*). In light of such data, Mesulam (*33*) has suggested that the amygdala acts as a "neural gateway" for the binding of incoming sensory information and affective valence. In specific phobia, the amygdala may be overly tuned to respond to incoming sensory information related to the target of their phobia.

Given the need to limit the spatial extent of data acquisition to increase temporal resolution for hemodynamic responses in the amygdala in the current study, we were unable to image the more posterior thalamic nuclei implicated in this pathway (*27*), and therefore unable to directly test the notion that accelerated amygdala activation in phobic fear may involve the thalamo-amygdalar pathway outlined by LeDoux. However, the data from this study are congruent with the notion that phobic fear has a rapid onset and that the amygdala is a key neural component of this response.

The amygdala has been implicated in automatic processing of stimuli signaling potential threat. Amygdala activation is evident in response to backwardly masked fear faces (*11*) and masked faces that have been paired with an aversive stimulus (*34*). Patients with posttraumatic stress disorder exhibit accentuated activation of the amygdala in response to masked facial expressions of fear, suggesting that the amygdala may play a key role in mediating preconscious response biases evident in anxiety disorders (*35*). Consistent with the notion that an adaptive fear detection system will function independently of the current focus of attention, amygdala responses to fearful faces are unaffected by manipulation of spatial attention (*36*). These data are consistent with the notion that in its role as a detector of emotionally-relevant stimuli, the amygdala may act relatively automatically, independent of input from higher cognitive control (*30, 34, 3*).

Taken together, mounting evidence indicates that the amygdala plays a primary role in the rapid, automatic perception of threat-related stimuli. The present data demonstrate the advantages of using an imaging paradigm with fine-grained time resolution to examine the temporal unfolding of activation in specific brain regions, in this case the accelerated onset of amygdala activation in phobic fear. Our data illustrate the utility of understanding not just magnitude, but also timing of brain activation, to delineate differences in normal versus pathological emotional states. As with previous reports, we found that magnitude of activation was at best a marginally significant predictor of group differences in response to phobia-relevant pictures *(16, 17)*. However, consistent with previous behavioral (*5*) and physiological (*4*) findings, the data reported here indicate that phobia-relevant stimuli are distinguished by exacerbated rapidity of activation, not magnitude of activation. Whether this accentuated rapidity of amygdala responding in phobics plays a

causal role in the display of pathological fear and the failure to adequately regulate such fear, should be addressed in future research.

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