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## Ventromedial prefrontal cortex damage alters resting blood flow to the bed nucleus of stria terminalis

Julian C. Motzkin<sup>1,2,3</sup>, Carissa L. Philippi<sup>1</sup>, Jonathan A. Oler<sup>1</sup>, Ned H. Kalin<sup>1</sup>, Mustafa K. Baskaya<sup>4</sup>, and Michael Koenigs<sup>1,\*</sup>

<sup>1</sup>Department of Psychiatry, University of Wisconsin-Madison, 6001 Research Park Blvd., Madison, Wisconsin, 53719, USA

<sup>2</sup>Neuroscience Training Program, University of Wisconsin-Madison, 1300 University Ave., Madison, Wisconsin, 53706, USA

<sup>3</sup>Medical Scientist Training Program, University of Wisconsin-Madison, 750 Highland Ave., Madison, Wisconsin, 53705, USA

<sup>4</sup>Department of Neurological Surgery, University of Wisconsin-Madison, 600 Highland Ave., Madison, Wisconsin, 53792, USA

### Abstract

The ventromedial prefrontal cortex (vmPFC) plays a key role in modulating emotional responses, yet the precise neural mechanisms underlying this function remain unclear. vmPFC interacts with a number of subcortical structures involved in affective processing, including the amygdala, hypothalamus, periaqueductal gray, ventral striatum, and bed nucleus of stria terminalis (BNST). While a previous study of non-human primates shows that vmPFC lesions reduce BNST activity and anxious behavior, no such causal evidence exists in humans. In this study, we used a novel application of MRI in neurosurgical patients with focal, bilateral vmPFC damage to determine whether vmPFC is indeed critical for modulating BNST function in humans. Relative to neurologically healthy subjects, who exhibited robust rest-state functional connectivity between vmPFC and BNST, the vmPFC lesion patients had significantly lower resting-state perfusion of the right BNST. No such perfusion differences were observed for the amygdala, striatum, hypothalamus, or periaqueductal gray. This study thus provides unique data on the relationship between vmPFC and BNST, suggesting that vmPFC serves to promote BNST activity in humans. This finding is relevant for neural circuitry models of mood and anxiety disorders.

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\* Author for correspondence: mrkoenigs@wisc.edu, 608-263-1679 (phone), 608-263-9340 (fax).

### CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

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## Keywords

Prefrontal Cortex; Bed Nucleus of Stria Terminalis; Emotion; Lesion; Anxiety

## 1. INTRODUCTION

The ventromedial prefrontal cortex (vmPFC) plays a critical role in human social and affective processing. Dysfunction in this brain area is thought to be a key neural substrate underlying the pathophysiology of mood and anxiety disorders (Price, 1999, Critchley *et al.*, 2001, Milad *et al.*, 2006, Drevets *et al.*, 2008, Myers-Schulz and Koenigs, 2012). However, the precise mechanisms by which vmPFC dysfunction contributes to affective psychopathology are not fully understood. A leading neural circuit model proposes that vmPFC serves to regulate negative affect via top-down inhibition of brain regions involved in processing negative emotion—particularly the amygdala—and that pathologically elevated levels of negative affect in mood and anxiety disorders result from deficient vmPFC-mediated inhibition of amygdala activity (Quirk and Gehlert, 2003, Milad *et al.*, 2006, Rauch *et al.*, 2006). While this model is consistent with a considerable body of anatomical, behavioral, and neurophysiological data from rodent fear conditioning paradigms (Milad *et al.*, 2006), studies of human lesion patients suggest a more complex role of vmPFC in affective function. For instance, although vmPFC lesion patients exhibit increased amygdala activity in response to aversive stimuli (Motzkin *et al.*, in press), vmPFC damage has been shown to *reduce* the likelihood of developing PTSD and depression (Koenigs *et al.*, 2008, Koenigs *et al.*, 2008). These findings suggest that vmPFC may coordinate multiple neural processes critical for the expression of negative affect in humans. Beyond top-down inhibition of amygdala, vmPFC may also modulate activity in other regions, such as the bed nucleus of the stria terminals (BNST).

