Published in final edited form as:

Neuroimage. 2013 September ; 78: 217–223. doi:10.1016/j.neuroimage.2013.03.075.

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 pharmacofMRI 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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Keywords

Serotonin; ATD; amygdala; dMPFC; negative bias; aversive amplification

1. 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; Cools et al. 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. 2012a; Robinson et al. 2011b). 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. 2011; 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. 2012a; Robinson et al. 2011b).

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 *hyperactivity* 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 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. 2007), 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. 2011). 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.

2 Materials and Methods

2.1 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 DSMIV (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 hours, 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 hours following dietary manipulation). The fMRI task was always performed second due to scheduling constraints within the clinical and neuroimaging facilities.

2.2 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 2.25g 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.

2.3 Task

Trials (N=148) consisted of happy and fearful faces (presented for 1000ms) 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 3000–5000ms 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.

2.4 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 = 2000ms; echo time=30ms; FOV=22×22cm; slice thickness=3.5mm; slice spacing 0mm; 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=7200ms; echo time =3000 ms; inversion time=450; FOV=24×24cm; slice thickness =1.0mm; slice spacing=0mm; matrix=224×224 for spatial co registration and normalization. For each subject, both sessions were acquired on the same scanner (thus any effects 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 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 was set to the canonical hemodynamic response function and its temporal derivatives. Separate first level events were specified for each valence (fear, happy), congruency of word distractor (congruent, incongruent) and by the congruency of the prior trial (congruent, incongruent)(Etkin et al. 2006). As such, there were 8 events of interest (e.g. prior trial congruent, current trial incongruent, fear face). The first trial of each block (i.e. when there was no prior trial) was modeled as independent event leaving 36 trials per condition (ii, ci, cc, ic), 18 of which were fearful and 18 happy (note that this is the same number of trials used previously to reveal congruency effects (Etkin et al. 2010; Etkin and Schatzberg 2012; Jarcho et al. 2013)) Motion parameters created during the realignment phase were also included as ‘nuisance’ regressors in the first-level analysis such that any variance in the signal that was associated with motion was controlled for in the regression.

2.5 Event related analysis

For each subject, a regressor representing each of the eight trial types for each session was collapsed into a 2(ATD,BAL) × 2(fear,happy) flexible factorial design (16 regressors per subject; all were entered into the flexible factorial, but grouped by valence and treatment when specifying factor and conditions within the flexible factorial, thereby averaging across congruency). All whole brain peaks (MNI coordinates) are reported as $p < 0.001$ uncorrected and supported by voxel-level FWE corrected ROI analyses. An ROI was created by

generating a 5mm sphere around the dorsal peak identified in our prior paper (Fig 1a) as showing stress-potentiated connectivity with the amygdala ($xyz = 8, 34, 40$) (Robinson et al. 2012a) and the ventral node in the ventral medial prefrontal-amygdala circuit (5mm sphere at $xyz = 4, 48, -18$) previously highlighted by Linnman et al. (2012). Analysis examining the impact of treatment on the word distractor failed to reveal significant neural differences across treatment (see Supplementary materials), so results are restricted to the valence analysis.

2.6 Connectivity analysis

A generalized PPI (gPPI) GLM was then created for each session and for each subject. Specifically, SPM8 was used to extract the 1) eigenvariate BOLD signal (VOI extraction) from the amygdala seed used in our prior study ((Robinson et al. 2012a) (Automated Anatomical Labeling library (Tzourio-Mazoyer et al. 2002)); 2) separate psychological term for each event; and 3) PPI interaction terms (Psycho-Physiological interaction). For the first level model, one regressor representing de-convolved BOLD signal was included alongside each psychological and PPI interaction terms for each event type to create a gPPI model. The canonical hemodynamic response function was the basis function. At the second level, contrasts representing a PPI interaction term for each of the 8 events per session were then collapsed into a 2 (ATD,BAL) $\times$ 2(Fear,Happy) flexible factorial design as in the event-related analysis. For contrasts of interest, whole brain peaks are reported as $p < 0.001$ uncorrected and supported by voxel-level FWE corrected ROI analyses defined as in the event related analysis.

