Effect of Vagus Nerve Stimulation on Cerebrospinal Fluid Monoamine Metabolites, Norepinephrine, and Gamma-Aminobutyric Acid Concentrations in Depressed Patients

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Background: *Vagus nerve stimulation (VNS) has shown promising antidepressant effects in treatment-resistant depression, but the mechanisms of action are not known. Cerebrospinal fluid (CSF) studies in epilepsy patients show that VNS alters concentrations of monamines and γ-aminobutyric acid (GABA), neurotransmitter systems possibly involved in the pathogenesis of depression.*

Methods: *Twenty-one adults with treatment-resistant, recurrent, or chronic major depression underwent standardized lumbar puncture for collection of 12 mL CSF on three separate but identical procedure days during participation in the VNS D-02 clinical trial. All subjects remained on stable regimens of mood medications. Collections were made at baseline (2 weeks after surgical implantation but before device activation), week 12 (end of the acute-phase study), and week 24. Cerebrospinal fluid concentrations of norepinephrine (NE), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) were determined with high-performance liquid chromatography. Concentrations of GABA were assayed with mass spectrometry.*

Results: *Comparison of sham versus active VNS revealed a significant (mean 21%) VNS-associated increase in CSF HVA. Mean CSF concentrations of NE, 5-HIAA, MHPG, and GABA did not change significantly. Higher baseline HVA/5-HIAA ratio predicted worse clinical outcome.*

Conclusions: *Although several of the CSF neurochemical effects we observed in this VNS study were similar to those described in the literature for antidepressants and electroconvulsive therapy, the results do not suggest a putative antidepressant mechanism of action for VNS.*

Key Words: Cerebrospinal fluid, depression, GABA, mechanism, monoamines, neurotransmitters, norepinephrine, vagus nerve stimulation

Therapeutic brain stimulation through delivery of pulsed electrical impulses to the left cervical vagus nerve now has established safety and efficacy as an adjuvant treatment for medication-resistant epilepsy (Ben-Menachem et al 1994; Handforth et al 1998; Morris and Mueller 1999; Salinsky et al 1996; Schachter 2002). In the past 5 years, vagus nerve stimulation (VNS) has been investigated as an adjuvant therapy for treatment-resistant major depression. Open-label studies have produced promising results (Rush et al 2000; Sackeim et al 2001a, 2001b), especially when response and remission rates at longer-term (1- and 2-year) follow-up time points are considered (Marangell et al 2002; Rush et al 2002). Although primary outcomes from short-term (10 weeks) treatment with VNS did not differ statistically from those after sham treatment in a double-blind study (Marangell, unpublished data; George et al 2003), a comparison of longer-term outcomes has revealed statistical superiority of adjuvant VNS over naturalistic treatment (Cyberonics [Houston, Texas], unpublished data).

Several lines of research, including studies of sleep architecture, cerebrospinal fluid (CSF) components, and brain imaging, suggest that VNS has acute and chronic effects on various indices of key brain activity and neurotransmitter regulation that are implicated in seizure suppression and mood regulation (Vonck et al 2001). Ascending projections from the nucleus tractus solitarius (NTS) to the midline raphe and locus coeruleus (LC) are hypothesized to be among the pathways through which VNS exerts antiseizure and neuropsychiatric effects. Studies in rats during VNS reveal increased cellular activity in amygdala, cingulate, LC, and hypothalamus (Naritoku et al 1995). Further support for a role of noradrenergic neurotransmission comes from a report of suppression of VNS' antiseizure effects in animals after lesion of the LC (Krahl et al 1998). Although the basic mechanisms of action are unknown, a VNS-induced increase in NTS concentration of γ-aminobutyric acid (GABA) and/or decrease in NTS glutamate level could theoretically explain the antiseizure activity of VNS (Walker et al 1999). Consistent with this hypothesis, epilepsy patients sampled before and after 3 months of VNS showed significant increases in lumbar CSF concentrations of GABA and trend-level decreases in CSF glutamate (Ben-Menachem et al 1995). Other findings from the CSF study were trends toward VNS-induced increases in levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolites of dopamine and serotonin, respectively. A positron emission tomography study in epilepsy patients demonstrated significant VNS-induced modulation of blood flow in key brain structures thought to be involved in both seizures and emotion regulation (Henry et al 1998). Blood flow increases were seen in

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the rostral medulla, thalamus, hypothalamus, insula, and postcentral gyrus, all with greater activation on the right side. Bilateral decreases were seen in the hippocampus, amygdala, and cingulate gyrus.

The results of neurobiological studies conducted thus far with depressed patients receiving VNS have paralleled and extended those generated from the epilepsy population. A single photon emission computerized tomography imaging study conducted in six depressed patients found that, compared with normal control subjects, patients had reduced regional cerebral blood flow (rCBF) to left dorsolateral prefrontal, anterolateral temporal, and perisylvian temporal structures, including posterior insula, at baseline (Devous et al, unpublished data). After 10 weeks of open-label VNS, apparent resolution of the classic rCBF abnormalities in depressed patients was especially pronounced among those showing favorable clinical response (Devous et al, unpublished data). In depressed patients, synchronized blood oxygenation level–dependent functional magnetic resonance imaging (fMRI) (Bohning et al 2001) response to VNS was shown in areas regulated by the vagus nerve: orbitofrontal and parieto-occipital cortex bilaterally, left temporal cortex, hypothalamus, and left amygdala (Lomarev et al 2002). This fMRI technique was also used to show that acute immediate regional brain activity changes vary with the frequency or total dose of stimulation, and that VNS exerts a dose-dependent modulatory effect on other brain activities, such as hearing a tone (Lomarev et al 2002). Sleep electroencephalogram (EEG) studies in patients with treatment-resistant depression (Armitage et al 2003) have demonstrated that after 10 weeks of VNS, amplitude of sleep EEG rhythms was restored to near-normal levels, and patients manifested significantly less awake time and "light sleep" and significantly more stage 2 (deep) sleep. Results from both the epilepsy and depression samples thus far provide converging lines of evidence that VNS exerts measurable effects in brain regions and neurotransmitter systems implicated in mood disorders.

