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Common and specific amygdala-function perturbations in 2 depressed versus anxious adolescents

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ABSTRACT

Context: Few studies directly compare amygdala function in depressive and anxiety disorders. Data from longitudinal research emphasize the need for such studies in adolescents.

Objective: To compare amygdala response to varying attention and emotion conditions among adolescents with Major Depressive Disorder (MDD) or anxiety disorders, relative to adolescents with no psychopathology.

Design: Case-Control-Study.

Setting: Government Clinical Research Institute.

Participants: Eighty-seven adolescents matched on age, gender, intelligence, and social class: 26 with Major Depressive Disorder (MDD; 14 with and 12 without anxiety disorders), 16 with anxiety disorders but no depression, and 45 with no psychopathology.

Main Outcome Measures: Blood oxygenated level dependent signal in the amygdala, measured using event-related functional magnetic resonance imaging. During imaging, participants viewed facial expressions (neutral, fearful, angry, happy) while attention was constrained (afraid, hostility, nose width ratings) or unconstrained (passive-viewing).

Results: Left and right amygdala activation differed as a function of diagnosis, facial expression, and attention-condition both when comorbid MDD/anxiety patients were included and excluded (group-by-emotion-by-attention interactions: p-values$\leq$.03). Focusing on fearful-face-viewing events, anxiety and MDD patients both differed in amygdala responses from healthy participants and from each other during passive-viewing. However, both MDD and anxiety patients, relative to healthy participants, exhibited similar signs of amygdala hyper-activation to fearful faces when rating subjectively experienced fear.

Conclusions: Adolescent MDD and anxiety disorders exhibit common and distinct functional neural correlates during face processing. Attention modulates the degree to which common or distinct amygdala perturbations manifest in these patient groups, relative to healthy peers.

INTRODUCTION

Rates of anxiety and depression markedly increase in adolescence.[1,2] Comorbidity data[3-10] suggest that these conditions may share brain-based diatheses.[11-13] However, non-comorbid cases of anxiety and depression[2,10,14] raise questions about neural differences. In adults, biased amygdala engagement occurs in major depressive disorder (MDD)[15-18] and anxiety disorders.[19-24] For both conditions, increased amygdala activation has been reliably seen, suggesting shared neural-circuitry dysfunction. However, strong conclusions cannot be drawn, since few studies directly contrast patient groups with each other and with healthy individuals.

Vital questions emerge on commonalities and distinctions between adolescent MDD and anxiety disorders. Work is important in this age group, since most adult mood and anxiety disorders are preceded by adolescent disorders.[5,6] Similar functional perturbations could present in adolescent and adult mood and anxiety disorders; alternatively, unique perturbations could present in adolescence that ultimately evolve into adult profiles. Studies of adolescents begin to consider these possibilities by charting early-emerging correlates of mood and anxiety disorders. Since anxiety disorders differ from MDD in several ways,[1,2,10,25,26] specific neural correlates may be expected. Nevertheless, few neuroimaging studies compare adequately-sized samples of MDD and anxiety-disorder patients at any age, and studies in adolescents appear especially rare. As in adults, initial findings in anxious adolescents[27-30] and in individuals at risk for anxiety disorders[31] show altered amygdala function relative to healthy subjects, with signs of enhanced activation to fear-faces.[27,28,31]

To our knowledge, only two studies examined amygdala response to facial stimuli in adolescent MDD.[28,29] Their results are inconsistent, with one study finding increased[29] and the other decreased[28] amygdala activity relative to healthy participants. Findings from two other studies[32,33] suggest that biased amygdala function in individuals at risk for MDD occurs specifically when passively viewing emotional stimuli. Because neither study excluded subjects with anxiety disorders, the influence of anxiety remains unclear.

The primary goal of the current study is to compare amygdala engagement to face-emotion stimuli among three groups of adolescents: MDD patients, anxiety patients, and healthy subjects. Comparative analyses require "pure" groups, but prior research in adolescent MDD[28,29,33,34] includes anxious individuals. Thus, we study MDD patients both with comorbid anxiety included and excluded. Existing data support competing hypotheses. On the one hand, data in adults,[16-21,24] together with the strong cross-sectional, longitudinal, and familial relationships among adolescent and adult anxiety and MDD,[3-10,14] raise the expectation of overlapping amygdala dysfunction, consistent with a "shared diathesis" perspective.[11,12] Based on these data, one might expect similarly biased amygdala engagement in anxious and MDD adolescents, relative to healthy peers. On the other hand, preliminary data suggest that amygdala engagement in anxious and MDD adolescents might vary with changing emotional state and attention,[27,31-33] consistent with evidence of disorder-specific cognitive biases.[13,35,36]

METHODS

PARTICIPANTS

Eighty-seven adolescents were studied: 26 with MDD, 16 non-MDD with anxiety disorders, and 45 without psychopathology. MDD patients were initially recruited; others were then selected from larger pools to form three groups, matched on age, sex, social class, and IQ (Table 1). While groups did not differ statistically on these variables, anxiety patients included somewhat younger and more male subjects; we repeated all analyses covarying for age and sex. A prior report[27] included 7 of the 16 anxiety and 16 of the 45 healthy adolescents.

(Table 1)

Diagnoses were assessed using the Schedule-for-Affective-Disorders-and-Schizophrenia-for-School-Aged-Children (K-SADS).[37] As described previously,[27,29] MDD and anxiety patients were required i) to show persistent, impairing anxiety or depressive symptoms, respectively, during three weeks of supportive therapy, and ii) to meet previously-reported exclusion criteria.

All anxiety patients were without lifetime history of MDD; all non-anxious MDD patients were without lifetime history of anxiety. All were medication-free, and only one had past exposure to any anxiolytic, such as an SSRI. The study was approved by the NIMH-IRB. All participants/parents provided written informed consent/assent.

TASK

We used functional magnetic resonance imaging (fMRI) with a previously-described paradigm.[27,29,31,38] Briefly, participants viewed 32 faces (8 each of: neutral, fearful, angry, happy),[39-41] each presented for 4000 ms, four times in one 160-trial run, divided into four 40-trial epochs (32 faces, 8 fixation trials) and four ten-trial blocks (8 faces, 2 fixation trials). During three blocks, participants adopted different constrained attention states by rating the face stimuli on 5-point scales (1=not at all to 5=very): (1) "How hostile is this face?", (2) "How afraid are you of this face?", and (3) "How wide is the nose?". During the fourth block, participants passively viewed the faces (unconstrained attention). Order of face presentation and attention-conditions were randomized. Ratings and reaction times (RTs) were recorded.

MRI PROCEDURES

Whole-brain blood-oxygen-level-dependent (BOLD) fMRI data were acquired on one of two 3-T scanners in groups matched with regard to scanner (χ^2=4.05, df=2, p=.13). T2-weighted images were acquired in 23 axial slices parallel to the anterior-commissure/posterior-commissure line using an echo-planar single-shot gradient echo pulse sequence (matrix=64x64; repetition time (TR)=2000 milliseconds; echo time (TE)=40 milliseconds; field of view (FOV)=240 mm; voxels=3.75x3.75x5.0 mm). As reported previously,[27,29] high-resolution T1-weighted anatomical images were acquired.

