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The adaptive threat bias in anxiety: amygdala- dorsomedial prefrontal cortex coupling and aversive amplification

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Abstract

Functionally, anxiety serves to increase vigilance towards aversive stimuli and improve the ability to detect and avoid danger. We have recently shown, for instance, that anxiety increases the ability to a) detect and b) instigate defensive responses towards aversive and not appetitive face stimuli in healthy individuals. This is arguably *the key* adaptive function of anxiety, yet the neural circuitry underlying this valence-specific effect is unknown. In the present translational study, we sought evidence for the proposition that dorsomedial regions of the prefrontal (DMPFC) and cingulate cortex constitute the human homologue of the rodent prelimbic and are thus associated with increased amygdala responding during this adaptive threat bias in anxiety. To this end, we applied a novel functional connectivity analysis to healthy subjects (N=20) identifying the emotion of fearful and happy faces in an fMRI scanner under anxious (threat of unpredictable foot shock) and non-anxious (safe) conditions. We showed that anxiety significantly increased positive DMPFC-amygdala connectivity during the processing of fearful faces. This effect was a) valence-specific (it was not seen for happy faces), b) paralleled by faster behavioral response to fearful faces, and c) correlated positively with trait anxiety. As such we provide the first experimental support for an anxiety-mediated, valence-specific, DMPFC-amygdala *aversive amplification* mechanism in healthy humans. This may be homologous to the rodent prelimbic-amygdala circuit and may, given the relationship with trait anxiety, underlie vulnerability to anxiety disorders. This study thus pinpoints a key neural mechanism in adaptive anxiety and highlights its potential link to maladaptive anxiety.

Keywords

amygdala; dMPFC; functional connectivity; prelimbic; anxiety; threat bias

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1 Introduction

Anxiety is adaptive; its functional role is to facilitate sensory processing and prompt rapid activation of defense mechanisms (Baas et al. 2006; Cornwell et al. 2007); especially in response to threatening stimuli (Blanchard et al. 2011; Davis et al. 2009; Grillon 2008). We have recently shown, for instance, that threat of shock-induced anxiety selectively increases a) detection of (Robinson et al. 2011a) and b) psychophysiological defense responses towards (Grillon and Charney 2011) fearful faces while at the same time having no effect on detection or response to happy faces. This affective bias can significantly improve survival chances. For instance, when anxiously walking home at night, this bias might improve the ability to detect and respond to a mugger waiting in the dark. It is arguable, in fact, that this bias towards threat is *the key* adaptive function of anxiety. Yet the neural circuitry underlying this crucial effect is unknown. The present study thus aims to clarify the functional connectivity underlying the threat-specific affective bias in anxiety.

Recent research in rodents has highlighted a medial prefrontal cortical (prelimbic)-amygdala circuit which can increase behavioral responses to threat (Sierra-Mercado et al. 2011). More specifically, stimulation of the prelimbic region increases, via excitatory top-down neurons, activity within the amygdala and subsequent behavioral fear responses (Sierra-Mercado et al. 2011; Vidal-Gonzalez et al. 2006). A human homologue of this top-down *aversive amplification* mechanism could thus plausibly drive the selective bias towards fearful faces in anxiety. Milad et al. have demonstrated, on the basis of regions critical for fear conditioning in healthy individuals and in PTSD, that dorsal regions of the cingulate and medial-prefrontal cortex in humans are functionally equivalent to the rodent's prelimbic (Milad et al. 2009; Milad et al. 2007). This is consistent with the observation that dorsal regions of the prefrontal cortex and cingulate show positive connectivity with the amygdala (Etkin et al. 2011) and with the observation that the same dorsal regions (specifically the dorsomedial prefrontal cortex; DMPFC) are most consistently activated by induced anxiety in healthy individuals (Mechias et al. 2010). However, the functional connectivity between these regions during anxiety and, specifically, their relationship with the adaptive threat bias is unknown. We thus sought evidence for the proposition that DMPFC-amygdala connectivity can promote a bias towards threat in anxious, healthy, humans.

We adapted the task used in our prior behavioral and psychophysiological studies for fMRI. Healthy volunteers identified fearful and happy faces while they experienced anxiety induced via threat of unpredictable foot-shock. We predicted an enhanced behavioral response to fearful, but not happy, faces during threat (Robinson et al. 2011a). Moreover, as stimulation of the prelimbic in rodents underlies an increase in amygdala-mediated aversive behavior during anxiety, we expected to see *positive* coupling between the DMPFC (Mechias et al. 2010; Milad et al. 2007) and the amygdala in a functional connectivity analysis (Etkin et al. 2011; Vidal-Gonzalez et al. 2006) and, more specifically, *increased* positive coupling between the DMPFC and the amygdala during processing of fearful faces under induced anxiety (Robinson et al. 2011a).

