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Abstract

1. Context. Considerable work implicates abnormal neural activation and disrupted attention to facial-threat cues in adult anxiety disorders. However, in pediatric anxiety, no research has examined attention modulation of neural response to threat cues.

2. Objective. To determine whether attention modulates amygdala and cortical responses to facial threat cues differentially in adolescents with generalized anxiety disorder (GAD) and healthy adolescents.

3. Design. Case-control study.

4. Setting. Government clinical research institute.

5. Participants. Adolescent volunteers, 15 with GAD and 20 diagnosis-free.

6. Main Outcome Measure(s). Blood oxygenation level-dependent (BOLD) signal, as measured via functional magnetic resonance imaging (fMRI). During fMRI scans, participants completed a face-emotion rating task that systematically manipulated attention.

7. Results. While attending to their own subjective fear, patients, but not controls, showed greater activation to fearful than to happy faces (small volume corrected p's < .05) in a distributed network including the amygdala, ventral PFC (vPFC), and anterior cingulate cortex (ACC). Right amygdala findings appeared particularly strong. Functional connectivity analyses demonstrated positive correlations among the amygdala, vPFC, and cingulate.

8. Conclusions. Findings provide the first evidence in juveniles that GAD-associated patterns of pathological fear-circuit activation are particularly evident during certain attention states. Specifically, fear-circuit hyperactivation occurred in an attention state involving focus on subjectively-experienced fear. These findings underscore the importance of attention and its interaction with emotion in shaping function of the adolescent human fear circuit.

Introduction

Pediatric generalized anxiety disorder (GAD) confers major risk for adult psychopathology.[1] Numerous mechanisms may underlie this developmental association; one possibility is that early neural dysfunction associated with anxiety persists into adulthood. Functional magnetic resonance imaging (fMRI) research in anxious adults indicates dysfunction in a circuit involving peri-amygdala regions, ventral prefrontal cortex (vPFC), and the anterior cingulate cortex (ACC).[2, 3] Identifying comparable patterns in pediatric GAD would provide a critical step toward linking childhood anxiety and adult disorders.

Cognitive perturbations, particularly attention to subjectively experienced threats, are central to the emergence of anxiety.[4-6] fMRI research shows that variations in state anxiety modulate associations between attention and activation in a "fear circuit" encompassing the amygdala, vPFC, and ACC.[7-9] Nevertheless, it remains unclear how this pattern of associations among attention, anxiety, and neural function evolves during development. The primary hypothesis emerging from neuroscience suggests that early developmental disruption of amygdala-PFC circuitry mediates the emergence of attention biases toward threats and subsequent anxiety disorders.[10] If so, then attention to threats should potentiate fear circuit activity differently in youth with and without GAD.

Virtually no research examines associations among pediatric anxiety, attention to threat, and neural activity. The first published fMRI study of threat-cue processing in pediatric anxiety patients found more amygdala activation to fearful vs. neutral faces in patients than controls during a block-design passive face-viewing task.[11] Surprisingly, given data in adults, amygdala activation to fearful faces did not occur in healthy adolescents.[12] Regardless, this study did not constrain attention as recent adult studies have done by examining patterns of activation within

clearly-defined attention states.[13, 14] The only other published fMRI study in pediatric anxiety disorders used an event-related attention-orienting paradigm. This study found no between-group differences in amygdala activity to angry faces, but it did find enhanced right-sided vPFC activation in GAD vs. healthy adolescents.[15] Taken together, these studies implicate the vPFC and amygdala in pediatric anxiety.

Enhanced attention to internal threat is pathognomonic for anxiety disorders.[4, 6] We developed a paradigm that explicitly manipulates attention focus toward and away from internal threat responses.[13] Using behavioral measures, we found evidence of perturbed attention both in adolescents with anxiety disorders and those with parental panic disorder.[5] Specifically, high-risk youth reported more fear and showed slowed response times in response to evocative faces. Moreover, in fMRI work among healthy individuals, we found this paradigm to engage a fear circuit encompassing the amygdala, vPFC, and ACC.[13] The current study uses this paradigm to test the hypothesis that attention modulation of the amygdala-based fear circuit differs between adolescent GAD patients and controls. Specifically, we hypothesize that under conditions where attention is directed towards internal fear states, activation in this circuit to fearful vs. happy faces is greater in GAD than healthy subjects. Moreover, we hypothesize that this pattern of amygdala hyperactivation is associated with parallel increases in PFC activation.

Methods

Subjects. Fifteen medication-free adolescents with DSM-IV anxiety disorder diagnoses participated, 13 meeting full criteria for current GAD on the Kiddie-Schizophrenia-and-Affective-Disorders-Schedule (K-SADS)[16] and two with current GAD where it was unclear whether their anxiety was confined to other anxiety disorders. Comorbidity (see Table 1)

resembled patterns in other samples.[17] Twenty healthy adolescents, matched to patients on age, gender, and IQ, served as controls. Assessment with the K-SADS[16] confirmed that all controls were healthy. Other inclusion criteria for patients comprised: clinically significant anxiety on the Pediatric Anxiety Rating Scale[18] (PARS; score ≥10); significant impairment[19] on the Child Global Assessment Schedule (CGAS<60); and persistent anxiety during 3 weeks of supportive therapy. Exclusion criteria comprised: current Tourette's syndrome, major depressive disorder (MDD), obsessive compulsive disorder (OCD), or conduct disorder; exposure to trauma; suicidal ideation; lifetime history of mania, psychosis, or pervasive developmental disorder; and IQ<70. The study was approved by the NIMH Institutional Review Board and all participants/parents provided written informed consent/assent.

fMRI Task. As described in detail elsewhere, the face-attention paradigm[13] acquired data in four epochs. During three epochs, subjects adopted different attention states, requiring them to make ratings of face stimuli on one of three 5-point scales (ratings ranged from 1=not at all to 5=very): "*How afraid are you*?", "*How hostile is the face*?", "*How wide is the nose*?". During the fourth epoch, subjects passively viewed stimuli. Epochs alternated during repeated viewing of 32 faces (eight stimuli from each of four emotion categories: afraid, happy, neutral, angry) drawn from three widely-used stimulus sets.[21-23] Each of these 32 faces was presented four times through Avotec Silent Vision Glasses (Stuart, FL), once in each of the four epochs/attention states. Order of face presentation and order of attention-state epochs was randomized. Rating and response time for each trial were recorded as behavioral data, using a 5-key MRI-compatible glove device (MRI Devices, Waukesha, WI). The task used a rapid-event-related-mixed/hybrid design, with 32 interspersed "blank" trials, each 4000 msec in duration.

Stimulus presentation occurred during one 160-trial run (14-minute, 42-seconds), with four epochs/rating blocks comprising 10 randomly ordered 4000 msec events (eight face and 2 "null-event" trials). Instructions for epochs/rating blocks were presented for 3000 ms before each epoch. An inter-trial interval varying from 750-1250 ms followed each event.

Procedures. We used T2* imaging (axial plane, 23 slices) on a 3-Tesla GE-scanner (64x64 matrix; TR=2000 ms, TE=40 ms, FOV=240 mm; 3.75 x 3.75 x 5 mm voxels). Images were acquired in 23 contiguous slices parallel to the AC-PC line. High-resolution T1 weighted anatomical images were acquired to aid with spatial normalization (180 1-mm axial slices, FOV=256, NEX=1, TR=11.4 ms, TE=4.4 ms, matrix=256x256, TI=300 ms, bandwidth=130 Hz/pixel, 33 kHz/256 pixels).

fMRI pre-processing. We discarded data from participants (4 patients, 3 controls) moving ≥ 1 voxel in any plane. We conducted analyses with SPM software (SPM99, Wellcome Department of Neurology). We corrected functional data for slice timing and motion, co-registered them to the anatomical data, and spatially normalized and resliced them into isotropic 1 mm voxels. After completing these pre-processing steps, we evaluated the quality of the normalization procedure via visual inspection of fMRI images.

Data Analysis: We estimated event-related response amplitudes at the individual subject level for each event type in each attention set using the General Linear Model (GLM). The waveform for each event-related response was a rectangular pulse (4s duration) convolved with the HRF specified by SPM99. We generated contrast images for each subject using pair-wise comparisons of event-related responses across event types. We then divided each contrast image by the subject-specific voxel time series means, yielding values proportional to percentage fMRI

signal change.[24] Each contrast image was then smoothed with an isotropic Gaussian kernel (FWHM =11.4)..

