Epilepsia, **45**(9):1064–1070, 2004
Blackwell Publishing, Inc.

Brain Blood-flow Alterations Induced by Therapeutic Vagus Nerve Stimulation in Partial Epilepsy: II. Prolonged Effects at High and Low Levels of Stimulation

*Thomas R. Henry, †Roy A. E. Bakay, *Page B. Pennell, *Charles M. Epstein, and ‡John R. Votaw

*Departments of *Neurology, †Neurosurgery, and ‡Radiology, Emory University School of Medicine, Atlanta, Georgia, U.S.A.*

Summary: *Purpose:* To measure vagus nerve stimulation (VNS)-induced cerebral blood flow (CBF) effects after prolonged VNS and to compare these effects with immediate VNS effects on CBF.

Methods: Ten consenting partial epilepsy patients had positron emission tomography (PET) with intravenous $[^{15}O]H_2O$. Each had three control scans without VNS and three scans during 30 s of VNS, within 20 h after VNS began (immediate-effect study), and repeated after 3 months of VNS (prolonged study). After intrasubject subtraction of control from stimulation scans, images were anatomically transformed for intersubject averaging and superimposed on magnetic resonance imaging (MRI) for anatomic localization. Changes on t-statistical maps were considered significant at $p < 0.05$ (corrected for multiple comparisons).

Results: During prolonged studies, CBF changes were not observed in any regions that did not have CBF changes during immediate-effect studies. During both types of studies, VNS-induced CBF increases were similarly located in the bilateral thalami, hypothalami, inferior cerebellar hemispheres, and right postcentral gyrus. During immediate-effect studies, VNS decreased bilateral hippocampal, amygdalar, and cingulate CBF and increased bilateral insular CBF; no significant CBF changes were observed in these regions during prolonged studies. Mean seizure frequency decreased by 25% over a 3-month period between immediate and prolonged PET studies, compared with 3 months before VNS began.

Conclusions: Seizure control improved during a period over which some immediate VNS-induced CBF changes declined (mainly over cortical regions), whereas other VNS-induced CBF changes persisted (mainly over subcortical regions). Altered synaptic activities at sites of persisting VNS-induced CBF changes may reflect antiseizure actions. **Key Words:** Vagus nerve stimulation—Complex partial seizures—Blood flow—Positron emission tomography.

Prolonged, intermittent electrical stimulation of the left cervical portion of the vagus nerve was established as efficacious therapy of medically refractory complex partial seizures, in prospective, randomized, controlled trials (1,2). Subsequent clinical investigations reported evidence of incremental seizure reductions over months of ongoing vagus nerve stimulation (VNS) and VNS efficacy in generalized epilepsies (3–5). In experimental epilepsy models, cervical VNS aborted seizures when stimulation was applied during a seizure, reduced seizures between periods of stimulation (so long as interstimulation periods were not longer than ∼10 min), and retarded amygdalar kindling (6–9). Physical or chemical lesions of parasympathetic vagal efferents distal to the cervical site of stimulation did not prevent antiseizure effects of VNS (9,10). The therapeutic mechanisms of VNS in epilepsy are likely to occur in the brain, without participation of peripheral parasympathetic systems, but remain largely unknown.

Cervical VNS is likely to cause increased transsynaptic neurotransmission in the nucleus of the tractus solitarius and at other medullary vagus nerve terminals and to cause altered synaptic activity in sites receiving projections from these areas. The medullary complex of the vagus projects to the parabrachial nucleus (which projects to the thalamus and insular cortex), cerebellum, hypothalamus, amygdala, and other regions (11,12). Functional neuroimaging can measure cerebral blood flow (CBF) noninvasively, to map anatomic pathways that are activated or deactivated by transsynaptic neurotransmission. Localized, rapidly reversible CBF alterations primarily reflect changes in the local intensity of transsynaptic neurotransmission (in the absence of seizures, arterial thromboembolism, and other brain vascular dysfunctions) (13). Our prior study of acute VNS effects on CBF (14) showed that within the first 24 h of stimulation, VNS causes regional synaptic activity to

Accepted May 9, 2004.
Address correspondence and reprint requests to Dr. T.R. Henry at Department of Neurology, Emory University, Woodruff Memorial Research Building, Suite 6000, 1639 Pierce Drive, Atlanta, GA 30322, U.S.A. E-mail: thenr01@emory.edu

