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Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects

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Abstract

Stimulation of the vagus nerve in the neck can reduce seizures in epilepsy patients, and may be helpful in treating depression. PET studies have shown that vagus nerve stimulation (VNS) in epilepsy patients causes acute dose (intensity) dependent changes in regional cerebral blood flow. We sought to use the newly developed VNS synchronized fMRI technique to examine whether VNS BOLD signal changes depend on the frequency of stimulation. Six adults with recurrent depression were scanned inside a 1.5 T MR scanner. Data were acquired at rest, with the VNS device on for 7 s, and also, for comparison, while the patient listened to a tone for 7 s. In two separate back-to-back sessions, the VNS stimulation frequency was set to either 5 or 20 Hz. Data were transformed into Talairach space and then compared by condition. Compared to 5 Hz, 20 Hz VNS produced more acute activity changes from rest in regions similar to our initial VNS synchronized fMRI feasibility study in depression. Brain regions activated by hearing a tone were also greater when VNS was intermittently being applied at 20 Hz than at 5 Hz. In depressed adults, left cervical VNS causes regional brain activity changes that depend on the frequency of stimulation or total dose, or both. In addition to the acute immediate effects of VNS on regional brain activity, this study suggests further that VNS at different frequencies likely has frequency or dose dependent modulatory effects on other brain activities (e.g. hearing a tone).

Keywords: Vagus nerve stimulation; Depression; FMRI

1. Introduction

Vagus nerve stimulation (VNS; Zabara, 1985, 1992) with the Neurocybernetic Prosthesis (NCP®) Generator (Cyberonics, Inc., Houston, TX) has shown beneficial clinical effects in treating epilepsy (Ben-Menachem et al., 1994; Handforth et al., 1998), and has recently shown promise in treating patients with major depression (George et al., 2000a,b; Rush et al., 2000; Sackeim et al., 2001).

Vagus nerve stimulation is applied through an electrode wrapped around the left vagus nerve in the neck. The electrode is connected to a subcutaneous pulse generator that can deliver intermittent electrical current for variable on and off times at different intensities, frequencies, or pulsewidths. Thus, VNS can be administered with a range of at least five different use parameters (intensity, frequency, pulsewidth, on-time, off-time). Scientists do not fully understand the neurobiological effects of these different use parameters, either alone or in combination, although VNS at different intensities has different effects on emotional memory (Clarke et al., 1999) and pain perception (Ness et al., 2000; Kirschner et al., 2000).

Following the discovery by Zabara in 1985 that VNS could stop seizures, work was done to better understand the use parameters. Researchers used animal studies with EEG and EMG to determine the use parameters most likely to be effective for epilepsy (Zabara, 1992),

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which were then employed in initial clinical trials in epilepsy (Ben-Menachem et al., 1994; Handforth et al., 1998) and then adopted for the current clinical studies in patients with depression (George et al., 2000a,b; Rush et al., 2000; Sackeim et al., 2001). Still, there is incomplete understanding of the regional neurobiologic effects of different VNS use parameters. More information is needed about the effects of different VNS parameters on regional brain activity to help set optimal dosing in clinical use.

Several groups have used positron emission tomography (PET) to investigate the chronic effects of VNS (Ko et al., 1996; Garnett et al., 1992). Another group has studied acute effects of VNS by comparing the differences between PET images acquired before and during VNS stimulation (Henry et al., 1998, 1999). Unfortunately, the low temporal resolution of PET limits its observations to integrated effects over at least a minute (longer than most VNS trains), and its dependence on radioactive tracers makes it unsuitable for repeated use. We recently demonstrated the feasibility of performing functional magnetic resonance imaging (fMRI), with its relatively high spatial and temporal resolution, during VNS (Bohning et al., 2001). This technique can reveal the exact location and level of the brain's immediate response to VNS, and allows for the examination of the regional brain effects of different VNS settings, possibly leading to more effective treatment regimens.

