ARCHIVAL REPORT

Ventromedial Prefrontal Cortex Is Critical for the Regulation of Amygdala Activity in Humans

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Background: Dysfunction in the ventromedial prefrontal cortex (vmPFC) is believed to play a pivotal role in the pathogenesis of mood and anxiety disorders. Leading neurocircuitry models of these disorders propose that hypoactivity in the vmPFC engenders disinhibited activity of the amygdala and, consequently, pathologically elevated levels of negative affect. This model predicts that a selective loss or diminution of function of the vmPFC would result in heightened activity of the amygdala. Although this prediction has been borne out in rodent lesion and electrophysiologic studies using fear conditioning and extinction paradigms, there has not yet been a definitive test of this prediction in humans.

Methods: We tested this prediction through a novel use of functional magnetic resonance imaging in four neurosurgical patients with focal, bilateral vmPFC damage.

Results: Relative to neurologically healthy comparison subjects, the patients with vmPFC lesions exhibited potentiated amygdala responses to aversive images and elevated resting-state amygdala functional connectivity. No comparable group differences were observed for activity in other brain regions.

Conclusions: These results provide unique evidence for the critical role of the vmPFC in regulating activity of the amygdala in humans and help elucidate the causal neural interactions that underlie mental illness.

Key Words: Amygdala, anxiety, emotion, fMRI, lesion, prefrontal cortex

The ventromedial prefrontal cortex (vmPFC) is a key neural substrate of human social and affective function (1–3) and is considered central to the pathophysiology of mood and anxiety disorders (4,5) However, the precise mechanisms by which the vmPFC contributes to affective processing are not fully understood. The predominant neural circuitry model proposes that the 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 (6–8). Multiple lines of convergent evidence support this inhibitory model of vmPFC function. In rodents, infralimbic cortex (the purported homologue of human vmPFC) has been shown to mediate sustained extinction of conditioned fear through inhibition of the amygdala (7,9,10). In humans, functional imaging studies have demonstrated that activity in the vmPFC and amygdala is inversely related during the extinction of conditioned fear (11) and during the volitional suppression of negative emotion (12–14), with the inverse coupling between the vmPFC and the amygdala commonly disrupted in mood and anxiety disorders (6,7,13). Anatomic tracing studies in rodents and nonhuman primates have identified direct projections from the vmPFC to inhibitory interneurons within the amygdala, indicating a viable anatomic substrate for the observed functional relationship (15,16).

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Although these findings are consistent with the proposal that the vmPFC plays a critical and causal role in regulating activity of the amygdala, human vmPFC lesions are commonly associated with changes in personality and behavior (e.g., social disinhibition, blunted affect) that are notably distinct from changes typical of anxious and depressive psychopathology (17,18). Further, focal vmPFC damage has been shown to reduce the likelihood of developing posttraumatic stress disorder and depression (19,20), consistent with previous studies indicating that metabolism in the subgenual cingulate region of the vmPFC is increased (not decreased) in depression (21). Thus, it remains unknown whether the disruption of vmPFC function would significantly disinhibit amygdala activity in humans. In the present study, we addressed this empirical gap through a novel application of functional magnetic resonance imaging (fMRI) to patients with focal, bilateral vmPFC lesions. Using this unique approach, we show that the vmPFC exerts a causal influence on amygdala activity in humans.

Methods and Materials

Participants

The target 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 (Figure 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 magnetic resonance imaging performed after surgery, although vasogenic edema largely resolved, there were persistent T2-weighted signal changes, consistent with gliosis, in the vmPFC bilaterally. All experimental procedures were conducted >3 months after surgery, when the expected recovery was complete. At the time of testing, all patients had focal, stable magnetic resonance imaging signal changes and resection cavities and were free of dementia and substance abuse.

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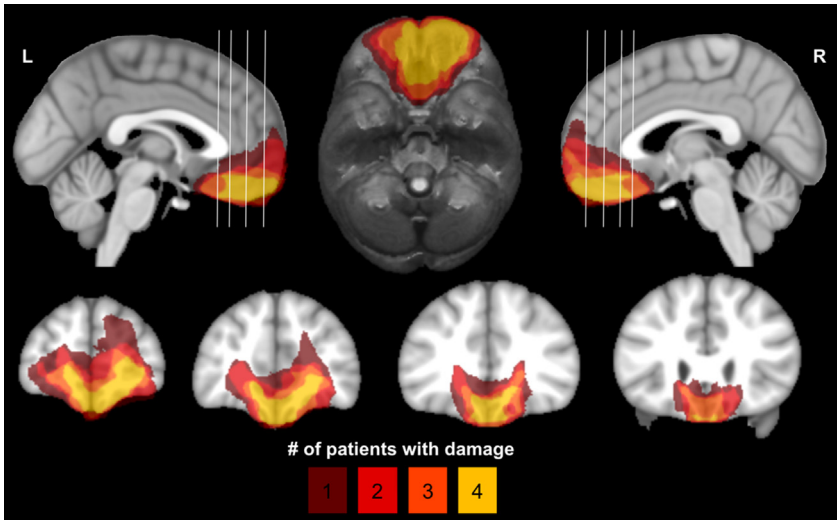

Figure 1. Lesion overlap of patients with ventromedial prefrontal cortex lesions. Color indicates the number of overlapping lesions at each voxel. All patients with ventromedial prefrontal cortex lesions had damage to the medial one third of the orbitofrontal cortex and the ventral one third of the medial surface of prefrontal cortex bilaterally. This area includes Brodmann areas 11, 12, 24, 25, and 32 and the medial portion of 10 below the level of the genu of the corpus callosum as well as subjacent white matter. L, left; R, right.