(a) increase in the rostral, dorsal-central medulla, (b) increase in the right postcentral gyrus, (c) increase bilaterally in the hypothalami, thalami, and insular cortices, and in cerebellar hemispheres inferiorly, and (d) decrease bilaterally in the hippocampi, amygdalae, and posterior cingulate gyri. Published studies of prolonged VNS effects on CBF, performed after months or years of ongoing VNS, showed far more restricted volumes of CBF activation (15,16). These reports are difficult to interpret, because one study included patients with prior cerebral resection (15), and the other included patients who had seizures during positron emission tomography (PET) imaging (16). In the current study, we looked for evidence of VNS-induced alterations in synaptic activity during prolonged stimulation and compared these prolonged VNS studies with immediate-effect studies in the same patients.

METHODS

Subjects and VNS techniques

The 10 subjects were patients participating in the E05 Protocol [previously reported (1)], by using the NeuroCybernetic Prosthesis (NCP) system to provide VNS. The Emory University Institutional Review Board approved the E05 Protocol and the VNS-activation PET protocol. The Emory University Radiation Safety Committee approved the VNS-activation PET protocol. Informed consent was obtained from each volunteer.

Among our 10 patients, five were in the High-Stimulation group (receiving levels of stimulation that were believed to be most effective in epilepsy therapy) and five were in the "active control" or Low-Stimulation group (receiving lower levels of stimulation likely to be suboptimal in epilepsy therapy, but causing perceptible left cervical sensations during VNS) of the E05 protocol for VNS, sponsored by Cyberonics, Inc. In each group, three patients were women and two were men. Ages ranged from 26 to 51 years (mean, 38 years) in the High-Stimulation group and from 23 to 40 years (mean, 32 years) in the Low-Stimulation group. As required by the E05 protocol, each patient had medically refractory complex partial seizures, occurring 6 or more times per month. One patient in each group also had reported secondarily generalized seizures within the preceding 2 years. Each group had four patients with mesial temporal lobe epilepsy, and one patient with seizures of dorsolateral frontoparietal origin. No patients had undergone cerebral resection. No patients changed antiepileptic drug (AED) regimens during the 3 months between brief and prolonged PET imaging, or during the 3-month pre-VNS baseline period, as expected under the E05 protocol.

The brief PET imaging session occurred within the first 20 h after initiation of VNS, as previously described (14). For each patient, the prolonged PET session occurred 12 weeks after the brief PET session. For the entirety of these 12 weeks, the patients in the Low-Stimulation group had the same stimulation parameters as were used during the brief PET session. For the Low-Stimulation group, each patient received 130-μs pulses at 1 Hz in trains of 30 s duration, with 180 min between trains of stimuli; output current levels of the five patients in the Low-Stimulation group were 0.5, 0.5, 1.0, 1.0, and 1.25 (mean, 0.85) mA, which were the lowest currents necessary for perception of cervical stimulation. During the 12 weeks between the brief and prolonged PET sessions, the patients in the High-Stimulation group received sequentially increasing output current of VNS, as tolerated by the individual and not to exceed 3.5 mA. For these 12 weeks, the High-Stimulation patients received 500-μs pulses at 30 Hz in trains of 30 s duration, with 5 min between trains of stimuli; output current levels of the five patients in the High-Stimulation group were 0.25, 0.25, 0.25, 0.75, and 1.0 (mean, 0.5) mA during the brief PET session, and were 0.25, 0.75, 1.25, 1.75, and 2.5 (mean, 1.3) mA during the prolonged PET session.

Emission tomographic data acquisition

Each subject had CBF imaging sessions within 20 h after VNS began (immediate-effect study), and again after 3 months of VNS (prolonged study). Each imaging session consisted of three control scans without VNS and three scans during 30 s of VNS. Both the brief and the prolonged PET sessions were performed >12 h after the last complex partial seizure and >1 month after the last generalized seizure (as determined by subjects' reports). No seizures occurred during imaging sessions (as determined by subjects' reports, continuous observation by a neurologist, and continuous scalp EEG recording).