Data from subjects moving >2.5 mm in any plane were discarded. Subsequent analyses were conducted with SPM99 and Matlab 6.1 routines. Functional data were corrected for slice timing and motion, anatomically co-registered, and spatially normalized to the SPM99 Montreal

Neurologic Institute (MNI) T1-weighted template. We used SPM99 to maximize parallels with prior work.[27,31,42] Nevertheless, group analyses implemented in SPSS15.0 avoid problems created by outdated aspects of SPM99.

DATA ANALYSIS

Behavioral Data

Ratings and RTs confirm participants' task compliance and evaluate group differences in behavior. Due to an equipment malfunction, data for three participants were not recorded. Data were analyzed with analyses of variance (ANOVAs) with diagnostic group as the between-subjects factor and face-emotion and attention-condition as within-subjects factors. To minimize Type-I errors the Greenhouse-Geisser correction was applied.[43]

fMRI Data

We estimated event-related-response amplitudes at the individual-subject level for each face-emotion type in each attention-condition using the General Linear Model (GLM). The waveform for each event-related response was a rectangular pulse (4 seconds) convolved with the SPM99 hemodynamic response function (HRF). We generated contrast images using pair-wise comparisons across event types. We then divided each contrast image by subject-specific voxel time series means.[44]

Group-level analyses use random effects models.[45] Prior findings document amygdala abnormalities on this task in pediatric anxiety[27] and bipolar[42] disorder. Hence, we used a region-of-interest (ROI) strategy focused on the amygdala, defined using standard criteria[46] on the MNI template. All subjects had BOLD activity data in >65% of ROI voxels. BOLD signal changes for each event vs. fixation baseline were averaged across all amygdala voxels and were submitted in SPSS15.0 to multi-factorial analyses of complex two- and-three-way interactions.

Our primary hypothesis was that overall between-group amygdala differences vary as a function of both face-emotion type and attention. We tested this with omnibus three-way group-

by-face-emotion-by-attention-condition interactions, in repeated-measures ANOVAs for each amygdala, with one 3-level between-subject factor (group) and two 4-level within-subject factors (emotion, attention). Two analyses were conducted, using Greenhouse-Geisser correction: (1) including 14 comorbid anxiety patients in the MDD group (n=26) and (2) including only non-comorbid MDD cases (n=12). Focused post-hoc analyses decomposed significant three-way interactions. These post-hoc analyses compared amygdala activation (1) to fearful faces specifically viewed across different attention-conditions and (2) across all face-types specifically in the passive-viewing condition. This post-hoc approach extends prior findings.

Data from three studies in anxiety patients,[27] youths at risk for anxiety,[31] or at risk for depression[33] had led us to expect between-group differences to fearful faces, specifically, relative to other face-types, viewed in particular attention-conditions: we expected hyper-activation in anxiety when participants monitored subjective fear, relative to passively viewing fear-faces. This prediction was first investigated by a two-factor repeated-measure ANOVA testing the significance of group-by-attention-condition interactions for amygdala activation to fearful faces, relative to fixation, across all four attention-conditions. This was followed by three-group ANOVAs (Brown-Forsythe test when variances unequal) and two-group t-tests for the a-priori-defined "fearful-afraid-vs.-fearful-passive" contrast.

Prior research also suggested that specific anxiety-related and depression-related biases manifest during passive-viewing.[27,28,31,33] Based on McClure et al.[27], Pérez-Edgar et al.[31] and Monk et al.[33], we expected greater amygdala activation to fearful faces passively viewed in MDD than anxious and healthy individuals. However, prior studies generate inconsistent data concerning amygdala response to other face-emotion types, viewed passively. Thus, we performed a two-factor repeated-measure ANOVA including all face-emotion classes to test the significance of a group-by-face-emotion interaction in passive-viewing; post-hoc tests focused on contrasts of fearful versus other emotions.

Finally, although the current study focused on the amygdala, secondary analyses examined the orbitofrontal cortex (OFC), guided by previous research.[15,16,20,27,34,47-49] Procedures followed those for the amygdala by extracting values for entire ROIs,[27,29] defined using standard, validated anatomical criteria, as delineated in previous research.[46] Of note, the OFC ROI used here encompasses both medial and lateral inferior-frontal expanses of prefrontal cortex (PFC). Due to susceptibility-related signal loss, two individuals were excluded, yielding n=26 MDD, n=15 anxiety, and n=44 healthy adolescents.

In addition to ROI analyses, supplementary voxel-based techniques generated coordinates of between-group peak-activation differences. As we entered this work with relatively clear, regionally-based, a priori hypotheses and we wanted to minimize Type-II-errors in this three-group study, we treated results from our ROI-based analyses as primary. Nevertheless, findings from voxel-based analyses replicated those in whole-structure ROI approaches while also informing future work; they are accordingly summarized using MNI coordinates.

RESULTS

SAMPLE CHARACTERISTICS & BEHAVIOR

Table 1 displays sample demographic and clinical characteristics; Table 2 displays behavioral performance during scanning. These behavioral data revealed the expected face-emotion-by-attention-condition interactions for ratings ($F[4.6,368.6]=63.6$; $p<.001$) and RTs ($F[5.5,446.9]=15.4$; $p<.001$). Both ratings and RTs for the “afraid” and “hostile” questions were highest for angry and fearful faces and lowest for happy faces; “nose” ratings and RTs were highest for happy and angry faces and lowest for neutral faces. No two- or three-way interactions with group were found for either ratings or RTs ($p=.15$-to-$=.76$). No significant main-effects of group emerged on ratings ($F[2,81]=1.2$; $p=.32$) or RTs ($F[2,81]=1.6$; $p=.20$). Similar findings were revealed when excluding comorbid MDD/anxiety patients [available upon request].

Absence of group-effects indicates that all groups similarly altered behavior across emotion and attention-conditions.

(Table 2)

IMAGING

Amygdala activation

We tested our primary hypothesis using repeated-measures ANOVAs for BOLD responses in each amygdala. These analyses revealed the expected three-way group-by-face-emotion-by-attention-condition interaction in left and in right amygdalae (Table 3).

(Table 3)

Three-way interactions indicate that between-group differences vary with both face-emotion and attention-condition. These were decomposed in post-hoc tests focusing on a-priori anticipated group-differences. Specifically, differences were expected (1) in select attention-conditions in fearful-face viewing events and (2) when fearful faces versus other face-emotions were passively viewed.

Fearful-face viewing.