As a normal comparison (NC) group, 19 healthy adults with no history of brain injury, neurologic or psychiatric illness, or current use of psychoactive medication were recruited. From the full NC group, we selected a subsample of 10 subjects who were more closely matched to the patients with vmPFC lesions in age and gender to corroborate results from the larger NC sample. Demographic and neuropsychological data for the vmPFC and NC groups are summarized in Table 1.

Event-Related fMRI Task

We assessed amygdala function in two separate fMRI experiments: an event-related task scan involving the presentation of aversive and neutral pictures and a resting-state scan in which subjects passively viewed a fixation cross. During the fMRI task, adapted from a previous paradigm shown to elicit strong amygdala activation in healthy subjects (22), subjects viewed 64 unique images drawn from the International Affective Picture System (23), divided evenly among pictures with aversive and neutral content (Figure S1 and Table S1 in Supplement 1). Aversive stimuli consisted of 32 negative or unpleasant and arousing images, based on published norms (23,24) (valence, 2.01 ± .39; arousal, 6.25 ± .7). Neutral stimuli consisted of 32 images with neutral valence and low arousal ratings (valence, 4.96 ± .21; arousal, 2.95 ± .77). All images were preceded by one of three visual cues ("X," "O," or "?"). The "X" and "O" cues indicated that the subsequent image would be aversive or neutral, respectively, whereas the "?" cue provided no information regarding the emotional content of the image (equal likelihood of aversive or neutral content). Each experimental trial consisted of a cue presented for 2 sec, followed—after a jittered interstimulus interval (range, 2–8 sec)—by a 1-sec picture presentation. After a second jittered interstimulus interval (range, 5–9 sec), subjects had 4 sec to rate their emotional response to the image using a scale ranging from 1 ("very positive") to 4 ("very negative") (Table S2 in Supplement 1). Before scanning, subjects were informed of all cue-picture contingencies and completed a practice task consisting of 16 unique trials (4 per cue-picture pair) to ensure task comprehension.

Magnetic Resonance Imaging Data Acquisition

All structural magnetic resonance imaging and fMRI data were acquired using a 3.0 tesla GE Discovery MR750 scanner equipped with an eight-channel radiofrequency head coil array (GE Healthcare, Waukesha, Wisconsin). High-resolution T1-weighted anatomic images were acquired using an inversion recovery spoiled gradient recalled acquisition in the steady state (GRASS) sequence (repetition time [TR] = 8.2 msec; echo time [TE] = 3.2 msec; $\alpha = 12^{\circ}$; field of view [FOV] = 256 mm × 256 mm; matrix = 256 × 256; in-plane resolution = 1 mm × 1 mm; slice thickness = 1 mm; 1024 axial slices). To facilitate lesion segmentation, we collected a separate T2-weighted fluid attenuated inversion recovery scan (TR = 8650 msec; TE = 136 msec; $\alpha = 0^{\circ}$; FOV = 220 mm × 220 mm; matrix = 512 × 512; in-plane resolution = .43 mm × .43 mm; slice thickness = 5 mm; gap = 1 mm; 25 axial slices).

Baseline resting cerebral blood flow (CBF) was estimated using a three-dimensional fast spin echo spiral sequence with pseudocontinuous arterial spin labeling (pcASL) (25–27) and background suppression for quantitative perfusion measurements (TR = 4653 msec;

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)
NC age >50 ($n = 10$)	59.8 (4.7)	8 M, 2 F	16.8 (2.3)	113.1 (7.2)	39.2 (5.4)	12.6 (2.7)	3.7 (2.9)	29.6 (5.0)
p (vmPFC vs. NC)	.16	.63	.51	.25	.56	.73	.11	.44
p (vmPFC vs. NC age >50)	.95	.99	.64	.14	.54	.64	.13	.28

Means are presented with SDs in parentheses.

BDI-II, Beck Depression Inventory-II (69); Edu, years of education; IQ, intelligence quotient estimated by the Wide Range Achievement Test 4, Blue Reading subtest (67); F, female; M, male; NC, normal comparison; Pos/Neg Aff, scores from the Positive and Negative Affect Schedule (68); STAI-T, trait version of the Spielberger State Trait Anxiety Inventory (70); vmPFC, ventromedial prefrontal cortex.

TE = 10.5 msec; post-labeling delay = 1525 msec; labeling duration = 1450 msec; eight interleaved spiral arms with 512 samples at 62.5-kHz bandwidth and 38 4-mm-thick slices; number of excitations = 3; scan duration = 4.5 min).