Beyond our main analysis of between-group differences in task-related activation, we also performed an analysis of functional connectivity.[25] For each subject, functional connectivity was examined between each amygdala region of interest (ROI) and the entire brain. The mean EPI time series over the ROI was extracted, mean corrected, and then normalized (root-mean-square). The resulting time series was entered into a general linear model as the sole regressor of interest. The data for the model comprised the smoothed, spatially normalized whole-brain EPI data. Both high- and low-pass filtering were employed (using a 128 sec cut-off and the SPM-provided canonical HRF, respectively). Regression coefficients, corresponding to the voxel-wise regressor of interest, were entered into a second-level analysis.

For all group-level analyses, we used a random effects model focused on ROIs.[26] Because we entered the study with an *a priori* hypothesis, we defined ROIs *a priori* and used the Gaussian random field threshold (α=.05). To adjust for multiple comparisons, we applied the small volume correction within each region. We defined ROIs to include bilateral amygdala, ventromedial prefrontal cortex (vPFC), and anterior cingulate cortex (ACC) using standard anatomical criteria[27] on a single MNI template. These ROIs were applied to all normalized brains at the group level.

Statistical Tests: Analyses of between-group differences in behavioral data relied on repeated measure ANOVAs. For functional connectivity analyses of fMRI data, we used t-tests generated from a group-level random effects model. These tests examined amygdala connectivity in the entire data set of 35 adolescents, as well as between-group differences in connectivity. For analyses of group differences in task-related fMRI activation, we selected one

key contrast, on an *a priori* basis. This contrast was restricted to the "*How afraid*?" attention state and it compared activation during fear-face, relative to happy-face, viewing (i.e. the "*Afraid-fear vs. Afraid-happy*" contrast). Selection of this contrast was based on two factors.

First, all prior pediatric and adult anxiety fMRI studies have restricted analyses to a single attention set. Our explicit focus on the "*How afraid?*" set was based on our previous behavioral research with this task.[5] Second, we focused specifically on the contrast of fearful vs. happy faces since prior work most consistently demonstrates amygdala activation, as well as anxiety-related between-group differences in amygdala activation, specifically for fear faces. Moreover, research in youth suggests neutral expressions constitute a suboptimal baseline.[11] Happy faces, which constitute a less ambiguous alternative to neutral faces, have been used successfully as comparison stimuli in prior studies of clinical patients.[28, 29]

Tests proceeded in two stages. First, we examined group-level differences for the "*Afraid-fear vs. Afraid-happy*" contrast. Primary random effects analyses with small volume correction (α=.05) focused on the bilateral amygdala; additional analyses examined activation in the vPFC and ACC. Second, for peak activations that surpassed this initial threshold, we conducted a subsequent analysis using data from all attention sets in the face-task to determine whether findings were specific to the "How afraid" rating condition. This analysis subjected subject-level contrast values in locations identified as peaks in the initial analysis (each presented in MNI coordinates) to a series of three-factor ANOVAs to test for group-by-attention-state-by-face-type interactions. These ANOVAs had one two-level between-subject factor (group [GAD vs. healthy]) and two four-level within-subject factors (attention state [Afraid, Hostility, Nose-width, Passive] and face-emotion-type [Fear, Happy, Angry, Neutral]).

Results

Behavior: Behavioral data indicated no between-group differences in ratings, $F(1,33)=1.61$, $p=.21$, or reaction times, $F(1,33)=0.26$, $p=.62$ (see Table 1). For illustrative purposes, we present in Table 1 data from both the fear-rating and nose-rating conditions. Between-group differences were expected in the former condition, where a non-significant trend was evident, $F(1,33)=3.32$, $p=.07$, but not the latter condition, $F(1,33)=.08$, $p=.78$. Patients and controls exhibited comparable patterns of behavioral response across conditions, with highest fear ratings for angry faces (Greenhouse-Geisser corrected $F(1.95,64.42)=43.95$, $p<.001$; p's < .001 for all pairwise comparisons between face types) and highest nose-width-ratings for happy faces (Greenhouse-Geisser corrected $F(2.49,82.26)=15.05$, $p<.001$; p's < .001 for all pairwise comparisons between face types). Of note, given the absence of between-group behavioral differences, interpretation of between-group differences in neural activation cannot be attributed to between-group differences in performance.

Task-Related Amygdala Activation: We tested the hypothesis that the "*Afraid-fear vs. Afraid-happy*" contrast would elicit more activity in patients' than controls' amygdalae. This hypothesis was supported by significant group-by-emotion-type interactions indicating that GAD patients showed significantly greater relative bilateral amygdala activation to fearful vs. happy faces in this attention state than healthy adolescents (see Table 2 and Figure 1).

Follow-up ANOVAs on the BOLD responses in the two identified peak suprathreshold voxels within the right amygdala revealed significant three-way interactions (group x attention state x face emotion) ($p's \leq .05$; see Table 2 and Figure 1). No significant interactions were

evident in peak voxels within the left amygdala. For the right amygdala, post-hoc analyses decomposed the significant three-way interactions.

Figure 1a presents the topography of the peak activations in the right amygdala where the three-way interactions emerged. Post-hoc analyses generated comparable profiles for the two peak voxel activations; for illustrative purposes, Figures 1b and 1c display bar graphs for the post-hoc analyses in one of these activations, examining right amygdala activation in each condition, relative to the null-event baseline. One post-hoc analysis revealed a significant group-by-emotion interaction in the "*How afraid*" attention set, $F(2.36,77.70)=4.05$, $p=.02$. As shown in Figure 1b, group differences in right amygdala activation emerged during fear-face viewing. A similar trend occurred during angry-face viewing, but not during happy or neutral-face viewing.

Another post-hoc analysis showed this group-by-emotion interaction to be restricted to the "*How afraid*" attention state, $F(2.16,71.30)=4.87$, $p=.009$). Figure 1c presents data for a post-hoc analysis examining fear-face viewing across the four attention states. As shown, enhanced right amygdala activation in GAD was evident only in the "*How afraid*" state.

Finally, we found no significant correlations between activation in the right amygdala voxels for the "Afraid-fear vs. Afraid-happy" contrast and severity of anxiety, as rated on the PARS (p's $> .05$).

Task-Related PFC Activation: In secondary analyses, we tested the hypothesis that the "*Afraid-fear vs. Afraid-happy*" contrast also would elicit more activity in patients' than controls' vPFC and ACC regions. Results indicated that GAD patients showed significantly greater activation in voxels within the right vPFC and ACC to fearful vs. happy faces than did healthy adolescents (see Table 2). Subsequent three-way repeated measures ANOVAs for each

significant voxel evaluated the degree to which these between-group differences occurred specifically during the "*Afraid-fear vs. Afraid-Happy*" contrast.

These ANOVAs revealed a significant 3-way interaction in the ACC (see Table 2, Figures 2a and 2b). As shown, ACC-related, between-group differences in activation showed parallels with those observed in the right amygdala in that they were strongest in the "*How Afraid*?" attention state, $F(3,99)=2.57$, $p=.06$ (see figure 2c). Similarly, a significant 3-way interaction was apparent in the right vPFC (see Table 2). As it did for the amygdala and ACC, the magnitude of the between-group difference in vPFC activation differed markedly between the "*How Afraid*?" and *Passive-Viewing* attention states (compare Figures 1c and 3c). Parallels also emerged between the amygdala and vPFC in responses to angry faces (compare Figures 1b and 3b).

Functional connectivity analyses: These analyses focus on the right amygdala, given stronger evidence of task-specific group differences for this structure, relative to the left amygdala. Results examining patterns of functional connectivity in the entire sample of 35 subjects revealed a strongly positive correlation between activity in the right amygdala and right vPFC, with no significant negative correlations in ROIs (see Table 3). These findings suggest that performance of the task paradigm is associated with strong functional connectivity within a distributed amygdala-vPFC network. Group differences in connectivity between the right amygdala and vPFC, however, were not evident.

Given that this is the first study to examine amygdala connectivity in adolescents, we also present results for between-group comparisons at locations beyond our ROIs. Table 3 presents results using a $p<.001$ uncorrected threshold with an extent threshold of 10 voxels. This analysis revealed multiple areas of positive and negative correlation. A between-group difference

emerged in functional connectivity between the insula and right amygdala (38, 12, -16; t = 4.81, p<.001, uncorrected), with greater connectivity evident for patients than for healthy subjects (see Figure 4). Degree of connectivity appeared to relate to severity of anxiety; within the patient group, magnitude of connectivity correlated significantly and negatively with total PARS score, r=-.51, p=.05. Moreover, a series of between-group differences also emerged within posterior cortical regions (all p's<.001, uncorrected), mostly within the cingulate gyrus but also the precuneus and lingual gyrus. These differences reflected stronger negative correlations with right amygdala activity in patients than controls. No further between-group differences were evident using either the SVC-focused ROI analyses with α=.05 or the exploratory approach.