Based on prior research,[27,31,33] we predicted between-group differences in amygdala response to fearful faces with hyper-activation in anxiety patients during afraid ratings. As expected, significant bilateral group-by-attention-condition interactions emerged when comorbid MDD/anxiety patients were included (left: $F[5.8,244.0]=6.4$, $p<.001$, Figure 1a; right: $F[5.5,231.0]=2.5$, $p=.03$) or excluded (left: $F[5.6,197.8]=6.1$, $p<.001$; right: $F[5.3,185.9]=2.3$, $p=.05$). Anxiety patients showed the predicted amygdala hyper-activation when rating subjectively-experienced fear to fearful faces but not when passively viewing these faces.

In the a-priori defined "fearful-afraid-vs.-fearful-passive" contrast, significant between-group differences were evident only in left amygdala (comorbid MDD/anxiety patients included:

F[2,25.8]=4.7; p=.02, Figure 1b; comorbid MDD/anxiety patients excluded: F[2,25.6]=5.3, p=.01; Figure 1c). Data from this contrast supported the "shared-diathesis" perspective. Thus, both anxiety ($t_{18.5}$=2.2, p=.04; see also Figure 1d) and MDD (with and without anxiety: t_{69}=3.2; p=.002; without anxiety: t_{55}=3.2; p=.002, see also Figure 1e) patients showed greater amygdala activation than healthy peers, with no significant differences between patient groups.

(Figure 1)

We also compared groups on other "fearful-face" contrasts (e.g., afraid-nose, hostile-nose; compare Figure 1a). This revealed consistent evidence of increased activation in anxious, relative to healthy subjects, somewhat less consistent evidence of enhanced activation in MDD, relative to healthy subjects, and in anxiety patients relative to MDD patients (results available on request). Further analyses did reveal between-group differences during afraid-rating to show some degree of emotion-specificity: no between-group differences emerged for afraid-rating events with neutral or happy faces (p-values>.35).

Passive-viewing.

As noted previously, prior studies most consistently yielded disorder-specific biases under unconstrained attention-conditions.[27,28,31,33] Thus, we were particularly interested in between-group comparisons in this condition. Across passive-viewing face-types, significant group-by-face-emotion interactions emerged in left (F[5.5,230.3]=3.2, p=.006; Figure 2a) and right (F[5.4,226.8]=3.2, p=.04) amygdala. Similar results occurred when excluding comorbid MDD/anxiety cases (left: F[5.4,188.1]=3.4, p=.005; right: F[5.3,186.1]=2.2, p=.05).

Post-hoc tests focused on fearful versus other face-emotions, as prior research did not generate more specific hypothesis. The interactions reflected amygdala activation differences for the "fearful-passive-vs.-happy-passive" contrast, both when comorbid MDD/anxiety patients were included (left: F[2,84]=6.6, p=.002, Figure 2b; right: F[2,84]=5.1, p=.008) or excluded (left: F[2,70]=6.5; p=.003, Figure 2c; right: F[2,70]=4.6, p=.01). Consistent with the "disorder-

specificity" perspective, opposite patterns emerged in patient groups: anxiety patients showed activation and MDD patients showed deactivation for fearful versus happy faces. This difference was significant whether MDD/anxiety patients were included (left: t_{40}=3.3, p=.002, Fig. 2b; right: t_{40}=2.8, p=.008) or excluded (left: t_{26}=3.1, p=.004, Fig. 2c; right: t_{26}=2.4, p=.02). Both patient groups also showed significantly different responses from healthy controls, with hyper-activation in anxiety (left: t_{59}=2.2, p=.03; right: t_{59}=2.6, p=.01) and hypo-activation in MDD (left only: with or without comorbid anxiety disorder: t_{69}=-2.2, p=.03; without comorbid anxiety disorder: t_{55}=-2.4, p=.02).

(Figure 2)

Of note, post-hoc results also showed that between-group differences reflected responses to "passive-happy" events, independent of the response to "fear-faces". Comparing groups on the "neutral-passive"-vs.-"happy-passive" contrast revealed amygdala hyper-activation in anxiety, relative to both healthy and MDD subjects, similar to the "fearful-passive"-vs.-"happy-passive" contrast. However, healthy and MDD subjects did not differ (p-values=.09). Further analyses demonstrated the between-group differences for happy faces to be specific to passive-viewing: no between-group differences emerged during afraid- or hostility-ratings (p-values>=.37). Finally, we repeated all analyses using amygdala ROIs while covarying for age and sex. No differences in results occurred (available upon request).

OFC activation

Secondary analyses examined group differences in OFC in the a-priori defined "fearful-afraid-vs.-fearful-passive" contrast. Results were largely consistent with those emerging in the amygdala-based analyses, both when comorbid MDD/anxiety patients were included (left OFC: F[2,82]=3.2, p=.05, Figure 3a) or excluded (left OFC: F[2,68]=2.7, p=.08, Figure 3b). Anxiety patients showed significantly enhanced left OFC activation relative to healthy subjects (t_{57}=2.2, p=.04; Figure 3c); a non-significant trend emerged for the MDD vs. healthy comparison, but only

when comorbid MDD/anxiety patients were included (t_{68}=1.8, p=.07). No significant differences emerged between the anxiety and the MDD groups.

(Figure 3)

We also examined group differences in the "fearful-passive-vs.-happy-passive" contrast that evidenced "disorder-specificity" in amygdala response. Between-group differences were also found in the right OFC, both when comorbid MDD/anxiety patients were included F[2,82]=4.2, p=.02, Figure 4a) or excluded F[2,68]=5.3, p=.007, Figure 4b). Anxiety patients showed significantly greater activation than MDD patients (with and without comorbid anxiety: t_{39}=2.1, p=.04; without comorbid anxiety: t_{25}=2.5, p=.02) and than healthy controls (t_{57}=3.2, p=.002). MDD patients, however, did not differ from healthy controls.

(Figure 4)

Repeating the OFC-related analyses covarying for age and sex did not change the results with one exception. The significance of the difference between anxiety and MDD patients in the "fearful-passive-vs.-happy-passive" contrast was diminished when comorbid MDD/anxiety patients were included (F[1,37]=2.8, p=.10), but not when considering MDD alone (F[1,23]=5.1, p=.03).

COMMENT

The current study generates two key findings. First, when adolescents viewed faces expressing fear and focused their attention on internally experienced fear, relative to passive viewing, both anxiety and MDD patients exhibited greater amygdala activation than healthy peers. Second, distinct emotion-specific amygdala responses in MDD and anxiety disorders occurred during passive viewing, where patients also significantly differed from healthy peers.

The degree to which MDD and anxiety disorders represent nosologically distinct conditions remains unclear. Particularly intense debate occurs regarding youth. This arises in light of longitudinal data demonstrating strong but relatively non-specific associations over time

among MDD and anxiety disorders in adolescents and in adults.[5,6,50,51] The current data suggest that adolescent anxiety disorders and MDD exhibit neural commonalities but also demonstrable differences, depending on the specific attention and emotion states engaged during fMRI. From a theoretical perspective, this suggests that adolescent anxiety disorders and MDD involve complex, overlapping yet distinguishable patterns of amygdala-related biases. For some biases, related to subjective-state monitoring, similar perturbation of amygdala engagement and associated psychological processes may occur in MDD and anxiety. For other, spontaneously-elicited psychological processes engaged during unconstrained, passive viewing of faces, disorder-specific biasing may occur. Viewed broadly, these data support the view of neural distinctions between MDD and anxiety as complex and nuanced but clearly demonstrable.