2 Materials and methods

Right-handed volunteers (N=23; 3 subjects were subsequently excluded due to response box failure; final N=20; 8 females) aged between 18–50 successfully passed a screening procedure. Physical and mental health of the participants was determined by a physical examination performed by a physician, a clinical interview conducted by a trained psychologist using the Structured Clinical Interview for the DSM-IV (First et al. 2002), and self-report of medication and drug use confirmed by urine toxicology analysis. Exclusion criteria included: psychotropic drug exposure within 3 weeks, major medical or neurological

illness; illicit drug use or alcohol abuse within 1 year; lifetime history of alcohol or drug dependence; psychiatric disorders; current pregnancy or breast feeding; structural brain abnormalities on MRI; general MRI exclusions. Participants provided informed consent as approved by the NIMH Combined Neuroscience IRB and were monetarily compensated for their time. Subjects completed the Spielberger state/trait anxiety questionnaire (Spielberger) (mean trait 32, mean state 26).

2.1 Task design

A schematic of the paradigm is presented in Figure 1. The task was programmed in eprime (Psychology Software Tools, Inc,USA). A face stimulus (1000ms) (Ekman and Friesen 1976) was followed by a jittered (2000–4000ms) fixation cross. Subjects identified the emotion of the face ("happy" or "fearful") during the stimulus presentation using a response box (left and right button counterbalanced) with their right hand. Thirty-seconds of fixation at the start and end of each run served as a baseline for fMRI analysis. Reaction time (RT) and performance accuracy were analysed in 2(threat,safe)*2(fearful,happy) ANOVAs, simple effects were Bonferonni adjusted.

2.1.1 Threat procedure—The threat procedure was based upon previous translational psychophysiology paradigms (Grillon et al. 2008; Grillon et al. 2009) adapted for neuroimaging (e.g., (Cornwell et al. 2007; Hasler et al. 2010). There were 3 scanning runs. Each run consisted of 3 alternating (counterbalanced) safe and threat conditions (14 faces per condition) separated by a 4000–6000ms jitter. The words "You are now safe from shock" or "You are now at risk of shock" were presented at the start of each condition. A blue border surrounded safe trials and a red border surrounded threat trials. Prior to the task, subjects went through a shock work-up procedure to control for individual differences in shock tolerance. A single unpredictable shock was delivered to the foot (DS7A; Digitimer,UK) during one of the three threat conditions on each run (3 per subject). Efficacy of the manipulation was verified at the end of each run by asking subjects to retrospectively rate how afraid, anxious and happy they felt (on a scale of 1–10) during each condition.

2.2 Functional imaging procedure

A GE Signa HDXT 3-Tesla 940 scanner was used to acquire structural and functional images. The functional sequence comprised 3 EPI sessions of 255 volume acquisitions: flip angle 90°; repetition time=2000ms; echo time=30ms; FOV=22×22 cm; slice thickness=3.5mm; slice spacing 0mm; matrix=64×64 sagittal slices with ASSET to increase coverage area. The first 10 volumes from each session were discarded to allow for magnetisation equilibrium prior to acquisition. The structural sequence comprised an MPRAGE anatomical reference image: flip angle 10°; repetition time=7200ms; echo time=3000 ms; inversion time=450; FOV=24×24cm; slice thickness=1.0mm; slice spacing=0mm; matrix=224×224 for spatial coregistration and normalisation.

2.2.1 Image analysis—Images were pre-processed and analyzed using SPM8 (Functional Imaging Laboratory, Institute of Neurology, UK). Preprocessing consisted of within-subject realignment, coregistration, segmentation, spatial normalization and spatial smoothing. Functional scans were coregistered to the MPRAGE structural image, which was processed using a unified segmentation procedure combining segmentation, bias correction and spatial normalization; the same normalisation parameters were then used to normalise the EPI images. Finally the EPI images were smoothed with a Gaussian kernel of 8 mm full-width at half-maximum. The canonical hemodynamic response function and its temporal derivative were used as covariates in a general linear model.

2.2.1.1 Event related analysis: At the first level we built a model for each subject based on event-related onsets (0 duration) for our variables of interest: 1) fearful faces under threat[TF]; 2) happy faces under threat[TH]; 3) fearful faces under safe[SF]; and 4) happy faces under safe[SH]. We also included 'nuisance' variables (0 duration): 1) threat block onset/offset; 2) safe block onset/offset; 3) movement parameters; 4) shock onset. Aside from these events, the task consisted of jittered fixation periods, including 30s of fixation at the beginning and end of the task, which constituted the implicit baseline. At the second level, we entered the activity associated with the 4 variables of interest for each subject into a 2(threat)*2(face) flexible-factorial design. A whole-brain voxel-wise analysis was performed alongside region-of-interest (ROI) analyses for 1) a sphere with a 5mm radius around the MNI rDMPFC peak 6,38,38 identified by Mechias et al (2010), 2) the 8mm ventral prefrontal cortex (vPFC) sphere (±24,34,−12)used by Indovina et al. 2011 and 3) for the anatomically-defined amygdala (Tzourio-Mazoyer et al. 2002). The Mechias et al. peak was selected on the basis that it was identified in meta-analysis across multiple instructed threat studies and thus plausibly more robust, and less subject to bias, than a peak identified by any one individual study. Following this, to more precisely specify interactions, standardized betas from the 6, 38, 38 peak identified by Mechias et al (2010) were extracted for each trial type using a custom script and analyzed in a 2(threat,safe) *2(fear,happy) ANOVA in SPSS. Simple effects were Bonferonni adjusted.