Comment

Two main fMRI findings emerged from this study. First, group differences in right amygdala activation varied with participants' attentional focus. GAD patients exhibited greater activation than healthy subjects during fearful- versus happy-face viewing when attending to subjective fear. Second, between-group differences in amygdala response emerged against a backdrop of strong co-activation in a distributed "fear circuit" for the sample as a whole. Functional connectivity analyses demonstrate strong relationships between changes in amygdala activity and activity throughout a ventrally and medially distributed circuit. Moreover, analyses of task-related changes in PFC demonstrated between-group differences in the ACC and vPFC that paralleled those found in right amygdala. Notably, between-group differences occurred only when participants' attention focused on subjective fear.

In addition to these fMRI results, we found that rating behavior varied as a function of attention-task demands and stimulus features, as in prior studies.[5, 13] Rating and reaction-time

patterns were identical to those observed previously. No difference was evident, however, between GAD patients and healthy adolescents, despite between-group differences in neural activation. Nevertheless, while statistically non-significant, the patterns in the current study resembled those observed previously,[5] with higher ratings and slower reaction times during the fear-attention condition in GAD patients than in healthy adolescents.

Controversy surrounds the interpretation of between-group differences in fMRI activation as they relate to between-group differences in task performance. Some view group differences in task performance as experimental confounds.[30] From this perspective, the absence of group differences in task performance facilitates meaningful interpretations of observed group differences in neural responses. In particular, differences in activation cannot be dismissed as artifacts of differential compliance with or capacity to perform the behavioral task. Thus, matched task performance represents a potential strength. Other researchers, however, view differences in task performance as necessary for interpreting differences in activation.[31] Clearly, both positions make cogent arguments that bear careful consideration.

It is important to emphasize that one prior behavioral study in adolescents, using the task employed in the current imaging study, found associations between anxiety diagnosis and task performance.[5] However, this study differed in several ways from the current one, including population sampled, sample size, and settin. Thus, our failure in the current, smaller fMRI study to detect statistically significant behavioral differences is not entirely surprising. Indeed, it is consistent with the possibility that fMRI activation more sensitively indexes anxiety-related disruptions in amygdala function than does behavioral perturbation.[32, 33]

As noted above, a prior fMRI study[11] also found greater amygdala activation during fearful face viewing in youth with anxiety disorders, relative to healthy comparisons. The

authors suggested that fearful faces evoke amygdala responses because they are novel and imply that a threat is emanating from an ambiguous source; this interpretation is consistent with some findings in adults.[34, 35] However, since this previous study relied on a block design and collected no behavioral data during scanning, it left open questions about factors that may contribute to amygdala hyperactivity. By constraining attention, the present study adds an element that may help more clearly elucidate cognitive mechanisms contributing to amygdala hyperactivity. Specifically, right amygdala hyperactivity to fearful faces in pediatric GAD occurred when attention was directed to personally relevant, emotionally salient aspects of a stimulus but not in other attention states.

As illustrated in the bar graphs in Figure 1, we also found evidence of negative BOLD signal responses in the amygdala when participants viewed certain face types in varying attention states. Controversy persists concerning interpretations of such negative BOLD values; although some recent evidence suggests that they indicate reductions in neural activity,[36] this perspective is not universally accepted. Similar negative BOLD values have been obtained in studies of face processing examining amygdala activation in adults, particularly when participants focused their attention selectively,[37] and in healthy youth during passive fearful-face viewing.[11] In light of these findings, it is possible that the deactivation evident in Figures 1b-1e partially reflects the influence of attention state on amygdala functioning. Other interpretations, however, are plausible; consequently this issue warrants further study.

In contrast to the paucity of fMRI studies in adolescents, considerable research examines amygdala response to evocative faces in adult psychopathology. This work provides relatively consistent evidence of enhanced amygdala reactivity to fearful and other negatively-valenced facial expressions in a range of mood and anxiety disorders. Increased amygdala response is

found consistently in adults with MDD, post-traumatic stress disorder (PTSD), and social anxiety disorder.[2, 38, 39] Interestingly, enhanced response to fearful faces does not occur in OCD, consistent with nosological distinctions between OCD and other anxiety disorders.[40]

Substantive questions remain, however, concerning the specificity of relationships among amygdala activity, face processing, and diagnosis. Findings in adults show between-group differences in amygdala activity for both fearful and angry faces; some evidence of hyper-activation to angry faces also emerged in the current study. Further, as of this writing, no study in adults has directly compared neural activation during evocative-face viewing among healthy subjects and groups of patients with varied mood or anxiety disorders, nor has any study of anxious adults carefully controlled attention. Such research is needed to elucidate whether specific disorders show distinctive patterns of neural responsivity to emotionally salient cues.

Although adults with acute disorders consistently show amygdala hyperactivity, it remains uncertain how elevated activity relates to pathophysiology. For example, amygdala hyperactivity may represent a correlate of anxious states, a complication of chronic psychopathology, or a risk factor. Treatment has been shown to modulate amygdala hyperactivity, consistent with the possibility of state effects.[28, 38, 41] Other studies, however, support trait-marker hypotheses. For example, a polymorphic variant of the serotonin transporter associated with risk for MDD predicts enhanced amygdala activation to evocative faces in asymptomatic, high-risk individuals.[42] For the current sample, the lack of a correlation between PARS rating and amygdala activation could suggest trait effects. Finally, prior data in another study using this task demonstrate an association between parental panic disorder and behavioral response to evocative faces in the “How afraid” attention state.[5] These data also suggest that trait effects influence response to evocative face-viewing in some attention states.

Studies in adults implicate ventral and medial PFC regions, as well as the amygdala, in various forms of psychopathology. Evidence is perhaps strongest in adult MDD, where lesion studies, brain imaging data, and post-mortem investigations document abnormalities in relatively broad expanses of the PFC, particularly ventral and medial components.[43] Moreover, functional connectivity analyses suggest that amygdala abnormalities in MDD reflect dysfunction in a neural circuit encompassing these PFC regions.[44, 45] Less evidence implicates the PFC in anxiety disorders. However, consistent with signs of enhanced vPFC/ACC activation in anxious participants in the current study, we detected, in another study, enhanced right vPFC activation in adolescent GAD.[15] Thus, these two studies document consistent evidence of enhanced vPFC activity in pediatric GAD.

The current results for the PFC reveal both consistencies and inconsistencies with data in adults. As in studies of adults, our analyses detected a vPFC region where strong positive amygdala connectivity emerged.[25, 44, 45] However, we failed to detect negative connectivity between more dorsal PFC-based regions and the amygdala, although we did detect relatively strong negative connectivity in GAD patients with many posterior cortical regions. The different topography found in the current study, relative to studies in adults, may relate to differences in task methods or to functional developmental changes within amygdala-PFC circuitry.

As noted above, we also found some evidence of task-related between-group differences in cortical neural response and connectivity. Specifically, as in the right amygdala, between-group differences restricted to the "*How afraid*?" condition emerged in the ACC and vPFC. Given the known role of both PFC sub-regions in modulating attention in varying contexts,[46] one interpretation of these results is that attention-related between-group differences in amygdala response to fear faces might be "gated" by differential PFC modulation of the amygdala.

Alternatively, the co-activation of amygdalar and prefrontal cortical regions could indicate disruption of reciprocal projections between the two regions rather than a modulatory impairment.

Moreover, connectivity analyses suggested the presence of greater coupling in GAD patients between the amygdala and insula. These findings, too, implicate perturbations in PFC-amygdala circuitry in pediatric GAD. We did not include the insula as an ROI for a priori analyses because of the paucity of research on this structure in pediatric anxiety patients. However, some evidence in adults indicates that the insula may participate in evaluating rewarding or punitive properties of stimuli and feeling emotions.[47, 48] Further, one recent study in healthy adults showed that anxiolytic medication decreased activation to negative faces in bilateral amygdala and insula.[41] Consequently, findings of connectivity between these two regions are of interest. For other PFC regions, data documenting strong amygdala-PFC connectivity suggest that the observed between-group differences in amygdala response emerge in a task inducing strong functional coupling between the amygdala and ventral expanses of the PFC known to exhibit rich anatomical interconnections.