3 Results

3.1 Manipulation check

Complete data were unavailable for 4 participants due to difficulties with blood extraction. A significant two-way ATD by time interaction was seen in the TRP/ LNAA ratio ($F(1,14) = 36.5, p < .0001$) driven by a 81.9% decrease in the TRP/ LNAA ratio between T0 (0.16) and T1 (0.03) on the ATD visit ($F(1,14) = 210, p < .0001$) but no significant change in the TRP/ NAA ratio between T0 (0.17) and T1 (0.19) on the BAL visit ($F(1,14) = 1.2, p = .29$). In addition, the T0 ratios were the same on both visits ($F(1,14) = .01, p = .76$), but T1 ratios were significantly decreased on the depletion vs. placebo visit ($F(1,14) = 50, p < .0001$). All functional imaging differences can thus be attributed to a tryptophan decrease on the depletion day only.

3.2 Prefrontal and amygdala activity

Event related whole brain analysis revealed a treatment by valence interaction in the dorsal ACC/dorsal medial PFC ($xyz = 18, 24, 32, t = 3.5, p(\text{uncorrected}) < 0.001$). A full breakdown of this whole brain interaction is presented in Table 1. The region of interest analysis confirmed a significant interaction in the dorsal medial PFC region identified in our prior paper ($xyz = 10, 32, 44, t = 2.3, p(\text{FWE}) = 0.038$; fig 1b), but no effect in the ventral node ($p > 0.9$). Breaking this dorsal interaction down further (fig 1c), ROI analysis showed significantly increased activity during fearful relative to happy faces under ATD ($xyz = 10, 32, 44, t = 3.3, p(\text{FWE}) = 0.003$) but not BAL ($p(\text{FWE}) > 0.5$). Moreover, there was a trend towards significantly greater response under ATD than BAL during fearful ($xyz = 6, 38, 38, t = 3.4, p(\text{FWE}) = 0.085$) but not happy faces ($p(\text{FWE}) > 0.4$). Thus we demonstrate that ATD induces a negative bias (increased the neural response to fearful, but not happy faces) in the cortical dorsal amplification node. Coinciding with this, the amygdala was significantly more active during ATD than BAL ($xyz = 34, 2, -24, t = 2.6, p(\text{FWE}) = 0.04$), but consistent with prior work (Cools et al. 2005; Daly et al. 2010; Robinson et al. 2012a; van der Veen et al. 2007), the amygdala showed only a weak, trend level, preference for fearful faces in the

treatment by valence interaction ($xyz=30,4, -18, t=2.1, p(\text{uncorrected})=0.02$; Supplemental figure 1).

3.3 Prefrontal-amygdala connectivity

We next examined whether this ATD-induced affective bias in the dorsal medial PFC was also associated with altered connectivity with the amygdala. In the whole-brain voxel-wise analysis, there was a trend towards a treatment by valence interaction in dorsal medial PFC-amygdala connectivity ($xyz=-18,46,30, t=2.8, p(\text{uncorrected})<0.003$). Consistent with the amygdala activity, whole brain analyses also revealed greater connectivity between the amygdala and a region of the dorsal medial PFC during ATD relative to BAL ($xyz=4,24,26, t=3.7, p(\text{uncorrected})<0.001$). However, this was driven by a significant increase in connectivity for fearful (Fear ATD vs. BAL contrast: $xyz=4,24,26, t=3.2/xyz=2\ 24\ 38, t=3.2, p(\text{uncorrected})<0.001$), but not happy faces ($p(\text{uncorrected})>0.09$) under ATD. This was also associated with a whole-brain trend towards *even greater* amygdala-dorsal ACC/dorsal medial PFC connectivity during fearful relative to happy faces under ATD (fear vs. happy under ATD contrast $xyz=20,16,26, t=2.9, p(\text{uncorrected})<0.002$). ROI analyses using our prior dorsal medial PFC peak confirmed that increased dMPFC-amygdala connectivity overlapped with that seen in both our prior study (fig 1d). Specifically, we extend our prior stress-potentiated increase in aversive dMPFC-amygdala coupling to encompass ATD-potentiated aversive coupling for fearful faces (fear ATD vs. BAL contrast $xyz=6,30, 38, t=2.35, p(\text{FWE})<0.04$; fig 1d), but not happy faces ($p(\text{uncorrected})<0.2$). Extracting mean betas across the ROI revealed that this was an increase in *positive* coupling (a mean coupling value of 0.5 for fear faces under BAL to 0.9 for fear faces under ATD) that was not present for happy faces (fig 1e). By contrast no significant interactions were seen in the ventral node (all $p>0.9$).