The present investigation was undertaken to further explore possible neurotransmitter mechanisms of VNS antidepressant activity through an evaluation of selected neurochemical concentrations in human lumbar CSF. Incorporating a CSF sampling component into the multi-center, VNS clinical trial ("D-02") for depression provided an opportunity for measuring changes in the neurochemicals of interest in a double-blind, randomized, sham-controlled fashion. On the basis of the findings in epilepsy patients reported by Ben-Menachem et al (1995), we hypothesized that 10–12 weeks of active VNS would be associated with increased CSF concentrations of GABA and 5-HIAA in patients with depression.

Methods and Materials

VNS Clinical Trial Design and Patient Characteristics

The present study was conducted in a subgroup of depressed patients who were enrolled in the "Multicenter, Pivotal, Safety and Efficacy Study of the NeuroCybernetic Prosthesis (NCP) System in Patients with Depression (D-02)." The larger VNS trial was conducted at 21 academic sites in North America and had a double-blind, randomized, parallel-groups, sham-controlled design. Preliminary results from the D-02 study have been presented (Marangell, unpublished data), and a detailed report is in preparation for publication (J. Rush, personal communication, July 2004). Relevant study details will be briefly summarized here.

Inclusion criteria required that adult patients present with a recurrent or chronic major depressive episode (bipolar or unipolar) that had failed to respond to at least two but not more than six adequate trials of standard antidepressant treatment. Eligible patients were taking stable doses of psychotropic medications for at least 4 weeks before the first of two baseline visits, which were performed before surgical implantation of the VNS device. An average baseline total score of 20 or higher on the 24-item version of the Hamilton Rating Scale for Depression (HAM-D) (Hamilton 1960) was also required for implantation and randomization. After a 2-week surgical recovery period, patients in the active treatment group had the neurocybernetic prosthesis system turned on by an unblinded programmer at their study site. Patients assigned to the control group underwent identical "sham" programming procedures. All clinical assessments were conducted by blinded raters, and extensive efforts were made at each participating clinic to protect the treatment blind. Initial stimulation parameters reflected the range of settings used in an open, pilot study of VNS for depression (Rush et al 2000): output current adjusted to comfortable tolerance (typically less than 1.5 mA), frequency 20 Hz, pulse width 500 μsec, cycling continuously with 30 sec on and 5 min off. The "acute" treatment phase of the study was 12 weeks in duration, including 2 weeks postimplantation recovery, 2 weeks of stimulation titration, and 8 weeks of fixed-dose VNS. Every effort was made to maintain patients on stable doses of their psychotropic medications during the acute phase.

At week 12, all subjects entered the "long-term" phase of the trial. For those who had been in the active VNS group, stimulation continued with the option for change in stimulation parameters if response criteria were not met. Those in the sham control group who continued to be depressed were crossed over to active stimulation in a blinded fashion. Patients in the acute-phase sham group therefore established a new "baseline" (i.e., prestimulation) set of symptom ratings at week 12 and in essence began a 12-week course of VNS adjuvant treatment and schedule of assessments, which mirrored that completed by the active VNS group during the first 12 weeks. Efforts to avoid unnecessary changes in psychotropic medications or their dosages continued during the long-term phase of the study. Long-term study visits were conducted at monthly intervals after 4 months of active stimulation and were then extended to quarterly intervals after a full year of stimulation.

CSF Protocol Design

Participation in the study of CSF correlates of VNS was offered to subjects enrolled in the D-02 clinical trial at three sites (Butler Hospital Mood Disorders Research Program, Brown University, Providence, Rhode Island; Psychopharmacology Research Program, University of Arizona Health Sciences Center, Tucson; and Baltimore Veterans Affairs Medical Center, University of Maryland, Baltimore). Because full approval for this "add-on" study was not obtained from the three participating institutional review boards until after enrollment had already commenced for the primary VNS clinical trial, only 32 consecutively enrolled subjects (out of the total 45 enrolled in D-02 from these three sites) were considered for participation in the CSF study. Morbid obesity, prior lumbar spine or disk surgery, and chronic lower back pain ($n = 4$) were conditions leading to exclusion from the CSF study. Seven subjects declined participation in the CSF study, indicating either a preference to avoid risks related to lumbar puncture and/or an already substantial time commitment to the primary VNS treatment trial visits and time devoted to participation in other "add-on" studies that were offered. A total of 21 subjects