Intermittently stimulating a single nerve cell electrically at different frequencies produces drastically different changes in neuronal behavior. Low frequency stimulation induces long term depression (LTD); while intermittent high frequency stimulation produces long term potentiation (LTP). Similarly transcranial magnetic stimulation (TMS) of the cortex at different frequencies can temporarily inhibit (Wassermann et al., 1998), excite (Sawaki et al., 1999) or even arrest function (Epstein, 1998). Knowing whether VNS at different frequencies or doses evokes similar spatial patterns of brain activation would greatly advance knowledge of the basic mechanisms of action of VNS, accelerate the exploration of the effects of different stimulation parameters and regimen, and, possibly, extend its application to other disorders.

In this current preliminary study, we wished to examine whether different frequencies of VNS have differing brain effects. We therefore used interleaved VNS and Blood Oxygen Level Dependent (BOLD) fMRI to measure the regional cerebral blood flow related changes resulting from intermittent applications of 7 s of either 20 Hz or 5 Hz VNS in patients with major depression.

2. Subjects and method

2.1. Subjects

Nine subjects, who had participated in a recent VNS clinical study for treatment-resistant depression (Rush et al., 2000), were initially recruited for this study and brought in for scanning. Four of them participated in our previous VNS fMRI study (Bohning et al., 2000). Of these initial nine, three had to be excluded from the final data analysis because the device would not restart while in the MR scanner, leaving six subjects for the final analysis. There were three men and three women; their ages ranged from 43 to 59 years (mean 49.5 ± 6.1 S.D. years; Table 1). These subjects had their NCP pulse generators implanted in an MRI-compatible fashion, i.e. with the lead pins oriented along the long axis of the body (Maniker et al., 2000, and unpublished independent determination by DEB). They varied markedly in their mood state at the time of scanning, and their clinical response to VNS. The time since the start of their therapeutic VNS varied from 8 to 19 months (mean 14.2 ± 4.1 S.D. months). They were on diverse chronic VNS settings as well as taking a variety of antidepressant medications. The subjects signed a written informed consent that was approved by the Medical University of South Carolina's Institutional Review Board for Human Research.

Table 1
Subject demographics

Patients	Age	Months from implantation	$HDRS^{a}_{28}$	
			Before NCP[b] implantation	On day of fMRI
Pt1	43	16	42	13
Pt2	56	8	39	23
Pt3	50	11	26	15
Pt4	44	17	35	26
Pt5	45	14	44	6
Pt6	59	19	31	21

[a] $HDRS_{28}$, 28 item Hamilton Depression Rating Scale.
[b] NCP, the Neurocybernetic Prosthesis.

2.2. General experimental design

Subjects were scanned in a 1.5T clinical MR scanner (Signa Horizon LX, SR 77 gradients, software Rel. 8.3 M5, GE Medical Systems, Inc. Milwaukee, WI) with a send/receive RF head coil. Prototype software provided by GE was used for the multi-slice single-shot gradient-echo EPI-fMRI acquisitions (64×64 matrix, FOV = 270 mm, $\alpha = 88^\circ$, TE = 40.0 ms, slice thickness = 8.0 mm, gap = 0.0 mm, with fat saturation). Fifteen contiguous 8 mm thick axial slices were acquired parallel to the Anterior Commissure-Posterior Commissure (AC-PC) Line, and centered so that the AC-PC line was in slice number 5. A set of T1-weighted structural images (TE 20 ms, TR 600 ms) was acquired with the same slice coverage for anatomical reference. Details of the method were published earlier (Bohning et al., 2001).

Two fMRI scanning sessions were performed on each subject in a randomized counterbalanced method across subjects, with subjects having to exit the MR scanner between the runs in order to have the VNS device reset. Three of six subjects received 5 Hz during the first session and three during the second session. In each session, the VNS generator was programmed to deliver a 7 s on–108 s off (nominal) stimulation cycle. Because of the device's clock cycle, and the fact that the VNS device ramps up and down, this created an effective stimulation cycle of 13 s on and 103 s off, 116 s for the entire cycle. The pulse width was 500 μs; the current (intensity) settings, were left at the patient's treatment level setting, and ranged from 0.25 mA to 1.25 mA (mean 0.79±0.27; see Table 3). After completing the entire MRI scan (lasting about 90 min), their device was reset to the individual's original parameters.