3.4 Behavioral findings

As with prior work (Daly et al. 2010; Fusar-Poli et al. 2007; Passamonti et al. 2012), separate behavioral analysis of reaction time and valence in treatment $\times$ congruency $\times$ valence ANOVAs revealed no significant valence $\times$ treatment interaction in RT ($F(1,18)=0.24, p=0.6$) or error rate ($F(1,18)=0.004, p=0.9$), and no significant impact of valence on either RT ($F(1,18)=0.30, p=0.6$) or error rate ($F(1,18)=0.82, p=0.4$), thereby indicating that the above functional imaging findings are un-confounded by performance differences. Thus, imaging results likely reflect altered neural preference for faces of different valences, rather than generic ‘response’ or ‘error’ effects. For a discussion of the impact of the word distractor on both behavior and neural activation (which was not impacted by ATD), refer to the supplemental material.

4 Discussion

In line with hypotheses, serotonin reduction increased dorsal ACC/mPFC activity during the processing of fearful relative to happy faces alongside increased *positive* dorsal ACC/mPFC-amygdala connectivity in the same circuitry identified in our prior study using threat of shock to induce anxiety (Robinson et al. 2012a). These findings therefore tie together two lines of translational research to provide clear support for the proposition that serotonin can modulate the dorsal ACC/mPFC-amygdala ‘aversive amplification’ circuit (Robinson et al. 2012a).

Our key finding is that serotonin reduction via acute tryptophan depletion (Crockett et al. 2011) increased activity of the dorsal ACC during fearful relative to happy face processing; a neural bias which was not present under placebo. This increase in event related activity to fearful faces was, moreover, accompanied by a corresponding increase in positive

connectivity between the dorsal ACC/mPFC region and the amygdala. This mimics the effects of induced anxiety (via threat of electric shock) on the neural correlates of fearful face processing (Robinson et al. 2012a). Namely, both induced anxiety and serotonin reduction selectively recruit the dorsal prefrontal-amygdala circuit (Robinson et al. 2012a) during the processing of fearful, but not happy, faces. This is consistent with the clear role that serotonin reduction plays in pathological anxiety (Dayan and Huys 2009; Robinson et al. 2012b) and suggests that the effect of serotonin is to *inhibit* aversive responding in this circuit under *non-anxious* conditions. Such an inhibitory mechanism concurs with a range of recent psychological (Cools et al. 2008b; Crockett et al. 2009), computational (Dayan and Huys 2008) and psychophysiological studies (Robinson et al. 2012b), all of which have argued that the role of serotonin is to inhibit aversive responding. The present study extends these prior findings to provide fMRI evidence of a translational neural circuit - the putative human homologue of the rodent prelimbic-amygdala circuit (Milad et al. 2006; Sierra-Mercado et al. 2011) - which may be the target of such serotonergic inhibition. Specifically, when serotonin is reduced this circuit demonstrates *increased* neural response to fearful faces, indicating that it has been 'disinhibited' by serotonin reduction. The mechanism by which this disinhibition occurs is unclear, but one possibility is that ascending afferents from the dorsal raphe nucleus to the prefrontal cortex release serotonin which inhibits prefrontal responding (Amat et al. 2005; Cools et al. 2008a) and that serotonin depletion disables these ascending connections. Indeed A) excitation of the dorsal raphe in rodents leads to increased serotonin and subsequent increased inhibition of the neurons in the prelimbic circuit (Puig et al. 2005), and B) chronic serotonergic medication reduces connectivity between the dorsal mPFC and subcortical structures in healthy humans (McCabe et al. 2011). As such, under optimal conditions, the dorsal prefrontal-amygdala circuit may be inhibited by serotonergic dorsal raphe innervations, but this control is removed by i) serotonin reduction (as seen here), ii) anxiety induced by threat of unpredictable shock (Robinson et al. 2012a; Robinson et al. 2011b; Robinson et al. 2012b), or iii) pathological anxiety (Graham and Milad 2011; Shin et al. 2011; Shin et al. 2005).