Disorder-Specificity

Our study finds evidence of specifically perturbed amygdala engagement in adolescent MDD and anxiety disorders, manifest in select attention states for specific face-emotions. This conclusion emerges from our omnibus approach to between-group contrasts. Such a statistical approach is necessarily complex: it rests on tests of three-way, group-by-emotion-by-attention interactions. Significant interactions emerge because between-group differences in anxious and MDD adolescents occur only when viewing fearful versus happy faces passively but not when viewing other emotions or when viewing these same emotions in other attention states.

Disorder-specificity was expected during passive-viewing, given prior research.[27,28,31,33] However, differences between the current and these prior studies complicate cross-study comparisons. These differences encompass clinical features of samples, task-stimulus features, and task-related cognitive processes. Nevertheless, the finding that disorder-specificity emerges during passive-viewing is consistent with other work.[27,28,31,33] This suggests that disorder-specific findings emerge when subjects are allowed to engage information processing strategies elicited naturally, during passive-viewing, an instance where task instructions do not constrain attention.

Further work is needed specifying the precise psychological nature of these disorder-specific processes that may emerge spontaneously.

Despite consistency across the current and prior studies, questions remain. For example, both Monk et al.[33] and the current study revealed MDD-related between-group differences in amygdala response during passive-viewing; however, Monk et al. found amygdala *hyper*-activation in at-risk adolescents viewing morphed faces showing varying blends of fear; the current study found amygdala *hypo*-activation in MDD-affected subjects viewing faces showing full displays of fear. Thus, these inconsistencies may be due to methodological differences.

Other questions emerge related to developmental perspectives. Due to strong longitudinal and family-based aggregation among MDD and anxiety disorders manifest in adolescents and adults,[3-10,14] one might expect brain imaging findings in adult MDD and anxiety[16-21,24] to parallel the findings observed here, in adolescents. Nevertheless, few imaging studies contrast anxious and MDD adults with any paradigm; none use paradigms similar to the one used here, which shows that different conclusions emerge concerning between-group comparisons as a function of relatively subtle task-related features. As with inconsistencies in work with adolescents, the dearth of studies directly comparing anxious and MDD adults emphasizes the need for more research on the nature of perturbed amygdala engagement in risk for and expression of MDD and anxiety. In pursuing such work, the current findings highlight the need to consider the sensitivity of group differences to variations in attention-conditions across fMRI paradigms.

One finding calls for particular attention. MDD-related deactivation specifically to passively-viewed happy faces represents a major contributor to the disorder-specific between-group differences in the "fearful-passive-vs.-happy-passive" contrast. Given the tendency in prior research to focus on hyper-activation, this finding for deactivation may appear intuitively surprising and in need of replication. Nevertheless, prior research consistently finds that between-group differences during passive-viewing observed with the current paradigm at least partially reflect anomalous patterns of amygdala deactivation in one or another unique subgroup.[27,31]

Moreover, prior work demonstrates the importance of happy faces, specifically, as an optimal comparison condition, while also suggesting that happy faces index reward-related processes uniquely perturbed in MDD but not anxiety disorders.[27,33] Finally, despite some divergence between the current findings and associated hypotheses emerging from prior studies,[27,31,33] our findings documenting disorder-related specificity during passive-viewing extend other work. For example, Thomas et al.[28] also used passive-viewing, though no other attention manipulation, and found amygdala hyper-activation in anxious children and amygdala deactivation in MDD children.

Shared-Diathesis

The current study also provides evidence of amygdala perturbations common to both adolescent MDD and anxiety disorders. These data suggest that at least some adolescent anxiety disorders share an underlying neural diathesis with adolescent MDD. Importantly, as with disorder-specificity, disorder-common manifestations occurred to particular face-emotion types, when viewed in specific attention states. Support for this conclusion again emerges from our focus on necessarily complex tests of three-way interactions. Thus, both patient groups had greater amygdala activation than healthy peers only when viewing fearful faces specifically. These differences occurred particularly when focusing on subjectively-experienced fear, relative to passively viewing the same fearful faces or relative to viewing happy or neutral faces in various attention states. Prior research[27,31] had led us to expect amygdala perturbations in anxiety patients specifically when viewing fear-faces and rating fear; the current study extends this observation to MDD, with or without anxiety.

Findings from our secondary analyses in the lateral OFC also provide some support for both the "disorder-specificity" and the "shared-diathesis" perspectives. This pattern is consistent with prior work implicating a distributed neural circuitry devoted to emotional modulation of perception and behavior.[27,52-55] Taken together, findings suggest that adolescent anxiety disorders

and MDD can exhibit neural commonalities but also distinctions, depending on the specific attention and emotion states engaged.

Development

Common and specific neural perturbations were not affected by sex and age. However, the current study was not specifically designed to examine questions of sex and age-specificity across adolescence and adulthood, questions which require large samples of adolescents and adults. Prior research does indicate differences in patterns of neural responses under varying emotion/attention conditions between healthy adolescents and adults, though no prior work has directly compared samples of MDD or anxious and healthy adolescents and adults.[38,56,57] The current work now sets the stage for such large, comparative studies among adolescents and adults with anxiety and mood disorders. Studies directly comparing these groups are needed, given the demonstrated effects of subtle task variations on between-group differences. Such studies, which may reveal similar or unique functional perturbations across pathologies and age groups, are particularly important in light of improved etiological/pathogenic models and treatment options.[58]

Behavioral Data

In addition to the fMRI results, we found expected variations in task performance as a function of attention-condition and face-emotion type, as shown previously.[27,31,32,38] However, groups did not differ on task performance. Thus, the current paper, when combined with others on amygdala function in both adults and adolescents[27,31,33,59] firmly establishes the fact that between-group differences in amygdala function emerge even in the absence of between-group differences in task performance. The observed amygdala differences in the current study specifically were independent of rated anxiety and are not epiphenomena of between-group differences in experienced anxiety or other task-performance differences. Some research, however, suggests that differences in task performance facilitate interpretation of differences in

neural activation.[60] From this perspective, the failure of a task to elicit expected between-group differences in behavior might suggest that the underlying psychological process engaged by the task is not directly relevant for the condition being studied.

In the current paper, the failure to observe between-group differences in behaviour, in the context of between-group differences in neural response, emerges for a task that is clearly disorder-relevant. Disorder-relevance reflects the definition of clinical anxiety as a condition characterized by excessive subjectively-reported anxiety. Comparable results emerge in another study of anxious adolescents,[30] using another disorder-relevant paradigm that engages threat-attention interactions during orienting, another process previously linked to clinical anxiety. This study also found between-group differences in the amygdala in the context of no between-group differences in behaviour. Moreover, the study utilized stimuli presented too rapidly to be perceived, in terms of their capacity to be rated as elicitors of subjectively-experienced anxiety.

