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

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1                   **Title: Common and specific amygdala-function perturbations in**  
2                   **depressed versus anxious adolescents**

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**ABSTRACT**41  
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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:  $p\text{-values} \leq .03$ ). 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.

67

## INTRODUCTION

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

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

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

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

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## METHODS

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### PARTICIPANTS

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(Table 1)

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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<sup>27</sup> included 7 of the 16 anxiety and 16 of the 45 healthy adolescents.

Diagnoses were assessed using the Schedule-for-Affective-Disorders-and-Schizophrenia-for-School-Aged-Children (K-SADS).<sup>37</sup> As described previously,<sup>27,29</sup> 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.

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

### 123 TASK

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

133

### 134 MRI PROCEDURES

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

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

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

## 148 DATA ANALYSIS

### 149 *Behavioral Data*

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

155

### 156 *fMRI Data*

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

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

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



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

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

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

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

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

209

210

## RESULTS

211

### SAMPLE CHARACTERISTICS & BEHAVIOR

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

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

223 (Table 2)

224

225 IMAGING

226 *Amygdala activation*

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

230 (Table 3)

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

236

237 *Fearful-face viewing.*

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

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

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

252 (Figure 1)

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

260

261 *Passive-viewing.*

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

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

273 specificity” perspective, opposite patterns emerged in patient groups: anxiety patients showed  
 274 activation and MDD patients showed deactivation for fearful versus happy faces. This difference  
 275 was significant whether MDD/anxiety patients were included (left:  $t_{40}=3.3$ ,  $p=.002$ , Fig. 2b; right:  
 276  $t_{40}=2.8$ ,  $p=.008$ ) or excluded (left:  $t_{26}=3.1$ ,  $p=.004$ , Fig. 2c; right:  $t_{26}=2.4$ ,  $p=.02$ ). Both patient  
 277 groups also showed significantly different responses from healthy controls, with hyper-activation  
 278 in anxiety (left:  $t_{59}=2.2$ ,  $p=.03$ ; right:  $t_{59}=2.6$ ,  $p=.01$ ) and hypo-activation in MDD (left only: with  
 279 or without comorbid anxiety disorder:  $t_{69}=-2.2$ ,  $p=.03$ ; without comorbid anxiety disorder:  $t_{55}=-$   
 280 2.4,  $p=.02$ ).

281 (Figure 2)

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

291

### 292 **OFC activation**

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

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

301 (Figure 3)

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

309 (Figure 4)

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

315

### 316 COMMENT

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

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

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

335

### 336 **Disorder-Specificity**

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

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

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

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

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

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



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

385

### 386 *Shared-Diathesis*

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

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

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

405

#### 406 **Development**

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

418

#### 419 **Behavioral Data**

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

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

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

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

447

#### 448 *Limitations*

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

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

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

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

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

493

494

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**FIGURE LEGENDS**

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688

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

691 a. Bar graphs of left amygdala activation to fearful-faces relative to fixation (error bars reflect

692 standard errors) displaying the group [healthy controls, MDD (with and without anxiety

693 disorder), anxiety disorder alone]-by-attention-condition interaction. A similar activation pattern

694 was found for the right amygdala and when excluding comorbid MDD/anxiety patients (not

695 shown in Figure).

696 b and c. Bar graphs of left amygdala activation to fearful-faces during afraid-ratings versus

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

698 activation among both anxiety patients and MDD patients (MDD with and without anxiety

699 disorder (b.), MDD alone (c.)) compared to healthy controls, with no difference between anxiety

700 and MDD patients.

701 d and e. The “fearful-afraid-vs.-fearful-passive” contrast evidences significantly greater left

702 amygdala activation in (d.) anxiety alone patients compared to controls (Montreal Neurological

703 Institute (MNI) coordinates: -20, -2, -20,  $p=.001$  (shown in figure); -10, -4, -16,  $p=.002$ ; MNI

704 coordinates are small volume corrected (svc)) and (e.) MDD alone patients compared to controls

705 (MNI coordinates: -20, 4, -16,  $p=.007$ ; svc). Highlighted areas indicate regions where the

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

707 uncorrected threshold was set at  $p=.0005$  (d.) and  $p=.005$  (e.)).