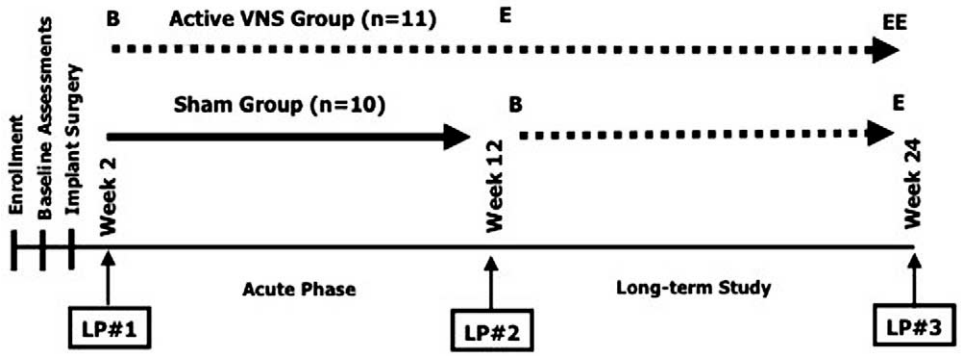

Figure 1. Lumbar punctures (LP) for cerebrospinal fluid collection in relation to vagus nerve stimulation (VNS) D-02 study timeline. B, prestimulation baseline; E, poststimulation end point (after 10 weeks active VNS); EE, extended end point (after 22 weeks active stimulation).

provided written informed consent for participation in the CSF study and had at least two lumbar punctures. Subjects in the CSF study were modestly remunerated for time and effort devoted to the additional procedures.

Subjects volunteering for the CSF study underwent three standard lumbar punctures during the D-02 protocol (see Figure 1). The first CSF collection took place after surgical implantation and recovery but before the device was turned on (week 2 visit). The second CSF sample was obtained at the week 12 visit (i.e., end of the acute phase of the D-02 study) before those in the sham treatment group were crossed over to active VNS. The third sample was obtained at the week 24 visit in the long-term phase of the D-02 study, representing the time point when the crossed-over group (hereafter referred to as SHAM-VNS group) had received a 12-week trial of active stimulation, and the group that originally received active treatment (hereafter referred to as VNS-VNS group) had received 22 weeks of continuous stimulation.

Lumbar puncture procedures were standardized across the three participating sites and were performed for collection of CSF at approximately 12 noon. Patients were instructed to follow a standard carbohydrate breakfast, adhere to their usual morning regimen of caffeine intake, and to avoid strenuous activity or exercise in the 24-hour period before each lumbar puncture. After a brief period of bedrest (minimum 30 min), patients were placed in a sitting/leaning-forward position on the edge of the bed. The sitting position was adopted as the standard for this and other CSF studies in our laboratory (Carpenter et al 2002, 2003, 2004a, 2004b) as a result of subjects' feedback that it is perceived as subjectively less stressful than the left lateral decubitus position. After intradermal injection of 1% lidocaine, a 20-gauge introducer needle was used to penetrate the skin and superficial tissue. The introducer is necessary because the Sprotte 24-gauge pencil-point spinal needle is very thin and has a relatively dull tip. The spinal needle was then inserted through the introducer to the L4-L5 interspace. A total of 12 mL of CSF was collected in a single tube, inverted, and immediately centrifuged (15 min), divided into .5-mL aliquots, and frozen at −80°C until assayed. Mixture and centrifuge of the entire 12-mL CSF specimen was performed to separate any red blood cells from CSF (aliquot from tube bottom discarded) and to eliminate possible flow gradient in CSF analytes.

Clinical Outcome Measures

Clinical assessments conducted at the week 2, week 12, and week 24 visits included the 24-item HAM-D and the Clinical Global Impressions (CGI) ratings for Severity of Illness (Likert scale of 1–7, with 1 = "normal, not at all ill" and 7 = "among the most extremely ill patients") and Improvement (also a Likert scale of 1–7, with 1 = "very much improved" and 7 = "very much worse") (Guy 1976). Baseline-to-end-point percent change scores were calculated for HAM-D total scores and CGI Severity ratings. End point CGI Improvement ratings were dichotomized as follows: "very much improved/much improved" (score 1 or 2) or "minimal improvement or worse" (score 3–7).

Biochemical Assays

Concentrations of CSF 5-HIAA, 3-methoxy-4-hydroxyphenylglycol (MHPG), and HVA were determined with modified versions of previously described high-performance liquid chromatographic (HPLC) methods (Leckman et al 1995). Intra- and interassay coefficients of variation were between 5% and 11%. The concentration of CSF norepinephrine (NE) was determined by HPLC with amperometric detection after alumina extraction of .5 mL of CSF; the interassay coefficient of variation of this method is 7.5% (Leckman et al 1995). Concentrations of CSF GABA were determined by stable isotope dilution mass spectrometry, as described elsewhere (Struys et al 1999).

Statistical Analyses

Descriptive statistics, t tests, and χ^2 tests were used to characterize the demographic and clinical features of the entire study sample (n = 21) and the two subgroups. The first set of CSF analyses examined the two parallel treatment groups (VNS or SHAM) during the acute phase. Seventeen of the 21 subjects (n = 8 in the SHAM group, n = 9 in the VNS group) had CSF samples collected at both of the first two lumbar punctures in the protocol, so they were included in these analyses. For each treatment group, a series of paired t tests was used to compare mean baseline (week 2) and end point (week 12) concentrations for each of the five CSF analytes and the ratio of HVA to 5-HIAA. T tests were used to compare the baseline mean analyte concentrations across treatment groups. Analysis of covariance (ANCOVA) was subsequently used to determine the effect of treatment condition on week 12 concentration of each analyte while covarying for baseline (week 2) concentrations. Analyses of covariance, again controlling for baseline values, were also used to examine the effect of treatment condition on symptom ratings during the acute phase.

