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Serial Vagus Nerve Stimulation Functional MRI in Treatment-Resistant Depression

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Vagus nerve stimulation (VNS) therapy has shown antidepressant effects in open acute and long-term studies of treatment-resistant major depression. Mechanisms of action are not fully understood, although clinical data suggest slower onset therapeutic benefit than conventional psychotropic interventions. We set out to map brain systems activated by VNS and to identify serial brain functional correlates of antidepressant treatment and symptomatic response. Nine adults, satisfying DSM-IV criteria for unipolar or bipolar disorder, severe depressed type, were implanted with adjunctive VNS therapy (MRI-compatible technique) and enrolled in a 3-month, double-blind, placebo-controlled, serial-interleaved VNS/functional MRI (fMRI) study and open 20-month follow-up. A multiple regression mixed model with blood oxygenation level dependent (BOLD) signal as the dependent variable revealed that over time, VNS therapy was associated with ventro-medial prefrontal cortex deactivation. Controlling for other variables, acute VNS produced greater right insula activation among the participants with a greater degree of depression. These results suggest that similar to other antidepressant treatments, BOLD deactivation in the ventro-medial prefrontal cortex correlates with the antidepressant response to VNS therapy. The increased acute VNS insula effects among actively depressed participants may also account for the lower dosing observed in VNS clinical trials of depression compared with epilepsy. Future interleaved VNS/fMRI studies to confirm these findings and further clarify the regional neurobiological effects of VNS.

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INTRODUCTION

Depression is a major public health problem. Approximately, 60% of patients treated with antidepressants do not achieve remission (O'Reardon and Amsterdam, 1998; Fagiolini and Kupfer, 2003). Neuroimaging studies have described the correlates of therapeutic interventions in an effort to better understand the neurobiological characteristics associated with clinical response. Although this area is relatively new and findings differ, many studies suggest that limbic and frontal brain regions change over time during successful treatment of depression regardless of the therapy. Such changes have been found with sleep deprivation (Wu and Bunney, 1990; Ebert *et al*, 1991, 1994; Wu *et al*, 1992), medications (Buchsbaum *et al*, 1997; Mayberg *et al*, 2000; Nobler *et al*, 2000; Drevets *et al*, 2002), placebo (Kleinschmidt *et al*, 1999), electroconvulsive therapy (ECT) (Nobler *et al*, 2001), transcranial magnetic stimulation (TMS) (Teneback *et al*, 1999; Nahas *et al*, 2001b), deep brain stimulation (DBS) (Mayberg *et al*, 2005), cognitive behavioral therapy (Goldapple *et al*, 2004), or interpersonal therapy (Brody *et al*, 2001).

Vagus nerve stimulation (VNS) is approved by the US Food and Drug Administration (FDA) to treat refractory partial-onset seizures and, more recently, treatment-resistant depression. Antidepressant VNS effects were initially found in an open acute study (Rush *et al*, 2000; Sackeim *et al*, 2001) and long-term follow-up (Marangell *et al*, 2002; Nahas *et al*, 2005). Despite lack of a significant effect greater than placebo in a double-blind acute study (Rush *et al*, 2005a), of which this study was a part, the naturalistic 1- or 2-year follow-up study of adjunctive VNS therapy in two independent cohorts (D01 (Nahas *et al*, 2005) and D02

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(George *et al*, 2005; Rush *et al*, 2005b)) showed the antidepressant response increasing over time with initial improvements largely sustained. Long-term results showed delayed and consistent improvement with substantially lesser relapse rates than other studies of similarly treatment-resistant subjects (Prudic *et al*, 2004). The mechanisms of action underlying these intriguing VNS response patterns remain unexplained.

Chae *et al* (2003) reviewed single photon emission computed tomography (SPECT) (Ring *et al*, 2000; Vonck *et al*, 2000; Van Laere *et al*, 2002; Barnes *et al*, 2003) and positron emission tomography (Garnett *et al*, 1992; Ko *et al*, 1996; Henry *et al*, 1998, 1999) investigating the acute and/or chronic effects of VNS for epilepsy and depression. A recent SPECT study suggested that 4 weeks of active VNS therapy in depression is associated with decreased activity in hippocampus and amygdala and increased activity in left prefrontal cortex (Zobel *et al*, 2005). Researchers have also used functional MRI (fMRI) to investigate VNS in epilepsy (Narayanan *et al*, 2002; Sucholeiki *et al*, 2002; Liu *et al*, 2003) and depression (Maniker *et al*, 2000; Bohning *et al*, 2001; Lomarev *et al*, 2002; Mu *et al*, 2004). Initial studies of depressed participants showed the feasibility of performing VNS-synchronized fMRI studies and compared the location and amount of blood oxygenation level dependent (BOLD) signal change caused by acute VNS for 7 s with the period when the device was not firing (Bohning *et al*, 2001). At least five adjustable parameters likely contribute to the immediate- and longer-term effects of VNS: intensity, frequency, pulse width, 'on' time, and 'off' time. Recent fMRI studies have demonstrated that the frequency (Lomarev *et al*, 2002) and pulse width(Mu *et al*, 2004) of VNS delivered to depressed adults produces dose-dependent modulatory effects on acute brain activity (eg, less than a minute). Little is known, however, about neurobiological effects of VNS over time beyond a period of 3 months (Henry *et al*, 2004) and dynamic regional brain activity changes that may correlate with depressive symptoms' severity. We used interleaved VNS/fMRI to serially scan depressed participants in a VNS clinical trial investigating how VNS parameters affect global and regional brain activity as a function of disease severity and time (Harrison *et al*, 2003).

METHODS

This study investigated the effects of adjunctive VNS on BOLD response over time among participants at the Medical University of South Carolina (MUSC) in a double-blind acute and open long-term follow-up clinical trial (D-02) for treatment-resistant depression (Rush *et al*, 2005a). Participants had not responded adequately to at least 2, but not more than 6, research-qualified medication trials of different antidepressant classes. All VNS-implanted outpatients enrolled at MUSC ($n = 18$) were approached for this serial VNS/fMRI study; one declined because of claustrophobia. All 17 others with either non-psychotic major depressive disorder ($n = 14$) or non-psychotic, depressed-phase bipolar disorder ($n = 3$) gave written informed consent approved by the Institutional Review Board for Human Research.