The BNST is a basal forebrain structure that is considered to be a component of the “extended amygdala” complex, in light of similarities in development, connectivity, and cytoarchitecture to the adjacent central nucleus of the amygdala (Heimer *et al.*, 1997). The BNST and vmPFC are strongly interconnected (Avery *et al.*, 2014), and BNST activity has been linked to anxiety-related behavior (Davis and Whalen, 2001, Walker *et al.*, 2003, Kalin *et al.*, 2005, Straube *et al.*, 2007, Mobbs *et al.*, 2010, Somerville *et al.*, 2010, Somerville *et al.*, 2013). Moreover, a previous neuroimaging study in non-human primates found that bilateral orbitofrontal cortex (OFC) lesions (which included regions of vmPFC) were associated with reduced BNST metabolism and reduced anxious behavior in a human intruder paradigm (Kalin *et al.*, 2007, Fox *et al.*, 2010). In addition, across the lesioned and non-lesioned monkeys, the level of BNST metabolism positively correlated with the degree of anxious behavior. These findings suggest that vmPFC/OFC may play a crucial role in generating or maintaining negative affect by promoting BNST activity. To explore this hypothesis in humans, we employed a magnetic resonance imaging (MRI) measure of resting cerebral blood flow in a sample of neurosurgical patients with circumscribed bilateral vmPFC lesions. We hypothesized that, consistent with the results of the non-human primate study (Fox *et al.*, 2010), humans with bilateral vmPFC damage would exhibit reduced BNST blood perfusion, which would in turn correlate with self-report measures of

negative affect and anxiety. Furthermore, we used rest-state fMRI in the healthy adult comparison group to assess functional connectivity between BNST and vmPFC.

## 2. METHODS

### 2.1. Participants

The lesion group consisted of four adult neurosurgical patients with extensive bilateral parenchymal damage, largely confined to the vmPFC—defined as the medial one-third of the orbital surface and the ventral one-third of the medial surface of prefrontal cortex, bilaterally (Fig. 1). Each of the four patients underwent surgical resection of a large anterior cranial fossa meningioma via craniotomy. Initial clinical presentations included subtle or obvious personality changes over several months preceding surgery. On post-surgical MRI, although vasogenic edema largely resolved, there were persistent T<sub>2</sub>-weighted signal changes, consistent with gliosis, in the vmPFC bilaterally. All experimental procedures were conducted more than three months after surgery, when the expected recovery was complete. At the time of testing, all patients had focal, stable MRI signal changes and resection cavities and were free of dementia and substance abuse. Nineteen healthy adults with no history of brain injury, neurological or psychiatric illness, or current use of psychoactive medication were recruited as a normal comparison (NC) group. Demographic and neuropsychological data for the vmPFC and NC groups are summarized in Table 1.

### 2.2. MRI data acquisition

All structural and functional MRI data were acquired using a 3.0 T GE Discovery MR750 scanner equipped with an 8-channel radio-frequency head coil array (General Electric Medical Systems; Waukesha, WI). High-resolution T<sub>1</sub>-weighted anatomical images were acquired using an inversion-recovery spoiled GRASS [SPGR] sequence (TR=8.2ms, TE=3.2ms,  $\alpha=12^\circ$ , FOV=256×256mm, matrix=256×256, in-plane resolution=1×1mm<sup>2</sup>, slice thickness=1mm, 1024 axial slices). To facilitate lesion segmentation, we collected a separate T<sub>2</sub>-weighted FLAIR scan (TR=8650ms, TE=136ms,  $\alpha=0^\circ$ , FOV=220×220mm<sup>2</sup>, matrix=512×512, in-plane resolution=0.43×0.43mm<sup>2</sup>, slice thickness=5 mm, gap 1mm, 25 axial slices).

Baseline resting cerebral blood flow (CBF) was estimated using a 3D fast spin echo spiral sequence with pseudocontinuous arterial spin labeling (pcASL) (Dai *et al.*, 2008, Xu *et al.*, 2010, Okonkwo *et al.*, 2012) and background suppression for quantitative perfusion measurements (TR=4653ms, TE=10.5ms, postlabeling delay=1525ms, labeling duration=1450ms, eight interleaved spiral arms with 512 samples at 62.5 kHz bandwidth and 38 4-mm thick slices, number of excitations=3, scan duration=4.5min).