The next series of analyses included data from both the acute and the long-term phases of the D-02 protocol. Data from the first 10 weeks of VNS treatment (week 2 to week 12) in the active group were pooled with data from the first 12 weeks of active stimulation (weeks 12–24) in the group that crossed from SHAM

Table 1. VNS CSF Study Patient Characteristics

	Total (n = 21)	SHAM-VNS (n = 10)	VNS-VNS (n = 11)
Gender			
Male	10 (47.6)	3 (30.0)	7 (63.6)
Female	11 (52.4)	7 (70.0)	4 (36.4)
Age (y)	48.1 ± 8.5	48.7 ± 8.3	47.6 ± 9.1
Unipolar	18 (85.7)	9 (90.0)	9 (81.8)
Bipolar	3 (14.3)	1 (10.0)	2 (18.2)
CGI Severity (1–7) Baseline Rating	4.9 ± 1.0	4.9 ± 1.0	4.9 ± 1.0

Data are presented as n (%) or mean ± SD.

VNS, vagus nerve stimulation; CSF, cerebrospinal fluid; SHAM-VNS, group crossed over at week 12 from sham to active VNS; VNS-VNS, group that received 22 weeks of continuous VNS; CGI, Clinical Global Impression Scale.

to VNS. For the SHAM-VNS group, week 12 clinical assessments and CSF analyte concentrations were used as the new prestimulation baseline values, and week 24 results were used for end point values. Eighteen of the 21 subjects (n = 9 from the VNS-VNS group and n = 9 from the SHAM-VNS group) had CSF collections at both a prestimulation baseline and a poststimulation end point. Paired t tests and ANCOVA were again used to examine change in mean values of CSF analytes during treatment in the pooled subject sample, as well as for comparison of the mean clinical ratings (HAM-D and CGI) from pre-VNS baseline to post-VNS end point.

Post hoc analyses were conducted with χ^2 tests to evaluate whether membership in either the acute-study VNS group (10 weeks active stimulation) or the group crossed over from SHAM to active VNS during the long-term phase (12 weeks active stimulation) was associated with positive changes in CSF HVA. Similarly, χ^2 tests were used to examine whether concurrent treatment with a dopaminergic medication (bupropion or stimulants) during active VNS was statistically associated with increases in CSF HVA.

Pearson correlation coefficients were generated to examine the relationships between change in depressive symptomatology during the 3-month VNS trial (absolute and percent change from baseline to end point in CGI severity and HAM-D total scores) and concurrent change in CSF concentrations of the five neurochemicals (calculated for each analyte as absolute change and as percent change from baseline to end point). Correlational analyses were also conducted on baseline and end point values of HVA, 5-HIAA, HVA/5-HIAA ratio, and the clinical outcomes variables.

Three principle hypotheses were tested regarding VNS-induced changes in CSF 5-HIAA, HVA, and GABA. All tests were two-tailed with the p value set at .05 for significance. Bonferroni correction factors were applied to control for multiple comparisons.

Results

Sample Characteristics and Clinical Outcomes

Clinical and demographic characteristics for the 21 subjects who participated in the CSF study are summarized in Table 1. The VNS and SHAM groups were comparable in size, mean age, affective polarity, and baseline severity of illness. Although the gender composition of the groups differed numerically, this was not statistically significant (χ^2 = 2.8, p = .12). As a group, the clinical and demographic characteristics of subjects participating in this CSF study were similar to those reported for the larger population of D-02 study subjects (n = 205), for whom preliminary efficacy data have been described (Cyberonics, data on file).

Concomitant psychotropic medications and the durations of each medication are listed in Table 2. One subject was taking no medications. The mean ± SD duration of medication was 652 ± 1009 days for the SHAM group (representing 37 medications) and 548 ± 780 days for the VNS group (representing 34 medications) in the acute phase. For pooled data (n = 18), the mean ± SD duration of psychotropic medication until the start of active VNS was 637 ± 938 days (representing 62 medications).

Clinical outcomes are presented in Table 3. Comparison of week 12 HAM-D total and CGI Severity scores (covarying for baseline values) revealed no significant treatment group effect during the acute phase. Paired t test analysis of the pooled groups data (n = 21; baseline and end point assessments related to the first 10 weeks of VNS in the VSN-VNS group and to the first 12 weeks of VNS crossover in the SHAM-VNS group) also showed no significant pre- to post-VNS change in HAM-D (t = 1.7, p = .10) or CGI scores (t = .9, p = .38) associated with the course of VNS. Separation of the two groups for subsequent paired t tests revealed that there was no change from baseline to end point in HAM-D or CGI scores for the SHAM-VNS group, but a nonsignificant trend toward lower poststimulation HAM-D scores was noted for the VNS-VNS group (t = 1.8, p = .09).

Acute Phase Results: Active VNS Versus Sham Treatment Conditions

Mean CSF analyte concentrations are presented in Table 4. Baseline (week 2) means were statistically similar across groups for HVA, MHPG, and NE, but there was a trend toward a higher 5-HIAA mean for the VNS group (t = 1.9, p = .07), and a significantly higher mean value was found for GABA in the VNS group (t = 2.3, p = .04). Examination of the CSF data from the acute phase demonstrated a significant treatment effect for HVA (F = 5.7, p = .03) but not for NE, MHPG, 5-HIAA, HVA/5-HIAA ratio, or GABA. When ANCOVA was repeated with presence/absence of concurrent bupropion or stimulants included in the model, no significant effect was attributable to the medications (F = .45, p = .51). Results of individual paired t tests indicated a nonsignificant trend-level increase in mean CSF HVA (from 34.6 ± 4.7 to 40.5 ± 5.3 pg/mL, t = 2.2, p = .06) in the active VNS group and no change in the SHAM group (Figure 2). The mean ± SD percent changes in CSF HVA concentrations were −9.4% ± 18.8% for SHAM and +20.8% ± 30.4% for VNS (t = 2.4, p = .03).