As an internal reference for comparing VNS response at different settings, a control auditory stimulus was used. During the MRI scan procedure, a 440 Hz tone was played through the scanner's sound system to headphones on the subject and interleaved in 7 s trains of 100 ms long pulses, 100 ms apart, on alternate 58.0 s epochs of the VNS epoch-TONE epoch stimulation cycle. During each cycle, 40 15-slice volumes of BOLD-EPI images were acquired with the TR = 2.9 s. The VNS-REST1-TONE-REST2 cycle was repeated 10 times for a total of 580 s or 9 min, 40 s (see Fig. 1). In summary, the experiment has a repeating block design with four epochs: VNS, Rest 1, Tone, Rest 2; each epoch is 10 images per slice/29 s; the block of four epochs is 40 images per slice/116 s. The VNS and TONE stimuli occur for 7 s at the start of the respective epochs.

2.3. VNS synchronization and fMRI scan acquisition

Before subjects entered the scanner, standard adhesive backed MRI-compatible electrodes (re-usable silver chloride, Coulbourn Instruments, Allentown, PA), were placed on the subjects' neck. Electrodes were positioned over the route of the implanted VNS leads, typically about 1.5 cm apart, just above and below the scar of the incision made during surgery for attaching the implanted VNS electrodes to the vagus nerve. Subjects were given earplugs and headphones, and instructed to lie quietly with their eyes closed and listen for the tone. While lying on the gantry outside of the scanner bore, the subjects inserted their head into the scanner head coil and adjusted their position until they were centered and comfortable. Their head was then stabilized with foam-padded Velcro restraints. Subjects were then moved into scanning position in the scanner bore. Bypassing the scanner's ECG processing hardware/software, the NCP System's pulse generator signal recorded from the patient's neck was fed to an isolated bioamplifier and high performance band pass filter (LabLink V System Model V75–05 and Model V75–48, respectively, Coulbourn Instruments, Allentown, PA), 2.0 kHz to 4.0 kHz. This system allows for synchronization of the MRI scanning cycle with the VNS generator cycle and has been previously described (Bohning et al., 2001).

2.4. Data processing

The reconstructed structural images and fMRI *k*-space raw data were transferred to a Sun workstation (Sun Microsystems, Inc., Mountain View, CA, USA), and the structural images were stored for anatomical reference. The raw fMRI data was first reconstructed into images using research software from GE (''epirecon'') and then subjected to a standard set of fMRI data processing steps, described below.

Motion Correction: Using MEDx 3.2 (Sensor Systems, Inc., Sterling, VA), a check was performed on each image set to determine if subject movement was less than 2 mm along all of the three major axes (*x*, *y*, *z*). All of the data sets met this test. In three data sets, movement in at least one of the three directions was between 1 and 2 mm, so the images were motion corrected and co-registered to the images in the acquisition

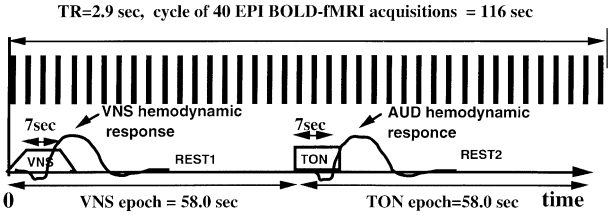

Fig. 1. Experimental paradigm of synchronized interleaving of TON and fMRI acquisitions. Each fMRI cycle is composed of two epochs: (1) 13 s (7 s peak) of VNS followed by 45.0 s of rest to measure the associated hemodynamic response and (2) 7 s on a 440 Hz tone (TON) followed by 51.0 s of rest.

midway through the first rest (REST1) epoch using the motion correction algorithm in MEDx, which is based on AIR (Woods et al., 1998).