Whole-brain functional scans (task and rest) were acquired using a T2*-weighted gradient echo echoplanar imaging sequence (TR = 2000 msec; TE = 22 msec; $\alpha = 79^{\circ}$; FOV = 224 mm × 224 mm; matrix = 64 × 64; in-plane resolution = 3.5 mm × 3.5 mm; slice thickness = 3 mm; gap = .5 mm; 38 interleaved axial oblique slices). Field maps were acquired using two separate acquisitions (TR = 600 msec; TE_1 = 7 msec; TE_2 = 10 msec; $\alpha = 60^{\circ}$; FOV = 240 mm × 240 mm; matrix = 256 × 128; slice thickness = 4 mm; 33 axial oblique slices). Resting-state functional images were collected while subjects lay still and awake, passively viewing a fixation cross for 5 min. The two task runs lasted 12.4 min each. Scans were acquired in the following order: pcASL, field map, rest, task, T1, T2–fluid attenuated inversion recovery.

Heart Rate Data Acquisition

Cardiac data were acquired at 100 Hz with a photoplethysmograph (GE Healthcare) affixed to the left index finger throughout the scan session. Heart rate data were available for 12 NC subjects and all 4 patients with vmPFC lesions.

Lesion Segmentation and Image Normalization

Individual vmPFC lesions were visually identified and manually segmented on the T1-weighted images. Lesion boundaries were drawn to include areas with gross tissue damage or abnormal signal characteristics on T1 or T2 fluid attenuated inversion recovery images. T1-weighted images were skull-stripped, rigidly coregistered with a functional volume from each subject, and diffeomorphically aligned to the Montreal Neurological Institute (MNI) coordinate system using a symmetric normalization algorithm (28) with constrained cost-function masking to prevent warping of tissue within the lesion mask (29). We created the lesion overlap map (Figure 1) by computing the sum of aligned binary lesion masks for all four patients with vmPFC lesions.

fMRI Task Preprocessing and Analysis

Data analysis was conducted using AFNI (30) and FMRIB Software Library (http://www.FMRIB.ox.ac.uk/fsl) software. Individual task runs were slice time corrected, field map corrected (31), motion corrected, smoothed with a 6-mm full width at half maximum Gaussian kernel, and scaled to percent signal change. Preprocessed task data were concatenated and analyzed using a general linear model (GLM) with separate regressors for each cue and picture type; the rating period; and several regressors of no interest, including six motion covariates from rigid-body alignment (32) and a fourth-order polynomial to model baseline and slow signal drift. Blood oxygen level–dependent (BOLD) signal was modeled by convolving each event with the AFNI default canonical hemodynamic response function (gamma function). Because the identity of the cue did not significantly alter amygdala responses to the aversive pictures in either group (Table S3 in Supplement 1), analyses were limited to aversive and neutral stimuli, regardless of cue. To avoid potential confounds introduced by subject motion, volumes in which >10% of voxels were time series outliers were censored before conducting the GLM; there were no group differences in the average proportion of censored volumes ($\chi^2 = 2.09$, $p = .15$) or in mean framewise displacement (NC, .06 ± .06 mm; vmPFC, .04 ± .02 mm; $W = 28$; $p = .44$). Resulting whole-brain maps of voxelwise β values for aversive and neutral pictures were aligned to MNI space and resampled to 3 mm^3 isotropic resolution for second-level analyses.

To identify brain regions responsive to aversive stimuli, we performed a whole-brain, two-tailed paired-sample *t* test between responses to aversive and neutral pictures in the full NC group. Resulting statistical maps were familywise error (FWE) corrected for multiple comparisons across the whole brain at the cluster level ($p_{FWE} < .05$), using a height threshold of $p < .001$ (33,34). A corrected $p_{FWE} < .05$ was achieved using a cluster extent threshold of 38 voxels (1026 mm^3), calculated using Monte Carlo simulations with 3dClustSim in AFNI. Significant clusters from the aversive > neutral contrast (10 total) were used as functional regions of interest (ROIs) for subsequent between-group analyses.

To visualize group-averaged BOLD responses to pictures within individual ROIs, we conducted a second GLM, replacing the canonical hemodynamic response function with a series of nine TENT functions to deconvolve the raw BOLD signal. This model yielded β values for each of 9 TRs 0–16 sec after picture onset. Because functional ROIs were derived using the canonical hemodynamic response function, estimated response data from the deconvolution model were used for display only.

In light of the small sample size of patients with vmPFC lesions, we used nonparametric Mann-Whitney *U* tests to evaluate our main a priori hypothesis regarding activity of the amygdala. Specifically, we focused our between-group analyses on percent signal change estimates extracted from functionally derived right and left amygdala ROIs (amygdala clusters from the aversive > neutral contrast in the NC group). We used functional ROIs to ensure that group comparisons were conducted within functionally homogeneous regions within the amygdala (i.e., regions that respond strongly to aversive relative to neutral stimuli) (35). However, to confirm that group comparisons within functionally derived amygdala ROIs reflected differences in amygdala activity per se, we conducted additional between-group tests using values extracted from atlas-defined anatomical ROIs in the right and left amygdala, created using the Talairach Daemon in AFNI. To examine subregions of the amygdala, we conducted follow-up analyses using hand-drawn, atlas-defined ROIs in the central nucleus of the amygdala (CeA) (36).

