Hyper-Reactive Human Ventral Tegmental Area and Aberrant Mesocorticolimbic Connectivity in Overgeneralization of Fear in Generalized Anxiety Disorder

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Article in The Journal of Neuroscience : The Official Journal of the Society for Neuroscience · April 2014
DOI: 10.1523/JNEUROSCI.4868-13.2014 · Source: PubMed

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Hyper-Reactive Human Ventral Tegmental Area and Aberrant Mesocorticolimbic Connectivity in Overgeneralization of Fear in Generalized Anxiety Disorder

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The ventral tegmental area (VTA) has been primarily implicated in reward-motivated behavior. Recently, aberrant dopaminergic VTA signaling has also been implicated in anxiety-like behaviors in animal models. These findings, however, have yet to be extended to anxiety in humans. Here we hypothesized that clinical anxiety is linked to dysfunction of the mesocorticolimbic circuit during threat processing in humans; specifically, excessive or dysregulated activity of the mesocorticolimbic aversion circuit may be etiologically related to errors in distinguishing cues of threat versus safety, also known as “overgeneralization of fear.” To test this, we recruited 32 females with generalized anxiety disorder and 25 age-matched healthy control females. We measured brain activity using fMRI while participants underwent a fear generalization task consisting of pseudo-randomly presented rectangles with systematically varying widths. A mid-sized rectangle served as a conditioned stimulus (CS; 50% electric shock probability) and rectangles with widths of CS ± 20%, ± 40%, and ± 60% served as generalization stimuli (GS; never paired with electric shock). Healthy controls showed VTA reactivity proportional to the cue’s perceptual similarity to CS (threat). In contrast, patients with generalized anxiety disorder showed heightened and less discriminating VTA reactivity to GS, a feature that was positively correlated with trait anxiety, as well as increased mesocortical and decreased mesohippocampal coupling. Our results suggest that the human VTA and the mesocorticolimbic system play a crucial role in threat processing, and that abnormalities in this system are implicated in maladaptive threat processing in clinical anxiety.

Key words: anxiety disorder; dopaminergic aversion system; fear generalization; human fear conditioning; mesocorticolimbic system; ventral tegmental area

Introduction

The midbrain ventral tegmental area (VTA) is a central part of the mesocorticolimbic dopaminergic system, which plays a pivotal role in motivation (Berridge and Robinson, 1998; Schultz, 2002). Previous studies demonstrate that this system may be one of the primary target sites of stress-induced disturbance. Early research in rodent models revealed that stress exposure activates dopaminergic innervations to the prefrontal cortex (PFC) (Thierry et al., 1976; Deutch and Roth, 1990). More recent studies demonstrate that chronic stress (e.g., social defeat) results in increased VTA dopamine transmission (Anstrom et al., 2009), excessive glucocorticoid signaling in the mesocortical dopamine system (Barik et al., 2013), and epigenetic control of this system (Niwa et al., 2013). Although previous research has linked an abnormal dopamine system to depression and addiction (Willner, 1983; Zacharko and Anisman, 1991; Cabib and Puglisi-Allega, 1996), little is known about its relationship with anxiety in humans.

Recent studies suggest that a distinct part of the mesocorticolimbic circuit plays a crucial role in negative motivation or aversion (Matsumoto and Hikosaka, 2009; Bromberg-Martin et al., 2010; Zweifel et al., 2011; Cohen et al., 2012). The VTA receives glutamatergic inputs from the lateral habenula and projects dopaminergic innervation to the medial PFC (mPFC) in rodent models (Lammel et al., 2012; Stamatakis and Stuber, 2012). The hippocampus partially controls VTA dopaminergic neuronal activity, forming the VTA–hippocampal loop...
that is crucial in motivation and memory (Lisman and Grace, 2005; Shohamy and Wagner, 2008). Therefore, normal VTA aversion signaling may depend on interconnections with the mesocorticolimbic system.