Taken together, these two studies dissociate individual differences in amygdala function and individual differences in the subjective experience of anxiety during scanning. Importantly, though, both studies demonstrate adolescent between-group differences in amygdala function using tasks previously linked to clinical anxiety. The current report specifically shows that between-group differences occur specifically during subjective-fear monitoring, the most clinically-relevant attention state engaged in the current study, but not in other attention states.

Limitations

Our findings must be viewed in light of four limitations. First, results are based on small sample-sizes. Because anxiety and MDD frequently co-occurs, it is difficult to gather large, non-comorbid samples. As a result, true positive effects might have been obscured. Given that type-II-error is more likely than type-I-error with small sample-sizes, negative findings should be interpreted with more caution than positive findings.

Second, additional aspects of our sample complicate interpretations. For example, findings emerging from analyses that included patients with comorbid MDD/anxiety raise the question of the degree to which anxiety-comorbidity influences or changes biased neural engagement in MDD, and whether findings can be attributed to MDD per se. It was not feasible to recruit sufficiently large samples of subjects in four mutually exclusive groups (MDD alone, anxiety alone, comorbid MDD/anxiety, and healthy controls). Similar concerns prevented us from recruiting sufficiently large samples of adolescents with specific anxiety disorders. However, we repeated all analyses with comorbid patients excluded from the MDD group; these analyses supported conclusions emerging from other analyses. Yet, some unanswered questions remain as our adolescent participants with "pure" anxiety or "pure" depression may develop heterotypic comorbidity in the future. Longitudinal studies conducting serial fMRI assessments might provide more definitive insights on the developmental trajectories of emerging comorbidity patterns. Similarly, because comorbidity among the anxiety disorders also complicates interpretations, future studies should examine brain imaging data in "pure" anxiety groups. However, such studies will face the problem that few cases with anxiety occur in the absence of comorbidity and that such samples may be unrepresentative, particularly of cases typically seen in clinical settings.

Third, our analysis is limited to amygdala and OFC regions, which may be perceived as a restricted view of (neural) dysfunction in anxiety and depressive disorders.

Fourth, the cognitive task used has advantages and disadvantages. Regarding advantages, prior work suggests that the task elicits disorder-specific profiles[27,31,33,42] Moreover, the task explicitly assesses neural activity engaged when participants report distress (i.e., experienced internal fear), a defining feature of anxiety disorders. On the other hand, ratings of distress engage a series of complex incompletely-specified psychological processes that require introspection and can be directed towards various environmental features. Because fearful faces signal threat but are not directly threatening, a task focusing attention on more general aspects of

threat might generate unique findings. Furthermore, in the passive-viewing condition, no information is generated concerning the cognitive processes engaged in each group. The use of only eight specific emotion events in each attention condition is also a limitation, as tasks with more replicates posses greater statistical power.[61] However, as the current analyses attempted to reveal between-group differences as a function of different emotion and attention conditions, we needed considerable variation on both factors. In an adolescent sample, for practicability reasons, this resulted in relatively few specific emotion events in each attention condition, to minimize task duration. Finally, this concern probably relates more to instances where studies fail to detect hypothesized between-group differences than to studies such as ours that confirm hypothesized differences. Thus, while the current paradigm appears to be sensitive to both commonalities and differences in the neural correlates of adolescent MDD and anxiety disorders, further refined tasks may generate more precise conclusions concerning the nature of these commonalities and differences.

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FIGURE LEGENDS

Figure 1. Amygdala activation to fearful-faces in anxiety and MDD patients relative to healthy controls for select attention-conditions.

a. Bar graphs of left amygdala activation to fearful-faces relative to fixation (error bars reflect standard errors) displaying the group [healthy controls, MDD (with and without anxiety disorder), anxiety disorder alone]-by-attention-condition interaction. A similar activation pattern was found for the right amygdala and when excluding comorbid MDD/anxiety patients (not shown in Figure).

b and c. Bar graphs of left amygdala activation to fearful-faces during afraid-ratings versus passive-viewing ("fearful-afraid-vs.-fearful-passive" contrast) showing significantly enhanced activation among both anxiety patients and MDD patients (MDD with and without anxiety disorder (b.), MDD alone (c.)) compared to healthy controls, with no difference between anxiety and MDD patients.

d and e. The "fearful-afraid-vs.-fearful-passive" contrast evidences significantly greater left amygdala activation in (d.) anxiety alone patients compared to controls (Montreal Neurological Institute (MNI) coordinates: -20, -2, -20, p=.001 (shown in figure); -10, -4, -16, p=.002; MNI coordinates are small volume corrected (svc)) and (e.) MDD alone patients compared to controls (MNI coordinates: -20, 4, -16, p=.007; svc). Highlighted areas indicate regions where the differences in BOLD activation between groups were significant (for displaying purposes, uncorrected threshold was set at p=.0005 (d.) and p=.005 (e.)).

Figure 2. Differential amygdala activation in MDD and anxiety patients during passive-viewing of fearful versus other face-emotion types.

a. Bar graphs of left amygdala activation to passively-viewed facial expressions relative to fixation (error bars reflect standard errors) among patients with MDD (with and without comorbid anxiety disorder), patients with anxiety disorder, and healthy controls displaying the group-by-face-emotion interaction in the passive-viewing condition. A similar activation pattern was found for the right amygdala and when excluding comorbid MDD/anxiety patients (not shown in Figure).

b and c. Anxiety patients and MDD patients (with and without comorbid anxiety (b.), MDD alone (c.)) showed opposite and significantly different left amygdala responses to fearful faces vs. happy faces passively viewed ("fearful-passive-vs.-happy-passive" contrast). MDD patients and anxiety patients each also differed from healthy controls in left amygdala activation in this contrast.

d. The "fearful-passive-vs.-happy-passive" contrast evidences significantly greater left and right amygdala activation in anxiety patients as compared to MDD patients even when MDD patients with comorbid anxiety are excluded (MNI coordinates left: -16, 2, -16, p=.014, svc; MNI coordinates right: 22, 0, -14, p=.001, svc). Highlighted areas indicate regions where the differences in BOLD activation between groups were significant (for displaying purposes, uncorrected threshold was set at p=.005).

729 Figure 3. OFC activation in the “fearful-afraid-vs.-fearful-passive contrast”.

730 a and b. Bar graphs of left OFC activation to fearful-faces during afraid-ratings versus passive-

731 viewing (“fearful-afraid-vs.-fearful-passive” contrast) showing significantly enhanced activation

732 among anxiety patients compared to healthy controls.