Generating Group Data in Talairach Space: Using the SPM96 module (Frackowiak et al., 1997) in MEDx 3.2, each person's functional data set was spatially normalized into Talairach space (Talairach and Tournoux, 1988) by deforming the image volume to match the standard SPM96 MR brain template with an affine transformation; input and output voxel dimensions were $4.22\times4.22\times8$ mm and $4\times4\times4$ mm, respectively. Each person's data was then high-pass filtered to remove slow signal drift, and spatially smoothed with an 8 mm kernel size. High pass filtration of individual data was done with the cutoff period of 232 s.

Identification of voxels with statistically significant levels of activation: using SPM96 in MEDx 3.2, a pixel-by-pixel non-paired bi-directional *t*-test was used to compare the individual fMRI time series data to a delayed boxcar model (VNS, REST1, TONE, REST2; REPEAT X 10). In each cycle, acquisitions 1–5 were taken as the VNS activation period and acquisitions 31–39 were taken as the resting period (REST2) at the end of the cycle. Given the BOLD time series data from our first VNS study, we were concerned about the lingering effects of VNS on blood flow immediately following device termination. Since a preliminary analysis revealed higher *Z*-values when the VNS was compared with REST2 than with REST1 (immediately following the VNS), or when VNS was compared with both REST1 and REST2, both VNS and the TONE were compared to REST2.

Identification of Activated Areas in Group Data: Nonthresholded individual Z-maps were averaged according to Bosch (2000). Using the SPM statistics functions in MEDx 3.2, a cluster analysis was also performed on the group *Z*-map data to identify areas of the brain showing significantly more or less BOLD-fMRI signal during the VNS and TONE conditions than during the REST2 condition (Friston et al., 1994). A cluster was defined as a group of spatially connected voxels with individual Z-score >3.09 (one-tailed $P<0.001$) or >2.326 ($P<0.05$), and a spatial extent threshold (Friston et al., 1997), based on the number and Z-scores of voxels in it, greater than $P<0.05$. These Z-maps were generated for the contrasts VNS-REST2 and TONE-REST2 both within individuals and for the group within and over the two frequencies (5, 20 Hz).

Quantitative analysis of overall brain reaction (both increases and decreases) to VNS and TONE was done based on the numbers of voxels with $Z>3.09$ and $Z<-3.09$ statistical level ($P<0.001$). A nonparametric paired randomization test and a two by two Chi square test of frequency of significant voxels in the volume of the brain were used to compare overall activation across the different VNS and TONE conditions.

3. Results

There were no adverse effects and none of the subjects noticed any aberrant VNS stimuli while in the scanner.

3.1. Group data, within run: 20 Hz (140 stimuli)

Fig. 2a,b show the 3D orthogonal view maximum intensity projection (MIP) of the results of the group VNS-REST2 and TONE-REST2 cluster analyses, respectively, in the VNS 20 Hz (140 stimuli) experiment. In the VNS-REST2 comparison (Fig. 2a), areas of significant activation (voxel $P<0.001$, extent $P<0.05$) can be seen bilaterally in the posterior part of the orbitofrontal cortex (mainly in gyrus rectus), more in the right hemisphere, and also in both frontal poles (mainly frontal superior gyrus). In addition, activity can be seen in the hypothalamus and the left globus pallidus. With reduction of statistical threshold (pixel $P<0.05$, extent $P<0.05$) bilateral activation in the thalamus was revealed. In the TONE-REST2 comparison (Fig. 2b) large areas of significant (pixel $P<0.001$, extent $P<0.05$) activation can be seen bilaterally in the auditory cortex. See also Table 2 for the exact coordinates.

3.2. Group data, within run: 5 Hz (35 stimuli)

For the VNS 5 Hz (35 stimuli; Fig. 2c), at the same level of statistical significance as above, there were no areas of significant activation of VNS-REST2. The same areas of activation were found during tone presentation in the VNS 5 Hz (35 stimuli) run (Fig. 2d) as was found in the VNS 20 Hz run, although the total amount of activation was reduced (see comparison below, Table 3, 324.7 vs. 366.3 voxels and 745.3 vs. 1291.2 voxels).

