frontiers in
HUMAN NEUROSCIENCE

SUPPLEMENTARY MATERIAL
published: 07 July 2010
doi: 10.3389/fnhum.2010.00056

The interaction between gaze and facial expression in the amygdala and extended amygdala is modulated by anxiety

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Received: 17 December 2009; paper pending published: 16 February 2010; accepted: 11 June 2010; published online: 07 July 2010.

Citation: Ewbank MP, Fox E and Calder AJ (2010) The interaction between gaze and facial expression in the amygdala and extended amygdala is modulated by anxiety. *Front. Hum. Neurosci.* **4**:56. doi: 10.3389/fnhum.2010.00056

AMYGDALA RELATIONSHIP WITH BEHAVIORAL RATINGS

Given that the relationship between the amygdala response and anxiety mirrored the relationship between behavioral ratings and anxiety, we investigated whether there was a direct relationship between behavioral ratings and amygdala response. A simple regression, including rating scores for each participant, revealed no correlation between amygdala activation and ratings for any of the contrasts (DA > AA: P = 0.19 svc; DF > DN: P = 0.36 svc; AF > AN: $P = 0.52$ svc). In addition, to determine whether anxiety alone was able to predict amygdala activity, we performed a further regression analysis looking at the relationship between amygdala activity and state anxiety, this time partialing out the influence of behavioral ratings. We found that all reported correlations between amygdala activation and state anxiety remained significant: DA > DN (32, 4, −26) $Z = 2.89$, $P < 0.05$ svc); DF > DN (36, 0, −24) $Z = 2.92$, $P < 0.05$); AF > AN: RH: (18, −2, −14) $Z = 3.68$, $P < 0.005$ svc); LH: (16, −6, −18) $Z = 3.69$, $P < 0.005$ svc). Thus, the results indicate that the amygdala activation found to angry and fearful faces appears to be related primarily to individual differences in anxiety rather than subjective ratings facial emotion; although, anxiety is clearly related to both the amygdala response and behavioral ratings.

SOCIAL ANXIETY

We also examined whether effects of state anxiety could be explained by variation in social anxiety, as measured by the fear of negative evaluation scale (FNE). We performed partial correlations to examine the relationship between amygdala activity and anxiety after partialing out the variance accounted for by FNE. This showed that the correlations between state anxiety and the amygdala response remained significant for all previous reported contrasts: direct gaze angry faces relative to averted gaze angry faces: (22, −6, −12, $z = 2.78$, $P < 0.05$ svc) and averted gaze fear minus averted gaze neutral faces: LH: (−16, −6, −18, $z = 2.96$, $P < 0.03$ svc); RH:(18, −2, −16, $z = 3.09$, $P < 0.02$ svc). The interaction between gaze and expression [(anger direct > anger averted) > (fear direct > fear averted)] in the right amygdala also remained significant (24, −4, −12, $z = 2.93$, $P < 0.04$ svc). FNE was unable to independently predict the amygdala response for any contrasts (P's > 0.14). The correlation between state anxiety and averted gaze neutral versus direct gaze neutral faces remained borderline significant ($P = 0.07$).

We noted that correlations between state anxiety and the amygdala response to angry faces were restricted to the right hemisphere, with fearful faces producing bilateral activation. In order to test this hemispheric difference we performed an additional analysis, comparing direct angry faces against averted fearful faces as a function of state anxiety. For direct anger > averted fear (DF > DA) we found no difference in either left or right amygdala (P's > 0.16), and no significant difference in the amygdalae response for averted fear > direct anger (P's > 0.52). Thus, any apparent lateralization of fear and anger in the amygdala was not statistically robust.

SEX DIFFERENCES

We investigated whether the effects of state anxiety were related to sex differences. Male ($n = 16$) and female participants ($n = 11$) did not differ in terms of state anxiety scores ($Z = -0.35$, $P = 0.72$). Partial correlations covarying out the influence of sex on the amygdala response to angry and fearful faces showed that correlations between state anxiety and the amygdala response remained significant – direct gaze angry faces relative to averted gaze angry faces: (22, −6, −12, $z = 2.90$, $P < 0.04$ svc); averted gaze fear minus averted gaze neutral faces: LH: (−16, −6, −18, $z = 3.08$, $P < 0.02$ svc); RH:(18, −2, −16, z = 3.11, $P < 0.02$ svc). The interaction between gaze and expression [(anger direct > anger averted) > (fear direct > fear averted)] in the right amygdala also remained significant (22, −6, −12, $z = 3.03$, $P < 0.03$ svc). The borderline correlation between state anxiety and the amygdala response to averted gaze neutral versus direct gaze neutral faces remained unchanged ($P = 0.06$).