733 c. The “fearful-afraid-vs.-fearful-passive” contrast evidences significantly greater lateral OFC

734 activation in anxiety patients compared to controls (MNI coordinates left: -50, 22, -2, p=.046

735 (shown in Figure), -14, 18, -10, p=.050, svc). Highlighted areas indicate regions where the

736 differences in BOLD activation between groups were significant (for displaying purposes,

737 uncorrected threshold was set at p=.005).

738

Figure 4. OFC activation in the “fearful-passive-vs.-happy-passive” contrast.

a and b. Bar graphs of right OFC activation to fearful-faces during passive viewing of fearful vs. happy faces “fearful-passive-vs.-happy-passive” contrast) showing significantly enhanced activation among anxiety patients compared to MDD patients and compared to healthy controls. c. The “fearful-passive-vs.-happy-passive” contrast evidences significantly greater right lateral OFC activation in anxiety patients as compared to MDD patients (with and without comorbid anxiety: MNI coordinates: 32, 24, -18, p=.005, svc; no suprathreshold voxels emerge for the anxiety versus MDD alone comparison). Highlighted areas indicate regions where the differences in BOLD activation between groups were significant (for displaying purposes, uncorrected threshold was set at p=.0005).

751 **TABLES**

752

753
754 Table 1. Demographic and clinical characteristics of subjects with MDD, anxiety disorder and no
755 psychopathology

Measure	Healthy Controls (n = 45)		MDD with and without anxiety disorder (n= 26)		MDD without anxiety disorder (n=12)		Anxiety disorder without MDD (n = 16)	
Age, mean (SD), y	13.93	(2.18)	14.08	(2.23)	14.20	(2.60)	12.77	(1.85)
IQ, mean (SD)	111.62	(13.57)	110.38	(18.05)	113.5	(21.82)	112.14	(14.53)
SES, mean (SD) 1	52.00	(23.34)	46.14	(19.34)	42.1	(22.35)	46.92	(24.62)
Female sex, No. (%)	24	(53)	15	(58)	7	(58)	5	(31)
DSM-IV diagnoses (current), No. (%)								
MDD	0		26	(100)	12	(100)	0	
Any anxiety disorder	0		14	(54)	0		16	(100)
GAD	0		10	(39)	0		8	(50)
Social Phobia	0		8	(31)	0		5	(31)
SAD	0		7	(27)	0		8	(50)
GAD alone	0		3	(12)	0		4	(25)
Social Phobia alone	0		1	(4)	0		3	(19)
SAD alone	0		2	(8)	0		4	(25)
Pediatric Anxiety Rating Scale (PARS), mean (SD)	n/a		15.32	(5.00)	13.42	(4.76)	16.44	(2.50)
Children's Depression Rating Scale (CDRS), mean (SD)	42.17	(8.43)	59.12	(13.00)	55.55	(13.40)	46.86	(4.45)
Clinical Global Impressions Scale (CGI), mean (SD)	n/a		4.73	(0.83)	4.67	(0.89)	4.19	(0.75)

[1] SES: Socioeconomic Status: Index generated from occupational and educational level of parents (theoretical range 20 - 137), higher values indicate higher SES

MDD - Major Depressive Disorder

GAD - Generalized Anxiety Disorder

SAD - Separation Anxiety Disorder

n/a - not applicable

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758 Table 2. Task performance by group

Behavioral Measures	Healthy controls (n = 45)	MDD with and without anxiety disorder (n = 25)	Anxiety disorder without MDD (n = 14)
Ratings, mean (SD)			
How hostile - Neutral faces	1.74 (0.61)	1.82 (0.56)	1.86 (0.88)
How hostile - Fearful faces	2.04 (0.83)	2.31 (0.89)	2.27 (1.08)
How hostile - Angry faces	3.17 (1.01)	3.42 (0.86)	3.34 (0.96)
How hostile - Happy faces	1.10 (0.18)	1.33 (0.42)	1.53 (0.71)
How afraid - Neutral faces	1.49 (0.64)	1.68 (0.69)	1.69 (0.83)
How afraid - Fearful faces	1.83 (0.77)	2.14 (0.99)	1.93 (1.01)
How afraid - Angry faces	2.38 (0.99)	2.52 (0.93)	2.76 (1.23)
How afraid - Happy faces	1.14 (0.24)	1.35 (0.49)	1.41 (0.64)
How wide is the nose - Neutral faces	2.19 (0.58)	2.12 (0.45)	2.16 (0.40)
How wide is the nose - Fearful faces	2.17 (0.54)	2.31 (0.62)	2.15 (0.49)
How wide is the nose - Angry faces	2.59 (0.65)	2.59 (0.60)	2.77 (0.50)
How wide is the nose - Happy faces	2.59 (0.53)	2.69 (0.53)	2.52 (0.48)
Reaction times (in ms), mean (SD)			
How hostile - Neutral faces	1820.19 (438.00)	1986.28 (377.51)	1894.08 (469.09)
How hostile - Fearful faces	2031.00 (495.40)	2104.97 (398.01)	1923.39 (373.43)
How hostile - Angry faces	1964.88 (400.65)	2000.80 (384.47)	2159.22 (445.21)
How hostile - Happy faces	1534.44 (351.09)	1656.25 (428.98)	1715.38 (278.31)
How afraid - Neutral faces	1692.53 (432.68)	1925.43 (435.90)	1713.75 (390.73)
How afraid - Fearful faces	1828.02 (421.88)	1968.81 (370.44)	1853.62 (414.40)
How afraid - Angry faces	1983.81 (443.39)	2057.27 (495.37)	2093.40 (564.12)
How afraid - Happy faces	1459.04 (370.12)	1732.17 (421.51)	1634.82 (368.52)
How wide is the nose - Neutral faces	1823.26 (363.40)	1918.47 (310.54)	2048.53 (308.56)
How wide is the nose - Fearful faces	1912.18 (345.08)	1991.08 (316.78)	1955.11 (260.15)
How wide is the nose - Angry faces	1971.25 (415.22)	2082.00 (365.71)	2145.36 (308.08)
How wide is the nose - Happy faces	1982.59 (394.67)	2111.49 (340.06)	2022.38 (271.16)

MDD - Major Depressive Disorder (n=14 with anxiety disorder, n=11 without anxiety disorder)