It should be noted that these findings are compatible with recent work supporting the proposition that ATD can *remove* a *negative* connection between *ventral* regions of the ACC and the amygdala present under placebo conditions (Passamonti et al. 2012). Rodent work has, in fact, shown that the prelimbic cortex has dissociable effects on fear processing relative to the adjacent infralimbic prefrontal cortex: the prelimbic circuit excites the amygdala, whilst the infralimbic inhibits the amygdala (Sierra-Mercado et al. 2011). In humans, connectivity analysis suggests that the same pattern is seen along a dorsal/ventral medial cortical divide, with more dorsal regions showing positive coupling with the amygdala, and more ventral regions showing negative coupling with the amygdala (Etkin et al. 2011). Thus, whilst the dorsal circuit may be an 'amplification' circuit, the ventral circuit might be an 'inhibitory' circuit. Dysfunction in both amplification *and* inhibition is seen in anxiety disorders (Richter et al. 2012), so from this perspective it is entirely plausible that serotonin both *promotes* the ventral inhibitory circuit and *inhibits* the dorsal amplification circuit, both of which protect against negative affective biases under healthy function. The negative bias seen following serotonin depletion (Cools et al. 2008a; Dayan and Huys 2008) may thus be the result of a shift in the balance from a ventral-mediated inhibitory condition (as seen in the prior study (Passamonti et al. 2012)) towards a disinhibited dorsal-mediated aversive amplification mechanism (as seen here); the ultimate affective bias being driven by the overall balance between these two circuits (Richter et al. 2012). The fact that the two different studies reveal activity changes in separate circuits (and that we do not see significant effects in the ventral circuit) may be driven by task demands (Robinson et al. 2012a). Specifically, although it was not impacted by treatment, there was a word distractor over the face in the present task which was absent in the Passamonti study. In this simpler face identification task (Passamonti et al. 2012) an inhibitory mechanism may have been

recruited to dampen the amygdala response to the fearful faces, as part of a serotonin-mediated ‘resilience’ mechanism in healthy individuals (Robinson 2011; Robinson et al. 2011a). Such a resilience effect, which serves to dampen the adverse impact of negative stimuli in healthy individuals under optimal conditions, has in fact been shown to be modulated by serotonin (Cools et al. 2008b; Robinson 2011; Robinson et al. 2011a), but future work is needed to clarify the circumstances under which each circuit is recruited (Robinson et al. 2011b). As an aside, it should be noted that in this prior study (Passamonti et al. 2012) (which to the best of our knowledge is the only other study examining prefrontal-amygdala functional connectivity in healthy individuals under tryptophan depletion) as well as others (Daly et al. 2010; Fusar-Poli et al. 2007), the effect was *also* restricted to the neural circuitry and unconfounded by behavioral differences in face emotion identification. One explanation for the lack of behavioral effects across all of these studies is that the subjects were healthy individuals, the tasks were simple, and that behavioral change seen in the pathological states of depression or anxiety disorders may require more chronic serotonin depletion. Indeed, in the reverse scenario, it often takes multiple weeks of serotonergic medication before psychological benefits (i.e. the removal of negative affective biases) are seen in patients, despite immediate pharmacological effects (Harmer et al. 2010; Harmer et al. 2009). As such, serotonin should be considered to modulate the *potential* of the circuit to drive behavioral bias, but it is ultimately the processing of external stimuli (e.g., threat of shock (Robinson et al. 2012a)) *within* this *modulated* circuit which drive the ultimate behavioral changes. Serotonin may act, that is to say, like a switch which shifts the focus of the circuit.