Image acquisition was performed with an ECAT Exact/921 tomograph (Siemens, Hoffman Estates, IL, U.S.A.), operating in 2D mode, with acquisition of activity over the entire brain simultaneously. For each scan 60 mCi of $[^{15}O]H_2O$ were administered as an intravenous bolus. For each VNS scan, a 30-s train of stimuli was synchronized to arrival in the brain of the bolus of $[^{15}O]H_2O$. The PET acquisition techniques used in the brief studies were further described elsewhere (14). The acquisition techniques for the prolonged studies were the same as for the brief studies, except as noted here. The NCP System was turned off for 1 h before the beginning of the prolonged scanning session and remained off during the session, except for single trains of VNS controlled by the investigators. At the start of each VNS scan, the magnet was used to trigger a single 30-s train of stimuli, timed so that the bolus of $[^{15}O]H_2O$ reached the brain at the onset of stimulation. The order of VNS-on and VNS-off scans was pseudorandom, with alteration from random so as to acquire three on scans and three off scans by the end of the prolonged PET session.

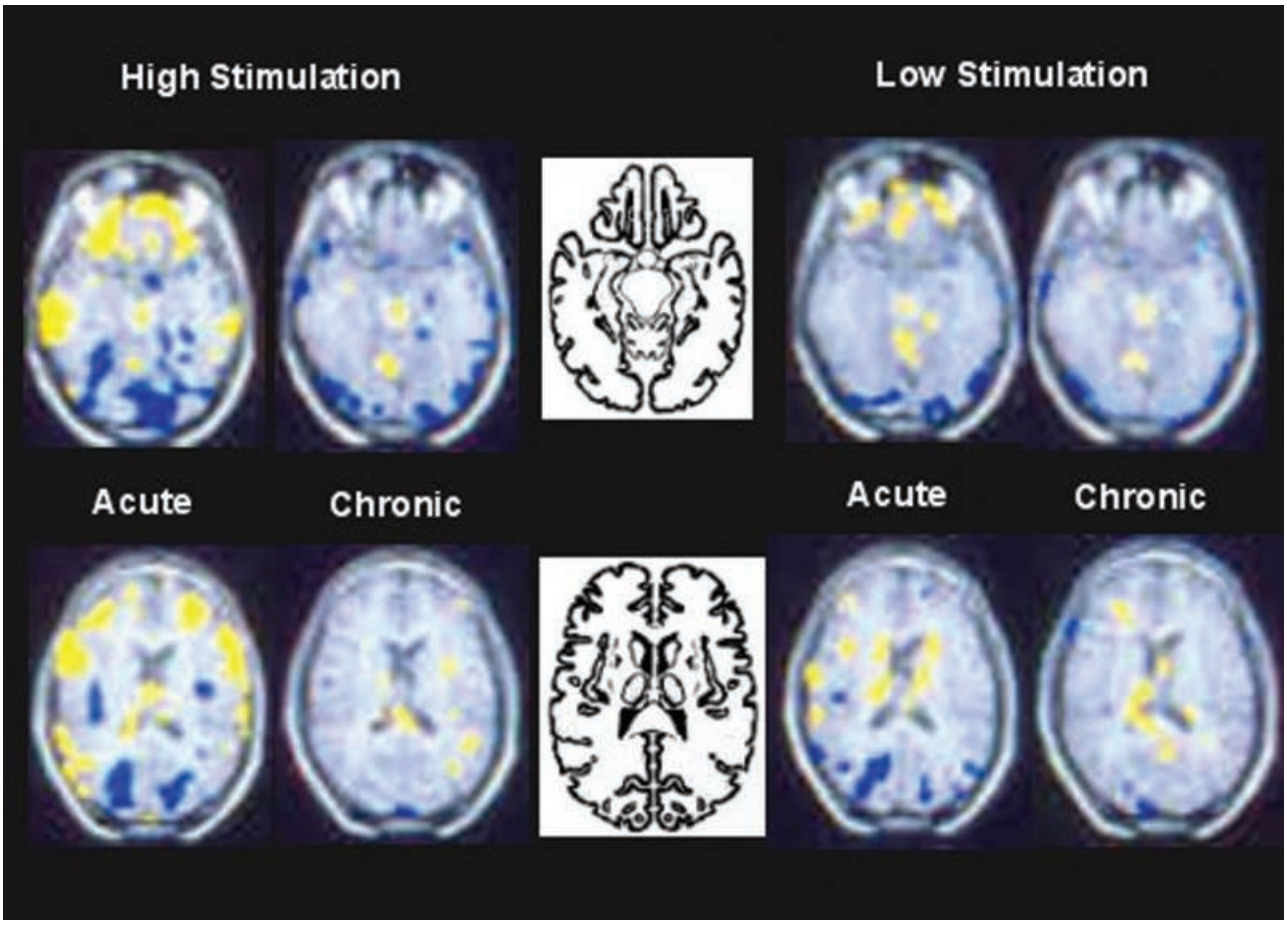

FIG. 1. Summary of vagus nerve stimulation (VNS) immediate and prolonged effects on regional cerebral blood flow (CBF). VNS-induced increases (yellow) and decreases (blue) in CBF are displayed on axial magnetic resonance imaging (gray scale) with subjects' left on image right. Colored pixels, sites with t-statistical differences between stimulated and nonstimulated periods that are significant at $p < 0.05$, not corrected for effects of multiple comparisons within each data set; the Table lists sites with changes significant at $p < 0.05$ after correction for multiple comparisons. Each row of images is at the same plane, shown schematically with cerebral contour lines at the center of the row. Horizontally adjacent image pairs were obtained immediately (left) or in the long term (right), in the High-Stimulation (pairs to the left of the schema on each row) and the Low-Stimulation (to the right) groups.

Emission tomographic data analysis

The PET data were analyzed as previously reported (14). In brief, images were reconstructed by using a ramp filter cutoff at the Nyquist frequency, with calculated attenuation correction and then 3D smoothing. Each image set consisted of 47 planes covering an axial field of view of 16 cm. The final isotropic resolution of the filtered images was 11.8 mm full width at half maximum. The PET images were normalized by the average radioactivity concentration within