708

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

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

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

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

728

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



739 Figure 4. OFC activation in the “fearful-passive-vs.-happy-passive” contrast.  
740 a and b. Bar graphs of right OFC activation to fearful-faces during passive viewing of fearful vs.  
741 happy faces “fearful-passive-vs.-happy-passive” contrast) showing significantly enhanced  
742 activation among anxiety patients compared to MDD patients and compared to healthy controls.  
743 c. The “fearful-passive-vs.-happy-passive” contrast evidences significantly greater right lateral  
744 OFC activation in anxiety patients as compared to MDD patients (with and without comorbid  
745 anxiety: MNI coordinates: 32, 24, -18,  $p=.005$ , svc; no suprathreshold voxels emerge for the  
746 anxiety versus MDD alone comparison). Highlighted areas indicate regions where the differences  
747 in BOLD activation between groups were significant (for displaying purposes, uncorrected  
748 threshold was set at  $p=.0005$ ).  
749  
750

751

**TABLES**

752

753

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

755 psychopathology

<b>Measure</b>	<b>Healthy Controls (n = 45)</b>	<b>MDD with and without anxiety disorder (n= 26)</b>	<b>MDD without anxiety disorder (n=12)</b>	<b>Anxiety disorder without MDD (n = 16)</b>
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) <sup>1</sup>	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)

<sup>1</sup> 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

<b>Behavioral Measures</b>	<b>MDD</b>		
	<b>Healthy controls (n = 45)</b>	<b>with and without anxiety disorder (n = 25)</b>	<b>Anxiety disorder without MDD (n = 14)</b>
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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761 Table 3. Statistical analyses of Regions of Interest (Omnibus repeated-measures ANOVA)

Effect §	Comorbid MDD/anxiety patients included §						Comorbid MDD/anxiety patients excluded &					
	Left Amygdala			Right Amygdala			Left Amygdala			Right Amygdala		
	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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**FIGURES**

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Figure 1.

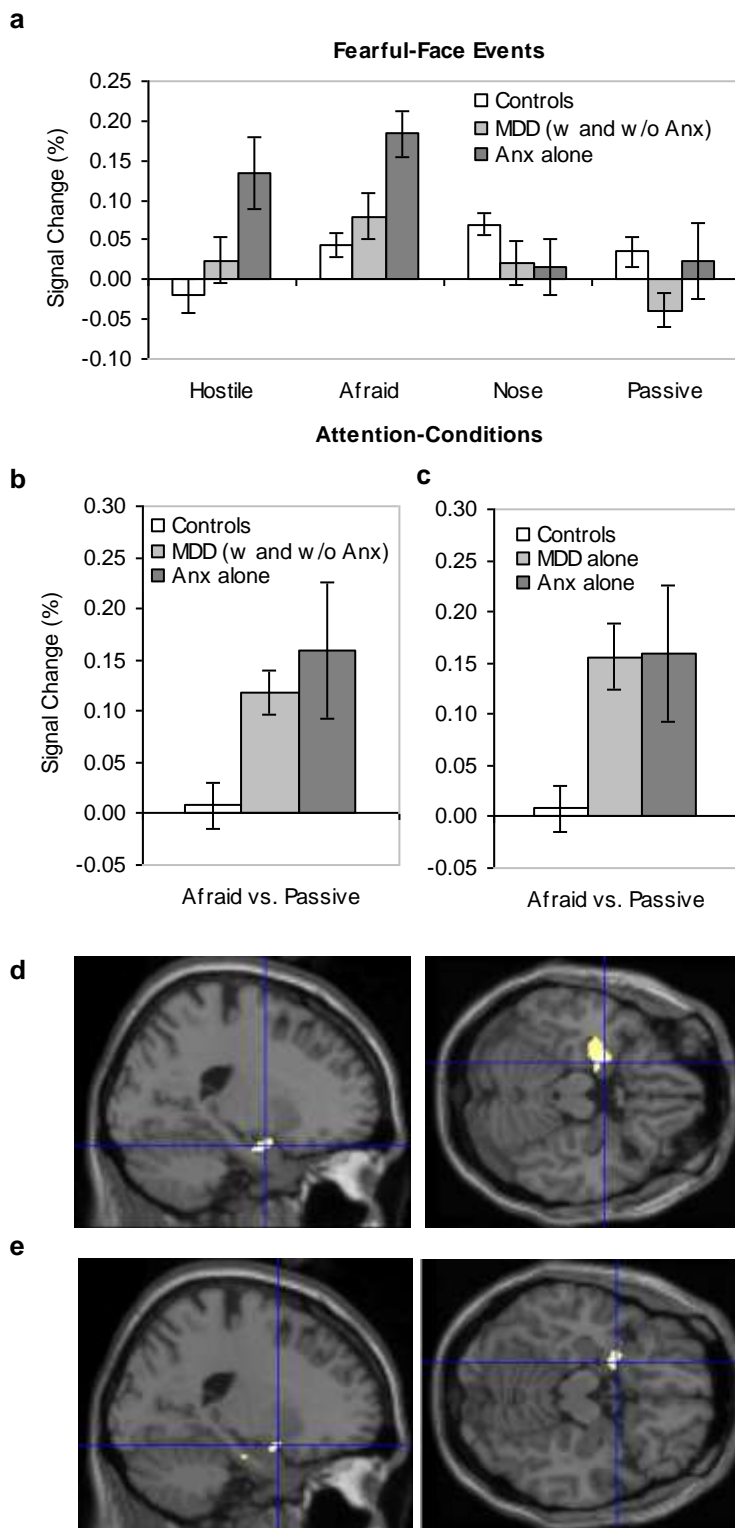
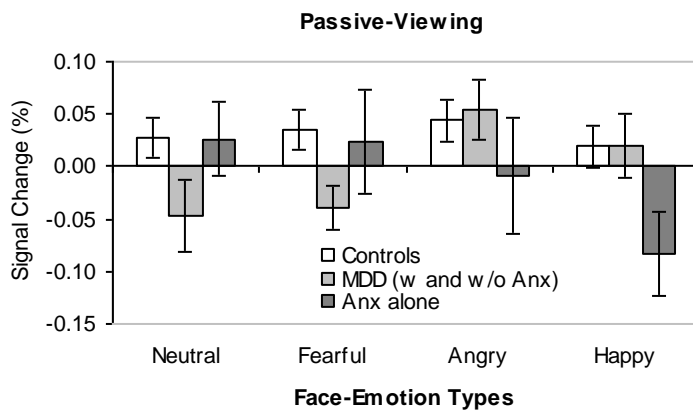
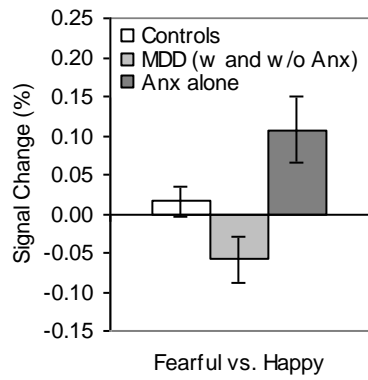