FEAR DEFINED AMYGDALA ROI

In order to compare the amygdala response to expression and gaze with that previously observed by Adams et al. (2003) we performed an additional analysis using a functionally defined amygdala ROI.

Table S1 | Regions showing activation across all participants, independent of anxiety.

Region	MNI coordinates			Voxels	Z
	x	y	z		
AVERTED FEAR > AVERTED NEUTRAL					
Left occipital cortex	–18	–82	0	48	3.86
Right occipital cortex/ primary visual cortex	32	–94	4	26	3.53
Right superior temporal sulcus	60	–42	8	34	3.30
AVERTED ANGER > AVERTED NEUTRAL					
Left fusiform gyrus	–40	–48	–14	72	4.37
Right occipital cortex/ primary visual cortex	32	–94	2	63	3.78
Right fusiform gyrus	42	–42	–14	34	3.59
DIRECT ANGER > DIRECT NEUTRAL					
Right fusiform gyrus	42	–40	–14	12	3.39
DIRECT FEAR > AVERTED FEAR					
Left amygdala	–22	–8	–16	73	4.37*
DIRECT ANGER > AVERTED ANGER					
Right medial frontal gyrus	14	6	56	94	4.17
Right posterior parietal lobe	24	–62	58	650	3.92
Right occipital cortex/primary visual cortex	6	–80	10	77	3.67

**P < 0.05 small volume corrected for amygdala ROI All other activations are significant at P < 0.001 whole brain uncorrected.*

Table S2 | Regions showing activation across all participants, independent of anxiety for averted relative to direct gaze fearful faces. All activations are significant at $P < 0.001$ whole brain uncorrected.

Region	MNI coordinates			Voxels	Z
	x	y	z		
AVERTED FEAR > DIRECT FEAR					
Right mid frontal gyrus	26	28	34	433	4.82
Superior occipital gyrus	40	–80	28	231	4.56
Left cerebellum	–32	–66	–34	502	4.09
Precuneus	4	–62	50	282	3.83
Left mid frontal gyrus	–38	14	30	143	3.80
Right superior temporal gyrus	68	–40	14	53	3.78
Precentral gyrus	68	–6	26	49	3.73
Inferior frontal gyrus	–54	24	0	91	3.73
Posterior cingulate cortex	10	–40	42	48	3.64
Temporoparietal junction	38	–56	32	27	3.62
Right anterior insula	28	28	10	17	3.61
Left superior temporal sulcus	–44	–48	16	79	3.61
Right occipital cortex/primary visual cortex	18	–98	6	59	3.55
Right pos/superior temporal sulcus	50	–58	12	100	3.48
Right mid/superior temporal sulcus	52	–36	–10	27	3.46
Right cerebellum	36	–24	28	19	3.41
Intraparietal sulcus	–46	–40	42	10	3.29
Thalamus	–6	–16	16	18	3.23

Using the same contrast as Adams et al. (fearful faces > baseline) we identified a region of the left, but not right amygdala ($P < 0.05$ uncorrected) that showed an increased response to fearful faces, consistent with that found by Adams et al. Percentage signal change for each subject (independent of anxiety) was then extracted using the peak voxel and entered in a 2 × 2 repeated measures ANOVA (expression × gaze). The ANOVA revealed no difference between ambiguous and unambiguous threat (averted anger and direct fear > averted fear and direct anger) in this region ($F < 1$). Indeed, consistent with activation patterns found using the anatomically

Table S3 | Regions in which activation showed a significant positive relationship with trait anxiety scores. All activations significant at $P < 0.001$ whole brain uncorrected.