Whole-brain functional scans were acquired using a T<sub>2</sub>\*-weighted gradient-echo echoplanar imaging (EPI) sequence (TR=2000ms; TE=22ms;  $\alpha=79^\circ$ ; FOV=224×224mm<sup>2</sup>; matrix=64×64, in-plane resolution=3.5×3.5mm<sup>2</sup>, slice thickness=3mm, gap=0.5mm, 38 interleaved axial oblique slices). Field maps were acquired using two separate acquisitions (TR=600ms, TE<sub>1</sub>=7ms, TE<sub>2</sub>=10ms,  $\alpha=60^\circ$ , FOV=240×240mm<sup>2</sup>, matrix=256×128, slice thickness=4mm, 33 axial oblique slices). Rest-state functional images were collected while subjects lay still and awake, passively viewing a fixation cross for 5 minutes. Scans were

acquired in the following order: pcASL, field map, rest, task, T1, T2-FLAIR. The fMRI task data from this scan session are presented in separate manuscripts (Motzkin *et al.*, 2014, Motzkin *et al.*, 2014).

### 2.3. Lesion segmentation and image normalization

Individual vmPFC lesions were visually identified and manually segmented on the T<sub>1</sub>-weighted images. Lesion boundaries were drawn to include areas with gross tissue damage or abnormal signal characteristics on T<sub>1</sub> or T<sub>2</sub> FLAIR images. T<sub>1</sub>-weighted images were skull-stripped, rigidly co-registered with a functional volume from each subject, then diffeomorphically aligned to the Montreal Neurological Institute (MNI) coordinate system using a Symmetric Normalization (SyN) algorithm (Avants and Gee, 2004) with constrained cost-function masking to prevent warping of tissue within the lesion mask (Brett *et al.*, 2001). We created the lesion overlap map (Fig. 1) by computing the sum of aligned binary lesion masks for all four vmPFC patients.

### 2.4. Cerebral perfusion analysis

Quantitative CBF images from pcASL were rigidly co-registered with a T<sub>2</sub>\*-weighted EPI volume from the task scan and normalized to MNI space. Normalized CBF volumes were scaled to whole-brain CBF (after masking out the lesion in vmPFC patients) and smoothed with a 6mm full-width at half-maximum (FWHM) Gaussian kernel. To test the main study hypothesis, we used regions-of-interest (ROIs) corresponding to the right and left BNST (Fig. 2). BNST ROIs were hand-drawn on the MNI template brain using neuroanatomical boundaries from the human brain atlas of Mai (Mai *et al.*, 2003). Comparing the field map data between groups, we found no significant differences in signal distortion or dropout in the region of BNST (Supplementary Fig. 1). To determine the specificity of between-group differences, we also examined group differences in mean unscaled whole-brain CBF, as well as differences in scaled CBF for additional subcortical ROIs that are known to be directly connected with vmPFC (amygdala, mediodorsal nucleus of the thalamus, hypothalamus, periaqueductal gray, and ventral striatum) as well as for several subcortical areas not densely or directly connected with vmPFC (lateral geniculate nucleus, caudate nucleus, putamen). Mediodorsal thalamus, hypothalamus, and lateral geniculate nucleus ROIs were generated using Talairach atlas labels from AFNI's built-in Talairach Daemon (Talairach and Tournoux, 1988). Striatal ROIs (ventral striatum, caudate nucleus, and putamen) were generated using spheres with a radius of 3.5 mm centered on coordinates corresponding to the inferior ventral striatum, dorsal caudate, and dorsal rostral putamen ROIs reported in a previous functional connectivity study (Di Martino *et al.*, 2008), which was based on a large-scale meta-analysis of striatal connectivity (Postuma and Dagher, 2006). The periaqueductal gray ROI was hand-drawn in MRICron software on the group average anatomical volume in MNI template space, based on neuroanatomical boundaries from the human brain atlas of Mai (Mai *et al.*, 2003). All between-group comparisons were assessed using non-parametric Mann-Whitney-Wilcoxon tests.