2.2.1.2 Connectivity analysis: For first level connectivity analysis we extracted, for each subject, a deconvolved time course averaged across the right amygdala cluster identified within the anatomical right amygdala ROI in the group event-related analysis (all trials>baseline). This timecourse was then included in a generalized PPI model alongside a psychological regressor and a PPI regressor for each of the four variables of interest (as above) as well as the movement parameters. The resulting PPI connectivity estimates were then taken into a 2(threat)*2(face) second level flexible-factorial design. Whole brain and rDMPFC ROI connectivity analysis proceeded as above, with activity representing estimates of connectivity with the right amygdala cluster. All imaging coordinates are presented as MNI coordinates.

3 Results

3.1 Manipulation check

Subjects rated themselves as significantly more anxious ($F(1,18)=114, p<0.001$;6.1vs1.6) and afraid ($F(1,18)=81, p<0.001$;5.4vs1.4), and significantly less happy ($F(1,18)=32, p<0.001$;3.5vs6.5) during threat of shock relative to safe conditions.

3.2 Behavioral

Consistent with hypotheses, there was a significant condition x valence interaction in RT ($F(1,19)=10.6, p=0.004$). Subjects were significantly faster to respond to fearful faces under threat relative to safe conditions ($F(1,19)=14.7, p<0.001$), despite no difference in the response to happy faces across conditions (F(1,19)=0.03,NS). Moreover, this led to a trend towards a negative bias (faster responses to fearful relative to happy faces) under the threat condition ($F(1,19)=4.1, p=0.058$), which was not present under the safe condition ($F(1,19)=1.3, p=0.26$; Figure 2A). By contrast, there was no condition x valence interaction in accuracy data (F(1,19)=0.51,NS), probably due to ceiling effects (mean accuracy 89%,SD8%).

3.3 Functional Imaging

3.3.1 Event Related

rDMPFC: Whole brain analysis revealed a condition x valence interaction (contrast:[TH-TF]-[SH-SF]) within the DMPFC (whole brain peak at 8,34,38,p(*uncorrected*)=0.001; Figure 2B) as well as dorsalateral PFC and the supramarginal gyrus (Table S1A). A small volume correction (see methods) around the peak highlighted in the meta analysis (Mechias et al. 2010) revealed the same (8,34,38), peak (T=3.28,p(*FWE voxel-level-corrected*)=0.004).

Extracting data from the Mechias peak (6,38,38) revealed a main effect of threat $F(1,19)=4.6, p=0.044$ driven by increased activation during threat relative to safe. Mean RT (across all trials) correlated with mean hemodynamic activity (across all trials) in the rDMPFC: faster responses were associated with increased heamodynamic activity (1-tailed Spearman's rho;$R=-0.42, p=0.03$; Figure 2D), indicating a linear relationship between the rDMPFC and behavior. The significant threat by valence interaction ($F(1,19)=8.9, p=0.007$) was, nevertheless, driven by lowest activity during happy faces under safe conditions (Figure 2C) and so did not explain the behavioral findings.

vPFC: No condition × valence interaction was seen in the vPFC ROI used by Indolova et al 2011, even at the liberal threshold of $p<0.003$ uncorrected.

Amygdala: Activity was greater across all trials>baseline in the anatomical right amygdala (peak voxel=24, 2,−12;T=3.68,p(*FWE voxel-level corrected*)=0.003; Figure 3Ai). There was also a trend towards increased activity during threat>safe (T=2.56,p(*uncorrected*)=0.006), a trend towards a condition x valence interaction (T=2.04,p(*uncorrected*)=0.022) and a trend towards increased activity for fearful faces under threat relative to safe (T=1.72,p(*uncorrected*)=0.044).

3.3.2 Connectivity

rDMPFC-amygdala: Whole brain flexible factorial analysis of a condition × valence interaction (contrast:[TF-TH]-[SF-SH]) in connectivity to a right amygdala seed (Figure 3Ai) revealed a significant connectivity difference in, most strongly, the DMPFC and dorsal anterior cingulate (Table S1B and figure 3Aii: whole brain DMPFC peak at 10,34,40,p(*uncorrected*)<0.001 and p(*FWE cluster-corrected*) =0.01). ROI analysis revealed an adjacent peak at 8,34,40 (T=3.78,p(*FWE voxel-level-corrected*)=0.001).