Our findings thus provide a key link between circuitry and pharmacology which could be potentially used to inform future, more direct, treatment or diagnoses of psychiatric disorders (Insel et al. 2010). Abnormalities within this dorsal prefrontal-amygdala circuit have been demonstrated in both mood and anxiety disorders (Bishop 2007; Graham and Milad 2011; Price and Drevets 2012) and, as such, it may be that hyperactivity within this circuit underlies, at least in part, the negative affective biases seen in these disorders (Robinson et al. 2012a). Furthermore, the link to serotonin in the present findings provides a mechanism by which the chronic serotonin depletion seen in depression and anxiety (Robinson et al. 2012b) may contribute to the -persistent detrimental focus on negative life events in both disorders. Moreover, on the flipside of the same coin, these findings suggests a mechanism – inhibition of a dorsal prefrontal-amygdala circuit - by which serotonergic medication may ultimately be able to break this negative focus. Future work examining means to ‘tune’ these circuits in psychiatric disorders will hopefully provide diagnostically relevant outcomes related to these circuits (Insel et al. 2010).

Before concluding, it is worth noting a number of caveats. Firstly, the PPI connectivity analysis adopted cannot provide information about directionality of neural circuits (Friston et al. 1997). We can say that as activity increases in one region, it also increases in the other. As such, we propose (in accordance with both rodent and human literature (Etkin et al. 2011; Sierra-Mercado et al. 2011)) that this reflects a top-down amplification mechanism, but future study with more complex techniques such as dynamic causal modeling (Friston et al. 2003) will be necessary to determine directionality. Secondly, this PPI technique only reveals the correlations between the regions but not the precise neural circuitry underlying these connections so future work is needed to trace the precise dorsal ACC –amygdala neural connections and any potential mediating nodes. Thirdly, scheduling constraints meant that the fMRI task always followed a startle study (presented in (Robinson et al. 2012b)), and although this study showed no significant main effects or interactions in subjective anxiety, state anxiety or pain; some unanticipated impact upon the present study cannot be ruled out and a counterbalanced order would be preferable for future studies. Fourthly, subjects have previously rated faces on a version of this task as equally emotionally salient (Robinson et

al. 2011b) and pictures of fearful/happy faces are inherently relatively low in arousal, but if the present population found one or other of the valences more arousing, this would be a potential confound that should be explored in future work via inclusion of a neutral (non-arousing) face set. Finally, although our sample completed a comprehensive psychiatric screen, we did not use a specific tool to identify axis II disorders, which can be difficult to diagnose. Such disorders can also alter amygdala response (Hazlett et al. 2012) and could constitute a potential confound if not excluded from our study.

4.1 Conclusion

Serotonin depletion promoted both activity and connectivity during the processing of fearful relative to happy faces in the dmPFC/dACC-amygdala 'aversive amplification' circuit. This circuit A) is a putative human homologue of the rodent prelimbic-amygdala circuit (Milad et al. 2007; Sierra-Mercado et al. 2011); B) is activated in healthy individuals undergoing threat of shock-induced anxiety (Robinson et al. 2012a); C) demonstrates abnormal activity in both depression and anxiety disorders (Eshel and Roiser 2010; Price and Drevets 2009) and D) provides a neural substrate for the diverse cognitive, computational and psychophysiological findings arguing that the role of serotonin is to inhibit aversive responses (Dayan and Huys 2008; Robinson et al. 2011a; Robinson et al. 2012b). Specifically, optimal serotonin levels may inhibit activity within the aversive amplification mechanism, but in the presence of reduced serotonin, this circuit is released, thereby inducing a bias towards aversive information. At a more practical level, this disinhibitory mechanism may lead to the psychological focus upon negative life experiences which pervade depression and anxiety, and which - on the flip side of the same coin - may be reversed by serotonergic medications. Given the prevalence and impact of such disorders, as well as the ubiquitous use of serotonergic medication in treatment, such clarification is of key importance. Within the not too distant future, it should be possible to use knowledge of the aberrant neural circuitry underlying pathology to diagnose and treat psychiatric disorders, (Insel et al. 2010) and the present findings provide an important step in this direction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institutes of Mental Health. We are grateful to Michael Jackson, Angie Wu, Joan Mallinger and Michael Franklin for enormous amounts of help and support.