the brain. Each subject's six PET image sets were averaged then coregistered with their magnetic resonance imaging (MRI) set. The MRI was translated three-dimensionally ("warped") into a common stereotactic system (17–19) to permit data for the five subjects of each (High-Stimulation or Low-Stimulation) group to be analyzed together. The images of each group were analyzed with volumes of interest (VOIs), placed on the MR images (without access to PET images during VOI placement) by one investigator (T.H.). The predetermined VOIs covered specified structures of the medulla oblongata and of subcortical and cortical cerebral gray matter that are involved in the somatosensory, autonomic, and limbic systems (as listed in Table 1), and were used to limit the search volume for t-statistic changes. Separate three-way analyses of variance (ANOVAs) were performed (with factors being stimulus condition, subject, and replication) for each group, and then contrast analyses were used to produce t-statistic images based on comparison between the stimulated and baseline conditions. Two-tailed t-statistic images were examined to determine whether blood-flow increases or decreases occurred within any of the predetermined VOIs, separately in the High-Stimulation and Low-Stimulation groups. Clusters of voxels with increases or decreases were then subjected to an analysis of cluster-based significance (20). This analysis was sensitive to the spatial extent as well as the degree of blood-flow change, and corrected for the fact that multiple *t* tests were generated within the area of search. Reported regions of

TABLE 1. *VNS-induced regional blood-flow alterations*

Structure	Side	Acute High VNS	Chronic High VNS	Acute Low VNS	Chronic Low VNS
Medulla, dorsal-rostral	Bilat.	↑	–	–	–
Cerebellar hemisphere, inf.	Left	↑	↑	↑	↑
Cerebellar hemisphere, inf.	Right	↑	↑	↑	↑
Cerebellum, vermis	Bilat.	–	–	↑	↑
Hypothalamus	Bilat.	↑	↑	↑	↑
Thalamus	Left	↑	↑	↑	↑
Thalamus	Right	↑	↑	↑	↑
Hippocampus	Left	↓	–	↓	–
Hippocampus	Right	↓	–	↓	–
Amygdala	Left	↓	–	↓	–
Amygdala	Right	↓	–	↓	–
Cingulate gyrus, post.	Left	↓	–	↓	–
Cingulate gyrus, post.	Right	↓	–	↓	–
Insula	Left	↑	–	↑	–
Insula	Right	↑	–	↑	–
Orbitofrontal cortex	Left	↑	–	–	–
Orbitofrontal cortex	Right	↑	–	–	–
Inf. frontal gyrus, inf.-post.	Left	↑	–	–	–
Inf. frontal gyrus, inf.-post.	Right	↑	–	↑	–
Entorhinal cortex	Left	–	–	↓	–
Entorhinal cortex	Right	↑	–	–	–
Temporal pole	Left	–	–	–	–
Temporal pole	Right	↑	–	–	–
Postcentral gyrus, inf.	Left	–	–	–	–
Postcentral gyrus, inf.	Right	↑	↑	↑	↑
Inferior parietal lobule	Left	↑	↑	↓	↑
Inferior parietal lobule	Right	↑	↑	↓	↑