Extracted data from the independently selected Mechias peak (6,38,38) revealed *positive* coupling during all trials (i.e. as rDMPFC activity increases, so does activity within the amygdala) and a main effect of threat ($F(1,19)=7.2, p=0.015$) driven by increased coupling under threat. Moreover, there was a threat by valence interaction ($F(1,19)=10.2, p=0.005$; Figure 3B) driven by increased coupling during fearful faces under threat relative to safe ($F(1,19)=30.0, p<0.001$), and no change during the processing of happy faces ($F(1,19)=0.07$,NS) thus paralleling the behavioral findings. Moreover, there was a significant negative correlation between coupling for fearful faces under threat and reaction time for fearful faces under threat. Thus, the strongest coupling was associated with the fastest responses (1- tailed Pearson's; $r=-0.4, p=0.04$; figure 3D). Coupling and reaction time did not correlate for any of the other trial types.

vPFC-amygdala: No condition × valence was seen in the vPFC ROI used by Indolova et al 2011, even at the liberal threshold of $p<0.003$ uncorrected.

3.3.3 Relationship with trait anxiety—Amygdala-rDMPFC coupling for fear faces under threat (and no other trial; safe fear, r=0.07; safe happy, r= 0.4; threat happy, r=0.2) correlated with trait anxiety (2- tailed Spearman's rho;r=0.5,p=0.03; Figure 3C). Similarly, there was a significant correlation between trait anxiety and A) the increase in coupling across conditions for fearful (threat fear minus safe fear; 1-tailed Spearman's rho;r=0.4,p=0.04) but not happy faces (r=−0.3); and B) the decrease in reaction time for fearful (2-tailed Spearman's rho;r=−0.5,p=0.04) but not happy faces (r=−0.3). Thus, the more vulnerable an individual is to anxiety, the greater the positive amygdala-rDMPFC coupling, the greater the anxiety-potentiated coupling and the faster the anxiety-potentiated speed for fearful faces. There was no correlation between trait anxiety and activity for any trial type in the regions of interest in the event-related analysis.

4 Discussion

This study sought to outline the neural mechanism underlying the adaptive bias towards threat in anxiety. Consistent with hypotheses, the dorsomedial prefrontal cortex showed positive connectivity with the amygdala but, more importantly, anxiety increased this connectivity as well as the response speed during the identification of fearful faces, while having no effect on behavior or connectivity during the identification of happy faces. This therefore provides the first experimental evidence of a valence-specific anxiety-mediated aversive amplification mechanism in humans which may be the homologue of the rodent prelimbic-amygdala circuit. Moreover, we demonstrate that the greatest anxiety-potentiated coupling between these regions is seen in those most vulnerable to anxiety disorders.

4.1 Top-down amplification in the adaptive anxiety threat bias

We have previously shown that anxiety can promote the detection of (Robinson et al. 2011a), and defensive responses towards (Grillon and Charney 2011) fearful face stimuli but the neural mechanism underlying this key adaptive function of anxiety remained unidentified. In the present study, we applied a novel functional connectivity approach to resolving this question and saw a pattern of positive connectivity between dorsomedial prefrontal cortex and the amygdala which was associated with a behavioral bias towards threatening stimuli. This pattern is consistent with a recent review of connectivity studies (across a wide array of different tasks), which, broadly speaking, showed a pattern of positive connectivity with the amygdala in dorsomedial prefrontal cortex (Etkin et al. 2011) and, perhaps more importantly, suggests that this region of the brain may perform a comparable function to the prelimbic region in rodents, which can actively excite the amygdala and increase fear responding (Sierra-Mercado et al. 2011; Vidal-Gonzalez et al. 2006). Moreover, we showed that this positive connectivity was significantly greater during the viewing of fearful faces under anxious (relative to non-anxious) conditions, and was associated with increased speed to identify fearful faces under anxious (relative to non-anxious) conditions. As such, this anxiety-potentiated coupling may represent the mechanism by which anxiety adaptively increases vigilance towards threat (Grillon 2008; Grillon and Charney 2011; Robinson et al. 2011a). That is to say, this pattern of positive connectivity is consistent with a mechanism of *aversive amplification*. It is not that the dorsomedial prefrontal cortex and amygdala are more active during anxious relative to non-anxious conditions; they are more *positively coupled* during anxiety. As such, when an emotional stimulus is relevant to the task being performed, dorsomedial regions of the prefrontal cortex may *amplify* amygdala responses. This is broadly consistent with the DMPFC regions activated when subjects are told to explicitly up-regulate their response to aversive stimuli (Ochsner and Gross 2005) and suggests that this effect is unique to aversive responses. Specifically, the same effect was *not* seen for matched happy face stimuli during this task arguing against a role in appetitive processing or a general salience effect.

4.2 Relationship with maladaptive anxiety

A key question is to what extent a mechanism like this may relate to vulnerability to anxiety disorders. Excessive, unchecked activity within the adaptive process of cell division can lead to a pathological state of cancer. Along the same lines, the adaptive threat-bias mechanism outlined in the present paper may, if experienced to excess or chronically, underlie the maladaptive threat-biases in disorders such as generalized anxiety disorder or post-traumatic stress disorder. The present data in fact support this proposition. Specifically, anxiety-potentiated coupling during fearful faces correlated positively with trait anxiety. That is to say, the more vulnerable an individual is to an anxiety disorder as measured by increased trait anxiety(Clark and Watson 1991), the more they recruit the putative top-down aversive amplification mechanism. Indeed a recent study showing hyperactivity within these same regions in the healthy twins of PTSD sufferers suggests that this may have a genetic basis (Shin et al. 2011). Thus, if the putative DMPFC-amygdala amplification mechanism is recruited too readily or chronically, it may lead to sustained attentional bias for threat and an interpretation of the world as a dangerous place. This may then increase anxiety, further promote the threat bias, and trigger a full-blown anxiety disorder in vulnerable individuals. This would then, in turn, explain why, once an individual suffers from an anxiety disorder, they show increased dorsal prefrontal responses to aversive stimuli alongside increased amygdala responses (Milad et al. 2009; Mobbs et al. 2010).