Long-Term Phase Results

For the VNS-VNS group, continuation of VNS for an additional 3 months did not result in significant changes (either from the

Table 2. Concomitant Psychotropic Medications for the 21 Study Subjects

Subject No.	Acute-Phase Treatment Group	Psychotropic Medications (Days[a])
1	SHAM	Mirtazapine (1391), gabapentin (237), clonazepam (175), olanzapine (114), zolpidem (83), thyroid (189)
2	SHAM	None
3	SHAM	Venlafaxine (35), carbamazepine (40), clonazepam (1913), quetiapine (218)
4	SHAM	Venlafaxine (260), gabapentin (452), dextroamphetamine/racemic amphetamine (270), thyroid (862)
5	SHAM	Mirtazapine (55), fluoxetine (391), trazodone (126)
6	SHAM	Bupropion (236), topiramate (764), citalopram (276), clomipramine (215)
7	SHAM	Fluoxetine (858), topiramate (493), olanzapine (858), alprazolam (4145)
8	SHAM	Venlafaxine (96), mirtazapine (369), nefazodone (186), buspirone (735)
9	SHAM	Venlafaxine (335), topiramate (463), clonazepam (1587)
10	SHAM	Sertaline (156), methylphenidate (66), naltrexone (66)
11	VNS	Mirtazapine (202), paroxetine (118), bupropion (202), methylphenidate (146)
12	VNS	Lamotrigine (55), olanzapine (272), hydroxyzine (42), clonazepam (273)
13	VNS	Citalopram (1161), trazodone (5668)
14	VNS	Lamotrigine (536), fluoxetine (64), buspirone (635), methylphenidate (978)
15	VNS	Clomipramine (52), topiramate (49), lorazepam (1263), methylphenidate (52)
16	VNS	Fluoxetine (706), lithium (1436), buspirone (492)
17	VNS	Sertraline (794), bupropion (805), lithium (105), trazodone (2836)
18	VNS	Mirtazapine (756), bupropion (634)
19	VNS	Mirtazapine (447), fluoxetine (356), dextroamphetamine (294), zolpidem (82), alprazolam (294)
20	VNS	Bupropion (1174), nefazodone (413), lithium (188)
21	VNS	Citalopram (140), quetiapine (416)

[a]Calculated as number of days from start date of first dose of the drug listed (regardless of starting dosage) to the date of lumbar puncture #1. For those in the SHAM group, an additional 70 days (approximately) would be added to each entry in the table above to represent the total duration of days subjects received each drug before initiation of active VNS therapy.

week 2 baseline or from the week 12 midpoint) in CSF concentrations of NE, MHPG, 5-HIAA, HVA/5-HIAA ratio, or GABA. Concentrations of CSF HVA decreased significantly during the extended VNS trial (from 36.5 ± 5.2 pg/mL at week 12 to 31.0 ± 3.9 pg/mL at week 24; $t = 2.7$, $p = .02$), essentially signaling a return to pre-VNS baseline CSF HVA concentrations.

Paired t test analyses showed no baseline-to-end-point changes in CSF neurochemicals or HVA/5-HIAA ratio for the SHAM-VNS group after the crossover phase. When data from the small group of seven subjects from the SHAM-VNS group who had completed all three lumbar punctures was examined with ANCOVA, a trend-level finding suggested that CSF HVA changes during the active stimulation were greater than those measured during the SHAM phase ($F = 3.3$, $p = .09$).

Pooled Data Results

When the data were pooled to yield the largest possible number of subjects for whom comparison of pre-VNS to post-VNS values was possible ($n = 18$), the finding of VNS-associated increase in CSF HVA remained significant ($t = 2.2$, $p = .04$). Chi-square tests with the pooled data revealed no difference in direction of CSF HVA change attributable to membership in either the acute-study VNS group (10 weeks active stimulation, $n = 9$) or the group crossed over from SHAM to active VNS during the long-term phase (12 weeks active stimulation, $n = 9$) ($\chi^2 = 1.0$, $p = .32$). Furthermore, direction of CSF HVA change during active VNS was not significantly associated with concurrent treatment with bupropion or stimulants ($\chi^2 = .1$, $p = .74$).

CSF Analyte Correlations with Clinical Outcomes

Vagus nerve stimulation–associated changes (absolute values) in the CSF analytes did not significantly correlate with clinical outcomes. After controlling for baseline values, baseline-to-end-point changes in CSF concentrations of NE were negatively correlated with corresponding changes in CGI severity scores ($r = -.56$, $p = .02$), but statistical significance did not persist after controlling for multiple comparisons. Similarly, a trend-level negative correlation noted between CSF NE change and change in HAM-D totals ($r = -.43$, $p = .08$) was lost after Bonferroni correction was applied. Baseline ratio of HVA/5-HIAA was significantly positively correlated with percent change in CGI severity ($r = .70$, $p = .0009$) but not with HAM-D outcome measures.