VNS, vagus nerve stimulation; upward-pointing arrows, significant blood-flow increases; downward-pointing arrows, significant blood-flow decreases; dashes, absence of significant blood-flow changes during VNS compared with the unstimulated state.

blood-flow alteration (see Table 1) are significant at the $p < 0.05$ level, corrected for effects of multiple comparisons within the same data set.

RESULTS

Changes in seizure frequency

During the first 3 months of VNS (the period between the brief and prolonged PET studies), the frequency of complex partial and secondarily generalized seizures decreased by 25% in the entire epilepsy group (compared with the pre-VNS, 3-month baseline period). Mean seizure frequency decreased by 35% in the High-Stimulation group and by 15% in the Low-Stimulation group, during the period between the brief and prolonged PET studies. AED regimens were not changed during the baseline period and during the first 3 months of VNS. No patient had significant changes in neurological and general medical conditions during the 3-month baseline period and the 3-month initial VNS period.

Blood Flow PET Findings

Single trains of VNS activated blood flow in several posterior fossa and cerebral structures bilaterally, following 3 months of chronic VNS, in both the High-Stimulation and the Low-Stimulation groups (see Table). In the chronic studies, the locations of significant CBF changes were similar between the High- and Low-Stimulation groups, but the volumes of significant changes tended to be larger in the High-Stimulation group. On comparing acute and chronic studies within each stimulation-level group, some sites had significant VNS-induced CBF change both acutely and chronically, but in general the spatial volumes of significant VNS-induced CBF change were reduced after 3 months of chronic VNS versus the volumes of significant CBF change that occurred acutely. No significant blood flow changes were induced by VNS in the chronic studies at sites that did not have CBF alterations in the acute studies (see Fig. 1).

Most subcortical sites that were activated by VNS acutely also were activated in the chronic condition (see Table). Acutely the High-Stimulation group had a site of significant blood flow increase in the dorsal, rostral medulla oblongata (the region where most vagus nerve afferent fibers terminate), but after chronic VNS this group had blood flow increases at that site, which were not statistically significant. The Low-Stimulation group had blood flow increases at this site during both acute and chronic studies, but these changes did not attain statistical significance. The High- and Low-Stimulation groups each had significant blood flow increases in the thalami, hypothalamus and cerebellar hemispheres, both acutely and chronically. The Low-Stimulation group had acute and chronic activation of the vermis.

Many cortical sites had activations (or deactivations) of CBF during acute VNS, but most of these regions did not have significant CBF changes on chronic studies (see Table). Insular activations and hippocampal, amygdalar and posterior cingulate deactivations, in both High- and Low-Stimulation groups, were significant with acute VNS, but no significant CBF changes occurred in these regions on chronic VNS. Other frontal and temporal lobe sites had acute activations, mainly in the High-Stimulation group, which were not present on chronic studies. Contrary to the trend for cortical regions to show little VNS-induced CBF change following chronic VNS, the right inferior postcentral gyrus (primary somatosensory region for left cervical stimuli) was significantly activated in both groups acutely and chronically; patients reported a left cervical sensation during trains of VNS, in both acute and chronic conditions. The right and left inferior parietal lobules also were significantly activated in both groups on chronic studies. A "sign change" occurred in the Low-Stimulation group, which had significant CBF decrease in the inferior parietal lobules acutely and significant CBF increase in the inferior parietal lobules chronically.

DISCUSSION

Therapeutic VNS induced bilateral thalamic, hypothalamic and cerebellar activations during both acute and chronic periods of VNS, in our study. While some parietal lobe activations persisted from acute into chronic periods, many cortical regions that had acute VNS-induced CBF alterations did not have CBF alterations during the chronic phase of VNS. No new sites of VNS-induced CBF alterations were observed in the chronic phase, beyond those observed acutely. Both the High- and Low-Stimulation groups demonstrated this pattern of persisting subcortical activations, and reduction over time in VNS-induced cortical blood flow changes. Changes in regional CBF effects of VNS between acute and chronic studies are unlikely to be due to alterations in VNS parameters between the two PET sessions, because VNS parameters were not changed over the intervening 3 months in the Low-Stimulation group. Changes in regional CBF effects of VNS between acute and chronic studies cannot be accounted for by alterations in antiepileptic drug therapy, as these medications were not changed over the intervening 3 months. Possibly the novel sensory stimuli of acute VNS might generate nonspecific alerting responses in cortical areas, which are not maintained chronically, accounting for reductions in cortical blood flow alterations over time.