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760

761 Table 3. Statistical analyses of Regions of Interest (Omnibus repeated-measures ANOVA)

	Comorbid MDD/anxiety patients included §						Comorbid MDD/anxiety patients excluded &					
	Left Amygdala			Right Amygdala			Left Amygdala			Right Amygdala		
Effect $	F-Value	df	p-Value	F-Value	df	p-Value	F-Value	df	p-Value	F-Value	df	p-Value
Main effect												
group (between subject effect)	0.98	2, 84	.38	1.25	2, 84	.29	0.52	2, 70	.60	1.40	2, 70	.25
emotion (within subject effect)	6.49	2.9, 240.1	<.001*	3.34	2.6, 219.2	.03*	4.45	2.8, 194.2	.006*	1.70	2.5, 177.4	.18
attention (within subject effect)	7.53	2.8, 238.5	<.001*	0.16	2.8, 236.2	.91	5.66	2.8, 196.1	.001*	0.15	2.6, 185.1	.91
2-way interaction												
group by emotion	2.16	5.7, 240.1	.05	1.80	5.2, 219.2	.11	2.02	5.6, 194.2	.07	1.94	5.1, 177.4	.09
group by attention	3.00	5.7, 238.5	.009*	0.88	5.6, 236.2	.50	2.74	5.6, 196.1	.02*	0.63	5.3, 185.1	.69
emotion by attention	1.21	7.4, 621.1	.30	1.69	6.6, 553.6	.12	1.45	7.2, 505.0	.18	1.31	6.0, 416.6	.25
3-way interaction												
group by emotion by attention	2.68	14.8, 621.1	.001*	2.01	13.2, 553.6	.02*	2.71	14.4, 505.0	.001*	1.90	11.9, 416.6	.03*

§ MDD patients with and without comorbid anxiety disorder (n=26), anxiety patients (n=16), healthy controls (n=45)

& MDD patients without comorbid anxiety disorder (n=12), anxiety patients (n=16), healthy controls (n=45)

$ Results of omnibus repeated-measures analyses of variance (Greenhouse-Geisser corrected)

group: MDD, Anxiety, Controls

emotion: neutral, fearful, angry, happy

attention: hostile, afraid, nose, passive

* significant at $P<0.05$

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763

FIGURES

Figure 1.

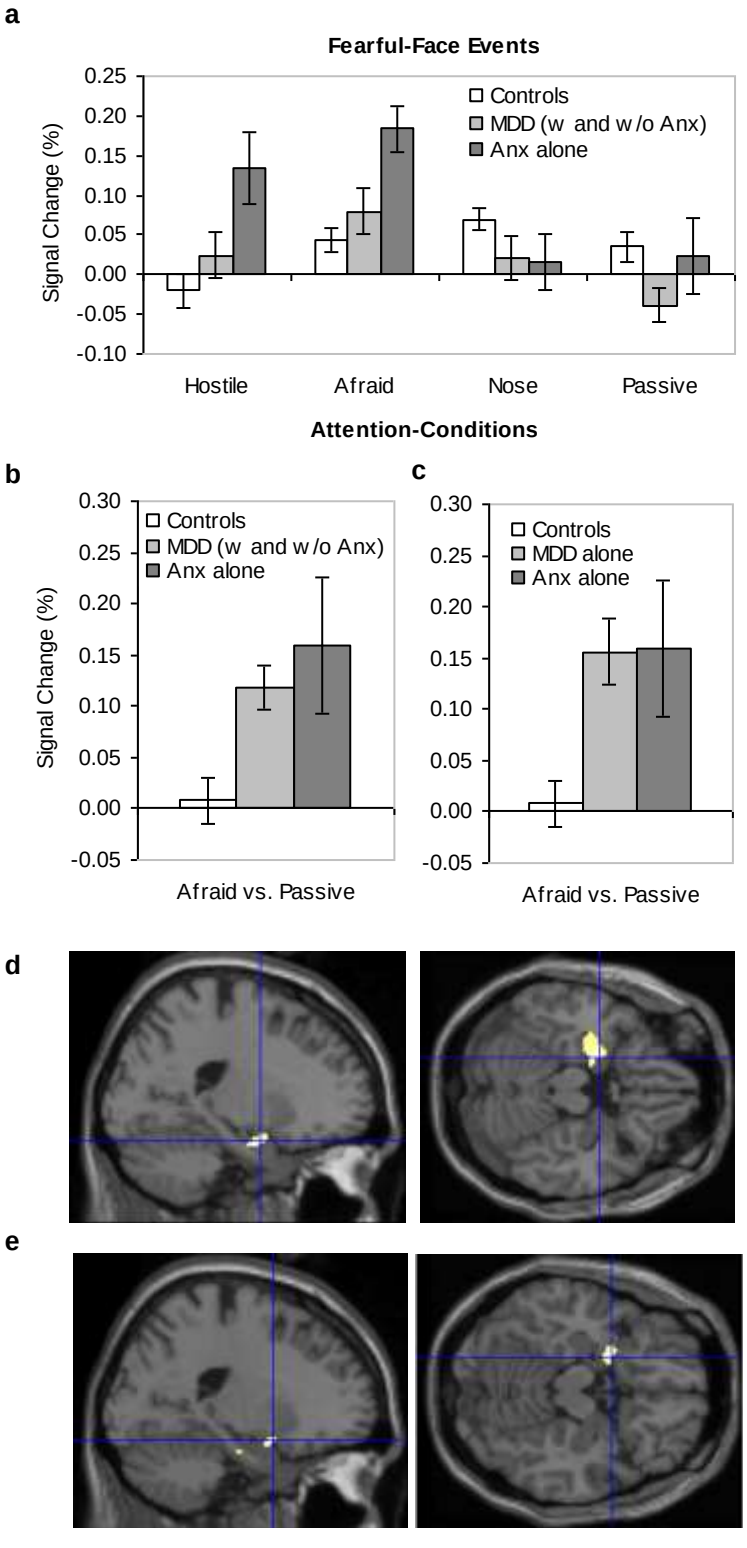

a
Fearful-Face Events
Signal Change (%)
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
Controls
MDD (w and w/o Anx)
Anx alone
Hostile
Afraid
Nose
Passive
Attention-Conditions
b
Signal Change (%)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Controls
MDD (w and w/o Anx)
Anx alone
Afraid vs. Passive
c
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Controls
MDD alone
Anx alone
Afraid vs. Passive
d
e

Figure 2.