Region	MNI coordinates			Voxels	Z
	x	*y*	*z*		
DIRECT FEAR > DIRECT NEUTRAL					
Precuneus	–12	–54	38	169	3.65
DIRECT ANGER > DIRECT NEUTRAL					
Left medial prefrontal cortex	–14	56	–4	17	3.42
DIRECT ANGER > AVERTED ANGER					
Left superior temporal sulcus	–44	–46	0	68	4.42
Right superior temporal sulcus	60	–50	14	144	3.80
Right anterior temporal lobe	52	14	–28	17	3.59
DIRECT NEUTRAL > DIRECT ANGER					
Right ventrolateral prefrontal cortex	38	44	4	67	3.56

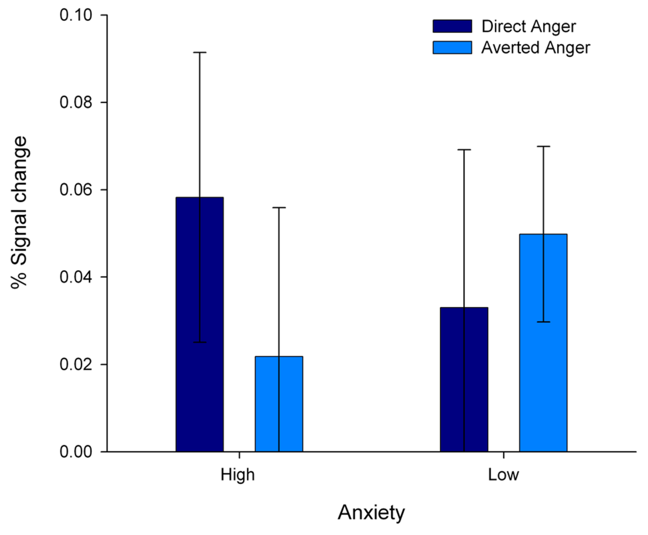

FIGURE S1 | We divided participants into high and low anxiety groups to illustrate the effects of gaze on the amygdala response to angry faces. A 2 × 2 ANOVA (gaze × anxiety) revealed no interaction between anxiety and gaze ($P = 0.22$). As can be seen **Figure S1**, the pattern of response in the low-anxious group shows a trend towards that found by Adams et al. (2003); however, the absence of a significant effect makes it difficult to draw any firm conclusions from this analysis.

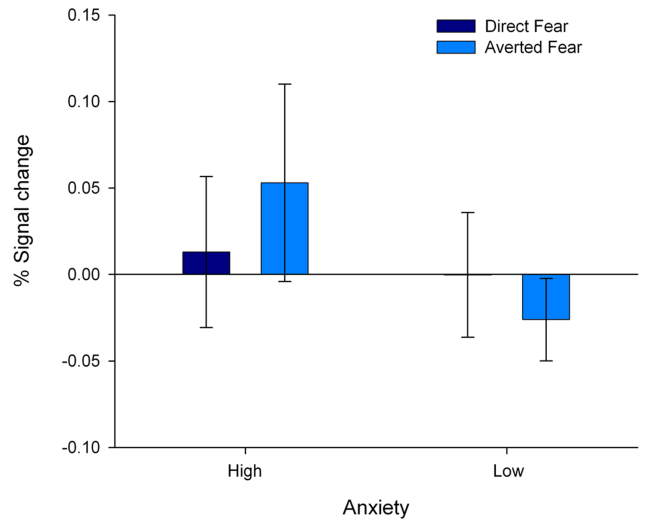

FIGURE S2 | Analogous to the anger contrasts, a 2 × 2 ANOVA (gaze × anxiety) on fearful faces revealed no interaction between anxiety and gaze ($P = 0.18$) (Figure S2). We note these patterns again suggest that the low anxious group show a trend towards effects that accord more closely with those of Adams et al. (2003) (i.e., a greater response to direct fear > averted fear) while high-anxious show the opposite pattern. However, the absence of a significant interaction makes it difficult to draw any firm conclusions.

defined amygdala ROI, paired comparisons revealed a borderline increase for direct gaze fear compared to averted gaze fear faces ($t(27) = 1.95$, $P = 0.06$), and no difference between averted and direct gaze anger faces ($P > 0.53$).

THE AMYGDALA's RESPONSE TO ANGRY AND FEARFUL FACES IN LOW AND HIGH ANXIOUS PARTICIPANTS

In order to test whether high and low anxious groups alone may replicate the findings of previous studies, e.g., Adams et al. (2003) Whalen et al. (2001), we compared the amygdala response to angry and fearful faces after dividing participants into high and low anxious groups using a median split. Whereas Whalen et al. (2001) found an increased amygdala response to fearful versus angry faces, a comparison of direct gaze fearful faces vs. direct gaze angry faces in the low anxious group only, revealed no significant difference in the amygdala ($P = 0.64$). It should be noted that dividing participants into high and low groups using a median split results in the exclusion of four participants (those with the median score), which reduces the power of the design. In addition, Whalen et al. (2001) used a high resolution acquisition sequence.

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