Clinical Assessments

All scanning sessions coincided with scheduled D-02 clinical trial protocol visits. Before the MRI scans on the same day, raters blinded to VNS active/placebo assignment conducted assessments, including the Number of Failed Antidepressants in current depressive episode (NFAD) at baseline according to the Antidepressant Treatment History Form (Sackeim, 2001) and the 24-item Hamilton Depression Rating Scale ($HRSD_{24}$) (Hamilton, 1967), the primary clinical efficacy outcome for acute (Rush *et al*, 2005a) and long-term follow-up (Rush *et al*, 2005b). The reliability of the placebo condition was high as described by Rush *et al* (2005a, b) despite a lack of physical sensation (Table 1).

Experimental Design and Procedure

The VNS/fMRI study was initiated simultaneously with clinical randomization of the D-02 trial. To allow for recovery from surgery, VNS was initiated 2 weeks after implantation of the MRI-compatible device (Cyberonics, Houston, TX) (Bohning *et al*, 2001). The VNS study involved two phases, the 10-week, randomized, controlled, masked trial during which concomitant medications were stable and participants were scanned three times: randomization and initial exposure to VNS (baseline); 2 weeks after VNS intensity adjustment ($\sim$week 2); and 8 weeks later ($\sim$week 10). The second phase was an open-label follow-up study with scans at week 13, $\sim$week 20), then quarterly (see study design in Figure 1).

In the MRI suite, and before participants entered the scanner, the unblinded VNS programmer for the clinical study (see Rush *et al* (2005a)) changed each subjects' VNS parameters from their ongoing settings to adapt to the imaging block design paradigm (pulse width 500 ms, pulse frequency 20 Hz, and duty cycle of 13.6 s 'on' (including 3.3 s ramp-up and 3.3 s ramp-down and 41 s 'off'). These settings were held constant throughout the imaging, about 1 h. The output current was maintained at the same level as the participant had been receiving except for the scan at baseline when the output current was set to a 'tolerable' level or 0 mA for those randomized to placebo.

Before and after each functional scan, the VNS generator signal was checked, which allowed synchronization of the fMRI scanning cycle with the VNS generator cycle (for a detailed method refer to Bohning *et al* (2001)).

All MRI scanning was performed using a 1.5 T clinical MRI scanner with a send-receive head coil (Intera, Philips Medical Systems, Bothell, WA, USA). A survey scan ascertained head location for subsequent anatomical and functional scans. A set of T1-weighted sagittal structural images encompassing the whole brain were acquired using the following parameters, TR = 625 ms, TE = 20 ms, slice thickness = 5 mm, gap = 1 mm, field of view = 256 mm, number of slices = 27, matrix = 256 × 256. Using the same slice coverage as the structural scans, a whole brain gradient echoplanar imaging (EPI) sequence was obtained for each participant, and employed the same scanning and reconstruction parameters as the structural scan except for a TR of 2837 ms, a TE of 45 ms, and a 128 × 128 matrix, resulting in a voxel size of $2 \times 2 \times 6\,mm^3$. The functional scanning session consisted of 400 images and lasted 18 min and 54 s.

Table 1 Scanning Schedule and Timeline for all Active VNS Scans

Subject	TIME	HDRS	NFAD	HDRS × NFAD	Intensity
1	1	28	2	56	0.25
	3	32	2	64	0.25
	11	19	2	38	0.25
	13	12	2	24	0.25
	22	14	2	28	0.25
	37	10	2	20	0.5
	55	21	2	42	0.5
2	1	24	5	120	0.25
	3	19	5	95	0.25
	11	22	5	110	0.25
	14	22	5	110	0.25
	54	31	5	155	0.75
	65	8	5	40	0.75
	88	10	5	50	0.75
3	3	19	7	133	0.5
	11	25	7	175	0.5
	42	21	7	147	0.75
	55	22	7	154	1
	67	10	7	70	1
	92	9	7	63	1
4	1	27	3	81	0.25
	4	29	3	87	0.5
	11	28	3	84	0.5
5	1	26	2	52	0.25
	3	21	2	42	1
	11	28	2	56	1
	24	24	2	48	1
6	3	27	3	81	0.25
	11	27	3	81	0.5
	34	27	3	81	0.25
	47	20	3	60	0.5
	65	11	3	33	0.5
7	11	26	2	52	0.25
	50	30	2	60	0.25
	67	31	2	62	0.25
	77	25	2	50	0.25
8	4	17	6	102	0.5
	11	30	6	180	0.5
	41	30	6	180	0.25
	56	29	6	174	0.75
	81	24	6	144	1
9	11	26	3	78	0.5
	49	16	3	48	1
	63	11	3	33	1
	72	12	3	36	1

The VNS epoch was synchronized at the beginning of each of the 10 cycles (scans 1–5). After scanning, VNS parameters were reset to those programmed when the participant reported for the scan.

Data Processing Methods

The fMRI image data were transferred to a Dell workstation, converted into Analyze format using MRICro, and analyzed with Statistical Parametric Mapping software, version 2 (Welcome Department of Imaging Neuroscience, London, UK). Data were reoriented to correspond with standard SPM format. Scans from each data set were co-registered to an image created from their mean, and corrected for motion using the standard SPM algorithm. Corrected images were reviewed for remaining motion, which was less than 0.5 mm along all three major axes (x, y, z) for all participants. Images were timing corrected using the center slice (seven of 15) as the reference slice in an ascending sequence, with an interscan interval of 2.837 s. Using the SPM2 algorithm, each data set was spatially normalized into Talairach space (Talairach and Tournoux, 1988) by adjusting images to conform to the SPM2 epi template through trilinear interpolation. Input and output voxel dimensions were $2 \times 2 \times 6$ and $4 \times 4 \times 4$ mm, respectively. Normalized data were spatially smoothed using a Gaussian filter width of $8 \times 8 \times 8$ mm.