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Figure 1. Functional Imaging effects

a) The threat-potentiated dMPFC-amygdala connectivity seen in our prior study (Fear threat vs safe contrast $t=3-3.5$) (Robinson et al. 2012) shows overlap with the b) treatment $\times$ valence interaction in event related activity ($t=1.5-2$) in our current study. This interaction was driven by c) increased activity in dMPFC for fearful faces under ATD (extracted betas averaged across region of interest for illustration). This was also associated with d) increased dMPFC-amygdala connectivity in the same region for fearful (fear ATD vs BAL PPI contrast $t=0.5-1.5$) but not e) happy faces (extracted betas averaged across region of interest for illustration). Crosshairs on all images point to peak threat potentiated dMPFC-amygdala coupling from Robinson 2012, numbers represent slices. ATD = acute tryptophan depletion; BAL= balanced placebo; L= left; R=right; dMPFC = dorsal medial prefrontal cortex.

Table 1

Whole-brain peak voxels for each contrast in the event related analysis. Coordinates represent MNI coordinates. F= fear face, H=happy face, ATD=acute tryptophan depletion, BAL=balanced placebo

Cluster size	Peak T	Peak p(unc)	x,y,z (mm)	AAL label
(ATD F-H) - (BAL F -H)				
89	3.61	<0.001	-28 -38 10	Hippocampus_L
40	3.47	<0.001	18 24 32	Cingulum_Mid_R
24	3.27	=0.001	-14 -44 4	Precuneus_L
87	3.2	=0.001	16 -56 -4	Lingual_R
22	3.17	=0.001	-8 -14 24	Thalamus_L
41	3.09	=0.001	-12 -38 30	Cingulum_Mid_L
23	3.08	=0.001	-24 36 4	Insula_R
70	3.06	=0.001	0 -6 34	Cingulum_Mid_L
3	3.05	=0.001	22 -44 16	Precuneus_R
6	3.04	=0.001	8 30 12	Cingulum_Ant_R
9	3.01	=0.001	-10 40 38	Frontal_Sup_Medial_L
2	3	=0.001	-42 -16 22	Rolandic_Oper_L
ATD (F-H)				
392	4.6	<0.001	-30 -40 10	Hippocampus_L
	3.28	=0.001	-28 -62 12	Calcarine_L
123	3.77	<0.001	-42 -8 34	Postcentral_L
45	3.63	<0.001	20 22 30	Frontal_Sup_R
85	3.61	<0.001	-12 -32 30	Cingulum_Mid_L
80	3.57	<0.001	10 28 50	Frontal_Sup_Medial_R
24	3.42	<0.001	22 -44 18	Precuneus_R
137	3.37	<0.001	-10 40 38	Frontal_Sup_Medial_L
	3.14	=0.001	-14 48 24	Frontal_Sup_L
42	3.3	=0.001	-28 -18 22	Insula_L
73	3.29	=0.001	34 -14 50	Precentral_R
	3.02	=0.001	24 -16 56	Precentral_R
14	3.26	=0.001	42 -50 -4	Temporal_Mid_R
20	3.21	=0.001	40 -36 30	SupraMarginal_R
5	3.18	=0.001	14 -30 30	Cingulum_Mid_R
16	3.13	=0.001	-12 -50 -20	Cerebellum_4_5_L
30	3.13	=0.001	36 -34 54	Postcentral_R
6	3.12	=0.001	-40 -26 28	Postcentral_L
8	3.06	=0.001	-30 8 18	Insula_L
6	3.06	=0.001	-30 -12 10	Putamen_L
10	3.06	=0.001	38 18 16	Frontal_Inf_Oper_R
3	3.05	=0.001	-48 -6 12	Rolandic_Oper_L