3.3. Individual data, between run comparison

We generated individual VNS 20 Hz *Z*-maps for each subject, on spatially normalized data. The numbers of statistically significant voxels are listed in Table 3, for both activations and deactivations. Individual response was heterogeneous, with differences across individuals in the amount and location of activated areas. In five of six patients with exactly the same fMRI data acquisition and processing, the degree of activation (number of voxels) was higher under the influence of 20 Hz VNS than 5 Hz VNS [Interestingly and perhaps of some importance, the only subject (Pt4) who demonstrated the opposite trend, with a decrease of the number of activation voxels (from 7 with 5 Hz to 1 with 20 Hz), was also the worst VNS treatment responder of the six subjects. This subject also demonstrated the highest degree of relative deactivation in comparison in the group (Table 3). As a result this subject's total reaction

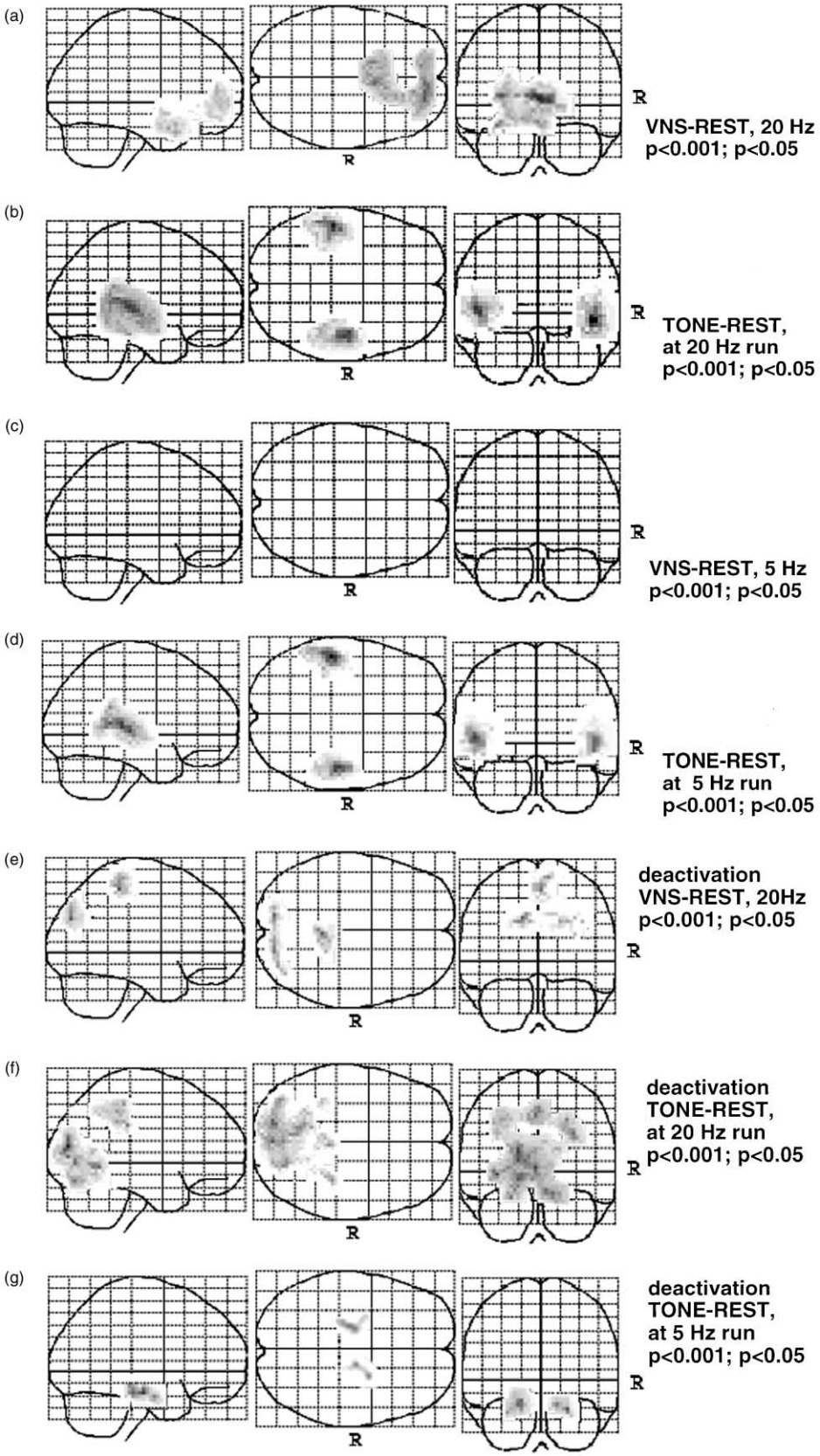

Fig. 2. SPM 3D maximum intensity projection views of clusters of activated and deactivated voxels (group data). Activated areas at: (a) VNS-REST2, 20 Hz experiment; (b). TONE-REST2 at 20 Hz VNS experiment (c) VNS-REST2, 5 Hz experiment; (d) TONE-REST2 at 5 Hz VNS experiment. Deactivated areas: (e) VNS-REST2, 20 Hz experiment; (f) TONE-REST2, 20 Hz experiment; (g) TONE-REST2, 5 Hz VNS experiment. *P*-values correspond for *Z*-value of individual voxel and cluster extent.

Table 2
Brain areas affected by the Vagus Nerve Stimulation (VNS) and tone

Condition	Activation clusters			Deactivation clusters		