## 2.5. fMRI preprocessing and analysis

Rest-state fMRI data analysis was performed using AFNI (Cox, 1996) and FSL (<http://www.fmrib.ox.ac.uk/fsl/>). EPI volumes were slice time corrected using the first slice as a reference (sequential acquisition, Fourier interpolation), field map corrected (Jezzard and Clare, 1999), and motion corrected by rigid body alignment to the first EPI acquisition. Next, images were deobliques and the first three volumes were omitted from the EPI time series. Data were then motion corrected (3dvolreg) and despiked to remove extreme time series outliers. Finally, the time series data were band-pass filtered ( $0.01 < f < 0.1$ ) and spatially smoothed with a 4mm FWHM Gaussian kernel. Two NC subjects were excluded from the rest-state analysis ( $n=1$  with excessive head motion  $>2\text{mm}$  (Power *et al.*, 2012),  $n=1$  due to errors in field map correction) for a total sample size of  $n=17$  NC subjects. Functional connectivity was assessed using the hand-drawn anatomical ROIs in right and left BNST as seeds. Functional connectivity was computed using a GLM with the mean resting-state BOLD time series extracted from each subject-specific ROI and eight regressors of no interest, including six motion covariates, and average time series from white matter and ventricles. To further control for subject motion, volumes in which more than 10% of voxels were time series outliers were censored in the GLM. Correlation coefficients were converted to z-scores via Fisher's r-to-z transform and corrected for degrees of freedom. Resulting z-score maps were aligned to MNI space and resampled to  $3\text{mm}^3$  isotropic resolution for subsequent second-level analyses.

To specifically examine whether the BNST ROIs used in this study exhibited significant functional connectivity with vmPFC in the NC group, we conducted a whole brain voxel-wise one-sample t-test against zero using z-transformed BNST connectivity maps from each subject. Group differences in right and left BNST functional connectivity were assessed using whole-brain voxel-wise two-sample t-tests. All statistical maps were FWE-corrected for multiple comparisons across the whole brain at the cluster level ( $P_{\text{FWE}} < 0.05$ ), using a height threshold of  $P < 0.001$  (Forman *et al.*, 1995, Carp, 2012). A corrected  $P_{\text{FWE}} < 0.05$  was achieved using a cluster extent threshold of 37 voxels ( $999\text{ mm}^3$ ), calculated using Monte Carlo simulations.

## 2.6. Relationship to anxiety measures

To investigate whether individual differences in anxiety were related to CBF in BNST, we regressed self-report measures of negative affect and anxiety on CBF values in BNST ROIs. Self-report scales were validated measures of trait anxiety (STAI-T) (Spielberger *et al.*, 1983), depression (BDI) (Beck *et al.*, 1996), and negative affect (PANAS-negative) (Watson *et al.*, 1988). Two separate linear regression models were conducted for each measure, one examining the strength of the relationship in the NC group alone, and a second examining the relationship across both the NC and vmPFC groups together. Regression analyses were considered significant at  $P < 0.05$ .

## 3. RESULTS

The vmPFC lesion patients exhibited significantly lower perfusion in the right BNST, as compared to NC subjects ( $W=65$ ,  $p=0.027$ ), supporting the hypothesis that vmPFC plays a

critical role in promoting BNST function. There was no significant difference between groups for the left BNST ( $W=50$ ,  $p=0.37$ ). Among the comparison ROIs, the groups differed in only one region—the right mediodorsal nucleus of the thalamus ( $W=72$ ,  $p=0.003$ ). This unpredicted result survives Bonferroni correction for the total number of comparison ROIs ( $\alpha=0.05/13=0.004$ ). Complete group ASL results are presented in Table 2. BNST ASL data for each individual subject are presented in Supplementary Fig. 2.