To test the specificity of observed effects to the amygdala, we conducted follow-up analyses on percent signal change values extracted from the eight remaining functionally derived non-amygdala comparison ROIs (e.g., bilateral visual cortex, lateral temporal cortex, thalamus), in which we predicted normal responses to pictures for the patients with vmPFC lesions. All group comparisons were corroborated with the subsample of 10 age-matched and gender-matched NC subjects to verify that group effects were not driven by potential differences in demographic variables. All tests were considered significant at $p < .05$.

Resting-State Functional Connectivity Analysis

Resting-state scans were preprocessed as in the task analysis, with despiking and band-pass filtering ($.01 < f < .1$) conducted before smoothing. There were 2 NC subjects excluded from the resting-state analysis ($n = 1$ because of excessive head motion [>2 mm] (37) and $n = 1$ because of errors in field map correction) for a total sample size of 17 NC subjects. Functional connectivity was assessed using task-derived left and right amygdala ROIs, masked by the anatomic amygdala ROI to exclude voxels outside the amygdala and aligned to native space, and confirmed using independent anatomically defined CeA ROIs. Functional

connectivity was computed using a GLM with the mean resting-state BOLD time series extracted from each subject-specific ROI and nine regressors of no interest, including six motion covariates, average time series from white matter and ventricles, and a second-order polynomial to model baseline signal and slow drift. To control further for subject motion, volumes in which >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* transformation and corrected for degrees of freedom. Resulting *z* score maps were aligned to MNI space and resampled to 3 mm^3 isotropic resolution for subsequent second-level analyses.

Because we had no a priori hypothesis regarding the particular brain regions where vmPFC damage would yield altered amygdala connectivity, we conducted an exploratory whole-brain voxelwise nonparametric comparison between amygdala connectivity maps in the NC and vmPFC groups. Resulting statistical maps were FWE-corrected for multiple comparisons across the whole brain at the cluster level ($p_{FWE} < .05$), using a height threshold of $p < .005$ (33,34). A corrected $p_{FWE} < .05$ was achieved using a cluster extent threshold of 90 voxels (2430 mm^3), calculated using Monte Carlo simulations.

Cerebral Perfusion Analysis

Quantitative CBF images from pcASL were rigidly coregistered with a T2*-weighted echo planar imaging 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 patients with vmPFC lesions) and smoothed with a 6-mm full width at half maximum Gaussian kernel. To rule out differences in baseline cerebral perfusion, we examined group differences in mean whole-brain CBF and differences in scaled CBF for all ROIs using nonparametric Mann-Whitney *U* tests.

Heart Rate Analysis

To assess cardiac responses to picture stimuli, we computed trialwise estimates of heart rate change for each subject, as previously described (38). Cardiac R-spikes were identified using interactive beat detection software. Trials with ectopic beats, missed beats, or periods of noisy signal (where beat detection failed), were excluded from further analysis (NC group, $n = 2$ with one excluded trial, $n = 1$ with two excluded trials, $n = 2$ with three excluded trials; vmPFC group, $n = 1$ with two excluded trials). R-R intervals were transformed into heart rate in beats per minute, averaged in 500-msec bins. Changes in heart rate were determined by subtracting the mean heart rate for 1 sec preceding each picture from the heart rate at each 500 msec after picture onset. As in previous studies, the maximum cardiac deceleration (i.e., heart rate decrease) during the first 3 sec of picture viewing was used as an index of the physiologic response to each picture (38). Group differences in cardiac deceleration were computed separately for aversive and neutral pictures using nonparametric Mann-Whitney *U* tests.

Results

fMRI Task

During the fMRI task, both groups rated aversive pictures as significantly more negative than neutral pictures, with no differences between groups in ratings for either emotion category (Table S2 in Supplement 1). Relative to neutral pictures, aversive pictures elicited robust bilateral amygdala activation in both NC subjects (Figure 2A and Table 2) and patients with vmPFC lesions (Figure 2B). To examine group differences in amygdala activity, we extracted percent signal change estimates from functionally derived right and left amygdala ROIs—clusters of suprathreshold amygdala voxels from the aversive > neutral contrast in the NC group (Figure 3A). In support of our main hypothesis, patients with vmPFC lesions exhibited significantly greater right amygdala activation to aversive pictures than NC subjects ($W = 6$, $p = .006$) (Figure 3B, Table 3). We observed similar group differences in activation to aversive pictures using an anatomically defined right amygdala ROI ($W = 13$, $p = .04$) and an anatomically defined right CeA ROI ($W = 13$, $p = .04$). This central finding was corroborated in a smaller sample of 10 NC subjects closely matched in age and gender to the patients with vmPFC lesions ($W = 2$, $p = .008$) (Table S4 in Supplement 1), suggesting that the findings were not driven by group differences in demographic factors. No significant group differences were observed in any left amygdala ROI (functional ROI, $W = 28$, $p = .46$; anatomic ROI, $W = 24$, $p = .29$; CeA ROI, $W = 24$, $p = .29$) (Figure 3, Table 3, and Table S4 in Supplement 1).