Our finding of a negative correlation between PARS scores and connectivity in the insula/amygdala is broadly consistent with findings from Pezawas and colleagues, who found reductions in positive connectivity between the ventral medial PFC and the amygdala in adults with an s-allelle of the 5HTTLPR gene. Moreover, Pezawas and colleagues also found an inverse correlation between the magnitude of positive connectivity and the amount of temperamental anxiety, consistent with the inverse correlation we found with PARS scores. Both sets of findings suggest that individual differences in anxiety reflect perturbations in expected positive coupling between ventral PFC and the amygdala.

The current findings are tempered by several limitations. First, results are based on relatively small samples. However, small sample size typically contributes to Type II rather than Type I error, thus potentially obscuring true positive effects. Given that we obtained positive findings, Type II error is less of a concern. Second, aspects of our sample limit generalizability. While we excluded patients with histories of MDD or trauma, most GAD patients exhibited comorbidity, as is typical.[49] Indeed, a recent multi-site study found only five patients among a total of 128 with anxiety disorders that presented with a "pure" form of GAD.[20] For the current study, we chose to focus on GAD, rather than other anxiety disorders, because longitudinal data indicate particularly strong associations between GAD and a range of adult conditions.[1] Future research might replicate this study, comparing results across groups with social phobia or separation anxiety disorder and GAD. Like the recent multi-site treatment study, we only enrolled patients who exhibited persistent GAD across a three-week period of supportive psychotherapy. This procedure has the advantage of limiting participants to adolescents exhibiting relatively severe and persistent anxiety, comparable to those in the community who require treatment. However, these subjects may not be representative of other cases of GAD in the community that may be milder or more transient.

Third, our cognitive task is limited on several fronts. In particular, one component of this task requires subjects to attend to their own feelings of fear. This manipulation is designed to engage psychological processes central to clinical disorders. While subjective report is important for both clinically focused and neuroscientific research,[50] methods that rely on introspection are subject to biases related to expectancy or subject demands. Currently, no "gold standard" definitively indexes how closely subjective ratings of fear relate to actual experience of fear.

However, considerable data support the validity of the procedures in the current paradigm. Prior studies show that this attention manipulation differentiates adolescents with acute anxiety or at risk for anxiety from unaffected and low-risk adolescents.[5] The current data add to existing findings by demonstrating an anxiety-related association between elevated amygdala activity and attention to one's own levels of fear. While our data suggest that our introspective attention manipulation engaged neural processes that reliably relate to clinical anxiety disorders, further study will be needed to more precisely specify the cognitive processes that occur during this type of introspection and their specific neural correlates.

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Figure Caption

Figure 1: Figure 1a illustrates the significantly greater activation in patients than controls ($p < 0.05$, small volume corrected) during the "how afraid are you" attention state for fearful vs. happy faces in the right amygdala (MNI coordinates 30 -6 -10). Figures 1b and 1c display bar graphs of activation in this voxel relative to the task null-event baseline for the post-hoc analyses in the same activation. Figure 1b illustrates the group-by-emotion interaction in the "*How afraid*" attention set, in which patients showed greater relative activation than controls to fearful faces. Figure 1c provides an illustration of the group-by-attention set activation for fearful faces and shows that the enhanced right amygdala activation in GAD was limited to the "*How afraid*" attention set. As figures 1d and 1e show, this pattern contrasts markedly with activation observed in the left amygdala, where although 3-way interactions were non-significant (Table 2), greater activation was evident in patients to angry, but not fearful, faces in the "*How afraid*" attention set. Fearful faces evoked greater activation in patients than controls, however, in the "*How hostile*" attention set.

Figure 2: GAD patients had significantly more activation than controls ($p < 0.05$, small volume corrected) during the "how afraid are you" attention state for fearful vs. happy faces within the cingulate (ACC) (MNI coordinates 4 4 44). In Figure 2b, a bar graph of activation relative to the task null-event baseline for post-hoc analyses in this voxel shows that patients showed greater relative activation than controls to fearful faces in the group-by-emotion interaction in the "*How afraid*" attention set. Figure 2c demonstrates that enhanced ACC activation in GAD emerged only in the "*How afraid*" attention set.

Figure 3. Significantly greater activation emerged in GAD patients than controls ($p < 0.05$, small volume corrected) during the "how afraid are you" attention state for fearful vs. happy faces in

the right ventrolateral prefrontal cortex (vPFC) (MNI coordinates 36 36 -6). The bar graphs in figures 3b and 3c show that enhanced activation in GAD was limited to fearful and angry faces in the "*How Afraid*" attention set.

Figure 4. Patients showed significantly greater connectivity than controls between activation in the insula (MNI coordinates 38 12 -16) and the right amygdala ROI, $p < .001$, as illustrated in figures 4a and 4b. As shown in Figure 4c, total score on the Pediatric Anxiety Rating Scale (PARS) correlated significantly and negatively (Spearman's $r=-.51$, $p=.05$) with magnitude of connectivity (presented in arbitrary units on the Y axis) between the right amygdala ROI and the insula (MNI coordinates 38 12 -16).

Table 1. Demographic and clinical characteristics of Generalized Anxiety Disorder patients and healthy controls and task ratings by group.

Measure	Patients (n=15) M(SD)	Controls (n=20) M(SD)
Age	11.67 (1.97)	12.19 (2.1)
IQ	112.5 (14.6)	115.6 (14.13)
Gender	8 male (53%)	9 male (45%)
DSM-IV Diagnoses (current)		
Generalized Anxiety Disorder	13 (87%)	0
Generalized Anxiety Disorder (probable)	2 (13%)	0
Comorbid Diagnoses (current)		
Separation Anxiety Disorder	5 (33%)	0
Social Phobia	6 (40%)	0
Specific Phobia	3 (20%)	0
ADHD	3 (20%)	0
Other Disorder	5 (33%)	0
Pediatric Anxiety Rating Scale (PARS)		
Number of anxiety symptoms	4.93 (.26)	--
Frequency of anxiety symptoms	3.53 (1.25)	--
Severity of anxiety symptoms	3.27 (.59)	--
Clinical Global Impressions Scale–severity at Week 0	4.20 (.78)	--

Behavioral ratings		
How afraid—neutral	1.95 (.76)	1.38 (.62)
How afraid—fearful	2.29 (1.04)	2.06 (.99)
How afraid—angry	3.03 (1.09)	2.44 (1.13)
How afraid—happy	1.54 (.61)	1.15 (.21)
Nose width—neutral	2.24 (.44)	2.22 (.55)
Nose width—fearful	2.30 (.52)	2.18 (.47)
Nose width—angry	2.77 (.71)	2.67 (.57)
Nose width—happy	2.52 (.52)	2.59 (.45)
Reaction times (in milliseconds)		
How afraid—neutral	1863.06 (520.37)	1791.07 (361.56)
How afraid—fearful	2012.61 (600.42)	1830.28 (331.78)
How afraid—angry	2187.24 (555.85)	2006.90 (440.44)
How afraid—happy	1831.81 (490.61)	1602.93 (359.40)
Nose width—neutral	2095.34 (342.67)	1915.35 (309.19)
Nose width—fearful	2049.32 (281.63)	2042.79 (387.21)
Nose width—angry	2118.70 (368.87)	2111.97 (411.11)
Nose width—happy	1977.96 (351.32)	2078.40 (487.60)

Table 2. Voxels (MNI coordinates) with significant emotion by group interactions within the "How afraid are you?" attention state and/or significant post hoc attention state by emotion by group interactions. All voxel-wise *t* values are significant at $\alpha = 0.05$ corrected for multiple comparisons within each region. Results of post hoc three way ANOVAs are Greenhouse Geisser corrected.