Scans acquired during the placebo phase were also analyzed.

Data Analysis

Identification of voxels with statistically significant activation during VNS in individual participants (f-maps using SPM2 General Linear Model; fixed effect). A pixel-by-pixel bidirectional *t*-test identified areas of significantly decreased or increased response to VNS *vs* rest (or placebo *vs* rest), using a delayed boxcar model (10 cycles of 40 scans). In each cycle, images 1–5 were considered the VNS activation period. Images 21–23, corresponding to a period of tone stimulation, are the subject of another analysis. The remaining images, considered rest periods provided comparison with VNS. Potentially confounding motion over time was regressed out, and a high-pass filter with a cutoff period of 232 s was applied to remove slow signal drift.

Using all participants' f-maps, a one-sample *t*-test determined areas of significant activation or deactivation in response to VNS compared with rest periods for all participants as a group. Only clusters of activation with *Z*-values corresponding to $p < 0.1$ and meeting an extended threshold of at least 15 voxels (960 mm^3) were considered in the group analyses.

Multiple regression analysis. To investigate effects of several clinical parameters on VNS response, a multiple linear regression mixed model was designed using multiple scans from each subject and the following covariates: (1) each participant's $HDRS_{24}$ at scanning ($HDRS_{24}$); (2) weeks since activation of VNS (TIME); (3) output current of VNS at each session, adjusted independently for clinical parameters (OUTPUT); and (4) severity of illness, based on the interaction between each participant's baseline NFAD

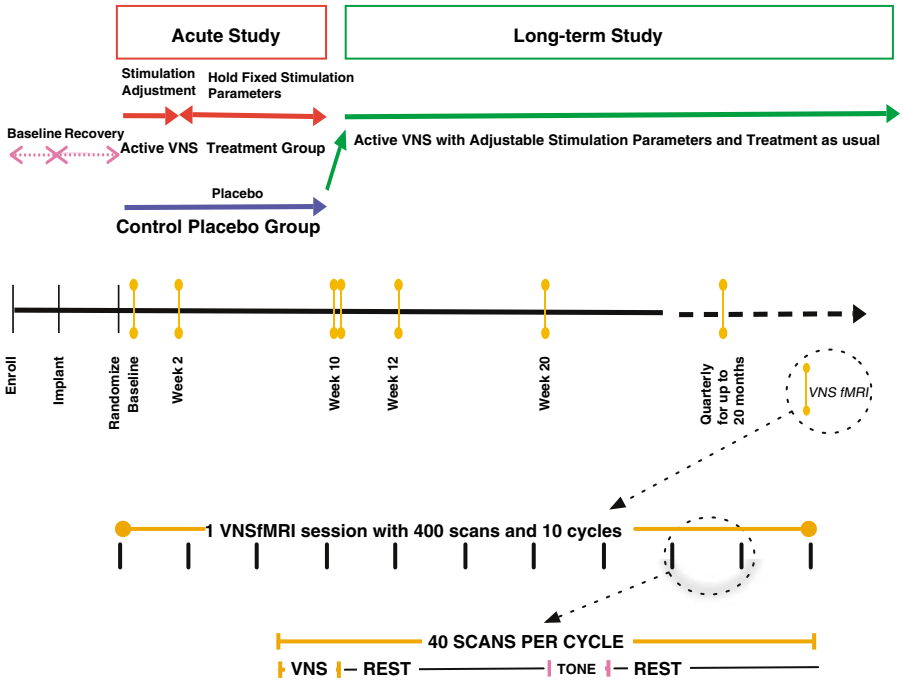

Figure 1 Study design and time points for serial VNS fMRI. Note the detailed a VNS fMRI scanning paradigm with 10 cycles and a total of 400 scans per session.

score and the current Hamilton Depression rating ($HDRS_{24} \times NFAD$). Despite us not being able to find any references for the combined ($HDRS_{24} \times NFAD$) variable, it was specifically chosen to differentiate among participants presenting with identical depression ratings but differing depressive histories and treatment resistance. Both of these variables have shown influence on clinical outcomes and thus likely to affect the brain activity and merit being considered and explored in our regression model (Sackeim, 2001; Nahas *et al*, 2005). Other variables likely playing an important role in the clinical outcomes to VNS therapy (ie depression sub-type, comorbid psychiatric conditions, age, specific concomitant medications, etc.) were considered but not included in this model owing to small sample sizes and unequal representation of the groups. These included gender, bipolar or unipolar depression, type of concomitant psychotropic medications. Individual participants' f-maps were entered into this analysis. Given that such an analysis would require a larger sample size than simple *t*-test to achieve the same power, only areas of activation meeting an extended threshold of a minimum of 10 voxels (640 mm^3) and *Z*-scores equivalent $\geqslant 2.5$ were considered significant (Harrison *et al*, 2003).

RESULTS

In all, 107 serial scans were acquired on 17 participants. Each participant underwent a minimum of three and a maximum of seven scans. In this analysis, we report 45 active VNS scans and nine placebo scans from nine participants. Seven of these nine participants were originally randomized to placebo then switched to active VNS therapy. Fifty-three scans were not used in this analysis and included technical difficulties (eg, participant movement >2 mm during scanning or poor quality, $n=26$), generator not restarting within scanner ($n=11$), generator not keeping pre-set on–off duty cycle within scanner ($n=16$), and one participant experiencing a panic attack at baseline scan and exiting this study. No patient experienced unintentional re-setting of VNS parameters into a higher range of nerve stimulation during fMRI sessions. The nine participants (six women) had a mean age, 46.8 (± 6.2) years and mean duration of current episode 71.2 (± 57.3) months (range, 9–194). The NFAD and VNS output current ranged from 2 to 7 (median 3) and 0.25 to 1.0 mA (median 0.5 mA), respectively. Median concomitant psychotropic drugs at each scheduled timeline ranged between 3 and 5.5. All corresponding structural high resolution T1 images revealed no overt regional abnormalities.