	Talairach cluster center, *x*, *y*, *z* (mm)	Size (voxels)	Brain structures	Talairach cluster center, *x*, *y*, *z* (mm)	Size (voxels)	Brain structures
VNS at 20 Hz ($P<0.001$; $P<0.05$)	12.2; 56.8; 5.4	251	Right Frontal pole	5.5;−87.7; 30.1	126	Occipital pole
	0.3; 5.9;−17.2	183	Posterior orbitofrontal cortex, extends to hypothalamus (0, 0, 0), left Gl. Pallidus (−16, 0, 4)	6.3;−35.1; 64.7	94	G. temporalis sup., medial surface
Tone at 20 Hz ($P<0.001$; $P<0.05$)	55.0;−22.9;−1.1	569	Right G. temporalis sup. and medius	−4.1;−80.3; 4.5	702	Occipital pole
	−52.4;−29.5; 7.6	431	Left G. temporalis sup, Brodman 42			
Tone at 5 Hz ($P<0.001$; $P<0.05$)	56.0;−25.2; 6.8	475	Right G. temporalis sup., Brodman 42	−25.0;−17.2;−24.1	76	Medial surface of the left temporal lobe
	−55.7; 31.7; 8.6	474	Left G. temporalis sup., Brodman 42	−25.0;−17.2;−24.0	76	Medial surface of the left temporal lobe
VNS, 20 Hz-5 Hz ($P<0.01$; $P<0.05$)	9.9; 58.7; 6.3	177	Right Frontal Pole			
Tone, 20 Hz-5 Hz ($P<0.01$; $P<0.05$)	−3.4;−25.0;−24.3	429	Pons			

Table 3
Numbers of different pixels activated by condition

	Intensity (mA)	VNS 5 Hz		VNS 20 Hz			Tone at 5 Hz		Tone at 20 Hz	
		> 3.09	> 3.09 < −3.09	> 3.09	> 3.09 + < −3.09	> −3.09/ < 3.09	> 3.09	> 3.09 + < −3.09	> 3.09	> 3.09 + < −3.09
Pt1	1.25	0	0	31	198	5.39	169	169	37	2753
Pt2	1.00	0	0	5016	6995	0.39	0	7	118	118
Pt3	0.75	4	112	4421	10197	1.31	349	518	498	519
Pt4	0.75	7	7	1	117	116.00	983	983	533	933
Pt5	0.50	0	33	98	98	0.00	58	58	72	203
Pt6	0.50	0	0	4	4	0.00	389	463	3214	3221
Mean	0.79	1.8	25.3	1595.2	2934.8	0.84	324.7	366.3	745.3	1291.2
RT[a]				0.006[c]	0.016[c]				0.17[d]	0.047[d]
CHI[b]				0.00001[c]	0.00001[c]				0.00001[d]	0.00001[d]