The VNS-induced activation and deactivation of synaptic processing in recognized central vagal pathways were also detected using other brain mapping techniques. Functional magnetic resonance imaging (fMRI) has been used to study VNS effects on CBF in epilepsy patients, with highly similar results to those of acute and chronic CBF PET studies (21–23). In contrast, single photon emission computed tomographic (SPECT) studies mainly found regional CBF decreases during VNS, which were located in the same areas that had CBF increases on PET and fMRI measurements (24–28). Differences in the direction of VNS-induced CBF alteration, on SPECT versus PET and fMRI studies, may be caused by differing temporal resolutions of these imaging modalities, and by different timing of the onset of image acquisition with regard to the onset of trains of VNS. The $[^{15}O]H_2O$ PET studies and fMRI studies achieved a 30-s or shorter window of CBF image acquisition, and this acquisition was timed to coincide with a 30-s train of VNS. The SPECT technique has a longer window (up to 10 minutes) of CBF-driven radioligand uptake (29), and in most of the reported studies the time from radioligand injection to cerebral arrival added delay of ligand uptake from the time of VNS onset. The fact that the similar sets of regions had VNS-induced CBF increases on PET and fMRI, versus CBF decreases on SPECT studies, may reflect image acquisition exclusively during stimulation in reported PET and fMRI studies, versus early poststimulation CBF measurement with SPECT.

Altered synaptic activities at sites with both acute and chronic VNS-induced CBF changes may reflect antiseizure actions of VNS, which are mediated by rapidly altered neurotransmission in these regions. This interpretation of the significance of our results would suggest that rapidly occurring subcortical effects may be more important in VNS antiseizure mechanisms than are rapidly occurring cortical effects. In an earlier investigation, the degree of bilateral thalamic activation on acute VNS activation PET studies correlated significantly with subsequent decreases in seizures over 3 months, among partial epilepsy patients who participated in this study (30). Based on the current study, we now know that these acute thalamic activations persist during chronic VNS. Two fMRI-based studies also observed greater seizure reduction in patients whose VNS effects included thalamic CBF activation (21,22). The PET and fMRI studies suggest that VNS acutely and chronically alters thalamic processing in some way so as to antagonize partial-onset seizures. Intrathalamic electrical stimulation can antagonize cortical seizures, in experimental and clinical observations (31) that support the concept of thalamic modulation of extrathalamic seizure generators. Some or perhaps many of the numerous sites of VNS-induced alterations in synaptic activity may not be relevant to antiseizure mechanisms, but may provide useful actions in conditions that involve these central autonomic, reticular and limbic sites. Depression, anxiety disorders, and chronic pain are among conditions that involve dysfunction at some of these sites, and are being studied in early clinical trials of VNS (32,33).

Thalamocortical relay neurons project to cortical columns over the entire cortex (11). These glutamatergic neurons control cortical neuronal membrane potentials, so as to alter degrees of synchronization among cortical neurons during wake-sleep states and in initiation of experimental seizures (34–36). Thalamocortical relay neurons themselves receive GABAergic synapses of thalamic interneurons, and thalamic afferent activities can indirectly alter membrane potentials of thalamocortical relay neurons (34). Intrathalamic processing controls seizures in idiopathic generalized epilepsies, and may also antagonize seizure generation in cortical regions in localization-related epilepsies. Perhaps VNS does not cause rapid polysynaptic transmission with immediate and direct seizure-antagonizing effects in cortical sites. Instead VNS might rapidly alter intrathalamic processes during VNS, with ongoing effects on intrathalamic activities for short periods between trains of stimulation, so as to both antagonize paroxysmal depolarization shifts (PDSs) in thalamocortical relay neurons and use thalamocortical relay neurons to antagonize PDSs in the cortical columns to which they project. Such phenomena would explain why the absence of cortical CBF changes during trains of VNS is associated with the presence of VNS antiseizure effects in cortical-onset seizures during chronic conditions, and why the presence of thalamic CBF increases during VNS is consistently (acutely and chronically) associated with decreased cortical-onset seizures. Established vagal anatomy and physiology support a diversity of VNS effects on thalamocortical relay neurons (12), such as rapidly transmitting, polysynaptic, ascending vago-solitario-parabrachial projections on thalamic interneurons (which affect activity of thalamocortical relay neurons), and diffusely altered cerebral norepinephrine and serotonin levels (with direct effects on thalamic neurons or effects on neurons that project to thalamic neurons). Functional CBF mapping studies do not provide specific information regarding the neurotransmitters and other modulators of synaptic activity that underlie these alterations. These imaging studies do provide reasons to further investigate intrathalamic processing and thalamocortical interactions during VNS, in experimental epilepsy models.