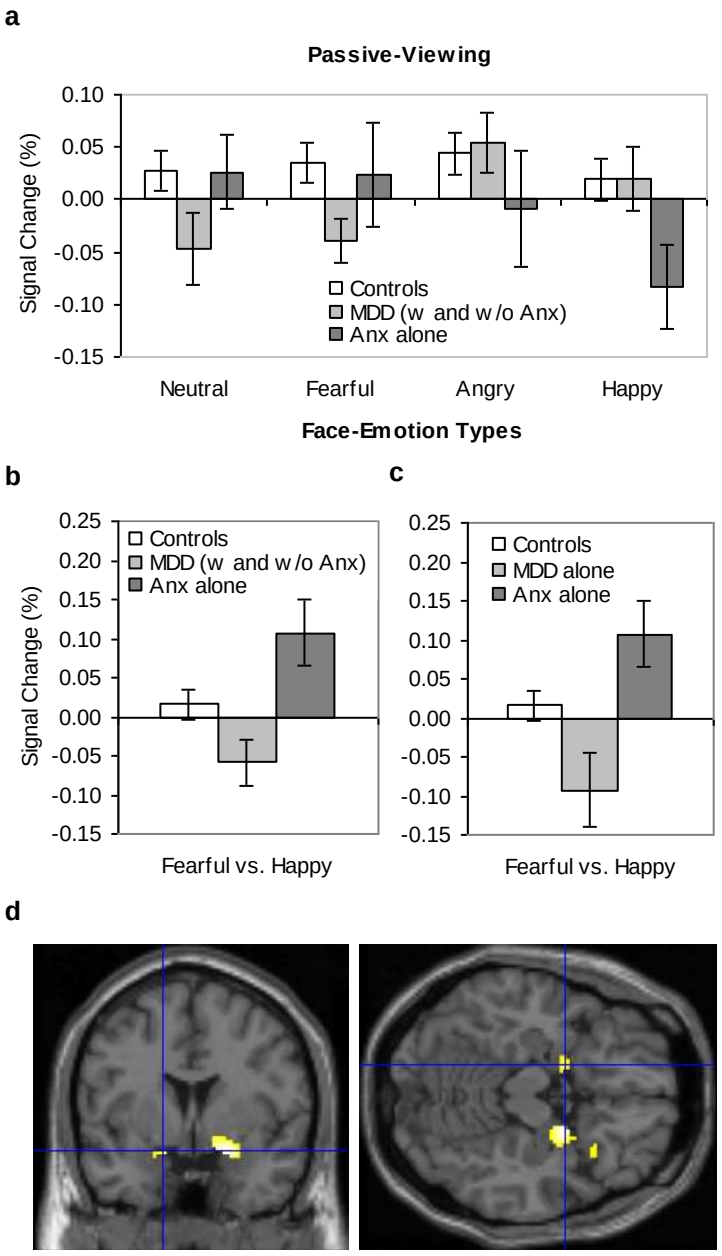

a
Passive-Viewing
Signal Change (%)
0.10
0.05
0.00
-0.05
-0.10
-0.15
Controls
MDD (w and w/o Anx)
Anx alone
Neutral
Fearful
Angry
Happy
Face-Emotion Types
b
c
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
Controls
MDD (w and w/o Anx)
Anx alone
Fearful vs. Happy
Controls
MDD alone
Anx alone
Fearful vs. Happy
d

Figure 3.

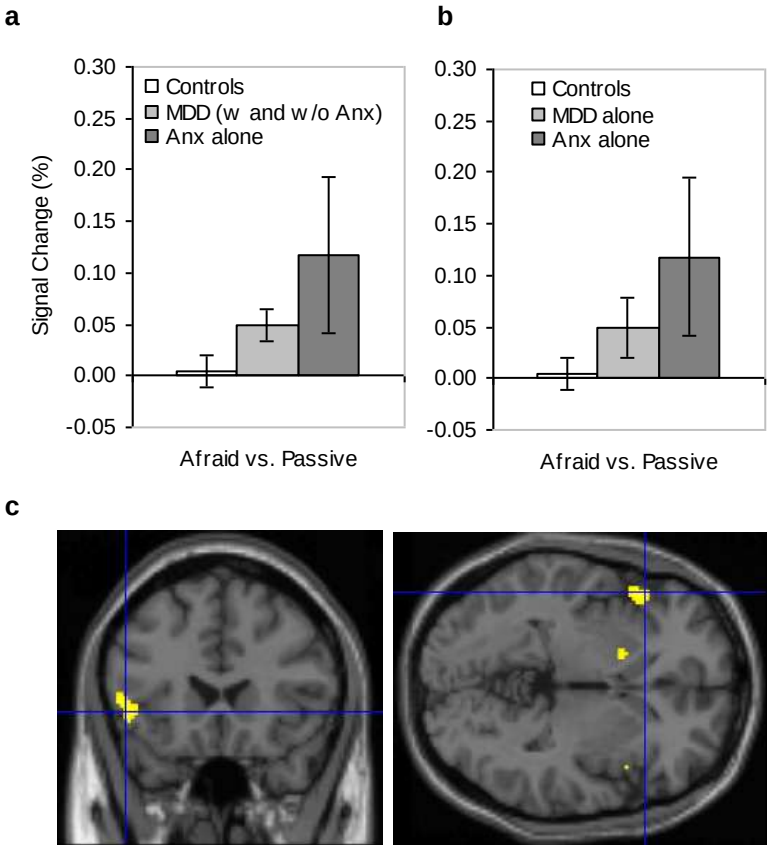
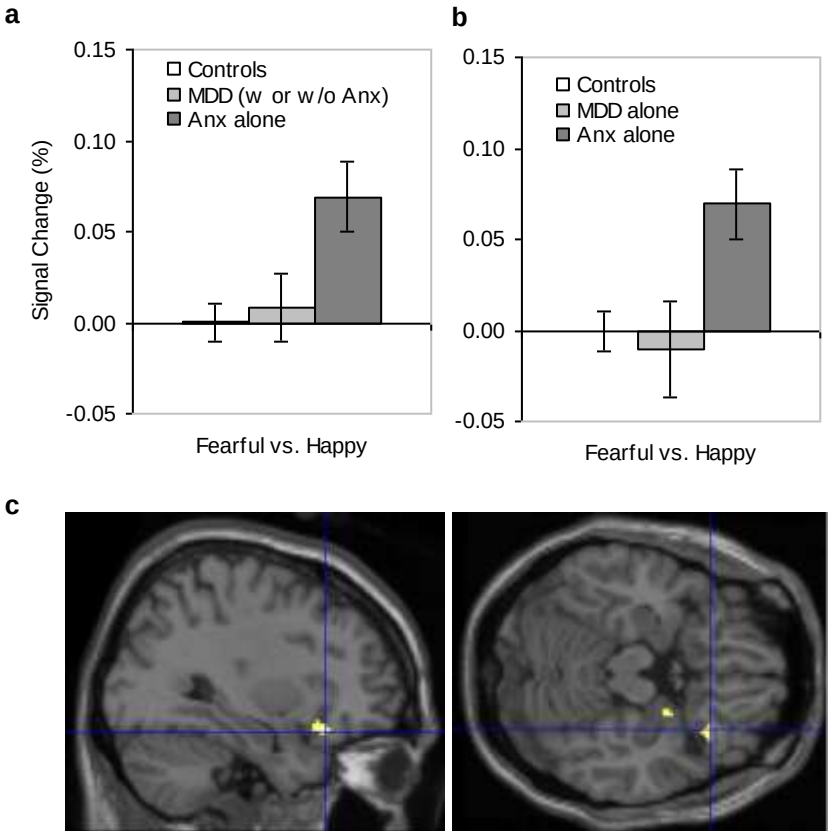

a
b
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Signal Change (%)
Controls
MDD (w and w/o Anx)
Anx alone
Afraid vs. Passive
Controls
MDD alone
Anx alone
Afraid vs. Passive
c

Figure 4.


a
b
0.15
0.10
0.05
0.00
-0.05
Signal Change (%)
Controls
MDD (w or w/o Anx)
Anx alone
Fearful vs. Happy
Controls
MDD alone
Anx alone
Fearful vs. Happy
c