Cluster size	Peak T	Peak p(unc)	x,y,z (mm)	AAL label
8	3.03	=0.001	-28 -38 58	Postcentral_L
5	3	=0.001	14 -4 40	Cingulum_Mid_R
BAL (F-H)				
NS				
F (ATD-BAL)				
886	5.07	<0.001	6 -18 32	Cingulum_Mid_R
	4.68	<0.001	-12 -44 8	Cingulum_Post_L
	4.62	<0.001	6 -34 14	Cingulum_Post_R
304	4.95	<0.001	36 -32 26	Insula_R
313	4.67	<0.001	32 -56 10	Calcarine_R
	3.61	<0.001	24 -62 26	Precuneus_R
	3.38	<0.001	22 -48 16	Precuneus_R
95	4.37	<0.001	4 8 60	Supp_Motor_Area_R
49	4.19	<0.001	28 8 -16	Olfactory_R
38	3.89	<0.001	-16 -60 32	Precuneus_L
	3.07	=0.001	-18 -54 24	Cuneus_L
340	3.84	<0.001	-34 -56 10	Temporal_Mid_L
	3.67	<0.001	-32 -72 2	Occipital_Mid_L
	3.64	<0.001	-36 -48 14	Temporal_Mid_L
12	3.84	<0.001	2 38 0	Cingulum_Ant_R
111	3.81	<0.001	52 -2 8	Rolandic_Oper_R
	3.25	=0.001	46 10 8	Frontal_Inf_Oper_R
31	3.79	<0.001	32 10 20	Insula_R
33	3.72	<0.001	12 18 40	Cingulum_Mid_R
33	3.66	<0.001	-36 -2 20	Insula_L
27	3.61	<0.001	32 -28 -8	Hippocampus_R
27	3.56	<0.001	-28 -36 10	Hippocampus_L
7	3.5	<0.001	36 -46 -6	Fusiform_R
25	3.49	<0.001	22 -76 -6	Lingual_R
8	3.35	<0.001	-20 -28 18	Caudate_L
4	3.32	=0.001	28 -44 -16	Fusiform_R
11	3.3	=0.001	-34 0 -2	Insula_L
14	3.29	=0.001	36 -66 -8	Occipital_Inf_R
7	3.28	=0.001	-44 -4 10	Rolandic_Oper_L
7	3.22	=0.001	-4 6 8	Caudate_L
19	3.19	=0.001	22 2 34	Frontal_Inf_Oper_R
8	3.13	=0.001	24 -74 20	Occipital_Sup_R
1	3.1	=0.001	20 -16 2	Thalamus_R
3	3.01	=0.001	34 -4 40	Precentral_R

Cluster size	Peak T	Peak p(unc)	x,y,z (mm)	AAL label
H (ATD-BAL)				
40	3.84	<0.001	24 -48 26	Precuneus_R
	3.29	=0.001	30 -36 26	Insula_R
28	3.41	<0.001	6 -34 10	Cingulum_Post_R
16	3.37	<0.001	36 -50 12	Temporal_Mid_R
35	3.33	<0.001	24 6 38	Frontal_Mid_R
3	3.08	=0.001	20 -16 2	Thalamus_R
1	3.04	=0.001	46 8 -18	Temporal_Pole_Sup_R
BAL (H-F)				
6	3.11	=0.001	2 44 0	Cingulum_Ant_R
3	3.01	=0.001	8 30 12	Cingulum_Ant_R
ATD (H-F)				
	NS			