To further explore the observed effect of vmPFC damage on BNST blood flow, we used rest-state fMRI to determine whether the BNST ROI used in this study is functionally connected with the vmPFC among the neurologically healthy subjects. As expected, the NC subjects exhibited significant rest-state functional connectivity between the right BNST and a single 116-voxel cluster within the vmPFC (Fig. 3). Importantly, the region of significant BNST functional connectivity was located in an area in which all four vmPFC patients had substantial damage. The left BNST seed exhibited a similar pattern of rest-state connectivity in the NC group, demonstrating significantly correlated activity in two clusters (45 and 91 voxels) located within the vmPFC. In a follow-up analysis examining group differences in functional connectivity, we observed no significant group differences in right BNST connectivity with any region outside of the area damaged in the vmPFC group. We did not specifically assess functional connectivity between the BNST and vmPFC in the lesion group because all four patients had significant damage to this region.

There were no significant relationships between any of the three self-reported measures of negative affect (BDI, STAI, and PANAS) and right or left BNST CBF, either within the NC group or across the full sample (all  $P$ 's  $> 0.10$ ).

## 4. DISCUSSION

In this study, we demonstrate a significant and rather selective reduction in right BNST perfusion in patients with bilateral vmPFC lesions. This finding supports our primary hypothesis, which was based on a non-human primate combined PET/lesion study, demonstrating reduced BNST metabolism following bilateral OFC lesions (Fox *et al.*, 2010). Our study is the first to use quantitative neuroimaging in human lesion patients to show an effect of vmPFC damage on resting cerebral perfusion, thereby providing unique and novel data regarding the causal relationship between vmPFC and BNST. More specifically, our data suggest that vmPFC normally serves to promote BNST activity, which in turn could enhance behavioral and physiological components of negative emotion (Davis and Shi, 1999, Walker *et al.*, 2003). This vmPFC-BNST interaction could explain in part why vmPFC damage in humans has been associated with blunted affect (Barrash *et al.*, 2000), diminished physiological arousal to emotionally evocative stimuli (Damasio *et al.*, 1990), and a reduced likelihood of developing PTSD and depression (Koenigs *et al.*, 2008, Koenigs *et al.*, 2008). A putative interaction between vmPFC and BNST is supported by our functional connectivity analysis (Fig. 3) as well as by previous human neuroimaging data documenting robust functional and structural connections between the two regions (Avery *et al.*, 2014). This vmPFC-BNST interaction could thus constitute an important addition to neural circuitry models of mood and anxiety disorders, which have predominantly focused



on the role of vmPFC in inhibiting amygdala activity (Quirk and Gehlert, 2003, Milad *et al.*, 2006, Rauch *et al.*, 2006).

The model of vmPFC-BNST interaction proposed above is based largely on previous research linking BNST activity to the expression of trait-like anxiety (Davis and Whalen, 2001, Walker *et al.*, 2003, Kalin *et al.*, 2005). Unlike the amygdala, which has been implicated in rapid, time-limited fear responses consistent with orienting towards potentially threatening stimuli, BNST is thought to be involved in generating and maintaining responses consistent with sustained anxiety (Walker *et al.*, 2003). Although direct axonal connections between vmPFC and BNST have not been well characterized in primates, high-resolution tracing studies in rodents have identified robust direct projections from infralimbic cortex—the putative homologue of human vmPFC—to BNST (McDonald *et al.*, 1999), in addition to well-documented indirect connections through other limbic regions like the amygdala, insula, and mediodorsal thalamic nucleus (McDonald *et al.*, 1999, Dong *et al.*, 2001, Dong *et al.*, 2001). Each of these areas in turn project to brainstem and hypothalamic nuclei involved in coordinating peripheral aspects of an emotional response (Heimer *et al.*, 1997, Tye *et al.*, 2011, Jennings *et al.*, 2013). Together, these regions form a network well-suited for the modulation and expression of behavioral and physiological components of emotion.