[a] RT *P*-value of randomization test comparing number of voxels (paired test).
[b] Chi^2, two by two Chi square test of frequency of significant voxels ($P<0.001$) in the volume of the brain.
[c] 0.006; 0.016; 0.00001; 0.00001, VNS 20 Hz VNS 5 Hz comparisons.
[d] 0.17[4]; 0.047[4]; 0.00001;.00001, TONE 20 Hz TONE 5 Hz comparisons.

to VNS (number of activated and deactivated voxels) was higher at 20 Hz similar to the other patients].

Tone presentation in the 20 Hz VNS run was also accompanied with greater activation than during the 5 Hz run, but this trend reached statistical significance only in the Chi square test. Activation was slightly lateralized to the right hemisphere in the 20 Hz experiment (569 vs. 431 voxels in acoustical clusters, Table 2) in comparison to the 5 Hz experiment (475 vs. 474 voxels). Total reaction of the brain (activation and deactivation) was significantly higher during the 20 Hz VNS run than during the 5 Hz run ($P=0.047$ and 0.00001 from randomization and CHI square tests respectively, Table 3). And again, only one of the patients (Pt4) demonstrated the opposite trend.

3.4. Individual data, examination of response heterogeneity and comparison with clinical variables

We performed scatter plots of these pixel activations on the 20Hz-REST2 comparison and ordinally ranked individual *Z*-maps with respect to the following clinical variables: time from implant, VNS clinical response during the clinical trial, and Degree of Depression (Hamilton

Score) at the time of scanning. In these few subjects no consistent trend could be detected for these variables.

4. Discussion

The present study in depressed adults using the VNS/fMRI technique has three preliminary findings. First, this study confirms the previous VNS/fMRI study (Bohning et al., 2001) in terms of the immediate neuroanatomy affected by VNS as seen with fMRI, including the hypothalamus and orbitofrontal cortex, both implicated in mood disorders. Second, this study is consistent with a frequency/dose effect of acute VNS on bilateral regional brain changes. Finally, there is an interesting suggestion of a dose dependent modulating effect of VNS on other brain activity during the off time between acute VNS.

Before discussing these results, it is important to point out some limitations of the study. These include the small sample size and the confound of intermingling frequency and dose. The subjects are a mixed cohort with treatment resistant depression who are taking multiple medications. They have divergent clinical responses and differing times from stimulation and variable VNS treatment settings. One cannot readily generalize these findings to other diseases without caution. We were able to detect local statistically significant rCBF changes associated with the VNS despite the fact that the patients were clinically heterogeneous. The paired study design with 5 and 20 Hz tested in two adjacent runs likely minimizes some of the impact of this clinical heterogeneity. Nevertheless, these results should be viewed with caution until they are replicated. It is also conceivable that these changes are not directly neuronal. We are measuring a surrogate of blood flow, and theoretically vagus effects on vascular flow could be a confound. This is unlikely however given the anatomical distribution and the timing of the changes.

Turning to the results of the paper, (1) in general, the VNS maps during the high frequency (20 Hz) run confirm our previous fMRI study and the known neuroanatomy of vagus nerve stimulation in depression. In the present study, 20 Hz VNS increased BOLD-fMRI response in the orbitofrontal cortex, frontal pole, hypothalamus, left pallidum, and, less significantly, the thalamus. Our previous study included the 20 Hz data of four of these subjects, so this should not be considered a truly independent replication. It should also be noted that the data analysis of our previous study differed in that the individual data sets were intensity normalized and averaged time point by time point without temporal smoothing to obtain a group data set. In general, however, the regional activation found in the current study is consistent with our previous fMRI findings (Bohning et al., 2001) and brain areas reactive to VNS when studied by [^{15}O]H_20 PET (Ko et al., 1996; Henry et al., 1999) or perfusion SPECT (Vonck et al., 2000; Van Laere et al., 2000; Ring et al., 2000). The different time domains over which the different imaging modalities sample brain information likely accounts for much of the inconsistencies across the studies. However, other likely important differences include study sample and diagnosis, time from implant, and concomitant medications.