VNS Active Scans Group Results, Acute VNS

When all 45 active VNS scans were grouped together (ignoring all other covariates such as time and depression state), we found significant BOLD increases during VNS in the bilateral superior temporal gyrus and left somatosensory cortex. We found significant BOLD decreases in the left middle frontal gyrus, left fusiform gyrus, left ventromedial frontal lobe, right cerebellum, and midbrain (Figure 2 and Table 2).

Placebo Scans Group Results

When nine placebo VNS scans were grouped together, we found significant BOLD increases during the no stimulation epoch compared with the rest in right orbitofrontal cortex

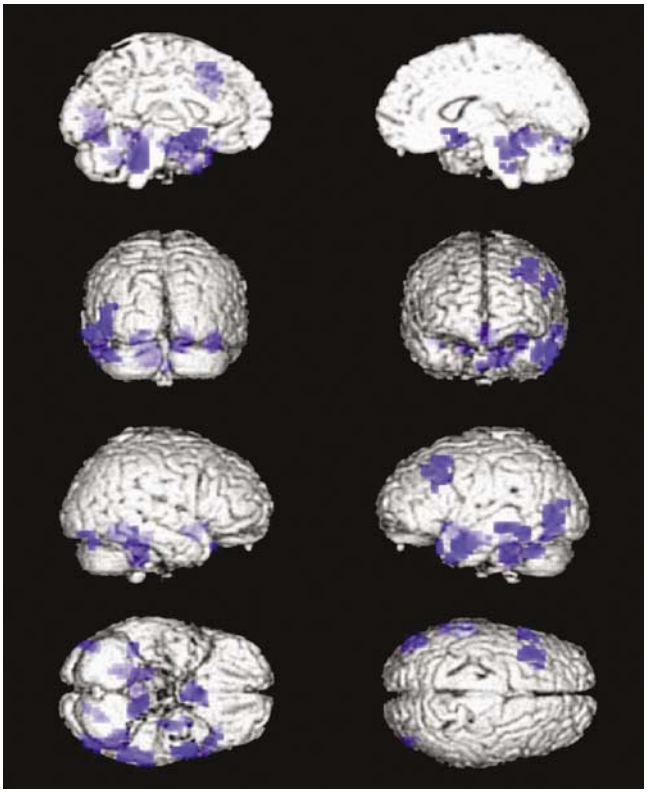

Figure 2 VNS-induced BOLD deactivations within all active 45 scans. $p = 0.01$.

and right parietal cortex. Interestingly, no significant deactivations were found.

Multiple Linear Regression Model

We report a statistically significant relationship between the dependent variable, regional BOLD activation, and at least one of the independent variables in this model.

TIME. No statistically significant BOLD increases were associated with TIME.

TIME from VNS activation or exposure to VNS was associated with significant BOLD immediate VNS-induced *decreases* in right insula (BA 13), right medial frontal gyrus, left frontal lobe (pre- and postcentral gyrus), right temporal lobe, right parietal lobe (supramarginal gyrus), left occipital lobe (BA 17), left parietal lobe (BA 2), and left cerebellum. Figures 3 and 4 represent the linear relationship of the parameter estimates of VNS-induced activations over time. These responses were extracted from the right medial frontal gyrus ($df = 44$, $F = 58.85$, $p < 0.0001$, $r^2 = 0.577$) and right insula ($df = 44$, $F = 17.252$, $p < 0.0001$, $r^2 = 0.286$).

$HDRS_{24}$. Depressive symptoms were associated with significant BOLD *increases* in right temporal lobe, right insula (Brodmann area (BA) 13), and left middle frontal gyrus. Figure 5 represents the linear relationship of the parameter estimates of VNS-induced activation function of $HDRS_{24}$ ($df = 44$, $F = 139.372$, $p < 0.0001$, $r^2 = 0.76$). Note: We subsequently ran the multiple regression analysis as described above with only three covariates: (1) $HDRS_{24}$; (2) TIME; and (3) OUTPUT (data not presented). At similar statistical thresholds, the results obtained from three covariates were very similar to the one summarized here with the exception of loss of right insula activation.

Depressive symptoms were associated with significant BOLD *decreases* in right occipital lobe (Cuneus) and right cerebellum (Pyramis).

$HDRS_{24} \times NFAD$. Severity of depressive illness was associated with significant BOLD *increases* in right cerebrum, occipital, right and left cerebellum.

Severity of depressive illness was associated with significant BOLD *decreases* in right inferior frontal gyrus, right cingulate gyrus (BA 32), left cingulate gyrus, right insula (BA 13), left middle frontal gyrus, left superior temporal gyrus, right parietal lobe (BA 7), left putamen, and left brainstem (pons).

Output current. VNS output current was associated with significant BOLD *increases* in left cerebellum, right parietal lobe (somatosensory BA 2), right superior frontal gyrus, and right middle frontal gyrus.

VNS output current was associated with significant BOLD *decreases* in left parietal lobe (precuneus), right posterior cingulate gyrus, and caudate.

DISCUSSION

This study used interleaved VNS/fMRI scanning to model dynamic regional brain responses to VNS as a function of time, the participant's depressive state, the underlying illness severity, and the stimulation output current. We found that 7 s trains of VNS at 20 Hz and 500 ms pulse width decreased BOLD–fMRI response in the right medial prefrontal cortex, anterior cingulate, and left anterior temporal pole and right somatosensory cortex. It also led to increased BOLD–fMRI response in the right superior temporal gyrus. These results are consistent with known vagus afferent projections (Henry, 2002; Craig, 2004) and previous VNS imaging studies(Maniker *et al*, 2000; Bohning *et al*, 2001; Lomarev *et al*, 2002; Mu *et al*, 2004). We only found BOLD increases in right orbitofrontal cortex and no significant decreases in the placebo group.