Unlike the previous lesion study in non-human primates (Fox *et al.*, 2010), we did not observe a significant relationship between BNST perfusion and measures of anxiety. There are several potential explanations for this null finding. One is that the ASL perfusion measure employed here may be too coarse of an index of BNST activity to correlate with specific aspects of emotional experience. Recent evidence indicates that BNST consists of distinct subregions with divergent roles in emotional expression. A study in rodents using optogenetics to activate and deactivate discrete subpopulations of BNST neurons found that focal stimulation of adjacent BNST subregions elicited opposing anxiolytic and anxiogenic effects (Jennings *et al.*, 2013, Kim *et al.*, 2013). Thus, the lack of an association between BNST perfusion and anxiety in the present may study reflect the conflicting effects of changes in blood flow on anatomically distinct and functionally antagonistic regions of BNST. Secondly, given that previous associations between BNST metabolism and anxiety phenotypes were observed using PET scanning under conditions designed to elicit anxious responses (Kalin *et al.*, 2005, Fox *et al.*, 2010), our approach of examining resting blood flow inside the MRI environment (with no anxiety-inducing stimulus) may be sufficient to detect overall differences in CBF that results from vmPFC damage, but not sensitive enough to observe the expected relationships between BNST function and anxious behavior. Finally, there was a narrow range of self-reported negative affect in our healthy adult comparison sample. All subjects were well within the subclinical range (Spielberger *et al.*, 1983, Watson *et al.*, 1988, Beck *et al.*, 1996), which may have limited our ability to detect a correlation between individual differences in BNST perfusion and self-reported anxiety. Future work in larger samples using more robust emotion induction paradigms and a broader range of anxiety levels will be necessary to more fully elucidate the relationship between BNST activity and psychopathology.

Several additional findings warrant further consideration. One is the unpredicted effect of vmPFC damage on mediodorsal thalamus perfusion. Among all comparison ROIs, only the

right mediodorsal thalamus exhibited a significant group difference. This finding can likely be explained by the fact that the mediodorsal nucleus is the region of thalamus that is the most densely interconnected with vmPFC (Ongur and Price, 2000). However, it is important to note that not all regions that are densely interconnected with vmPFC exhibited reductions in perfusion; no significant group differences were observed in either hemisphere for amygdala, ventral striatum, hypothalamus, or periaqueductal gray. This pattern of results suggests that vmPFC may play an especially critical role in modulating the activity of BNST and mediodorsal thalamus. Alternatively, the lack of significant findings in other brain regions could be due the small sample of vmPFC lesion patients, though the primate PET study also found no significant effect of OFC damage on amygdala metabolism (Fox *et al.*, 2010). Another noteworthy finding is that the significant reductions in BNST and mediodorsal thalamus perfusion were observed only in the right hemisphere. Even in a follow-up analysis, in which we refined our BNST ROI based on statistically significant ( $p < 0.005$ ) functional connectivity with the region of vmPFC exhibiting maximal overlap in the patient group, we see a similar result: vmPFC lesion patients had significantly lower perfusion in right BNST ( $W = 64$ ;  $p = 0.035$ ), whereas there was no significant group difference in left BNST ( $W = 48$ ;  $p = 0.46$ ). These lateralized effects may be due to the lesion characteristics of our vmPFC patient sample. Although all patients' lesions involved significant bilateral damage to vmPFC, each patient had slightly greater damage on the right side. Another possibility is that there may be some degree of asymmetry in the structure and function of the BNST. For example, fMRI studies have shown unilateral activation of BNST in anxiety-inducing paradigms (Straube *et al.*, 2007, Somerville *et al.*, 2010, Somerville *et al.*, 2013). Moreover, consistent with our findings, the PET study of non-human primates with bilateral OFC lesions reported a significant reduction in the metabolism of right, but not left, BNST (Fox *et al.*, 2010). Future work in larger patient samples with more heterogeneous vmPFC lesions could more conclusively determine the link between lateralization of vmPFC damage and BNST perfusion.

The sample size of vmPFC lesion patients was somewhat limited ( $n = 4$ ). For this study, we employed extremely stringent selection criteria for our target group; lesions had to involve substantial portions of vmPFC bilaterally, but could not extend significantly outside vmPFC. Furthermore, because the study involved fMRI, we could not include patients with metallic implants, such as aneurysm clips. To meet these criteria, we selected a group of patients who had all undergone surgical resection of large orbital meningiomas. So, although our sample size may be small by conventional vmPFC lesion patient standards (which typically feature  $n = 5$  to  $n = 12$  vmPFC lesion patients), it is unique with respect to the homogeneity of etiology, uniformity and selectivity of bilateral vmPFC lesions, and compatibility with MRI.

