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The role of serotonin in the neurocircuitry of negative affective bias: Serotonergic modulation of the dorsal medial prefrontal-amygdala 'aversive amplification' circuit

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ABSTRACT

Serotonergic medications can mitigate the negative affective biases in disorders such as depression or anxiety, but the neural mechanism by which this occurs is largely unknown. In line with recent advances demonstrating that negative affective biases may be driven by specific medial prefrontal-amygdala circuitry, we asked whether serotonin manipulation can alter affective processing within a key *dorsal* medial prefrontal-amygdala circuit: the putative human homologue of the rodent prelimbic-amygdala circuit or 'aversive amplification' circuit. In a double-blind, placebo-controlled crossover pharmaco-fMRI design, subjects (N = 19) performed a forced-choice face identification task with word distractors in an fMRI scanner over two separate sessions. On one session subjects received dietary depletion of the serotonin precursor tryptophan while on the other session they received a balanced placebo control diet. Results showed that dorsal medial prefrontal responding was elevated in response to fearful relative to happy faces under depletion but not placebo. This negative bias under depletion was accompanied by a corresponding increase in positive dorsal medial prefrontal-amygdala functional connectivity. We therefore conclude that serotonin depletion engages a prefrontal-amygdala circuit during the processing of fearful relative to happy face stimuli. This same 'aversive amplification' circuit is also engaged during anxiety induced by shock anticipation. As such, serotonergic projections may inhibit engagement of the 'aversive amplification' circuit and dysfunction in this projection may contribute to the negative affective bias in mood and anxiety disorders. These findings thus provide a promising explanation for the role of serotonin and serotonergic medications in the neurocircuitry of negative affective bias.

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Introduction

The neuromodulator serotonin has a long established role in the manifestation and treatment of psychiatric disorders. One key effect of serotonergic medications is to modulate 'negative affective bias', the persistent focus on negative life experiences, (Cools et al., 2008a, 2008b; Dayan and Huys, 2009; Harmer, 2008) which is seen in both depression and anxiety (Elliott et al., 2011; Reinecke et al., 2011). The neural mechanism mediating this effect is, however, unclear. Recent work in negative affective bias has evoked anxiety via threat of electric shock to instantiate bias in healthy individuals, manifest towards fearful relative to happy faces (Robinson et al., 2012a). This work has linked such biases to increased *functional connectivity* within a dorsal medial prefrontal cortex (dmPFC)-amygdala 'aversive amplification' circuit (Robinson et al., 2011b, 2012a). Serotonin may thus affect this circuitry to modulate affective bias, thereby contributing to mood and anxiety disorders. Here, we test this hypothesis by combining a procedure that reduces serotonin via acute tryptophan depletion (ATD) in healthy individuals (Crockett et al., 2012; Dayan and Huys, 2008; Robinson et al., 2012b), with procedures previously shown to reveal negative affective biases and associated perturbations in amygdala-based connectivity (Robinson et al., 2011b, 2012a).

This study attempts to tie together two parallel lines of negative affective bias related translational research. The first concerns amygdala–prefrontal interactions and their role in the processing of aversive stimuli. The amygdala activates in response to emotional faces (Adolphs, 2002) and amygdala *hyperac*tivity to fearful vs. happy faces is a marker of the negative affective bias in both depression (Siegle et al., 2007) and anxiety (Blair et al., 2008). However, amygdala responding is partly controlled by the medial prefrontal cortex (Price and Drevets, 2012; Quirk and Beer, 2006). Translational research has shown that the dmPFC/dorsal anterior cingulate (dACC) and amygdala comprise an 'aversive amplification' circuit which serves to potentiate responses to aversive stimuli, homologous to the rodent prelimbic-amygdala circuit (Milad et al., 2007; Robinson

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et al., 2012a; Sierra-Mercado et al., 2011). Specifically, rodent research demonstrates that prelimbic stimulation leads to increased activity within the amygdala and increased behavioral bias towards aversive stimuli (Sierra-Mercado et al., 2011). In humans, an increase in *positive* dmPFC/dACC–amygdala coupling (Robinson et al., 2012a) (as dmPFC activity increases, so does activity within the amygdala) corresponds with a comparable bias towards fearful relative to happy faces on a forced-choice facial emotion identification task (Robinson et al., 2011b).

The second line of translational research concerns converging cognitive (Robinson et al., 2011a), computational (Dayan and Huys, 2008), clinical (Eshel and Roiser, 2010), and psychophysiological (Robinson et al., 2012a) findings suggesting that functionally active serotonergic afferents can mediate negative affective bias by *inhibiting* aversive responding in humans. Studies have shown, in fact, that ATD can increase amygdala response to face stimuli (Cools et al., 2005; Daly et al., 2010; van der Veen et al., 2007) and dorsal ACC/mPFC response during executive processing (Evers et al., 2005; Roiser et al., 2008), but none of these studies examined the role of serotonin in connectivity between these regions. Rodent research shows that increased serotonin can *increase* neuronal *inhibition* within the prelimbic circuit (Puig et al., 2005) and recent evidence suggests that increasing serotonin via chronic serotonergic medication can *reduce* resting-state dorsal mPFC connectivity to subcortical structures in humans (McCabe et al., 2011), but no studies have examined serotonin–amygdala–dorsal prefrontal interactions as they pertain to affective bias in humans. We therefore sought to ask whether serotonin *reduction* can *increase activation* within the human homologue of the rodent prelimbic-amygdala 'aversive amplification' circuit during affective processing.