Discrimination of threat and safety is an essential aspect of aversion processing for adaptive behavior. Deficits in this process lead to an overgeneralization of conditioned fear (Kheirbek et al., 2012; Greenberg et al., 2013b; Lissek et al., 2013b); indeed, recent human studies suggest that fear overgeneralization is an important pathogenic marker of anxiety disorders, including panic disorder (Lissek et al., 2010) and generalized anxiety disorder (GAD) (Greenberg et al., 2013a; Lissek et al., 2013a). Therefore, given the crucial role of the mesocorticolimbic dopamine system in negative motivation and the impact of stress on this system, it is reasonable to postulate that maladaptive aversion processing in this system is related to the overgeneralization of conditioned fear in clinical anxiety. To the best of the authors’ knowledge, no human studies have previously tested this possibility.

The aim of this study was thus to test whether individuals with clinical anxiety manifest abnormal mesocorticolimbic reactivity during threat processing (i.e., fear generalization). We hypothesized that clinical anxiety is associated with excessive VTA aversion signaling. To this end, we tested individuals with GAD and healthy control participants with a fear generalization task (Greenberg et al., 2013a, b; Cha et al., 2014), which involves the presentation of a conditioned stimulus (CS; a mid-sized rectangle paired with electric shock) or generalization stimuli (GS) varying in perceptual similarity to CS systematically while collecting functional MRI.

Materials and Methods

MR data from the same participants have been used in a previous report, which provides detailed information on the participants, the study design, and the MRI data acquisition parameters (Greenberg et al., 2013b).

Participants. Fifty-seven participants from the community around Stony Brook, New York volunteered (all females; age, mean ± SD, 22.3 ± 4.5 years). Psychiatric diagnoses were based on the Structured Clinical Interview for DSM-IV Axis I Disorders and administered by trained clinical psychologists at Stony Brook University. This procedure confirmed the diagnoses of GAD in the patient group (n = 32) and absence of Axis I diagnoses in the control group (n = 25). Of 32 patients, 17 were comorbid with major depressive disorder and 15 were diagnosed solely with GAD. All healthy controls were free of current or past psychiatric comorbidity.

Motivation-elicited VTA activation (MNI, 3, −17, −12) (Ballard et al., 2011).

Figure 1. Fear generalization task. A period of fixation was followed by a red rectangle on a black screen (2000 ms). A mid-sized rectangle was paired with electric shock (500 ms duration, onset 1500 ms after cue onset) in 15 trials and unpaired in 15 trials (CS). For GS, rectangles with systematically varying widths were used (i.e., ±20%, ±40%, and ±60%) without paired electric shocks. Each stimulus type was presented 15 times in a pseudorandom order.
corrected \( p < 0.05 \) and deconvolved them. We then multiplied the time-series by a condition versus rest and convolved the time-series with the canonical hemodynamic response function in SPM. This procedure generated one interaction term per condition (i.e., GS/H11006 60%, GS/H11006 40%, GS/H11006 20%, and CS/unpaired), which were entered into a separate model. The model contained three regressors: the VTA time-series by “stimuli versus rest” interaction, “stimuli versus rest,” and the unmodulated VTA time-series. The primary objective of this analysis was to investigate group differences in mesocorticolimbic connectivity (i.e., correlation) modulated by GS versus rest, the contrast in which GAD patients showed a significant increase in VTA BOLD activation relative to controls. To this end, we entered individual PPI images of the “GS versus rest” contrast into a random-effect second level model containing subject, group, and condition. The secondary objective was to examine effects of conditioned fear on the mesocorticolimbic circuit in both groups. To address this, we created a second PPI using “CS versus rest” as a psychological regressor and then examined mean effects of group in a random-effect second level model. We corrected the results using a cluster extent method (AFNI’s 3dClustSim; 10,000 Monte Carlo simulations) with an \( \alpha \) of 0.005 for each ROI. Bilateral ROI masks were derived from the Automated Anatomical Labeling atlas. These include a broad medial prefrontal area (i.e., the medial frontal gyrus, the ventromedial PFC, and the ACC), the hippocampus, the ventral striatum, and the thalamus.

### Results

We first tested for generalization effects within the midbrain neural reactivity in individuals with GAD versus healthy controls (Table 1). After modeling effects of conditions using a general linear model in each individual, we investigated group differences in BOLD reactivity during generalization of conditioned fear. Individuals with GAD showed significantly greater midbrain VTA activation in response to GS compared with healthy controls at a small volume-corrected FWE \( p = 5 \times 10^{-5} \), but not CS (\( p > 0.48 \); one-tailed \( t \) test).