One limitation of the present study is the single neuroimaging measure (ASL) used to index BNST activity. Because resting cerebral blood flow is tightly coupled to cerebral metabolism (Fox and Raichle, 1986), ASL can be interpreted as a proxy measure of cerebral metabolism—and hence underlying neural activity—similar to PET (Xu *et al.*, 2010, Okonkwo *et al.*, 2012). However, the ASL data in this study only indicate resting state perfusion. Future fMRI studies could build upon the present results by determining whether



vmPFC damage also diminishes stimulus-evoked BNST activity in anxiogenic tasks (Alvarez *et al.*, 2011, Grupe *et al.*, 2013, Somerville *et al.*, 2013).

In sum, through a unique application of ASL cerebral perfusion imaging in patients with bilateral vmPFC lesions, we have demonstrated a role for vmPFC in promoting BNST function. This finding corroborates non-human primate data and yields novel insight on the brain circuitry underlying human emotion. Future work will be needed to more fully elucidate the role of vmPFC in the modulation of BNST function, as this circuit may be central to the dysregulated affect associated with anxious psychopathology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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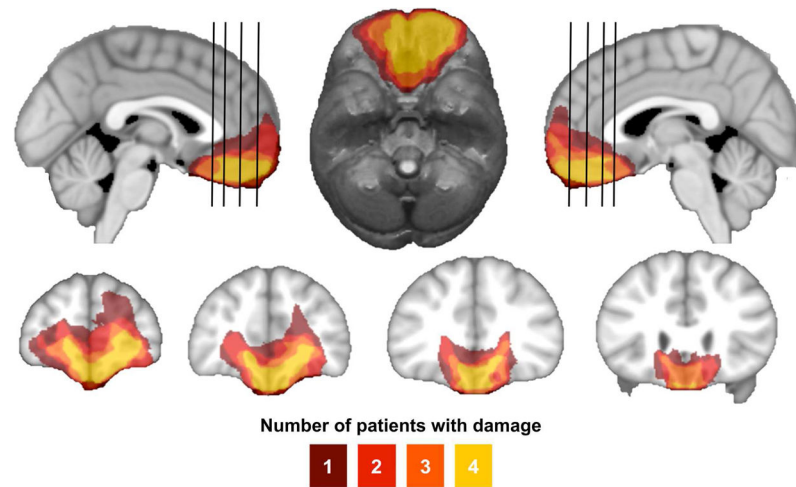
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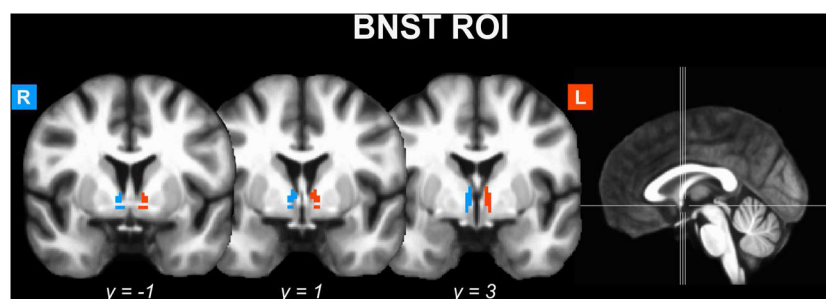
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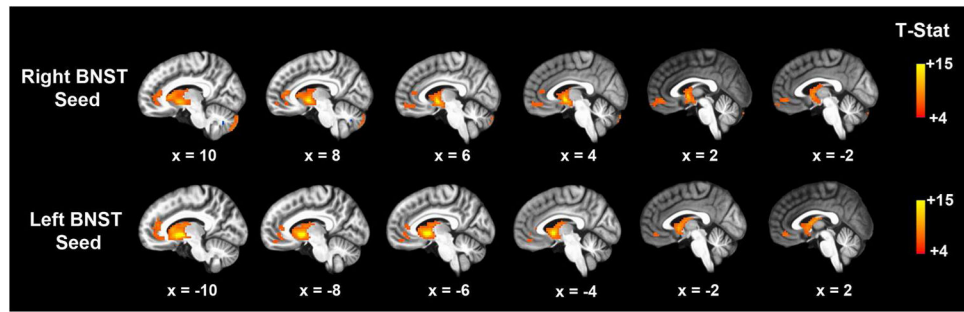


**Figure 1.**

Lesion overlap of vmPFC patients. Color indicates the number of overlapping lesions at each voxel. All vmPFC patients had damage to the medial one-third of the orbitofrontal cortex and the ventral one-third of medial surface of prefrontal cortex, bilaterally. This area includes Brodmann areas 11, 12, 24, 25, 32, and the medial portion of 10 below the level of the genu of the corpus callosum, as well as subjacent white matter.