Primary Analysis: "How Afraid" attention state (emotion x group)								Post hoc Analysis		
Contrast										
How afraid (Fear) vs. How afraid (Happy)	x	y	z	t_{32}	p	Brodmann Areas	Region	df	F	p
	18	-2	-16	3.72	.005	28	Right Amygdala	2.43, 80.38	3.54	.03
	30	-6	-10	3.37	.01			3.80, 125.44	2.75	.03
	-12	-6	-10	3.95	.003		Left Amygdala	4.47, 147.40	.57	.70
	-8	-4	-14	3.74	.004			3.31, 109.11	1.73	.08
	-16	-8	-8	3.65	.005			1.71, 54.70	1.14	.32

38	32	-2	3.53	.021	47	Ventral Prefrontal Cortex (vPFC)	4.13, 136.19	1.93	.10
36	36	-6	3.13	.047	47		4.02, 132.79	3.30	.01
8	4	34	4.41	.004	24	Anterior Cingulate (ACC)	4.07, 134.18	1.85	.12
4	50	16	3.29	.049	10		4.62, 152.30	.77	.56
4	4	44	3.53	.034	32		3.05, 100.57	3.05	.03
2	4	48	3.48	.038	32		4.28, 141.28	1.76	.14

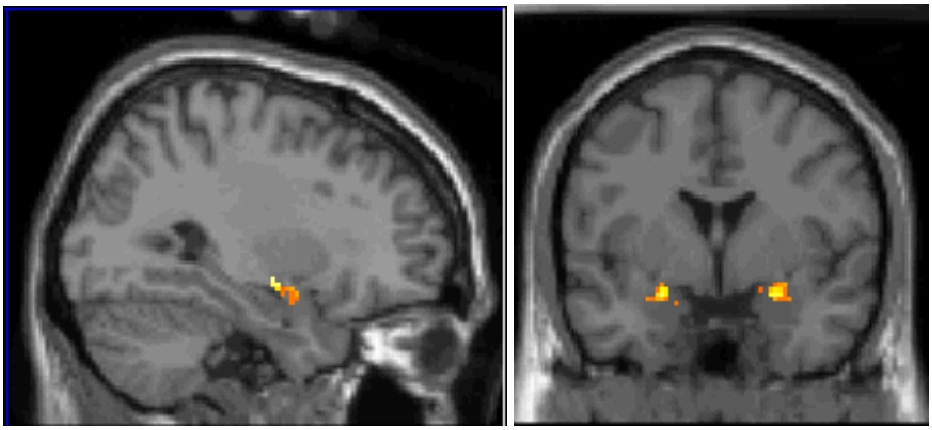
Table 3. Right Amygdala Connectivity. Voxels (MNI coordinates) with significant associations with the right amygdala.

Contrast	x	y	z	t	p	Region	Brodmann Areas	K
Connectivity in all participants, independent of group status								
	40	22	-10	4.25	0.028 (SVC[1])	Right vPFC[2]	47	40
Greater Positive Connectivity in Patients vs. Controls								
	38	12	-16	4.81	($p < .001$ uncorrected)	Insula	13	17
Greater Negative Connectivity in Patients vs. Controls								
	10	-58	18	4.81	($p < .001$ uncorrected)	Posterior Cingulate Cortex (PCC)	23	140
	12	-54	26	4.37		PCC	31	140
	-2	-56	28	3.66		PCC	31	140
	-16	-38	36	4.77		PCC	31	38
	-6	-56	12	4.09		PCC	23	34
	-8	-50	38	3.69		Left Precuneus	31	12
	32	-58	-4	4.38		Right Lingual Gyrus	19	20

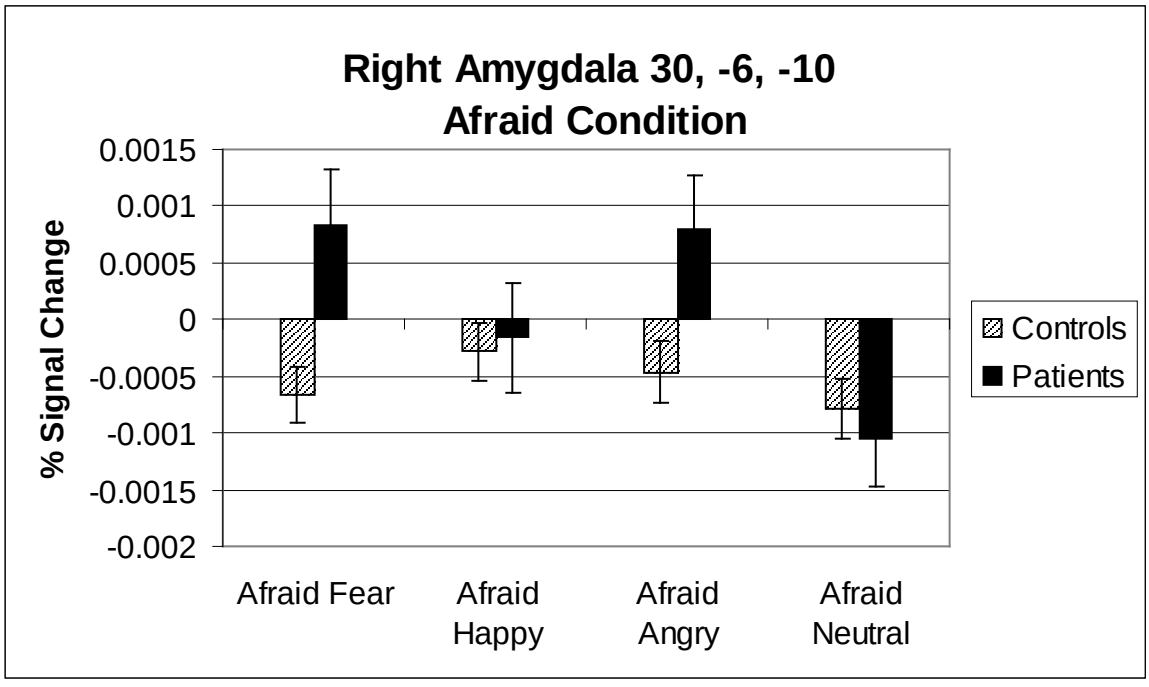
[1]Small volume corrected; [2]Ventral prefrontal cortex.

Figure 1.

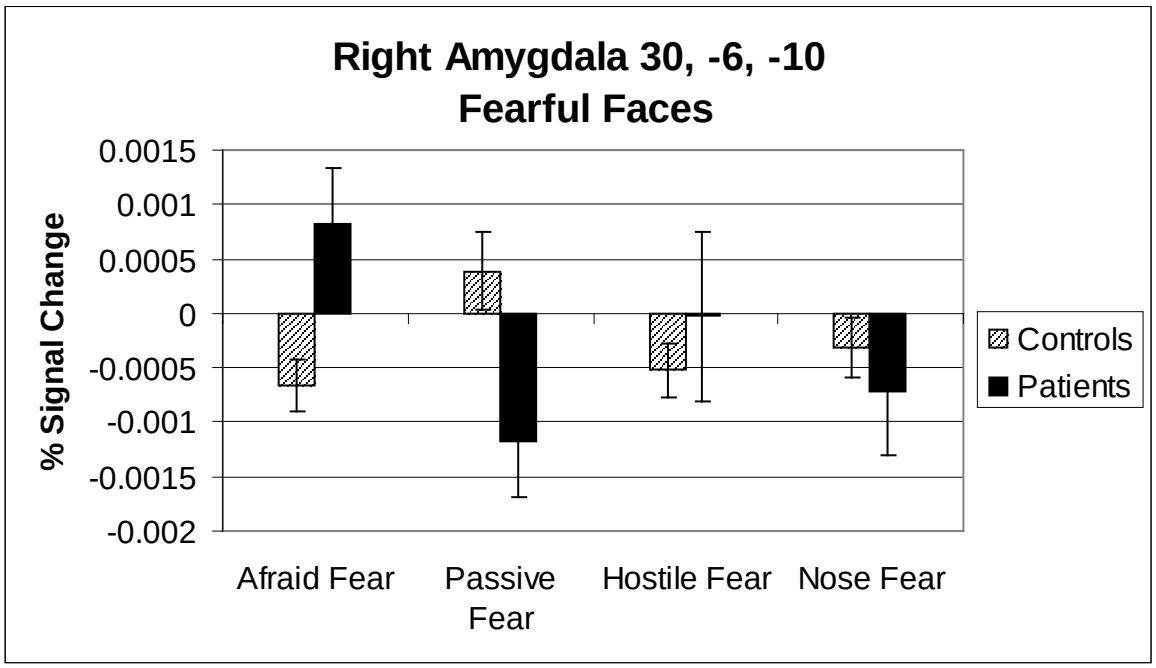
1a.