Subjects completed a forced-choice emotion identification task previously used to reveal negative affective biases in healthy individuals (Robinson et al., 2011b). The task comprised faces (fearful or happy) with superimposed emotional word ('FEAR' or 'HAPPY') distractors. Serotonin was reduced following the well-established acute tryptophan depletion (ATD) procedure (Crockett et al., 2012). Given that 1) ATD increases negative affective biases (Cools et al., 2008b; Hayward et al., 2005); 2) anxiety-induced negative biases in face identification on this task (Robinson et al., 2011b) are driven by dorsal ACC/mPFC-amygdala circuitry (Robinson et al., 2012a) and 3) increased serotonin *reduces* positive dorsal mPFC-subcortical connectivity (McCabe et al., 2011) we predicted that serotonin reduction would selectively increase response of the dorsal PFC-amygdala 'aversive amplification' circuit to fearful relative to happy faces.

Materials and methods

Participants

Participants were 19 paid healthy volunteers (mean age = 25; 7 females) who gave written informed consent approved by the NIMH Human Investigation Review Board and were free to withdraw from the study without penalty. Subjects went through a comprehensive medical screen. Inclusion criteria comprised 1) no past or current axis I psychiatric disorders as per Structured Clinical Interview for DSM-IV (SCID: First et al., 2002) administered by an experienced clinician, 2) no history of a psychiatric disorder in any first-degree relatives; and 3) no use of illicit drugs or psychoactive medications as per history and confirmed by a negative urine screen on the screening visit. Participants met with a psychiatrist prior to providing consent. All participants were asked to follow a detailed low protein diet during the day preceding the study. They arrived in the morning (between 8:00 and 10:30 am) where they gave written informed consent, gave a blood sample and consumed the dietary supplements. After a wait of approximately 3.5–4.5 h, subjects completed a psychophysiological startle paradigm (results presented elsewhere Robinson et al., 2012b; ATD increased anxiety-potentiated startle, but not fear-potentiated startle), before giving a second blood sample. Immediately after this blood sample, subjects moved to the fMRI scanner and completed the fMRI task (approximately 5–6 h following dietary manipulation). The fMRI task was always performed second due to scheduling constraints within the clinical and neuroimaging facilities.

Dietary manipulation

Details are presented elsewhere (Robinson et al., 2012b) but, briefly, subjects attended two double-blind, placebo controlled sessions separated by at least a week. On both visits they consumed tablets and a low protein diet. On the depletion day these tablets contained a balanced amino acids mix excluding tryptophan, while on the placebo visit, the pills contained lactose but the metabolic kitchen at NIH supplemented the low protein diet with a commercial protein mixture (containing 2.25 g of tryptophan; Nestle nutrition beneprotein whey powder (Nestle, Vevey, Switzerland)). This modified technique addressed two issues: 1) the meals avoided the hunger frequently seen in amino acid manipulation studies and 2) the commercial protein supplement avoided the change in tryptophan levels on the placebo visit which frequently confounds depletion studies.

Task

Trials (N = 148) consisted of happy and fearful faces (presented for 1000 ms) with either the word "HAPPY" or "FEAR" superimposed in red across the face (resulting in congruent and incongruent distractors) (Etkin et al., 2006; Robinson et al., 2011b). There was a 3000–5000 ms jitter between trials and the subjects identified the emotion of the faces using a response box. The task was programmed in Eprime and presented on a screen in the fMRI scanner room. Subjects viewed the paradigm by means of a mirror attached to the head-coil.

Functional imaging

3-Tesla functional images were acquired on two identical GE Signa HDXT 3-Tesla 940 scanners in the NIH NMR facility with a functional imaging sequence comprising 384 volume acquisitions: flip-angle 90°; repetition time = 2000 ms; echo time = 30 ms; FOV = 22 × 22 cm; slice thickness = 3.5 mm; slice spacing 0 mm; matrix = 64 × 64 sagittal slices with ASSET to increase coverage area. The first 5 volumes from each run were discarded to allow for magnetization equilibrium prior to acquisition. The structural sequence comprised an MPRAGE anatomical reference image: flip angle 10°; repetition time = 7200 ms; echo time = 3000 ms; inversion time = 450; FOV = 24 × 24 cm; slice thickness = 1.0 mm; slice spacing = 0 mm; matrix = 224 × 224 for spatial co registration and normalization. For each subject, both sessions were acquired on the same scanner (thus any effect of scanner is pooled with all other between subject differences); research has demonstrated that the data acquired across scanners is replicable (Gradin et al., 2010), can be pooled (Casey et al., 1998) and that the effect of different scanners dwarfs in comparison with between-subject effects (Costafreda et al., 2007). Images were pre-processed and analyzed using SPM8 (Functional Imaging Laboratory, Institute of Neurology, UK). Serious motion artifacts in the raw epi images were repaired at the slice level (via interpolation) using the ArtRepair toolbox for SPM. Preprocessing consisted of within-subject realignment (motion correction), coregistration, segmentation, spatial normalization and spatial smoothing. The EPI images were realigned for both sessions together, then independently coregistered to a structural MPRAGE obtained during each session which was processed using a unified segmentation procedure combining segmentation, bias correction and spatial normalization. The same normalization parameters were then used to separately normalize the EPI images from each session. Finally the EPI images were smoothed with a Gaussian kernel of 8 mm full-width at half-maximum. At the first level the basis function