### Table 1. Demographics and clinical characteristics across patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>GAD (n = 32)</th>
<th>Healthy control (n = 25)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>22.3 ± 5.14</td>
<td>21.3 ± 4.56</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>STAI-Trait ( a )</td>
<td>55.1 ± 10.08</td>
<td>37.5 ± 6.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BDI ( a )</td>
<td>22.3 ± 12.66</td>
<td>4.8 ± 3.54</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\( a \) Data from Greenberg et al. (2013a).

### Figure 2. Heightened VTA reactivity and altered mesocorticolimbic connectivity in GAD.

A. We observed heightened VTA reactivity in individuals with GAD to all stimuli, compared with healthy controls. Magnified views of the medial VTA cluster (thresholded at voxelwise \( p < 0.005 \) and 20 continuous voxels) are shown in a coronal (yellow panel) and a sagittal view (purple panel). Diagram represents the midbrain with the VTA shown in red (modified from Naidich et al., 2009). We found greater VTA activation in GAD to GS (\( p = 5 \times 10^{-5} \)), but not CS (\( p > 0.48 \); one-tailed \( t \) test). B. We performed psychophysiological-interaction analyses to examine the effects of a psychological modulator (i.e., either “GS-Rest” or “CS-Rest”) on VTA connectivity. The “GS-Rest” contrast increased VTA connectivity with the subgenual and rostral ACC, accumbens, and thalamus in patients, but not in controls, whereas this contrast increased VTA connectivity with the hippocampus, ACC, vmPFC, and thalamus in both groups, with an exception of connectivity with the accumbens, which was significant only in GAD patients. These results were significant at ROI-corrected \( p < 0.005 \). Mean differences of selected voxels were examined by Mann–Whitney \( U \) test (significances denoted in the bar graphs). Accu, Accumbens; hipp, hippocampus; rACC, rostral ACC; sgACC, subgenual ACC; thal, thalamus. *** \( p < 0.001 \).
repeated-measures ANOVA): activation of healthy controls’ VTA linearly tracked cue similarities to CS ($\beta = 0.57$, $p = 0.01$, a post hoc linear trend analysis), but not in patients ($p > 0.6$), which apparently resulted from a heightened reactivity to GS ($p = 0.00005$), but not CS ($p > 0.479$). We further examined whether individual variability of the VTA hyper-reactivity in patients was correlated with self-reported anxiety and depression scales. Trait anxiety in Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger, 2010) positively correlated with VTA reactivity to GS $\pm 60\%$ in patients (Spearman’s $r = 0.48; p = 0.005$) (Fig. 3). A positive correlation was also observed with the Beck Depression Inventory (BDI) (Beck et al., 1961) ($r = 0.39; p = 0.027$), which did not reach significance at Bonferroni-corrected $p$ of 0.0125 (i.e., by number of conditions). Partial correlations controlling for either STAI or BDI were marginally significant for STAI (Spearman’s partial $r = 0.31; p = 0.08$), but not for BDI ($r = 0.12, p = 0.50$). We found no significant correlations in other conditions ($p > 0.28$). These results suggest that anxiety is associated with elevated and indiscriminate VTA reactivity to GS.

The results of the hyper-reactive VTA in the GAD group raised the question of whether VTA activity would correlate with activity of the mesocorticolimbic circuit. To answer this question, we measured functional connectivity modulated by CS or GS using PPI analysis. We used a functionally defined VTA mask we measured functional connectivity modulated by CS or GS activity of the mesocorticolimbic circuit. To answer this question, we raised the question of whether VTA activity would correlate with abnormalities of this system are implicated in clinical anxiety. In healthy controls, the magnitude of activation in the VTA proportionally tracks the similarity of stimuli to CS, and mesocorticolimbic connectivity increases in response to GS. In individuals with GAD, however, the VTA is hyper-reactive to stimuli that resemble CS (i.e., GS) and mesocorticolimbic connectivity is significantly altered: increased in the ventral PFC and decreased in the hippocampus. These results suggest: (1) VTA activation and mesocorticolimbic connectivity are critical in threat processing; and (2) an abnormal mesocorticolimbic aversion system is implicated in the overgeneralization of conditioned fear in GAD.