4.3 Relationship with top-down inhibition

It is, however, important to acknowledge that many recent accounts of anxiety outline how increased detection of aversive stimuli can be a result of failed top-down *inhibition* rather than excessive amplification. Specifically, in the face of anxious distractors (in, for example, stroop tasks), individuals with either anxiety disorders or vulnerability to anxiety disorders fail to ‘top-down’ inhibit responses to aversive distractors (Bishop 2007; Etkin et al. 2011; Etkin et al. 2010; Mogg et al. 1993). This results in increased ‘attentional capture’ by these aversive stimuli and a failure to complete the non-emotional primary task (e.g. identification of word color in stroop tasks) (Bishop 2007). These effects have been linked in translational research to a failure to extinguish fear responses (Bishop 2007; Milad et al. 2009; Quirk and Beer 2006) driven by a failure of the infralimbic region–a region directly adjacent to the prelimbic - to inhibit amygdala responses to aversive stimuli (Milad et al. 2009; Quirk and Beer 2006). In humans, the functional equivalent of this infralimbic inhibition failure (Etkin et al. 2010; Indovina et al. 2011; Milad et al. 2009) is thought to be reduced activity in ventral regions of the prefrontal and cingulate cortex that results in reduced ability of these cortical regions to inhibit aversive responses in the amygdala (Bishop 2007; Milad et al. 2009; Milad et al. 2006; Quirk and Beer 2006). In the present study we did not see an inhibitory effect in the ventral prefrontal cortex, which is likely because the present task did not require the inhibition of any distractor. Subjects were asked to *identify* (and not inhibit) fearful and happy faces. If, by contrast, we had asked subjects to identify, say, houses whilst they were *also* shown fearful faces, then we would likely have seen reduced inhibition of these *distracting* responses to aversive faces in anxiety. To borrow an analogy from the introduction of this paper, if amplification is necessary to detect a mugger on the street, the inhibitory mechanism is necessary when a mugger appears on the TV whilst you are trying to complete your tax return. In the first instance, the mugger is a direct threat which could get in the way of getting home safely, while in the second instance, the mugger is a non-threatening distraction from the present task. It is likely that both *hyperactivity* within a top-down aversive amplification mechanism and *hypoactivity* within a top-down aversive inhibition mechanism may underlie trait vulnerability to anxiety disorders (such a pattern has, in fact, recently been shown in individuals vulnerable to the recurrence of mood disorders (Kerestes et al. 2011)), but that the precise effect observed is dependent upon demands of the task adopted. As an aside, it is worth nothing that these effects in anxiety

may be distinct from those in depression, in which recent findings show (albeit with a different reward and punishment reversal-learning paradigm) that an appetitive (and *not* aversive) specific deficit may be driven by attenuated activity within frontal-striatal pathways (Robinson et al. 2011b).

4.4 Limitations

It is important to note that the PPI connectivity analysis we adopted cannot determine direction of connections (Friston et al. 1997) or the neuronal pathways driving correlations. It will thus take further research to clarify directionality and circuitry. Moreover, although there are apparent discrepancies in labels for broadly similar activations across studies, it is important to recognize that the dorsal medial prefrontal cortex and cingulate are spatially close and there is substantial overlap across the clusters identified in the present connectivity findings and the clusters with nominally different peaks in other studies (Bishop 2007; Etkin et al. 2011; Knight et al. 2004; Mechias et al. 2010; Milad et al. 2007; Mobbs et al. 2009; Mobbs et al. 2010; Phelps et al. 2004; Shackman et al. 2011) to name but a few). It is thus possible that these studies all identify the same region and that differences in scanners, participants and task design drive at least some of the spatial discrepancies. Nevertheless, future comparison of different paradigms within the same sample, perhaps with higher resolution fMRI (e.g. 7T), combined with receptor-targeted PET imaging, may allow us to disentangle regional differences in function.