Table 3. Clinical Outcomes

	Total ($n = 21$)	SHAM-VNS ($n = 10$)	VNS-VNS ($n = 11$)
HAM-D 24 Total Score			
Week 2	28.2 ± 6.0	29.8 ± 6.1	26.8 ± 5.9
Week 12	23.5 ± 10.5	26.3 ± 8.2	21.0 ± 12.0
Week 24	20.6 ± 11.1	24.0 ± 11.8	17.5 ± 9.8
CGI Severity			
Week 2	4.9 ± 1.0	4.9 ± 1.0	4.9 ± 1.0
Week 12	4.7 ± 1.5	4.9 ± 0.9	4.5 ± 2.0
Week 24	4.2 ± 1.3	4.8 ± 1.1	3.7 ± 1.2
CGI Improvement			
Week 12			
Much/very much	2 (9.5)	0	2 (18.1)
Minimal to worse	19 (90.5)	10 (100)	9 (81.2)
Week 24			
Much/very much	4 (19.0)	1 (10.0)	3 (27.3)
Minimal to worse	17 (81.0)	9 (90.0)	8 (72.7)

Data are presented as mean ± SD or *n* (%).

SHAM-VNS, group crossed over at week 12 from sham to active VNS; VNS-VNS, group that received 22 weeks of continuous VNS; HAM-D 24, 24-item Hamilton Ratings Scale for Depression; CGI, Clinical Global Impression Scale.

Discussion

The main finding of this study was a significant increase in CSF HVA concentrations associated with active but not sham VNS during a 10-week treatment period. The finding was replicated at a trend level in a small group of subjects crossed over from sham to active VNS for 12 weeks and remained significant in the pooled sample of subjects when all pre-VNS CSF HVA concentrations were compared with post-VNS levels.

Several design limitations merit consideration in the interpretation of these results. Most notably, the patients we studied were typically taking multiple psychotropic medications, each known to be capable of altering animal extracellular fluid and/or human CSF concentrations of monoamines, their metabolites, and GABA. The design we used did not permit analysis of whether or how pharmacotherapy plus adjunct VNS might alter CSF concentrations of monoamines or GABA compared with VNS alone or with pharmacotherapy alone. The relatively small number of subjects, combined with the relatively large average number of concomitant psychotropic agents used by the subjects, also precluded meaningful comparisons between classes of drugs or between diagnostic subtypes (e.g., unipolar vs. bipolar depression). Several analyses examining the presence or absence of medications thought to primarily enhance dopaminergic neurotransmission (i.e., bupropion and stimulants) did not support the notion that these medications were responsible for the observed CSF HVA increases, but in all cases these drugs were used in combination with others, and the effects of all medications could not be systematically assessed. Maintaining stable doses of all psychotropic medications for at least 4 weeks before baseline assessment and throughout the subsequent VNS trial and CSF collection should have minimized medication effects on change measures; however, we cannot rule out the possibility that the

Table 4. CSF Analyte Concentrations

	Acute-Phase Group	Acute Phase (wk 2–12)[a]				Long-Term/Crossover (wk 12–24)[b]		
		LP #1	LP #2	p^c	p^d	LP #2	LP #3	p^e
HVA (ng/mL)	SHAM	26.9 (13.0)	24.6 (13.8)	.20	.03[f]	31.5 (18.3)	33.0 (21.2)	.46
	VNS	34.6 (14.2)	40.5 (15.8)	.06		36.5 (16.5)	31.0 (12.5)	.02[f]
5-HIAA (ng/mL)	SHAM	9.6 (3.0)	9.1 (4.2)	.38	.13	11.8 (6.8)	11.7 (6.9)	.88
	VNS	14.0 (5.7)	14.1 (4.5)	.97		14.1 (4.5)	14.0 (4.5)	.93
MHPG (ng/mL)	SHAM	13.6 (3.1)	13.7 (3.1)	.89	.67	13.3 (2.5)	12.3 (3.5)	.33
	VNS	13.2 (2.0)	13.0 (2.0)	.78		13.8 (2.4)	13.8 (3.3)	.96
NE (pg/mL)	SHAM	165.8 (77.4)	181.8 (118.4)	.40	.52	168.6 (109.9)	152.8 (80.8)	.50
	VNS	171.5 (107.2)	165.3 (77.5)	.82		156.1 (80.0)	158.5 (98.8)	.89
GABA (pmol/mL)	SHAM	89.8 (39.6)	120.6 (74.5)	.18	.68	120.1 (70.7)	105.4 (66.3)	.53
	VNS	150.8 (69.0)	158.8 (55.2)	.77		148.4 (49.9)	133.2 (39.6)	.28

Data are presented as mean (SD). CSF, cerebrospinal fluid; LP, lumbar puncture; HVA, homovanillic acid; VNS; vagus nerve stimulation; 5-HIAA, 5-hydroindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine; GABA, γ-aminobutyric acid.

[a]Number of subjects with data from both time points: $n = 8$ SHAM, n = 9 VNS.

[b]Number of subjects with data from both time points: $n = 9$ SHAM (crossed over to active VNS during this phase), $n = 10$ VNS (continued active VNS for another 10 weeks).

[c]Paired *t* tests comparing values from LP #1 and LP #2.

[d]Analysis of covariance for effect of treatment group, controlling for baseline value.

[e]Paired *t* tests comparing values from LP #2 and LP #3.

[f]$p < .05$.