Multiple mechanisms may underlie antiseizure effects of VNS. Evidence of thalamic activation in association with seizure reduction does not exclude additional mechanisms of VNS that are not mediated through thalamic processing. The degree of partial-onset seizure reduction during chronic VNS was positively associated with the reversal of asymmetry of [^{123}I]iomazenil activity between the ictal onset zones and homologous contralateral sites, in one recent investigation using SPECT and [^{123}I]iomazenil, performed before VNS and again after one year of VNS (37). The authors of this study interpreted their results to indicate that pathological reductions of $GABA_A$ receptor density in the ictal onset zone were reversed in VNS responders, i.e., that VNS can cause increased neuronal membrane expression of $GABA_A$ receptors chronically. Concentrations of ethanolamine, a constituent moiety of excitability-determining neuronal membrane phospholipids (which also control intracellular second messengers), were significantly increased in post-VNS compared with pre-VNS cerebrospinal fluid of VNS responders (38). Vago-solitarial projections to the locus coeruleus and raphe nuclei may permit VNS to increase global cerebral norepinephrine and serotonin concentrations, which likely would antagonize seizures (12); coeruleal ablation prevents VNS antagonism of rodent electroshock-induced seizures (39). These and other hypothetical VNS mechanisms are unlikely to be associated with any acute and chronic VNS effects that are detectable with CBF mapping techniques. Taken with these other studies, the results of CBF mapping studies suggest that rapidly altered intrathalamic synaptic activities are a component of VNS therapeutic mechanisms, and that multiple other mechanisms likely occur independently of thalamic activation.

Acknowledgments: We thank Cyberonics, Inc., for providing financial support for this study.

REFERENCES

1. Handforth A, DeGiorgio CM, Schachter SC, et al. Vagus nerve stimulation therapy for partial-onset seizures: a randomized, active-control trial. *Neurology* 1998;51:48–55.
2. Vagus Nerve Stimulation Study Group. A randomized controlled trial of chronic vagus nerve stimulation for treatment of medically intractable seizures. *Neurology* 1995;45:224–30.
3. Schachter SC, Saper CB. Vagus nerve stimulation. *Epilepsia* 1998;39:677–86.
4. Morris GL 3rd, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy: the Vagus Nerve Stimulation Study Group E01-E05. *Neurology* 1999;53:1731–5.
5. DeGiorgio CM, Schachter SC, Handforth A, et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. *Epilepsia* 2000;41:1195–200.
6. Fernández-Guardiola A, Martínez A, Valdés-Cruz A, et al. Vagus nerve prolonged stimulation in cats: effects on epileptogenesis (amygdala electrical kindling): behavioral and electrographic changes. *Epilepsia* 1999;40:822–9.
7. Takaya M, Terry WJ, Naritoku DK. Vagus nerve stimulation induces a sustained anticonvulsant effect. *Epilepsia* 1996;37:1111–6.
8. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. *Epilepsia* 1990;3(suppl 2):S7–19.
9. Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation. *Epilepsia* 1992;33:1005–12.
10. Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibers does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats. *Epilepsia* 2001;42:586–9.
11. Parent A. *Carpenter's human neuroanatomy*. 9th ed. Baltimore: Williams & Wilkins, 1996:1011.
12. Henry TR. Therapeutic mechanisms of vagus nerve stimulation. *Neurology* 2002;59(suppl 4):S3–14.
13. Jueptner M, Weiller C. Review: does measurement of regional cerebral blood flow reflect synaptic activity? *NeuroImage* 1995;2:148–56.
14. Henry TR, Bakay RAE, Votaw JR, et al. Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy, I: acute effects at high and low levels of stimulation. *Epilepsia* 1998;39:983–90.