5 Conclusion

We provide novel functional connectivity evidence for a putative dorsal top-down amplification mechanism in healthy individuals, whereby increased positive coupling between the amygdala and DMPFC may drive the adaptive threat bias in anxiety. This crucial anxiety-mediated, positive coupling-driven, threat bias has never previously been delineated and provides a translational link to the rodent prelimbic-amygdala circuit. Moreover, this coupling is stronger in those more vulnerable to anxiety disorders, indicating that hyperactivity within a putative adaptive mechanism may also contribute to the maladaptive state of anxiety.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Baas JMP, Milstein J, Donlevy M, Grillon C. Brainstem Correlates of Defensive States in Humans. Biological Psychiatry. 2006; 59:588–593. [PubMed: 16388780]

Bishop SJ. Neurocognitive mechanisms of anxiety: an integrative account. Trends in Cognitive Sciences. 2007; 11:307–316. [PubMed: 17553730]

Blanchard DC, Griebel G, Pobbe R, Blanchard RJ. Risk assessment as an evolved threat detection and analysis process. Neuroscience & Biobehavioral Reviews. 2011; 35:991–998. [PubMed: 21056591]

Clark LA, Watson D. Tripartite model of anxiety and depression: Psychometric evidence and taxonomic implications. J Abnorm Psychol. 1991; 100:316–336. [PubMed: 1918611]

Cornwell BR, Baas JMP, Johnson L, Holroyd T, Carver FW, Lissek S, Grillon C. Neural responses to auditory stimulus deviance under threat of electric shock revealed by spatially-filtered magnetoencephalography. Neuroimage. 2007; 37:282–289. [PubMed: 17566766]

Davis M, Walker DL, Miles L, Grillon C. Phasic vs Sustained Fear in Rats and Humans: Role of the Extended Amygdala in Fear vs Anxiety. Neuropsychopharmacology. 2009; 35:105–135. [PubMed: 19693004]

Ekman, P.; Friesen, WV. Pictures of Facial Affect. Consulting Psychologists; Palo Alto, CA: 1976.

Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends in Cognitive Sciences. 2011; 15:85–93. [PubMed: 21167765]

Etkin A, Prater KE, Hoeft F, Menon V, Schatzberg AF. Failure of Anterior Cingulate Activation and Connectivity With the Amygdala During Implicit Regulation of Emotional Processing in Generalized Anxiety Disorder. Am J Psychiatry. 2010; 167:545–554. [PubMed: 20123913]

First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders Patient edition (SCID-I/P, 11/2002 Revision). New York State Psychiatric Institute; New York: 2002.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and Modulatory Interactions in Neuroimaging. Neuroimage. 1997; 6:218–229. [PubMed: 9344826]

Grillon C. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology. 2008; 199:421–437. [PubMed: 18058089]

Grillon, C.; Charney, DR. Psychophysiology. 2011. In the face of fear: anxiety sensitizes defensive responses to fearful faces. In press

Grillon C, Chavis C, Covington MF, Pine DS. Two-Week Treatment With the Selective Serotonin Reuptake Inhibitor Citalopram Reduces Contextual Anxiety but Not Cued Fear in Healthy Volunteers: A Fear-Potentiated Startle Study. Neuropsychopharmacology. 2008; 34:964–971. [PubMed: 18800069]

Grillon C, Pine DS, Lissek S, Rabin S, Bonne O, Vythilingam M. Increased Anxiety During Anticipation of Unpredictable Aversive Stimuli in Posttraumatic Stress Disorder but not in Generalized Anxiety Disorder. Biological Psychiatry. 2009; 66:47–53. [PubMed: 19217076]

Hasler G, van der Veen JW, Grillon C, Drevets WC, Shen J. Effect of Acute Psychological Stress on Prefrontal GABA Concentration Determined by Proton Magnetic Resonance Spectroscopy. Am J Psychiatry. 2010; 167:1226–1231. [PubMed: 20634372]

Indovina I, Robbins TW, Núñez-Elizalde AO, Dunn BD, Bishop SJ. Fear-Conditioning Mechanisms Associated with Trait Vulnerability to Anxiety in Humans. Neuron. 2011; 69:563–571. [PubMed: 21315265]

Kerestes R, Ladouceur CD, Meda S, Nathan PJ, Blumberg HP, Maloney K, Ruf B, Saricicek A, Pearlson GD, Bhagwagar Z, Phillips ML. Abnormal prefrontal activity subserving attentional control of emotion in remitted depressed patients during a working memory task with emotional distracters. Psychological Medicine FirstView. 2011:1–12.

Knight DC, Cheng DT, Smith CN, Stein EA, Helmstetter FJ. Neural Substrates Mediating Human Delay and Trace Fear Conditioning. The Journal of Neuroscience. 2004; 24:218–228. [PubMed: 14715954]

Mechias M-L, Etkin A, Kalisch R. A meta-analysis of instructed fear studies: Implications for conscious appraisal of threat. Neuroimage. 2010; 49:1760–1768. [PubMed: 19786103]

Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, Zeidan MA, Handwerger K, Orr SP, Rauch SL. Neurobiological Basis of Failure to Recall Extinction Memory in Posttraumatic Stress Disorder. Biological Psychiatry. 2009; 66:1075–1082. [PubMed: 19748076]

Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL. A Role for the Human Dorsal Anterior Cingulate Cortex in Fear Expression. Biological Psychiatry. 2007; 62:1191–1194. [PubMed: 17707349]

Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: Implications for human brain imaging and anxiety disorders. Biological Psychology. 2006; 73:61–71. [PubMed: 16476517]