Figure 2.

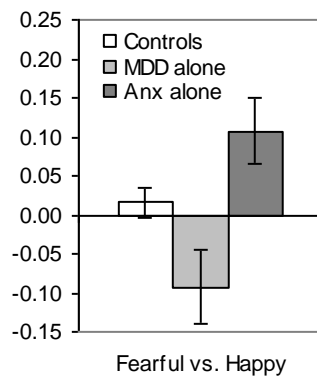
**a**



**b**



**c**



**d**

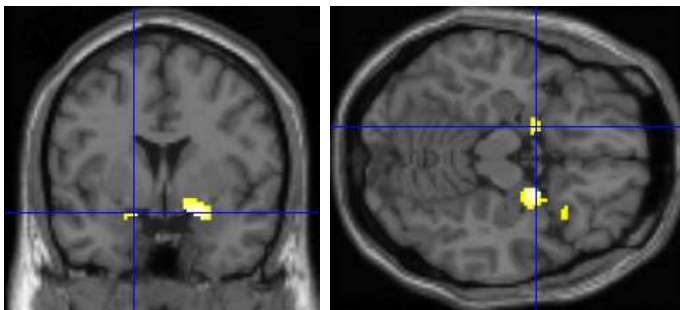




Figure 3.

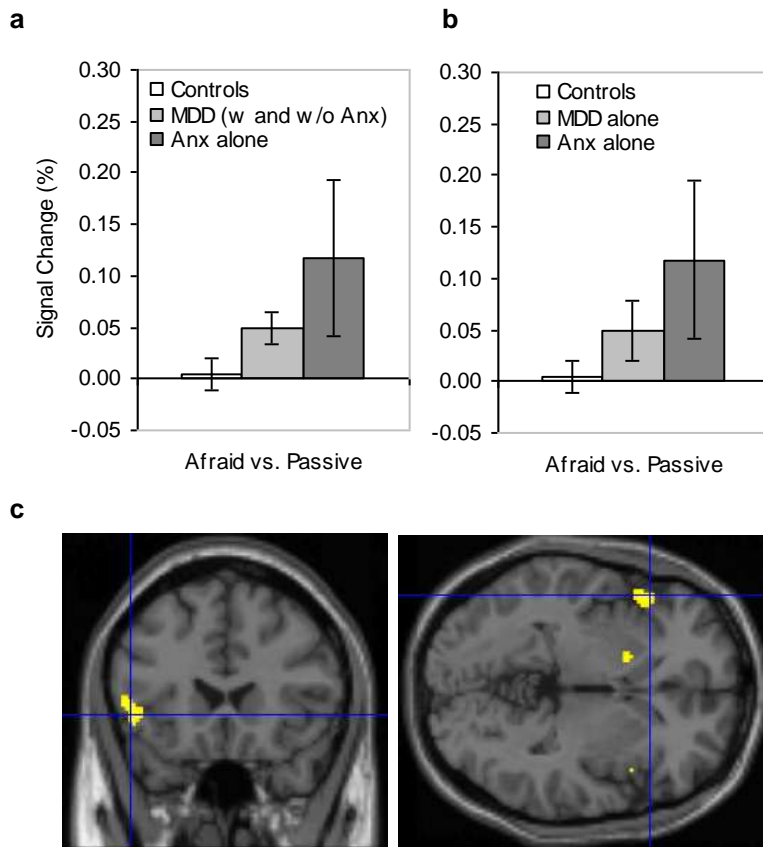


Figure 4.