In addition to this overall analysis, which replicates and extends previous work, we performed a multiple regression model, controlling for other parameters known to influence the VNS BOLD response. To our knowledge, this type of analysis has not been previously used for VNS imaging studies. Controlling for the important covariates, we found that VNS-induced brain changes differed as a function of duration of exposure to VNS, level of depression on the study day, and VNS output current used in the scanner.

TIME since VNS device activation: our data suggest that duration of exposure to VNS accounted for most of the medial prefrontal/limbic deactivations. Even the right insula became more deactivated over time, perhaps as a function of improved clinical outcome. When participants initially received VNS, the VNS induced limbic activation. However, over time and with adjustment for other important covariates, these areas became deactivated. In Figures 4 and 5, the linear relationship between VNS-induced

Table 2 SPM Output/Tailarach Coordinates

Condition	Region	Tailarach coordinates (*x*, *y*, *z*)	Cluster size (*n*)	*z*-score
Active VNS group (n = 45)				
Increases with VNS compared to rest				
	Left superior temporal gyrus	−56, 0, 0	182	4.21
	Left somatosensory cortex (BA1)	−36, −28, 60	24	3.40
	Right superior temporal gyrus	52, −16, 4	31	3.19
Decreases with VNS compared to rest				
	Right frontal oculomotor eye field (BA8)	36, 16, 52	44	4.07
	Right posterior cingulate gyrus (BA31)	8, −36, −32	60	4.05
	Right middle temporal gyrus (BA21)	56, 4, −28	42	3.90
	Right subgenual cingulate (BA25)	0, 8, −16	32	3.59
	Right inferior temporal gyrus (BA37)	48, −72, 0	41	3.42
	Right inferior temporal gyrus (BA37)	56, −44, −16	41	3.32
	Right cerebellum	44, −60, −32	18	3.30
	Right uncinate fasciculus	28, −4, −16	17	3.13
	Left cerebellum	−20, −52, −32	23	3.03
Placebo group (n = 9)				
Increases with placebo compared to rest				
	Right middle temporal gyrus (BA39)	52, −68, 16	36	4.49
	Right inferior frontal gyrus (BA47)	44, 40, −20	32	4.20
Decreases with placebo compared to rest				
		—		—
Multiple linear regression model				
Increases HDRS				
	Right temporal Lobe	20, −60, 20	71	3.98
	Right insula (BA13)	44, −4, 12	13	3.15
	Left middle frontal gyrus	−36, 4, 40	12	2.86
Decreases HDRS				
	Right cerebellum	24, −68, −32	12	3.22
	Right occipital lobe	12, −96, 4	39	3.14
	Right cerebellum	8, −72, −20	14	3.14
Increases time				
	—	—		—
Decreases time				
	Right insula (BA13)	36, 12, −4	134	3.78
	Right frontal lobe	24, −8, 56	68	3.76
	Right frontal lobe	40, 40, 8	67	3.58
	Right supramarginal gyrus	40, −48, 32	78	3.40
	Left postcentral gyrus	−36, −28, 64	38	3.29
	Right temporal lobe	40, 4, −24	19	3.20
	Right medial frontal gyrus	8, 8, 52	26	3.19
	Left occipital lobe (BA 17)	−16, −96, −12	16	2.97
	Left precentral gyrus	−56, 8, 8	14	2.86
	Left cerebellum	−12, −64, −32	14	2.71
	Left postcentral gyrus (BA 2)	−56, −20, 44	10	2.68

Table 2 Continued

Condition	Region	Tailarach coordinates (*x*, *y*, *z*)	Cluster size (*n*)	*z*-score
Increases HDRS × NFAD				
	Right cerebellum	24, −68, −32	18	3.2
	Left cerebellum	−48, −72, −24	10	3.07
	Right occipital lobe	12, −96, 4	10	2.88
Decreases HDRS × NFAD				
	Right parietal lobe (BA 7)	12, −76, 44	293	4.29
	Right insula (BA 13)	44, −4, 12	198	3.77
	Left middle frontal gyrus	−36, 4, 40	64	3.49
	Left temporal lobe	−20, −56, 16	128	3.26
	Left superior temporal gyrus	−52, −4, 0	10	2.98
	Left cingulate	−8, 0, 36	16	2.93
	Left, sub-lobar, extra-nuclear, white	−28, −20, 24	17	2.90
	Right inferior frontal gyrus	−8, 24, 20	11	2.72
	Left putamen	−24, 0, 8	14	2.70
	Right cingulate gyrus (BA 32)	12, 16, 32	20	2.59
	Left brainstem, pons	−8, −16, −20	10	2.52
Current output increases				
	Left cerebellum	−12, −64, −36	16	3.66
	Right parietal lobe (BA 2)	28, −36, 64	18	3.11
	Right superior frontal gyrus	0, 28, 48	11	2.90
	Right middle frontal gyrus	20, −12, 60	12	2.83
Current output decreases				
	Left parietal lobe, precuneus	−20, −64, 32	46	3.62
	Right cingulate gyrus	8, −20, 32	24	3.01
	Inter-hemispheric extending to right caudate	0, 12, 12	17	2.80

Z-sore >2.5.

parameters estimates became predominantly negative after week 30. This is also the time when pronounced clinical improvements in symptoms take place.

Depression level on scan day: the treatment resistance of each participant's depression helps explain the larger but complementary deactivation of medial and lateral prefrontal cortex and left superior temporal gyrus, whereas depressive symptoms alone contribute to activation of the right insula. This is important given the insula's role in vicero-autonomic and limbic function, and in somatic pain (Craig, 2003). Also of interest is the dynamic switch in the right insula's response to VNS. Acute VNS appears to primarily deactivate the right insula in mild-to-moderate depression but correlate with higher activation in relationship to increased severity of depressive symptoms (see Figure 6). This reversal in response may be in keeping with known anatomy of the vagus nerve and afferent parasympathetic pathways that provide sensory inputs to a hierarchically integrative network that extends to the insula via the thalamus and ultimately provides a means of 'introceptive' representations (Craig, 2004). It also provides a hypothesis explaining why VNS output current has been substantially lower in depression than epilepsy studies, despite an attempt in all studies to increase the VNS output intensity to the highest tolerated dose. Among participants with greater depression, VNS produces more activation in the right insula, an integrative center for pain perception. Depressed patients may perceive greater pain than epilepsy patients receiving VNS at the same parameter settings.