Mobbs D, Marchant JL, Hassabis D, Seymour B, Tan G, Gray M, Petrovic P, Dolan RJ, Frith CD. From Threat to Fear: The Neural Organization of Defensive Fear Systems in Humans. The Journal of Neuroscience. 2009; 29:12236–12243. [PubMed: 19793982]

Mobbs D, Yu R, Rowe JB, Eich H, FeldmanHall O, Dalgleish T. Neural activity associated with monitoring the oscillating threat value of a tarantula. Proceedings of the National Academy of Sciences. 2010

Mogg K, Bradley BP, Williams R, Mathews A. Subliminal processing of emotional information in anxiety and depression. J Abnorm Psychol. 1993; 102:304–311. [PubMed: 8315143]

Ochsner KN, Gross JJ. The cognitive control of emotion. Trends in Cognitive Sciences. 2005; 9:242–249. [PubMed: 15866151]

Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction Learning in Humans: Role of the Amygdala and vmPFC. Neuron. 2004; 43:897–905. [PubMed: 15363399]

Quirk GJ, Beer JS. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Current Opinion in Neurobiology. 2006; 16:723–727. [PubMed: 17084617]

Robinson O, Letkiewicz A, Overstreet C, Ernst M, Grillon C. The effect of induced anxiety on cognition: threat of shock enhances aversive processing in healthy individuals. Cognitive, Affective, & Behavioral Neuroscience. 2011a:1–11.

Robinson OJ, Cools R, Carlisi CO, Sahakian BJ, Drevets WC. Ventral Striatum Response During Reward and Punishment Reversal Learning in Unmedicated Major Depressive Disorder. Am J Psychiatry. 2011b appi.ajp.2011.11010137.

Shackman AJ, Salomons TV, Slagter HA, Fox AS, Winter JJ, Davidson RJ. The integration of negative affect, pain and cognitive control in the cingulate cortex. Nat Rev Neurosci. 2011; 12:154–167. [PubMed: 21331082]

Shin LM, Bush G, Milad MR, Lasko NB, Brohawn KH, Hughes KC, Macklin ML, Gold AL, Karpf RD, Orr SP, Rauch SL, Pitman RK. Exaggerated Activation of Dorsal Anterior Cingulate Cortex During Cognitive Interference: A Monozygotic Twin Study of Posttraumatic Stress Disorder. Am J Psychiatry. 2011 appi.ajp.2011.09121812.

Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable Roles of Prelimbic and Infralimbic Cortices, Ventral Hippocampus, and Basolateral Amygdala in the Expression and Extinction of Conditioned Fear. Neuropsychopharmacology. 2011; 36:529–538. [PubMed: 20962768]

Spielberger, CD. State-Trait Anxiety Inventory. John Wiley & Sons, Inc;

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage. 2002; 15:273–289. [PubMed: 11771995]

Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learning & Memory. 2006; 13:728–733. [PubMed: 17142302]

Highlights

- Anxiety adaptively increases vigilance to aversive stimuli
- We used fMRI to examine emotional face processing during induced anxiety
- Increased dMPFC-amygdala coupling was associated with increased aversive vigilance
- Correlates with trait anxiety and may thus drive anxiety disorder vulnerability
- May be the human homologue of the rodent prelimbic-amygdala circuit

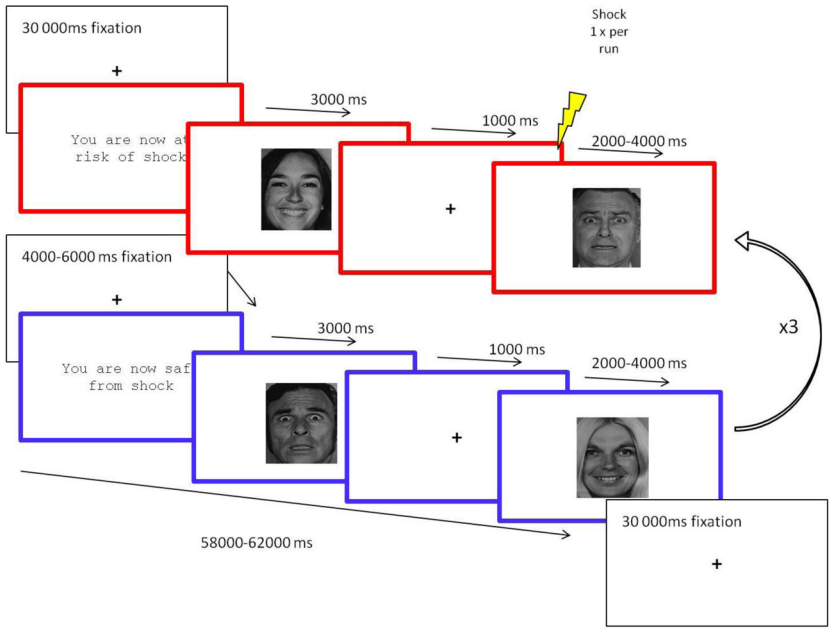

Figure 1. Task overview

Subjects (N=20) identified happy and fearful faces under alternating safe (non-anxious) and threat of shock (anxious) conditions.