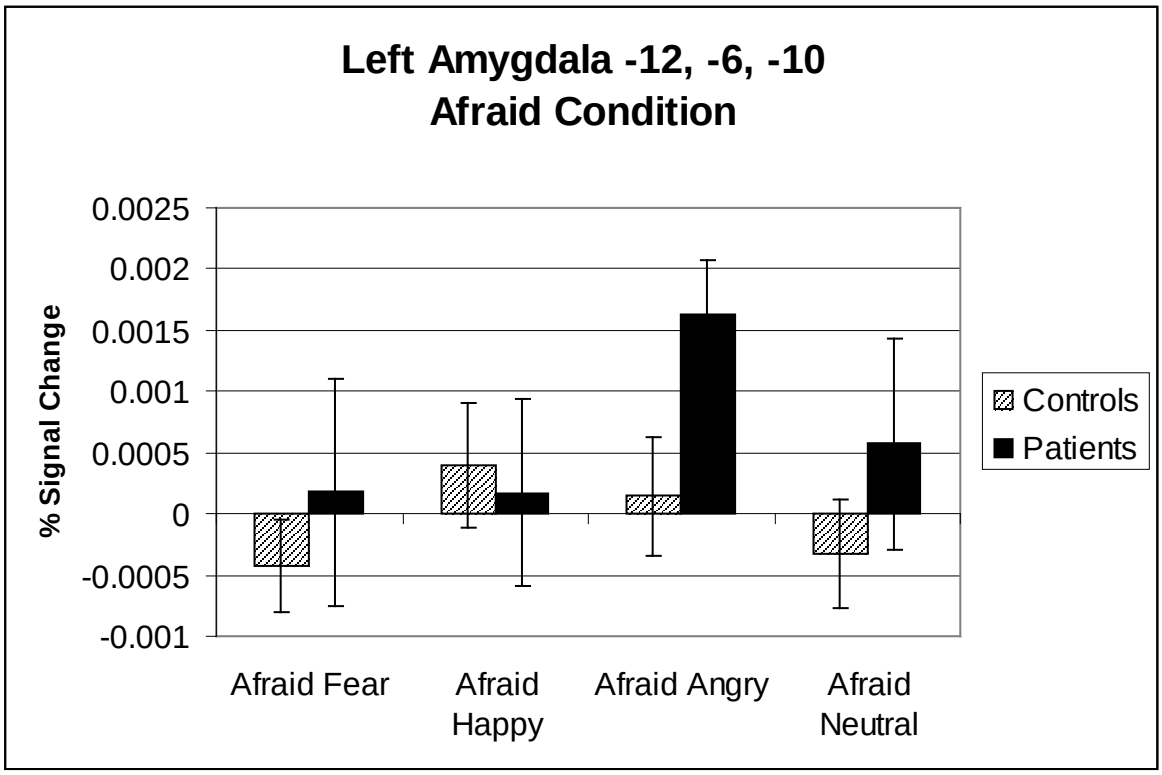
1b.



1c.


Right Amygdala 30, -6, -10
Fearful Faces
% Signal Change
0.0015
0.001
0.0005
0
-0.0005
-0.001
-0.0015
-0.002
Afraid Fear
Passive Fear
Hostile Fear
Nose Fear
Controls
Patients

1d.


Left Amygdala -12, -6, -10
Afraid Condition
% Signal Change
0.0025
0.002
0.0015
0.001
0.0005
0
-0.0005
-0.001
Afraid Fear
Afraid Happy
Afraid Angry
Afraid Neutral
Controls
Patients

1e.

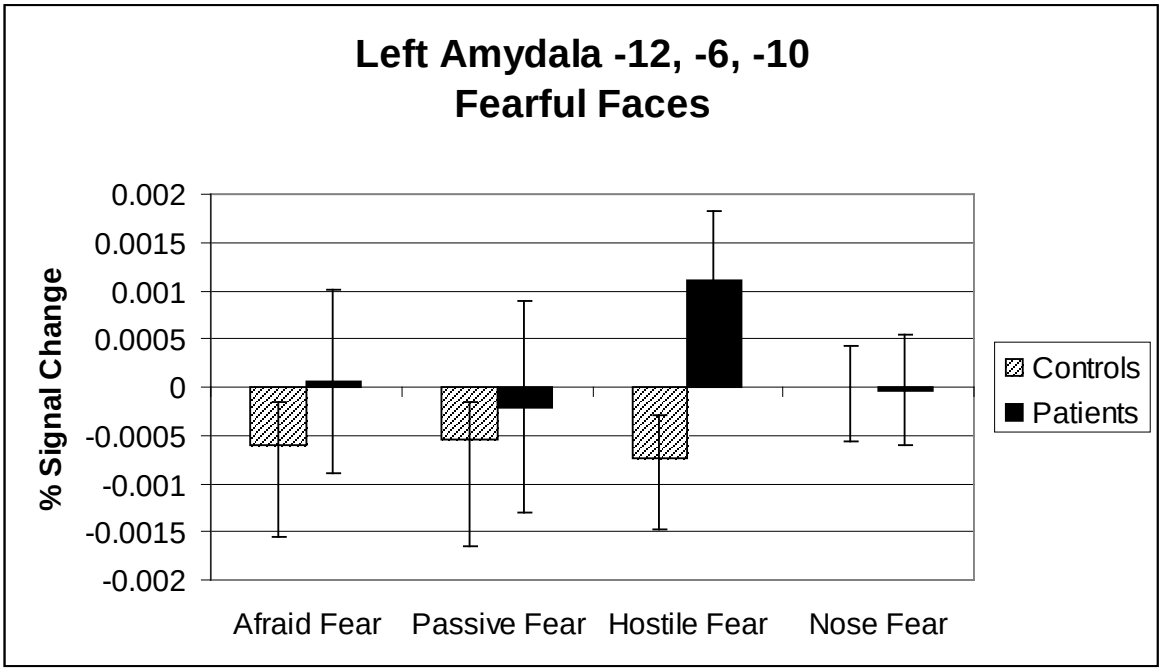
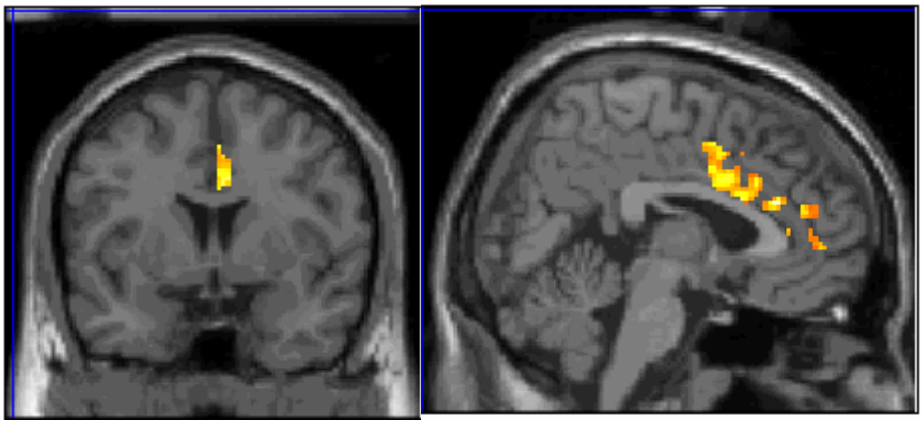
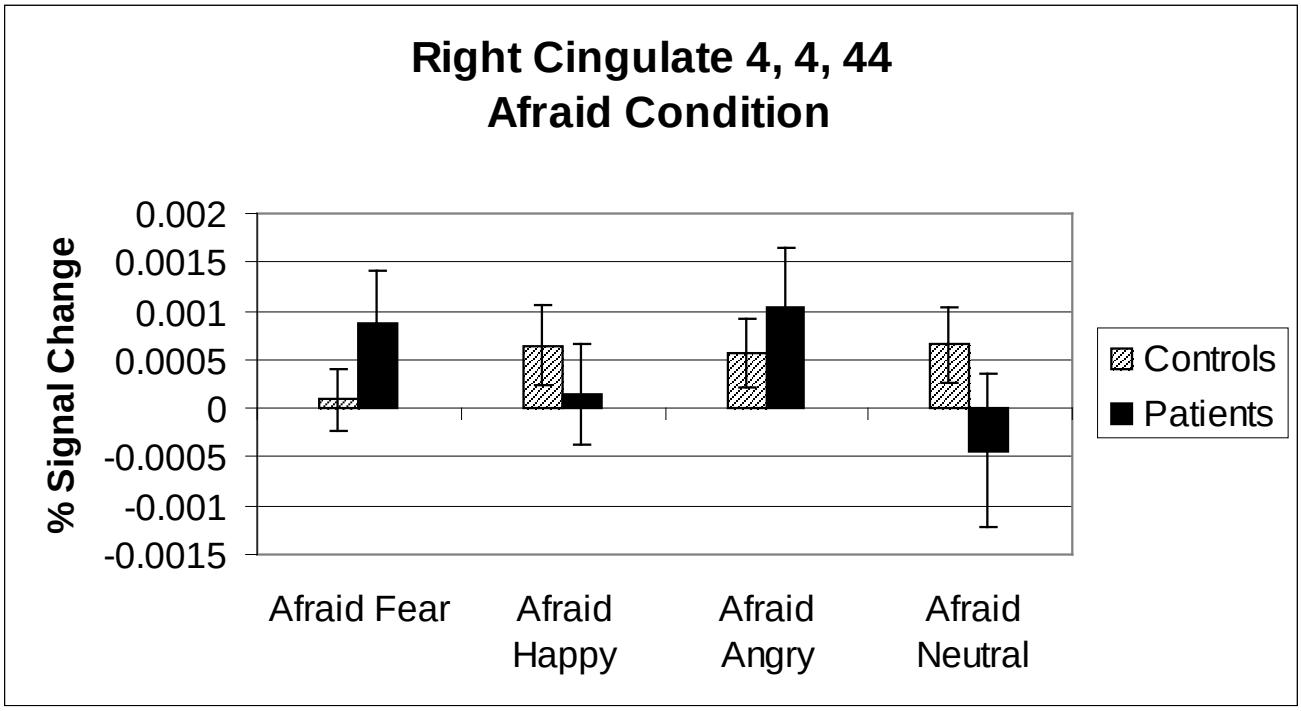