VNS output current: the patient's subjective perception of VNS greatly depends on the stimulation parameters, particularly the intensity of the stimulation (Sackeim *et al*, 2001). The increased activity in the right somatosensory area (likely neck and throat) associated with higher VNS output current provides an internal quality control. In addition, greater output current is associated with increased activity in the right middle frontal gyrus and decreased activity in the posterior cingulate.

Access to Mood-Regulating Networks

The vagus nerve, classically described as the 'wandering nerve', sends signals from the central nervous system to control the peripheral cardiovascular, respiratory, and

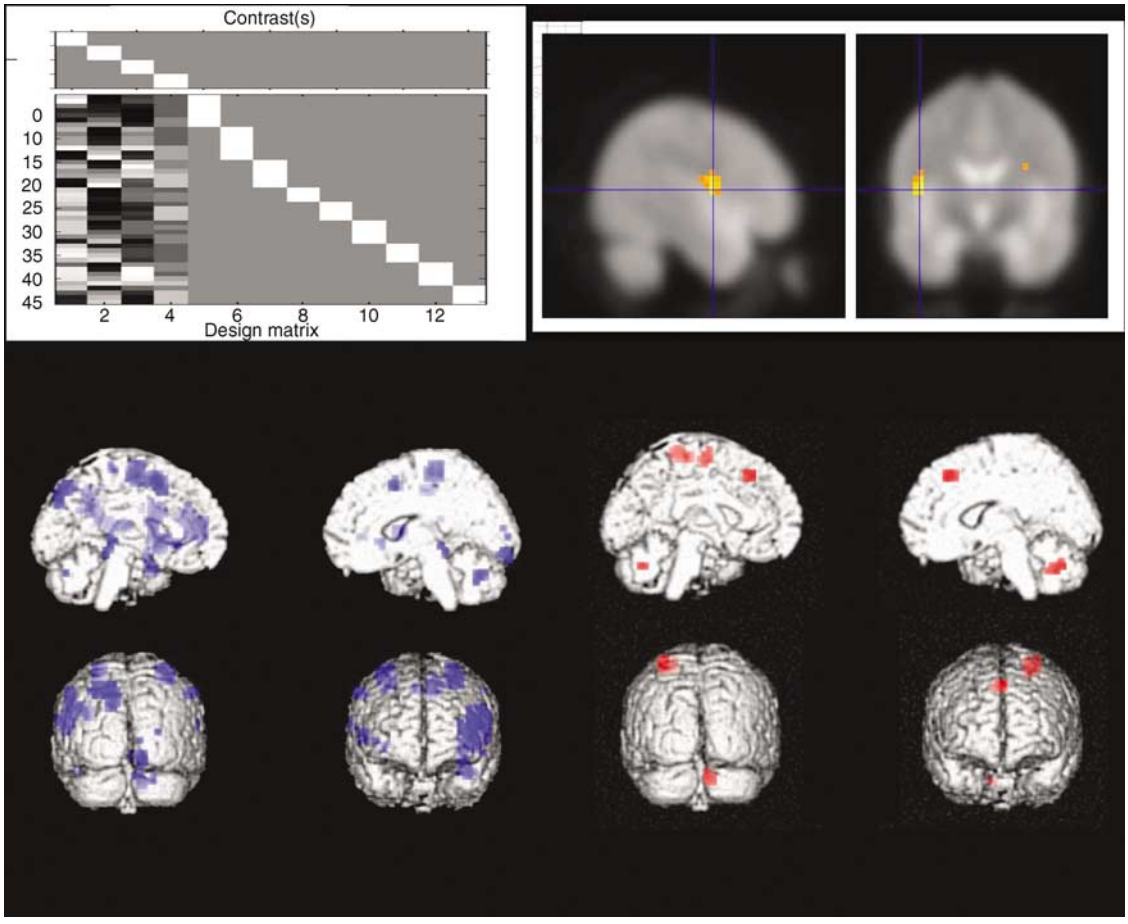

Figure 3 Design Matrix (top left) of the multiple regression model used and selected independent variables BOLD contrasts including: increases in insula associated with $HDRS_{24}$ (top right), decreases in medial prefrontal and limbic structures associated with TIME of Exposure to VNS therapy (bottom left) and increases in right somatosensory cortex associated with intensity of stimulation (bottom right).

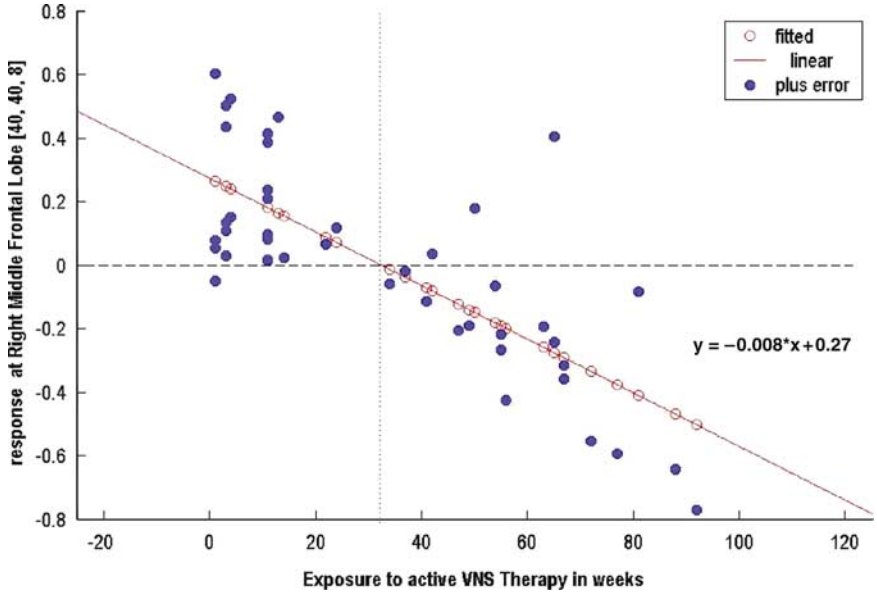

Figure 4 Relationship between VNS-induced parameters estimates response in the right medial prefrontal gyrus and exposure to active VNS therapy in weeks ($df = 44$, $F = 58.85$, $p < 0.0001$, $r^2 = 0.577$). Note that the linear response becomes predominantly negative after week 30.