To test the specificity of group differences to the amygdala, we conducted follow-up analyses in the eight remaining functionally derived ROIs from the aversive > neutral picture contrast (e.g., visual cortex, lateral temporal cortex, thalamus) and found no consistent group differences in the response to aversive or neutral pictures in the nonamygdala comparison ROIs (Table 3 and Table S4 in Supplement 1). To ensure that group differences in the amygdala were not due to baseline differences in amygdala perfusion after vmPFC damage, we estimated CBF using pcASL before both functional scans in all subjects. There were no significant differences between the NC subjects and the patients with vmPFC lesions in whole-brain CBF, and there were no differences in relative CBF for any ROI used in group comparisons, including right amygdala (Tables S5 and S6 in Supplement 1). Finally, it is unlikely that the observed findings are due to systematic group differences in the shape of the hemodynamic response because there were no apparent differences in the

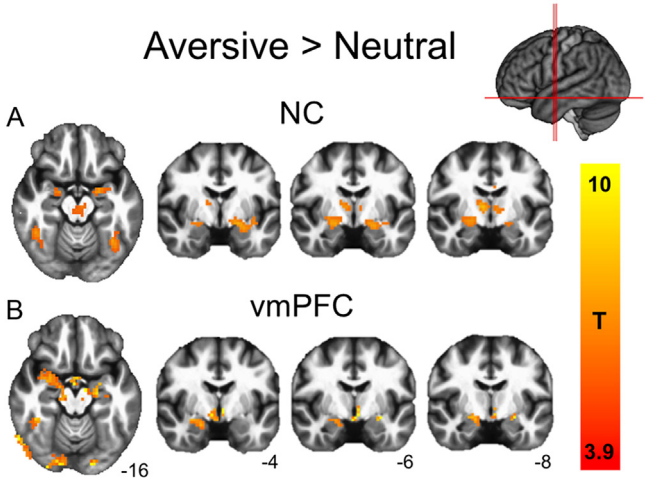

Figure 2. Neural responses to aversive > neutral pictures. **(A)** Subjects for NC ($p_{FWE} < .05$). **(B)** Patients with vmPFC lesions (displayed at corrected NC threshold of $T = 3.9$ for comparison). Both groups exhibited robust bilateral amygdala responses as well as responses in visual cortex, lateral temporal cortex, thalamus, and cingulate gyrus (Table 2 contains full cluster list). FWE, familywise error; NC, normal comparison; vmPFC, ventromedial prefrontal cortex.

Table 2. Cluster Maxima for Regions with Statistically Significant Increased BOLD Signal for Aversive Pictures Relative to Neutral Pictures

	NC Group							vmPFC Group	
				Peak Voxel					
Brain Region	BA	Cluster Size	p_{FWE}	*t*	x	y	*z*	*t*	*p*
R ITG	37	684	<.0001	12.50	47	−68	−3	3.47	.040
Thal		337	<.0001	11.16	−1	−27	−4	1.99	.141
L MTG	37	597	<.0001	8.84	−52	−69	6	3.07	.055
R Lingual	17	283	<.0001	7.00	17	−90	−3	1.36	.267
L Amyg	28	72	<.005	6.49	−19	−3	−12	2.23	.112
R Amyg	28	39	<.05	6.03	20	−6	−12	3.38	.043
R Precun	31	64	<.005	6.00	5	−48	33	−.85	.458
L MFG	9	68	<.005	5.23	−7	51	27	.90	.434
L ACC	24/32	72	<.005	4.86	−1	6	39	.87	.448
L PCC	23	62	<.005	4.58	−7	−24	27	−1.55	.219

Clusters ordered by *t* score, for the aversive > neutral contrast in the NC group. Corrected *p* thresholds indicate minimum FWE-corrected *p* value for each cluster. Uncorrected *p* values for the vmPFC group are derived from a voxelwise paired *t* test in the vmPFC group, estimated at the peak coordinates for the NC group.

ACC, anterior cingulate cortex; Amyg, amygdala; BA, Brodmann area; BOLD, blood oxygen level–dependent; FWE, familywise error; ITG, inferior temporal gyrus; L, left; Lingual, lingual gyrus; MFG, medial frontal gyrus; MTG, middle temporal gyrus; NC, normal comparison; PCC, posterior cingulate cortex; Precun, precuneus; R, right; Thal, thalamus; vmPFC, ventromedial prefrontal cortex.

estimated hemodynamic response in motor cortex to button press (Figure S2 in Supplement 1) or in visual and temporal comparison ROIs in response to aversive pictures (Figure S3 in Supplement 1).

Heart Rate Response to Pictures

To determine whether group differences in amygdala activity were accompanied by comparable differences in peripheral physiologic responses, we investigated stimulus-evoked reductions in heart rate in response to the picture stimuli. Consistent with previous studies using the same stimuli, both groups exhibited cardiac deceleration in response to aversive and neutral pictures (38,39). However, in contrast to the amygdala fMRI results, the magnitude of stimulus-evoked cardiac deceleration was significantly lower in patients with vmPFC lesions than in the NC subjects for aversive pictures (NC, −1.3 ± .67; vmPFC, −.52 ± .31; W = 6, p = .03) (Figure S4 in Supplement 1). We observed similar, although nonsignificant, reductions in cardiac deceleration in response to neutral pictures (NC, −1.4 ± .89; vmPFC, −.60