Left Amydala -12, -6, -10
Fearful Faces
% Signal Change
0.002
0.0015
0.001
0.0005
0
-0.0005
-0.001
-0.0015
-0.002
Afraid Fear
Passive Fear
Hostile Fear
Nose Fear
Controls
Patients

Figure 2.
2a.



2b.



2c.

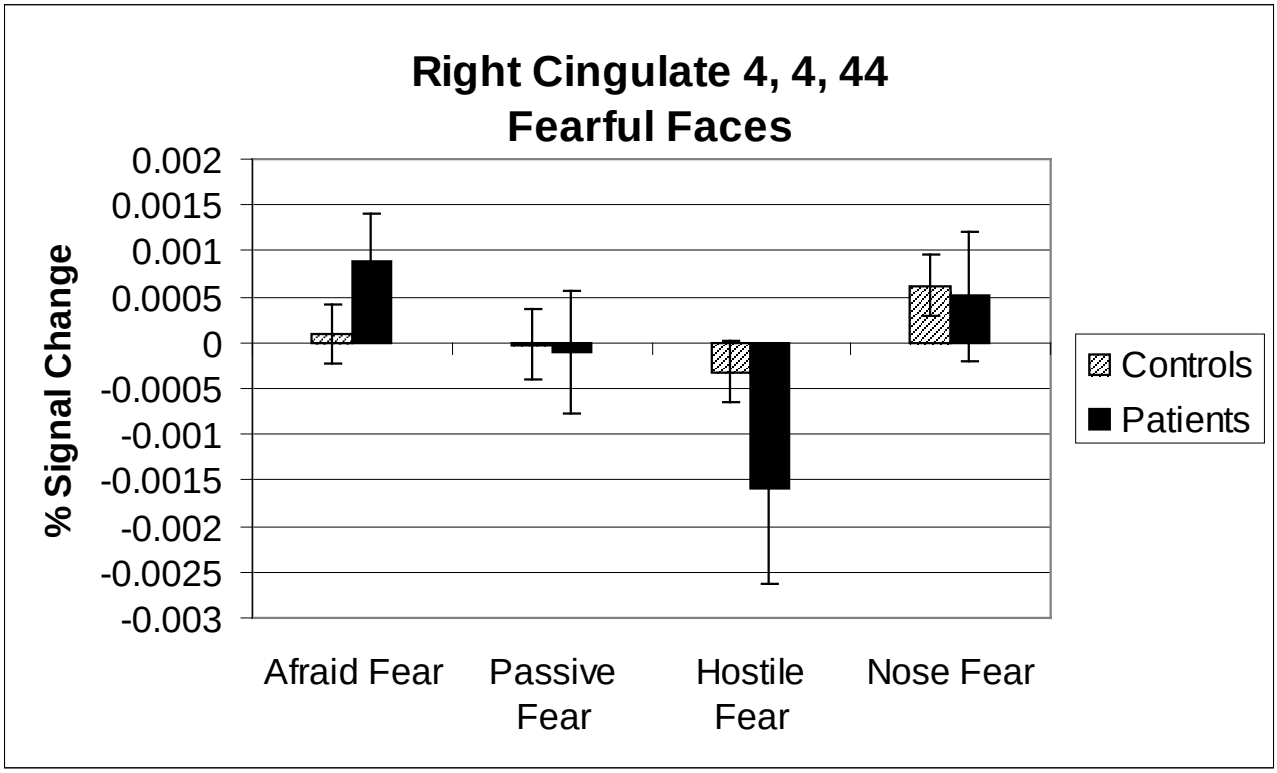
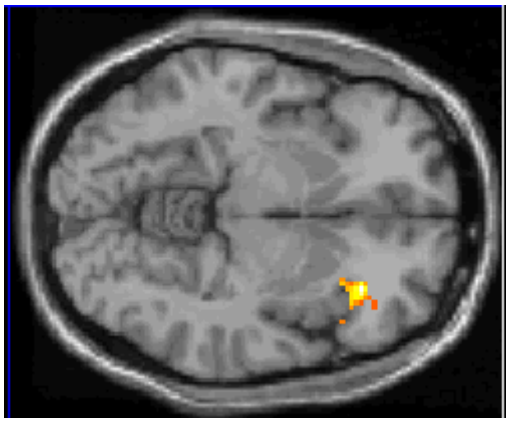
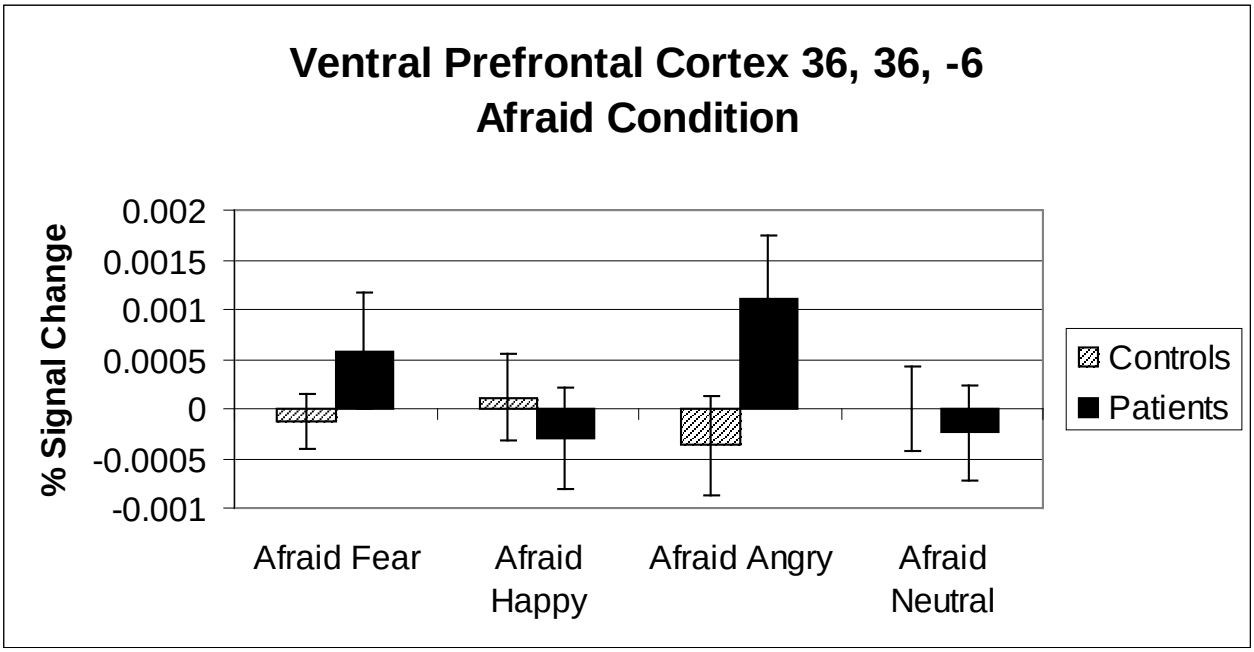

Right Cingulate 4, 4, 44
Fearful Faces
% Signal Change
0.002
0.0015
0.001
0.0005
0
-0.0005
-0.001
-0.0015
-0.002
-0.0025
-0.003
Afraid Fear
Passive Fear
Hostile Fear
Nose Fear
Controls
Patients

Figure 3.

3a.



3b.



3c.