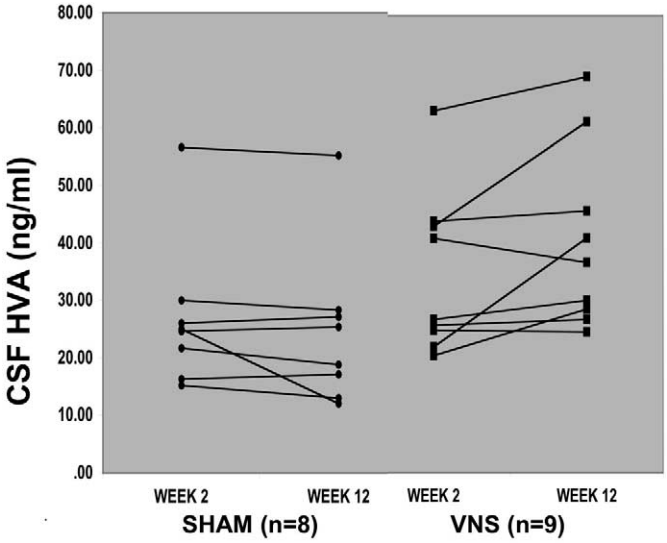

Figure 2. Acute phase data. Cerebrospinal fluid homovanillic acid (CSF HVA) levels (mean ± SD) for weeks 2 and 12, respectively, were 26.9 ± 13.0 ng/mL and 24.6 ± 13.8 ng/mL for SHAM subjects and 34.6 ± 14.2 ng/mL and 40.5 ± 15.8 ng/mL for vagus nerve stimulation (VNS) subjects. Analysis of covariance controlling for baseline values indicates a significant treatment effect ($F = 5.7$, $p = .03$). The mean ± SD percent changes in CSF HVA concentrations were −9.4% ± 18.8% for SHAM and +20.8% ± 30.4% for VNS. Week 2 CSF HVA group means were not significantly different from one another ($t = 1.2$, $p = .27$).

acute mean increase we measured in HVA after the VNS trial is attributable to the *combination* of VNS plus pharmacotherapy, rather than to VNS alone.

These limitations notwithstanding, an increase in CSF HVA after acute VNS is not unique to this investigation. A similar magnitude of VNS-associated increase in CSF concentrations of HVA was reported by Ben-Menachem and colleagues in their study of 16 epilepsy patients (Ben-Menachem et al 1995). Although CSF HVA was not found to be a significant predictor of change in seizure frequency, a 30.0% increase in mean CSF HVA concentration was reported for the group ($n = 16$) they studied (10 of whom received VNS with stimulation parameters similar to those applied for our patients in D-02), representing a trend-level increase from baseline ($p = .078$).

Because we were not able to assay CSF dopamine concentrations directly, we cannot interpret the observed changes in CSF HVA as reflective of changes in the synaptic concentrations of dopamine. Whereas 5-HIAA and MHPG concentrations in lumbar CSF are thought to include some contributions from spinal cord and peripheral nervous system sources, lumbar CSF HVA exclusively reflects brain metabolism of dopamine (Post and Goodwin 1978). Therefore, it is reasonable to conclude that central dopamine turnover, frequently interpreted as an index of dopaminergic neurotransmission, was somehow augmented during acute (10–12 weeks) VNS treatment. The return of CSF HVA values back to baseline levels in the group that continued with active VNS into the extended phase (24 weeks of stimulation) is consistent with a previous report describing acute changes in CSF 5-HIAA after antidepressant treatment that return to pretreatment baseline level after a mean of 30 weeks (Backman et al 2000). A time lag of several weeks between clinically appreciable change in symptomatology and measurable physiologic changes at the level of synaptic neurotransmission or receptor modulation is a well-documented phenomenon with antidepressant medications. It is possible that a similar process of acute biological perturbation, followed by induction of compensatory mechanisms that drive gradual return to a baseline homeostatic state, occurs in dopamine turnover after exposure to VNS. This dynamic might be one reason why the group that had CSF collections after a 10-week VNS trial showed more robust increases in CSF HVA than the group that had samples taken after 12 weeks of VNS therapy.

The relevance of the increases in CSF HVA concentrations we observed, particularly in light of the lack of robust clinical response to VNS, is not clear. The majority of studies in which basal CSF HVA levels in depressed patients and healthy control subjects are compared indicate trend-level or statistically lower levels among depressives (Goodwin and Jamison 1990), and some have shown lower levels to be associated with impulsive suicidal behavior (Cremniter et al 1999; Engstrom et al 1999), alcoholism (Sher et al 2003), and cocaine dependence (Roy et al 2002). Recently, researchers have reported relationships between CSF HVA concentrations, temperament (Chotai and Adolfsson 2002), and social dominance (Kaplan et al 2002).

Treatment with antidepressant medications has shown variable effects on CSF HVA (Altemus et al 1994; Bowden et al 1985; Little et al 1999; Martensson et al 1989; Potter et al 1985; Scheinin 1985; Sheline et al 1997), whereas treatment with electroconvulsive therapy (ECT) has consistently and robustly shown elevations (nearly 30% on average) of CSF HVA (Rudorfer et al 1992, 1988a, 1988b). Treatment with repetitive transcranial magnetic stimulation has been shown to decrease CSF HVA concentrations (Shimamoto et al 2001). Given the lack of convergence in findings from published studies of CSF HVA concentrations, it is premature to suggest that our observations reflect any putative antidepressant action of VNS. Indeed, a mechanism by which VNS influences dopamine functioning has not been established. Perhaps extrapyramidal motor systems, and thus dopamine, are activated through the NTS projections to the hypothalamus, thalamus, amygdala (central nucleus), bed nucleus of the stria terminalis, and the accumbens (Ter Horst and Streetland 1994). In light of the qualitative (electrical stimulation) and therapeutic (anticonvulsant and antidepressant) similarities between ECT and VNS, it is notable that the direction of change in CSF HVA after ECT is similar to that we observed with acute-phase VNS.