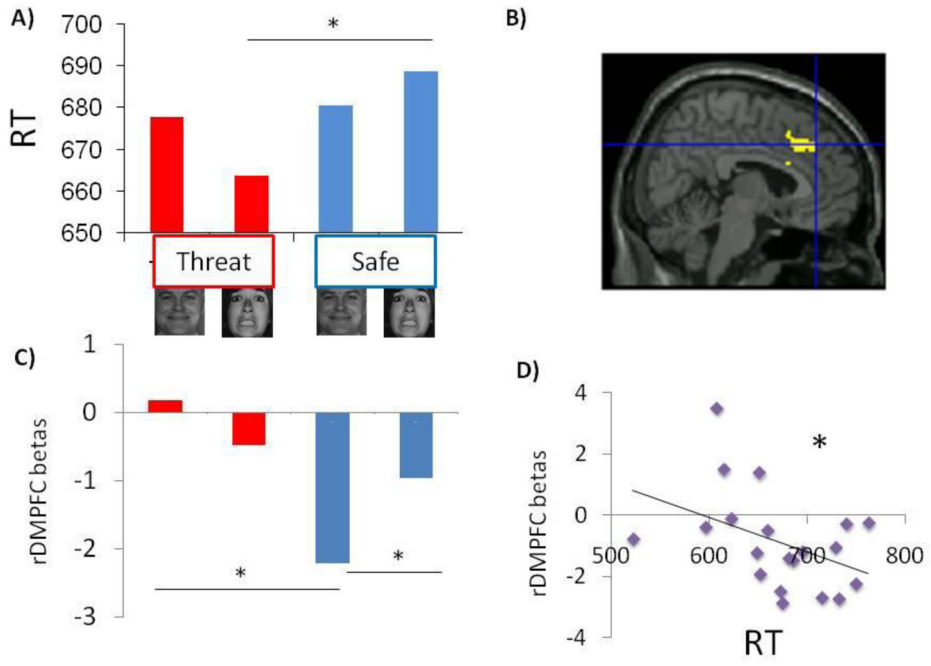

Figure 2. rDMPFC activity and aversive behavior

*=p<0.05

A)Anxiety induced by threat of shock significantly reduces reaction time (RT) to fearful, but not happy faces

B)rDMPFC shows a threat x valence interaction in activation. Crosshairs point to 6, 38, 38 (Mechias et al. 2010) image presented at P<0.005 uncorrected for display purposes.

C)Breakdown of task effects in rDMPFC fails to explain behavior

D)Collapsed across all trials, greater activity in rDMPFC is associated with reduced reaction time R=−0.42,p(one tailed)=0.03

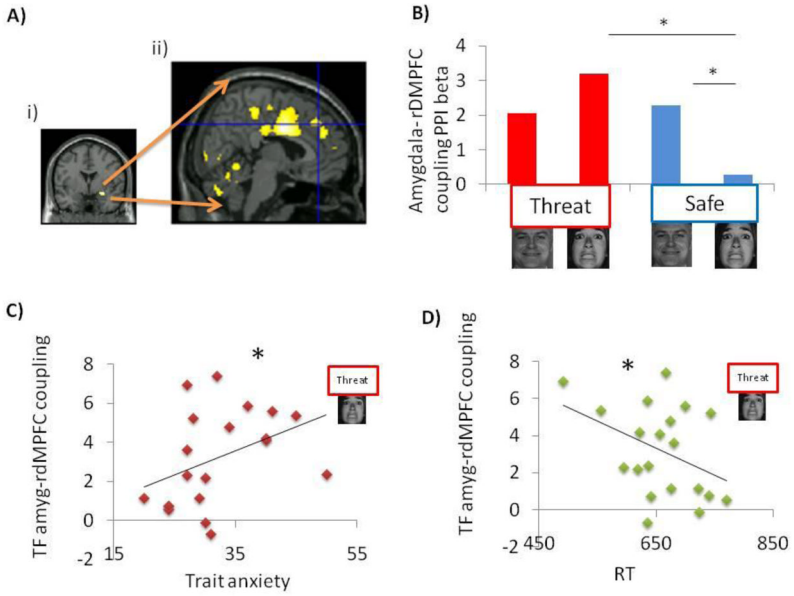

Figure 3. rDMPFC-amygdala coupling and relationship with trait anxiety

*=p<0.05

A)Amygdala is significantly active in all trials>baseline (i; image p(FWE corrected)<0.05) and shows a threat x valence interaction in connectivity with the rDMPFC (ii; image p(uncorrected)<0.001; crosshairs point to 6, 38, 38).

B)The positive rDMPFC-amygdala connectivity at 6, 38, 38 is significantly greater during the processing of fearful faces under anxious relative to non-anxious conditions.

C)The strongest positive coupling between the rDMPFC and the amygdala during the processing of fearful faces in anxious conditions is seen in those most at risk of anxiety disorders as measured by Spielberger trait anxiety (r=0.5, p(2-tailed)=0.03).

D)The strongest coupling is also seen during the fastest responses to fearful faces in anxious conditions (r=−0.4, p(1-tailed)=0.04).