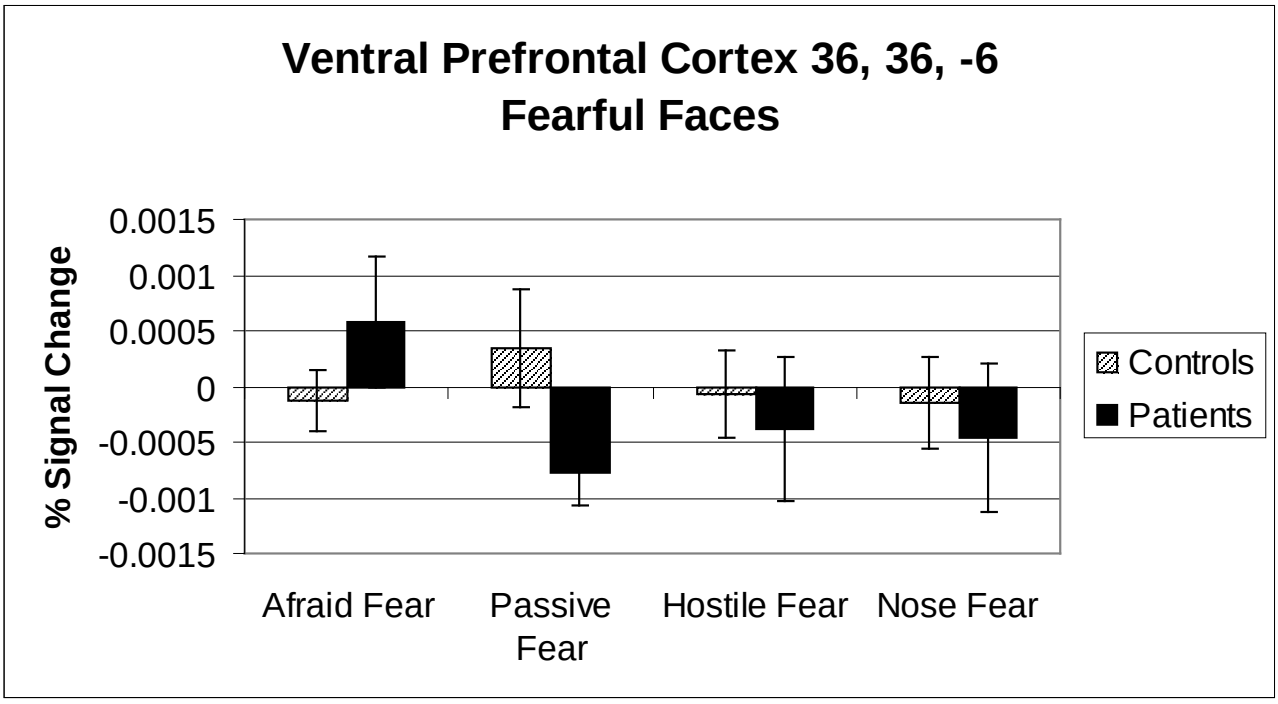
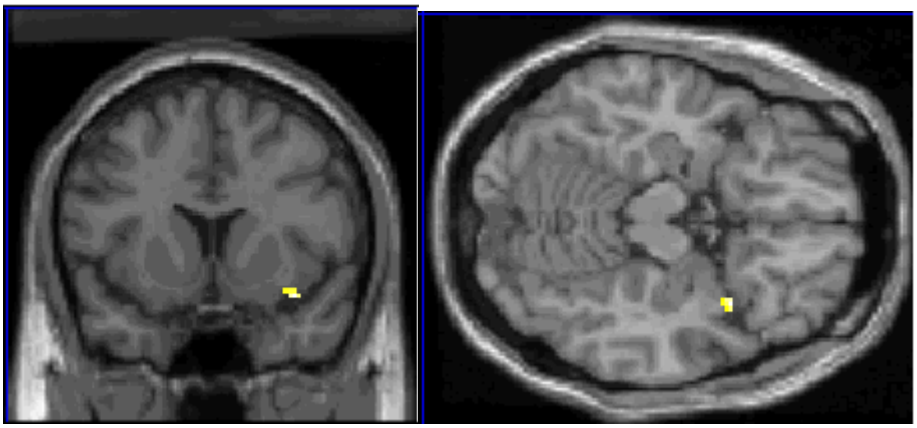

Ventral Prefrontal Cortex 36, 36, -6
Fearful Faces
% Signal Change
0.0015
0.001
0.0005
0
-0.0005
-0.001
-0.0015
Afraid Fear
Passive Fear
Hostile Fear
Nose Fear
Controls
Patients

Figure 4.

4a.



4b.

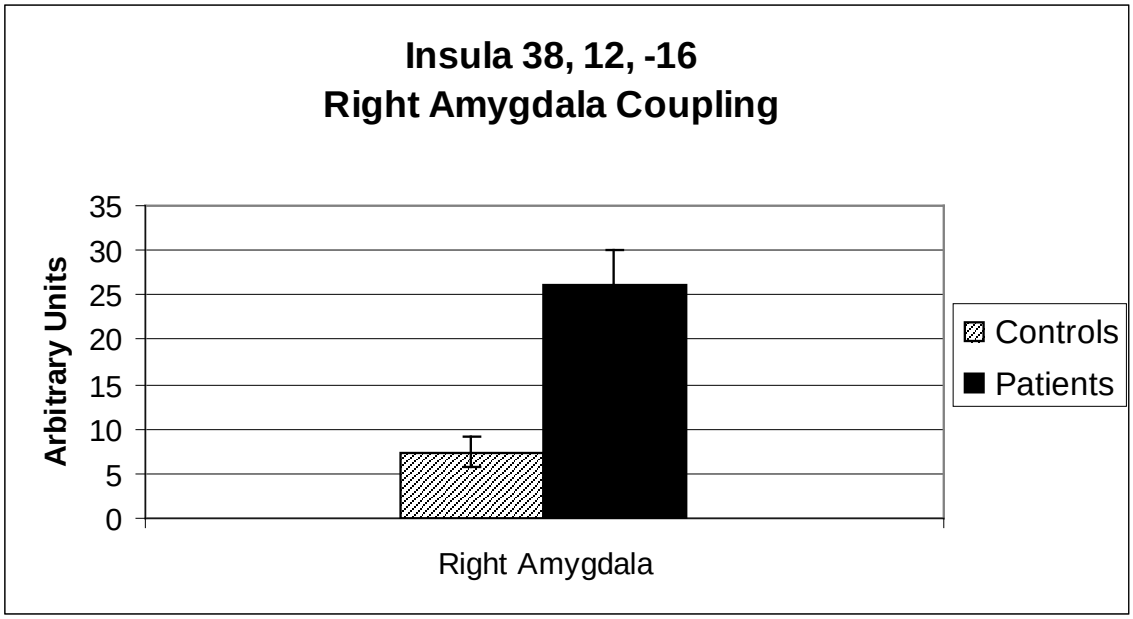
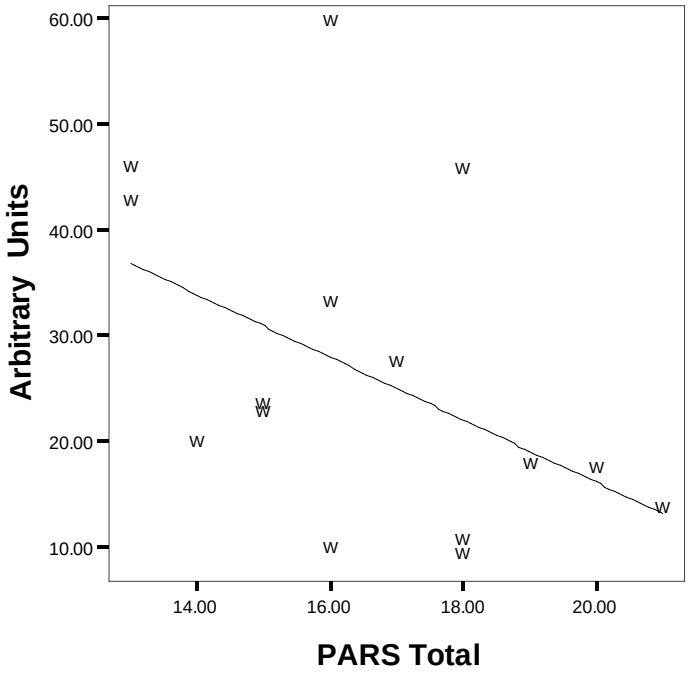


Figure 4c.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Arbitrary Units
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