gastrointestinal systems. However, 80% of its fibers are afferent, carrying information from the viscera to the brain (Foley and DuBois, 1937). The fibers enter the midbrain at the nucleus tractus solitaris (NTS) level. From the midbrain, they either loop back to the periphery in a reflex arc, connect to the reticular activating system, or reach the parabrachial nucleus and its connections to the NTS, raphe nucleus, locus ceruleus (LC), the thalamus, paralimbic,

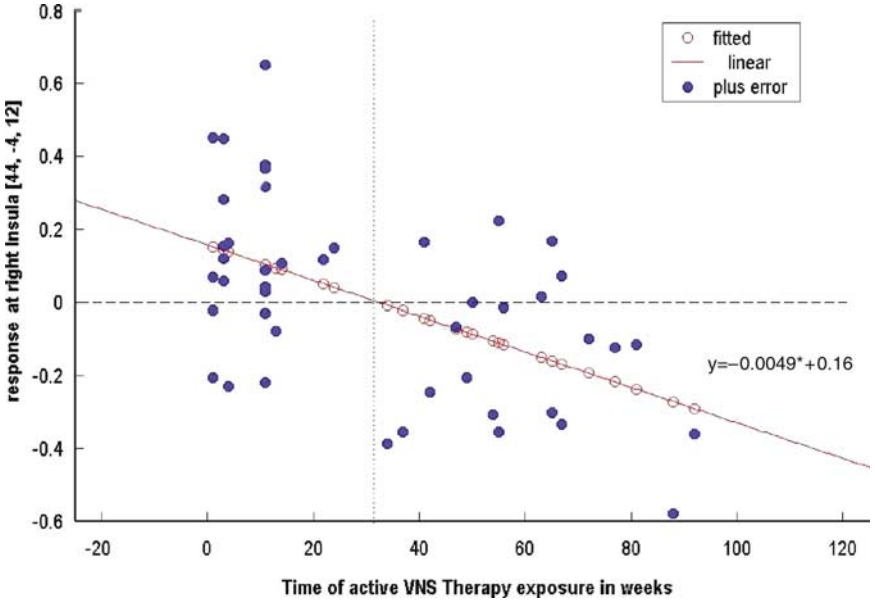

Figure 5 Relationship between VNS-induced parameters estimates response in the right insula and exposure to active VNS therapy in weeks (df = 44, F = 17.252, $p < 0.0001$, $r^2 = 0.286$). Note that here again, the linear response becomes predominantly negative after week 30.

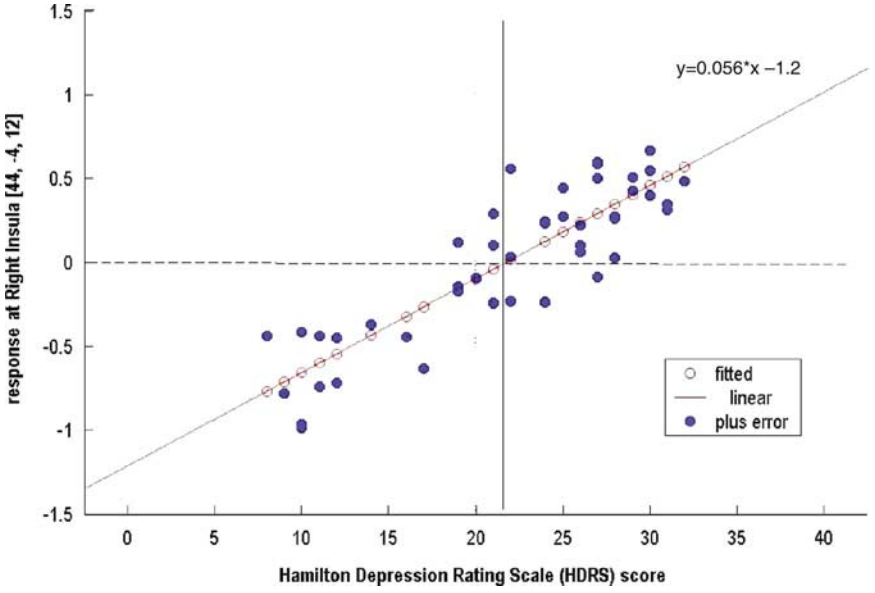

Figure 6 Relationship between VNS-induced parameters estimates response in the right insula and the depressive symptoms of each individual at the time of scanning as measured by HDRS (df = 44, F = 139.372, $p < 0.0001$, $r^2 = 0.76$). Note that the linear response becomes predominantly positive after a HDRS score of 22.

limbic, and cortical regions, including anterior insula and cingulate cortex (Hallowitz and MacLean, 1977). VNS modulates brain function through this route (Henry, 2002). The interplay between VNS and central nervous system monoamines has been demonstrated (Ben-Menachem *et al*, 1995; Naritoku *et al*, 1995; Krahl *et al*, 1998; Carpenter *et al*, 2004). The brainstem evidences specific acute markers of neuronal activity in vagus nerve nuclei, LC noradrenergic nuclei, and cochlear nucleus (Naritoku *et al*, 1995). Lesioning the LC interferes with the antiepileptic effect in rodents (Krahl *et al*, 1998). VNS induces specific nuclear fos immunolabeling in several forebrain structures, including the posterior cortical amygdaloid nucleus and cingulate retrosplenial cortex (Naritoku *et al*, 1995). Conversely, cerebral spinal fluid (CSF) studies involving epilepsy patients reveal increased γ-aminobutyric acid (GABA) as well as homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolites of dopamine and serotonin, respectively, and decreased glutamate after 3 months of treatment (Ben-Menachem *et al*, 1995). After 24 weeks in depressed subjects, VNS caused increases in CSF HVA but not in mean concentration of NE, 5-HIAA, 3-methoxy-4-hydroxyphenylglycol, or GABA (Carpenter *et al*, 2004).