Healthy controls and individuals with GAD alike showed VTA BOLD activation to the CS. This is in line with previous reports of

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Table 2. VTA connectivity modulated by psychological regressors

<table>
<thead>
<tr>
<th>Name</th>
<th>Cluster size</th>
<th>Peak z</th>
<th>MNI x,y,z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulator: GS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast: GAD &gt; healthy controls</td>
<td>61</td>
<td>3.77</td>
<td>45,-3,8</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>36</td>
<td>3.46</td>
<td>44,-2,8</td>
</tr>
<tr>
<td>Thalamus</td>
<td>84</td>
<td>4.48</td>
<td>36,-2,8</td>
</tr>
<tr>
<td>Accumbens</td>
<td>13</td>
<td>3.27</td>
<td>22,-4,8</td>
</tr>
<tr>
<td>Contrast: healthy controls &gt; GAD</td>
<td>34</td>
<td>4.78</td>
<td>95,-3,6</td>
</tr>
<tr>
<td>Hipocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulator: CS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast: GAD &gt; healthy controls</td>
<td>63</td>
<td>3.46</td>
<td>41,-8,10</td>
</tr>
<tr>
<td>Accumbens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast: mean positive effects</td>
<td>131</td>
<td>4.35</td>
<td>65,-4,16</td>
</tr>
<tr>
<td>Ventromedial PFC</td>
<td>290</td>
<td>4.15</td>
<td>80,-4,6</td>
</tr>
<tr>
<td>Medial PFC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC</td>
<td>42.1</td>
<td>5E-5</td>
<td>2,44,6</td>
</tr>
<tr>
<td>Hipocampus</td>
<td>33</td>
<td>3.81</td>
<td>65,-2,14</td>
</tr>
<tr>
<td>Thalamus</td>
<td>137</td>
<td>3.25</td>
<td>5E-4,10</td>
</tr>
</tbody>
</table>

*Voxelwise $p < 0.005$; all results at corrected $p < 0.005$ in each ROI.

Figure 3. Correlation between VTA BOLD reactivity to GS (60% different) and trait anxiety (STAI) in individuals with GAD. The effect was significant at an alpha level of 0.05 after Bonferroni adjustment for multiple conditions ($p < 0.0125$). Correlation was only significant in GS $\pm 60\%$, but not other GS or CS ($p > 0.14$).
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VTA activation during perception or anticipation of aversive stimuli (Fairhurst et al., 2007; Matsumoto and Hikosaka, 2009), as well as fear learning (Lammel et al., 2012; Brooks and Berns, 2013). Our results complement and expand on these findings by demonstrating that the normal VTA activates proportionally to the perceptual similarity of the CS. Moreover, VTA connectivity with prefrontal regions increases in response to conditioned fear. One possible explanation is that this increase in mesocorticolimbic connectivity represents the activation of VTA dopaminergic projections to the prefrontal system during threat processing. This idea is consistent with a recent animal model of dopaminergic aversion signaling, in which glutamatergic inputs from the lateral habenula excite dopaminergic neurons in the medial VTA, which in turn project to the mPFC, completing the mesocortical aversion circuit (Lammel et al., 2012). It should be noted, however, that the present fMRI results do not provide direct evidence of the involvement of dopamine or the directionality of connectivity.