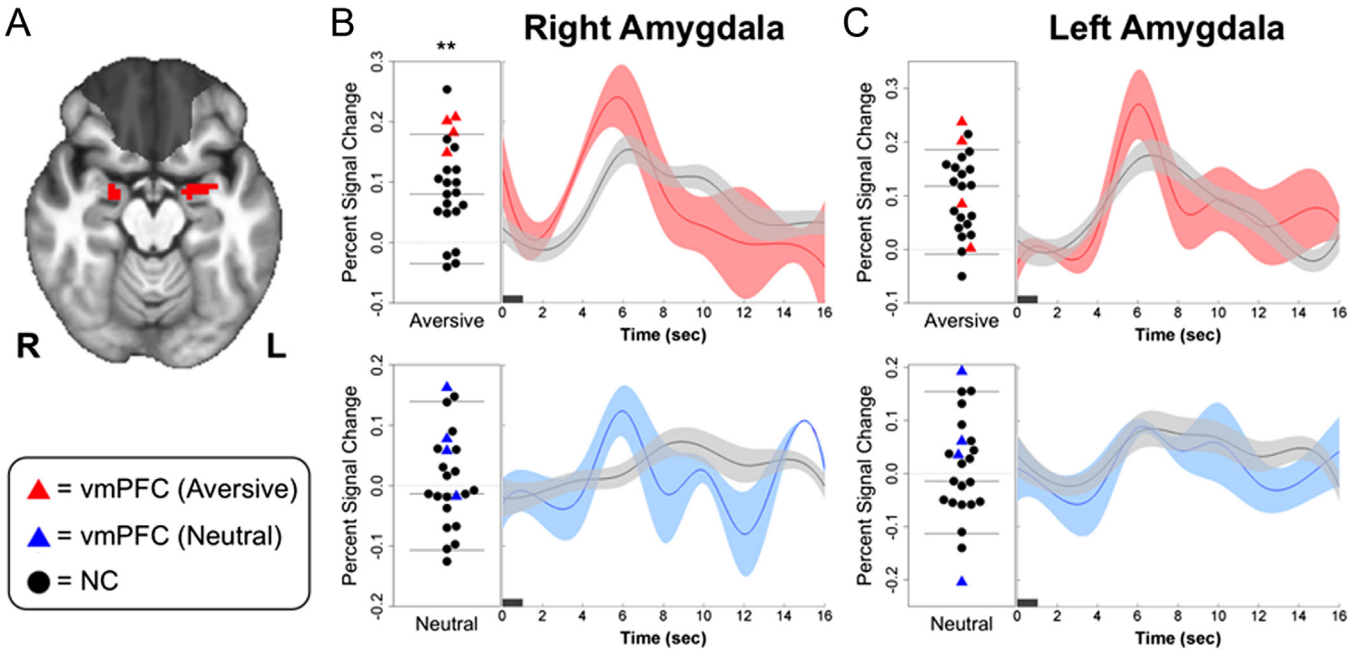

Figure 3. Greater right amygdala responses to aversive pictures in patients with vmPFC lesions. **(A)** Task-derived right (R) and left (L) amygdala regions of interest (red) used to extract mean percent signal change (PSC) estimates for group comparisons. Overlap of vmPFC lesions is shaded in gray for reference. **(B)** Left, Plots of right amygdala PSC for individual NC (black circles) and vmPFC (red and blue triangles) subjects in response to aversive pictures (top) and neutral pictures (bottom). Horizontal lines represent the mean and 95% confidence intervals of PSC values in the NC group. Right, Mean time series of right amygdala PSC in response to aversive and neutral pictures for vmPFC (red, blue) and NC (black) subjects (width of shaded area corresponds to ±1 SEM). **(C)** Plot and mean time series of PSC extracted from the left amygdala region of interest. Dark horizontal bars on time series plots indicate picture duration (1 sec). **p < .01. NC, normal comparison; vmPFC, ventromedial prefrontal cortex.

Table 3. Group Differences in Percent Signal Change to Aversive and Neutral Pictures in Functional and Anatomic Regions of Interest