Comparison with Other Imaging Studies of Antidepressant Treatments

The prefrontal/limbic pattern of activations observed in our study supports this general neuroanatomic framework. Moreover, VNS-induced effects are largely consistent with other published investigations of antidepressant mechanisms of action. Much of this literature has focused primarily on exploring pharmacologic interventions and their postulated effects on mood-regulating brain networks. Chronic antidepressant drug treatment, such as a serotonin reuptake inhibitors (SSRI) like sertraline (Buchsbaum *et al*, 1997; Nobler *et al*, 2000; Drevets *et al*, 2002), fluoxetine (Mayberg *et al*, 2000), or paroxetine (Brody *et al*, 2001), seems to reduce metabolism in the limbic areas and/or ventral ACC of subjects showing a persistent, positive treatment response. Clinical remission was also associated with decreased ACC activity in a study of subjects receiving either an SSRI or a norepinephrine-serotonin reuptake inhibitor (NSRI) (Holthoff *et al*, 2004), although these two classes of antidepressants may not share identical effects on brain metabolism.

Other brain stimulation modalities for treating depression have also shown dynamic modulation of prefrontal/limbic regions. ECT's antidepressant effect focuses on the dynamic interplay between the ictal and post-ictal phases (Rosenberg *et al*, 1988). During the ictal period, cerebral blood flow increases up to 300% of baseline and cerebral metabolic rate up to 200%; these measures decrease post-ictally. The degree of prefrontal and medial frontal deactivation immediately after ECT correlates with later clinical improvement. This inverse relationship holds true 2 months after ECT (Nobler *et al*, 2001). TMS, when applied repetitively over the prefrontal cortex, has also shown antidepressant effects (Kozel and George, 2002; Gershon *et al*, 2003) and is associated with local and transynaptic distal modulation of subcortical regions, including ACC and amygdala (Teneback *et al*, 1999; Speer *et al*, 2000; Nahas *et al*, 2001b; Strafella *et al*, 2001). Its antidepressive properties may also depend on the severity of underlying depression (Teneback *et al*, 1999) and the stimulation parameters (Kimbrell *et al*, 1999; Nahas *et al*, 2001b). A real-time assessment of brain activity with prefrontal interleaved TMS fMRI (Bohning *et al*, 1998) in a depressed cohort with concomitant medications, very similar to our cohort, has shown medial prefrontal deactivation (Li *et al*, 2003) not seen in healthy volunteers (Nahas *et al*, 2001a). Finally, an open study has reported antidepressant benefits of DBS posterior to the subgenual cingulate with concurrent deactivation of that region (Mayberg *et al*, 2005).

Progressive Adaptation

The deactivations of medial prefrontal cortex with VNS are similar to other antidepressant treatments, but they also suggest adaptation over time. Unlike pharmacologic interventions evidencing antidepressant effects after a few weeks of treatment (Mayberg *et al*, 2000), VNS clinical antidepressant effects occur later, after several months. The turning point for right insula, a key region in explicit subjective awareness (Critchley *et al*, 2004), and medial prefrontal activations seems to occur around 30 weeks of active VNS. If replicated in future studies, this particular characteristic may reflect VNS' relatively delayed time of effect. Of interest is a recent SPECT study showing limbic deactivations with only 4 weeks of VNS therapy (Zobel *et al*, 2005). Given the rapid response rate seen in this European study, the treatment resistance level may play an important role in how quickly VNS modulates these networks. In addition, SPECT and fMRI modalities differ in time their respective time resolutions and thus may explain some of the divergent results. Effective and progressive modulation of key brain regions may ultimately explain this distinctive therapeutic feature. Another distinctive clinical feature awaiting replication is the prolonged response and fewer relapses of depressed participants receiving adjunctive VNS (Nahas *et al*, 2005). Although our data do not fully explain this phenomenon, the progressive adaptation of crucial neuronal networks may complement animal epilepsy models in which VNS confers chronic progressive prophylactic effects, with seizure counts reduced more after chronic stimulation than after acute stimulation over less than a day. Similar research is needed in animal depression models.

Limitations

The 'magnetic switch' designed to allow epilepsy patients to self-administer an extra train of stimulation to help curb a full-blown seizure has limited our ability to successfully scan all participants. Despite a customized surgical implantation rotating the device about 45° counterclockwise to allow generator reactivation once the participant is parallel to the main magnetic field of the scanner, the VNS generator restarted in the scanner during only two-thirds of the sessions. This difficulty affected the statistical power of this study. Additionally, this fMRI paradigm had a relatively brief active-VNS epoch. The small number of participants with bipolar depression in the study precluded specific analysis differentiating MDD from BPAD responses. Given the adjunctive nature of this therapy, controlling for concomitant psychotropic medications is a limitation to this study. The median number was, however, relatively stable across the length of the study. Finally, the correlations presented here were derived from nine subjects but a total of 45 scans. And although this the largest imaging study reported in VNS literature, this does limit the interpretation of these data.

In conclusion, VNS/fMRI seems useful in studying the effects of VNS on brain activity both acutely and over time. VNS/fMRI may even allow in the future tailoring of stimulation parameters to optimally modulate specific regions and study progressive therapeutic adaptations. Although VNS and other antidepressant interventions share several general similarities, much work remains to fully elucidate the VNS mechanisms of actions.

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