(2) Although this is a small study, there appears to be a frequency/dose effect of VNS on acute blood flow changes. High frequency/dose VNS (20 Hz, at least 186 stimuli at 0.25 mA) increased BOLD-fMRI in numerous regions. At the same level of statistical significance, there was no brain activation at 5 Hz. The 5 and 20 Hz stimulation runs were identical and balanced by intensity and duration (train length), but because of the block design, the higher frequency run (20 Hz) also delivered more stimuli (140 stimuli) than did the lower frequency (5 Hz, at least 46 stimuli at 0.25 mA). Thus, differences in brain activity during the two runs may be due to frequency, or total stimuli, or both. Unfortunately, this design cannot distinguish these two parameters. Further studies are needed in order to tease apart the brain effects of these two different parameters (frequency, total number of stimuli).

Because of the small amount of activation seen in he 5 Hz VNS data at a reasonable level of statistical significance, we were not able to perform a formal analysis of whether VNS at different frequencies or doses affects different brain regions. This is a key question in the development of VNS as a potential therapy in other neuropsychiatric disorders. In an exploratory attempt at addressing this question, we generated group Z-maps for 5 Hz at $P < 0.1$ level, and overlaid them on the 20 Hz maps at the levels noted above. Although there were differences in regional activation, we are not convinced they are more than noise, given the need for the low significance level at 5 Hz. Future studies are needed using different VNS settings that each produce robust responses in order to address this very important question of whether VNS at different frequencies alters different brain regions.

(3) Finally, this study hints that the brain reacts differently in the off time between on times of VNS at different settings. That is, 20 Hz VNS (with more stimuli) had effects on the brain's response to hearing a tone (more activity overall, and more activity in the right auditory cortex), compared to during the 5 Hz stimulation. Specifically, although the VNS was not stimulating, and had not stimulated for almost 50 s, there were differences in the brain's response to the tone across the two runs. There was a trend for more activation elicited by the tone presentation in the 20 Hz experiment. Summing both activations and deactivations, there was a significant difference in reaction to the tone across the two runs. The results in this study are thus the first brain imaging results to confirm behavioral data showing lingering effects of VNS at different settings. For

example, using just behavioral measures without imaging, Clark and colleagues (1999) showed that epilepsy patients performed better at an emotional memory task at certain VNS intensity settings than at others. In similar work, Ness and colleagues found that the threshold to thermal pain decreased in patients with epilepsy across a wide range of VNS intensities (Ness et al., 2000). More behavioral and imaging studies, alone and in combination, are needed to elucidate this modulatory effect of VNS. A natural extension of our imaging results would be to formally test whether auditory perception is behaviorally better during VNS at 20 Hs than at 5 Hz.

Another important observation was the heterogeneity of individual response to VNS. Because of the large amount of information acquired, BOLD fMRI allows for the formal statistical analysis of brain activity within individuals. In this small sample single study, it is impossible to determine fully the sources of this heterogeneity. Potential causes of the heterogeneity range from the inherent variability of the VNS/fMRI scanning system, to the differences in our subjects with respect to age, gender, intensity, clinical condition at the time of scanning, time from implant, baseline VNS settings before and after the MRI study, concomitant medications, and co-morbid diagnoses. With a small sample of 6, preliminary ranking of individual VNS/fMRI responses with respect to these variables failed to reveal any trends. Future studies with larger sample sizes and covariate analyses are needed to examine these important issues and their influence on the individual VNS responses.

Future studies combining VNS and fMRI are needed to extend these important new findings of different regional brain activity when the vagus nerve is stimulated at different frequencies.

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