Individuals with GAD showed greater VTA reactivity to all GS compared with healthy controls. This led to a significant group difference in the VTA fear generalization gradient: a linear increase in VTA reactivity with increase in perceptual similarity to the CS (threat) in healthy controls, but indiscriminately enhanced VTA activation, to all stimuli regardless of perceptual similarity to CS (threat), in individuals with GAD. This effect is well in line with the model of overgeneralization of conditioned fear in pathological anxiety (Jovanovic and Ressler, 2010; Britton et al., 2011; Kheirbek et al., 2012; Greenberg et al., 2013a). Furthermore, variability of the patient-specific VTA hyper-reactivity to the GS least similar to CS was correlated with individual differences in trait anxiety. In animal models, excessive VTA dopaminergic activity is implicated in anxiety-like behaviors (Coque et al., 2011), and elevated VTA dopaminergic activity has been linked to vulnerability to social defeat in mouse models (Krishnan et al., 2007). Thus, our results seem to correspond to previous research in animal models providing a key link between abnormal VTA activity and anxiety. Future research may investigate whether the abnormal VTA BOLD reactivity reported here is indeed correlated with an increase in dopamine release or receptor activity.

We also found abnormal mesocorticolimbic connectivity in GAD. First, individuals with GAD showed heightened mesocorticolimbic coupling elicited by GS compared with healthy controls. Although speculative, one of the many potential neurobiological underpinnings of this maladaptive mesocorticolimbic coupling in clinical anxiety may be a disturbance of the “aversive” dopaminergic system because of excessive or prolonged stress (Coplan et al., 1996; Arbolesluis et al., 1999). In mouse models, chronic stress induces excessive glucocorticoid release in dopaminoreceptive neurons in the PFC (Barik et al., 2013). Chronic stress also leads to DNA hypermethylation of the tyrosine hydroxylase gene, a key regulator of dopamine synthesis, and this epigenetic influence is observed in mesocortical dopaminergic neurons (Niwa et al., 2013). Partially in line with this, recent human dopamine receptor imaging results suggest that acute stressors induce dopamine release in the mPFC and physiological arousal in healthy individuals (Nagano-Saito et al., 2013). Based on this literature, although speculative, the heightened mesocorticolimbic connectivity during fear generalization may indicate an aberrant dopaminergic mesocorticolimbic circuit in clinical anxiety.

Second, VTA–hippocampal connectivity elicited by GS was significantly attenuated in individuals with GAD compared with healthy controls. The hippocampus interacts with the VTA, forming a VTA–hippocampal loop that is crucial in motivational learning and memory (Lisman and Grace, 2005; Lodge and Grace, 2006; Shohamy and Wagner, 2008). Our results suggest that the attenuation of the VTA–hippocampal loop is associated with impaired threat-safety discrimination in clinical anxiety. This finding is in line with a recent animal model implicating impaired hippocampal pattern separation, perhaps via restrained neurogenesis under stress, to fear generalization and anxiety-related behaviors (Kheirbek et al., 2012). Together, our connectivity results support the idea that the abnormal mesocorticolimbic system contributes to overgeneralization of conditioned fear in GAD.

The present study advances the current understanding of the neural underpinnings of fear generalization. Several neuroimaging studies report neural correlates of fear generalization in humans, including the cingulate cortex, insula, striatum, and other limbic structures (Dunsmoor et al., 2011; Greenberg et al., 2013b; Lissek et al., 2013b). Studies of clinical anxiety document behavioral and/or neural evidence of fear generalization in anxiety disorders, such as panic disorder (Lissek et al., 2010) and GAD (Greenberg et al., 2013a; Lissek et al., 2013a). In particular, we have previously reported maladaptive vmPFC threat processing in GAD; that is, an indiscriminate vmPFC response to fear generalization stimuli (Greenberg et al., 2013a) showed correlations between circuit-wide structural and functional abnormalities (Cha et al., 2014). In the present study, we did not observe a direct relationship between vmPFC dysfunction and abnormal VTA reactivity or connectivity. However, given the extensive literature on the essential role of the two systems (perhaps mediated by the dorsolateral PFC) in adaptive behavior (Schoenbaum et al., 2009; Takahashi et al., 2009, 2011; Ballard et al., 2011; D’Ardenne et al., 2012), the coupling between these two structures may be important in the fear generalization and the pathophysiology of anxiety.

In conclusion, our findings in the present study provide novel evidence linking the abnormal mesocorticolimbic system to the overgeneralization of conditioned threat in GAD. Further investigation of the neurochemical substrates for these BOLD-based measures may contribute to the development of new treatment strategies targeting the dopaminergic mesocorticolimbic aversion circuit in anxiety disorders.

References
Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in mo-