	Region of Interest				Aversive Pictures				Neutral Pictures			
		Center of Mass										
Brain Region	Size	X	Y	Z	NC, Mean (SD)	vmPFC, Mean (SD)	W	*p*	NC, Mean (SD)	vmPFC, Mean (SD)	W	*p*
R Amyg (Func ROI)	39	23	−6.3	−9.6	.08 (.07)	.18 (.03)	6	.006[a]	.00 (.08)	.07 (.07)	20	.162
R Amyg (Anat ROI)	18	25	−3.8	−16	.09 (.08)	.19 (.07)	13	.044[a]	.02 (.08)	.05 (.12)	31	.611
R Amyg (CeA ROI)	6	23	−7.5	−15	.09 (.08)	.18 (.06)	13	.044[a]	.00 (.09)	.06 (.09)	23	.25
L Amyg (Func ROI)	72	−25	−2.7	−12	.10 (.07)	.13 (.11)	28	.456	.01 (.08)	.02 (.17)	31	.611
L Amyg (Anat ROI)	29	−23	−4.2	−17	.10 (.08)	.16 (.11)	24	.286	.02 (.09)	.03 (.14)	32	.667
L Amyg (CeA ROI)	6	−22	−7.5	−15	.12 (.09)	.19 (.11)	24	.286	.01 (.09)	.01 (.19)	31	.612
R ITG	684	46	−68	4	.34 (.13)	.42 (.11)	23	.25	.22 (.11)	.31 (.07)	20	.162
L MTG	597	−43	−71	6.6	.30 (.12)	.35 (.07)	26	.366	.20 (.10)	.25 (.02)	22	.218
Thal	337	.9	−20	−2.2	.14 (.08)	.18 (.02)	24	.286	.06 (.08)	.11 (.07)	23	.25
R Lingual	283	6.7	−87	1.3	.48 (.18)	.50 (.14)	31	.611	.38 (.14)	.44 (.08)	24	.286
L ACC	72	−1.5	15	32	.10 (.06)	.10 (.06)	40	.907	.02 (.09)	.07 (.06)	23	.25
L MFG	68	−8.5	51	26	.03 (.09)	.01 (.12)	41	.845	−.08 (.07)	−.04 (.06)	26	.366
R Precun	64	.1	−50	31	−.01 (.06)	−.02 (.04)	45	.611	−.06 (.07)	.01 (.03)	17	.097
L PCC	62	−1.3	−18	33	.05 (.05)	−.03 (.12)	56	.162	−.02 (.06)	.02 (.08)	27	.409

Cluster size in number of voxels ($3 \times 3 \times 3$ mm^3). Center of mass coordinates for each region of interest presented in Montreal Neurological Institute space.

ACC, anterior cingulate cortex; Amyg, amygdala; Anat ROI, anatomically defined region of interest; CeA ROI, central nucleus of the amygdala region of interest; Func ROI, functionally defined region of interest; ITG, inferior temporal gyrus; L, left; Lingual, lingual gyrus; MFG, medial frontal gyrus; MTG, middle temporal gyrus; NC, normal comparison; PCC, posterior cingulate cortex; Precun, precuneus; R, right; Thal, thalamus; vmPFC, ventromedial prefrontal cortex.

[a]*p* values for significant group differences.

± .30; W = 11, $p = .13$). There was no significant difference between groups in overall mean heart rate across the scan session (NC, 62.7 ± 9; vmPFC, 75.9 ± 12; W = 11, $p = .13$).

Resting State Functional Connectivity

To investigate whether group differences in amygdala activation during the task were also associated with group differences in amygdala resting-state functional connectivity, we conducted a secondary analysis using amygdala seed ROIs. Consistent with the results of the fMRI task, the resting-state functional connectivity analysis revealed greater connectivity between the right amygdala and a region of right anterolateral temporal cortex in patients with vmPFC lesions (Figure 4 and Figure S5 in Supplement 1). This finding was replicated using an independent anatomically defined CeA ROI (Figure S6 in Supplement 1).

Discussion

Through a novel application of fMRI in patients with bilateral vmPFC damage, we have demonstrated a critical role for the vmPFC in regulating amygdala activity. Specifically, we found that vmPFC lesions were associated with increased right amygdala reactivity to aversive stimuli and increased resting-state connectivity with anterior temporal cortex. These findings are directly relevant to neural circuitry models of emotion regulation and affective psychopathology.

One influential model of affective psychopathology proposes two key features: 1) vmPFC dysfunction results in disinhibition of the amygdala, and 2) the resultant amygdala hyperactivity engenders pathologically high levels of anxiety and negative affect (6–8). Although our results unequivocally support the first feature of this model, they seem to complicate the second feature, at least as it pertains to human affective processing. Using fear conditioning and extinction paradigms, an elegant set of rodent studies demonstrated a causal chain between activity in the infralimbic cortex (the purported homologue of human vmPFC), inhibition of the amygdala, and extinction of conditioned behavioral and physiologic fear responses (7,9,10,40). Human functional imaging studies have provided correlative data consistent with this model (6,7,11–14). However, the causal

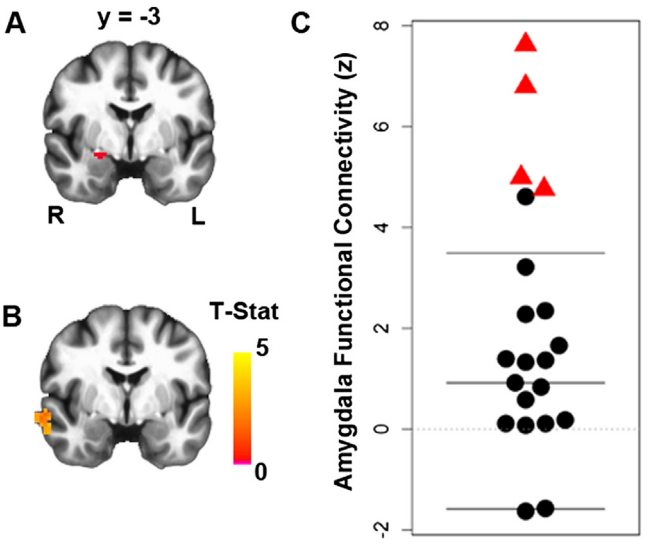

Figure 4. Greater right amygdala resting-state functional connectivity in patients with ventromedial prefrontal cortex lesions. **(A)** Right amygdala seed region (red). **(B)** Group difference map at corrected $p_{FWE} < .05$ showing greater right amygdala connectivity with a cluster in the ipsilateral anterior temporal lobe in the ventromedial prefrontal cortex lesion group (Figure S4 in Supplement 1 shows average amygdala connectivity maps from each group). **(C)** Plot showing distribution of connectivity values (*z* scores) in the significant cluster. FWE, familywise error; L, left; R, right.