**Figure 2.** BNST Regions of Interest (ROIs). Right (blue) and left (orange) BNST ROIs used to examine group differences in perfusion.



**Figure 3.**

Rest-state functional connectivity for the right and left BNST ROI in n=17 NC subjects. Significant functional connectivity was observed between the right BNST and a region of vmPFC in which all four vmPFC patients had damage (top row). A similar pattern of functional connectivity with the vmPFC was found for the left BNST ROI (bottom row). Coordinates presented (in mm) in MNI template space, thresholded at  $P_{FWE} < 0.05$ .



Table 1

Subject characteristics

	Age	Sex	Edu	IQ	Pos Aff	Neg Aff	BDI-II	STAI-T
vmPFC (n=4)	58.5 (6.2)	3 M 1 F	15.5 (4.1)	103.8 (12.4)	36 (8.4)	17.0 (8.7)	7.0 (3.2)	34.3 (9.5)
NC (n=19)	51.7 (9.9)	11 M 8 F	17.7 (3.5)	110.9 (7.2)	37.8 (4.9)	13.0 (2.4)	4.0 (3.3)	31.6 (6.0)
P (vmPFC vs NC)	0.16	0.63	0.51	0.25	0.56	0.73	0.11	0.44

Means are presented with standard deviations in parentheses. Edu, years of education; IQ, intelligence quotient estimated by the Wide Range Achievement Test 4, Blue Reading subtest (Wilkinson and Robertson, 2006); Pos/Neg Aff, scores from the Positive and Negative Affect Schedule (PANAS) (Watson *et al.*, 1988); BDI-II, Beck Depression Inventory-II (Beck *et al.*, 1996) ; STAI-T, trait version of the Spielberger State Trait Anxiety Inventory (Spielberger *et al.*, 1983).

**Table 2**

ASL cerebral perfusion data

ROI	NC mean	NC s.d.	vmPFC mean	vmPFC s.d.	W	p
<b>Whole Brain</b>	36.30	8.73	40.09	13.65	31	0.611
<b>BNST</b>						
L BNST	1.07	0.14	1.00	0.16	50	0.366
R BNST	<i>1.03</i>	<i>0.16</i>	<i>0.83</i>	<i>0.07</i>	65	<i>0.027</i>
<b>vmPFC network</b>						
L amygdala	1.11	0.15	1.23	0.11	20	0.162
R amygdala	1.1	0.15	1.01	0.19	46	0.557
L ventral striatum	1.43	0.21	1.31	0.13	51	0.324
R ventral striatum	1.36	0.23	1.14	0.07	58	0.116
L MD thalamus	1.28	0.09	1.23	0.05	51	0.324
R MD thalamus	<i>1.32</i>	<i>0.10</i>	<i>1.11</i>	<i>0.10</i>	72	<i>0.003</i>
L hypothalamus	1.08	0.16	1.01	0.09	46	0.557
R hypothalamus	1.07	0.18	0.91	0.04	61	0.067
Periaqueductal gray	0.94	0.12	0.96	0.11	33	0.725
<b>Non-vmPFC network</b>						
L caudate	1.24	0.21	1.17	0.23	45	0.611
R caudate	1.29	0.22	1.18	0.14	52	0.286
L putamen	1.26	0.23	1.26	0.17	36	0.907
R putamen	1.32	0.23	1.27	0.18	42	0.785
L LGN thalamus	1.04	0.17	1.08	0.08	28	0.456
R LGN thalamus	1.14	0.22	1.03	0.09	50	0.366

Significant group differences are in italics. L, left; R, right; BNST, bed nucleus of stria terminalis; LGN, lateral geniculate nucleus; MD, mediodorsal.