15. Ko D, Heck C, Grafton S, et al. Vagus nerve stimulation activates central nervous system structures in epileptic patients during PET $H_2{}^{15}O$ blood flow imaging. *Neurosurgery* 1996;39:426–31.
16. Garnett ES, Nahmias C, Scheffel A, et al. Regional cerebral blood flow in man manipulated by direct vagal stimulation. *Pacing Clin Electrophysiol* 1992;15:1579–80.
17. Woods RP, Mazziotta JC, Cherry SR. MRI-PET registration with automated algorithm. *J Comp Assist Tomogr* 1993;17:536–46.
18. Woods RP, Grafton ST, Holmes CJ, et al. Automated image registration, I: general methods and intrasubject, intramodality validation. *J Comp Assist Tomogr* 1998;22:139–52.
19. Woods RP, Grafton ST, Watson JD, et al. Automated image registration, II: intersubject validation of linear and nonlinear models. *J Comp Assist Tomogr* 1998;22:153–65.
20. Friston KJ, Worsley KJ. Assessing the significance of focal activations using their spatial extent. *Hum Brain Map* 1994;1:210–20.
21. Liu WC, Mosier K, Kalnin AJ, et al. BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. *J Neurol Neurosurg Psychiatry* 2003;74:811–3.
22. Narayanan JT, Watts R, Haddad N, et al. Cerebral activation during vagus nerve stimulation: a functional MR study. *Epilepsia* 2002;43:1509–14.
23. Sucholeiki R, Alsaadi TM, Morris GL 3rd, et al. fMRI in patients implanted with a vagal nerve stimulator. *Seizure* 2002;11:157–62.
24. Barnes A, Duncan R, Chisholm JA, et al. Investigation into the mechanisms of vagus nerve stimulation for the treatment of intractable epilepsy, using 99mTc-HMPAO SPET brain images. *Eur J Nucl Med Mol Image* 2003;30:301–5.
25. Ring HA, White S, Costa DC, et al. A SPECT study of the effect of vagal nerve stimulation on thalamic activity in patients with epilepsy. *Seizure* 2000;9:380–4.
26. Van Laere K, Vonck K, Boon P, et al. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. *J Nucl Med* 2000;41:1145–54.
27. Van Laere K, Vonck K, Boon P, et al. Perfusion SPECT changes after acute and chronic vagus nerve stimulation in relation to prestimulus condition and long-term clinical efficacy. *J Nucl Med* 2002;43:733–44.
28. Vonck K, Boon P, Van Laere K, et al. Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. *Epilepsia* 2000;41:601–9.
29. Lassen NA, Holm S. Single photon emission computerized tomography (SPECT). In: Mazziotta JC, Gilman S, eds. *Clinical brain imaging: principles and applications*. Philadelphia: FA Davis, 1992:108–34.
30. Henry TR, Votaw JR, Pennell PB, et al. Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. *Neurology* 1999;52:1166–73.
31. Fisher RS, Mirski M, Krauss GL. Brain stimulation. In: Engel J Jr, Pedley TA, eds. *Epilepsy: a comprehensive textbook*. Philadelphia: Lippincott-Raven, 1998:1867–75.
32. Chae JH, Nahas Z, Lomarev M, et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). *J Psychiatry Res* 2003;37:443–55.
33. George MS, Nahas Z, Bohning DE, et al. Vagus nerve stimulation therapy: a research update. *Neurology* 2002;59(suppl 4):S56–51.
34. Steriade M, Contreras D. Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neuroscience* 1995;15:623–42.
35. Bertram EH, Mangan PS, Zhang D, et al. The midline thalamus: alterations and a potential role in limbic epilepsy. *Epilepsia* 2001;42:967–78.
36. Chang BS, Lowenstein DH. Epilepsy. *N Engl J Med* 2003;349:1257–66.
37. Marrosu F, Serra A, Maleci A, et al. Correlation between GABA(A) receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. *Epilepsy Res* 2003;55:59–70.
38. Ben-Menachem E, Hamberger A, Hedner T, et al. Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. *Epilepsy Res* 1995;20:221–7.
39. Krahl SE, Clark KB, Smith DC, et al. Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia* 1998;39:709–14.