relationships among vmPFC activity, amygdala activity, and negative affect appear to be more complicated in humans. At least two lines of research argue for a more comprehensive model. One line of research involves patients with major depressive disorder. Numerous neuroimaging studies indicate that patients with major depressive disorder exhibit abnormally high levels of activity within the vmPFC (particularly in the subgenual cortex) (21,41–44). In addition, patients with major depressive disorder who are responsive to antidepressant medication or deep brain stimulation tend to exhibit decreased activity in both the subgenual vmPFC and the amygdala after treatment (21,44–46). Furthermore, activity within the subgenual vmPFC has been shown to correlate positively with negative affect in healthy subjects (47–49). The second line of research involves patients with vmPFC lesions. It is well established that damage to the vmPFC results in personality changes more reminiscent of psychopathy (e.g., blunted emotional experience, low emotional expressivity, impulsivity, lack of empathy, reckless decision making) than anxiety or depression (17,18,50). Critically, damage to the vmPFC has been shown to reduce (not increase) the likelihood of developing depression (20) and posttraumatic stress disorder (19). In addition, damage to the vmPFC is associated with diminished physiologic reactions (e.g., skin conductance responses) to aversive stimuli (51–53). Our heart rate data are consistent with these prior physiologic findings. Rather than observing increased cardiac deceleration in response to aversive pictures in the patients with vmPFC lesions (as the model might predict based on the amygdala hyperactivity in these patients), we observed reduced deceleration in the patients with vmPFC lesions relative to the NC subjects. However, the finding of similar group differences in cardiac deceleration in response to neutral pictures suggests a more general orienting deficit in the vmPFC group. Together, these findings suggest that the role of the vmPFC in affective processing is not simply the regulation of negative emotion through inhibition of the amygdala. Rather, the vmPFC appears to play a more multifaceted role that could include processes related to self-awareness and self-reflection (54,55), more direct modulation of emotion-related physiologic responses and negative affect, or both.

Anatomic tracing studies in rodents and nonhuman primates support comparable roles of the vmPFC and amygdala in generating emotion-related physiologic responses. Amygdala subnuclei (especially CeA) and areas within the vmPFC (especially Brodmann areas 24, 25, and 32) send dense, overlapping projections to brainstem and diencephalic nuclei directly involved in coordinating peripheral autonomic changes—lateral hypothalamus, bed nucleus of stria terminalis, parabrachial nucleus, and periaqueductal gray (56–60). Moreover, the vmPFC and amygdala are themselves densely and reciprocally interconnected (57). In addition to projections to intercalated interneurons that ultimately inhibit the CeA, the vmPFC shares reciprocal connections with the basolateral amygdala, which are thought to be critical for modulating the expression of negative affect (61,62). We observed similar effects of damage of the vmPFC on fMRI responses regardless of whether we used a whole-amygdala ROI or a CeA ROI (Table 3 and Table S4 in Supplement 1). Further research is necessary to delineate more clearly the specific contributions of vmPFC and amygdala subregions to human affective function.

Although we did not have any a priori hypothesis regarding lateralization, significant group differences in amygdala reactivity to aversive pictures were observed only in the right amygdala (Figure 3 and Table 3). Previous meta-analyses offer some support for a functional dissociation of the right and left amygdala in rapid, automatic stimulus processing and sustained stimulus evaluation, respectively (63–65). However, the laterality effects observed here may be due to the lesion characteristics of our sample of patients with vmPFC lesions. Although all lesions involved significant bilateral damage to the vmPFC, each patient had slightly greater damage on the right side (Table S7). Future work in larger samples with more heterogeneous vmPFC lesions is needed to determine more conclusively the link between lateralization of vmPFC damage and amygdala hyperactivity.

Future studies could also expand the scope of the present findings by using more diverse stimuli or task paradigms. One possibility would be to use a fear extinction paradigm, to allow more direct comparisons with rodent data (1,7). In addition, previous studies indicate that the amygdala responds to positive valence and may be more sensitive to stimulus arousal than to valence per se (64–66). To maximize our power to detect group differences in amygdala activation, we limited stimuli to aversive and neutral pictures and used a simple 4-point valence rating scale. Future studies could include images with positive valence and more detailed ratings of valence and arousal to determine whether changes in amygdala activity after damage to the vmPFC are specific to negative affect or more broadly related to subjective arousal.

In conclusion, we demonstrate a critical role for the vmPFC in regulating amygdala activity. Our findings provide unique evidence regarding the causal interactions among brain regions subserving emotion regulation in humans and offer novel support for the inhibitory influence of the vmPFC on the amygdala, as proposed in neurocircuitry models of affective dysfunction in mental illness.

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