The clinical correlates of CSF HVA concentrations have not been consistently established, but a sizable body of literature describes findings related to the ratio of CSF HVA to CSF 5-HIAA concentrations (De Bellis et al 1993a; Engstrom et al 1999; Hsiao et al 1987; Martensson et al 1989; Risby et al 1987; Soderstrom et al 2003). On the basis of the functional and anatomic relationships of the serotonin and dopamine neurotransmitter systems, it has been suggested that a relative lack of correlation between the two monoamine metabolites indicates disrupted interactions between the two systems and often characterizes nonresponse to antidepressant treatment (Hsiao et al 1987; Risby et al 1987). We did not find that baseline HVA concentrations were significantly related to any of our outcome measures, but we did observe that baseline ratios of HVA to 5-HIAA were highly significantly correlated with changes in global ratings of depression severity during VNS. The correlation can be interpreted as indicating that a higher baseline HVA/5-HIAA ratio (i.e., a ratio of metabolites favoring dopamine turnover) predicts worse clinical outcome after a 3-month trial of adjuvant VNS. In this regard, we can conclude that a CSF monoamine metabolite profile reported to characterize poor response to antidepressant pharmacotherapy is also associated with poor response to VNS.

Contrary to our hypothesis, CSF GABA levels did not change with VNS. One might speculate that VNS triggers differential biological responses and activates differential neurotransmitter systems, depending on the unique modulatory deficits of the population receiving the treatment. Perhaps the finding of increased CSF GABA among epilepsy patients (Ben-Menachem et al 1995) reflects in part some enhanced GABAergic activity from concurrent use of anticonvulsant medications. Alternatively, our modest sample size and the concurrent use of benzodiazepines in some depressed patients might have reduced our ability to detect a significant VNS-related change in CSF GABA. Baseline GABA mean concentrations were significantly different between active VNS and sham groups, but the statistical tests used to detect treatment effects used within-subjects comparisons and other controls for baseline values, which should have adequately compensated for this issue.

The use of a sham control and a within-subjects crossover phase provides a higher level of confidence that the results we observed were not attributable to pharmacotherapy, and the use of each patient's pretreatment baseline concentration as a control value obviates concern regarding possible confounding effects, such as age, gender, height, weight, or tobacco use. At $\alpha = .05$, this study had adequate power (.8) to detect a large effect size ($d = 1.05$) in the sham-controlled acute phase and a medium-large ($d = .7$) effect size in the pooled-subjects ($n = 18$) analyses (Cohen 1988). Although the sample sizes in this study were comparable to or larger than those of the previous studies examining the effects of antidepressant treatment on CSF neurochemicals (Altemus et al 1994; Backman et al 2000; De Bellis et al 1993a, 1993b), we cannot exclude the possibility that we might have failed to detect less robust changes in CSF analyte concentrations. One could speculate that if VNS acts similarly to some psychotropic drugs, a ceiling effect in CSF analyte concentrations might have already been reached during pharmacotherapy that could not be further enhanced by the subsequent addition of VNS.

Superimposing our CSF investigation on the larger, multisite D-02 clinical trial of VNS for severe and chronic treatment-resistant depression precluded the study of a sizable cohort of medication-free subjects and did not allow flexibility in the duration of the sham-controlled treatment trial. Perhaps future CSF and other mechanism-of-action studies in less severely depressed patients who are able to undertake VNS treatment as monotherapy will provide better opportunities for understanding the relevant biological correlates of VNS. Although not within the scope of this investigation, future studies might be designed and powered to explore possible effects of depressive subtypes, short- versus extended-term VNS therapy, various stimulation parameters, and various classes of adjunct therapies delivered with VNS.

In summary, this study found a significant VNS-induced increase in CSF HVA, similar in direction and magnitude to that reported after ECT. Additionally, we observed a correlation between baseline CSF HVA/5-HIAA ratio and response to VNS treatment, a biological prognosticator that has been described in the literature for antidepressant medications. Although understanding the exact mechanism of action of a therapeutic modality has not historically been paramount to its successful use in treating major depression, such understanding has provided critical insights into the pathogenesis of the disorder and the further refinements of effective treatments. More clinical and neurobiological data regarding the antidepressant effects of VNS will be of critical value in helping to clarify when, for whom, and how this innovative treatment should be delivered.

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Altemus M, Swedo SE, Leonard HL, Richter D, Rubinow DR, Potter WZ, et al (1994): Changes in cerebrospinal fluid neurochemistry during treatment of obsessive-compulsive disorder with clomipramine. *Arch Gen Psychiatry* 51:794–803.

Armitage R, Husain M, Hoffmann R, Rush AJ (2003): The effects of vagus nerve stimulation on sleep EEG in depression. A preliminary report. *J Psychosom Res* 54:475–482.

Backman J, Alling C, Alsen M, Regnell G, Traskman-Bendz L (2000): Changes of cerebrospinal fluid monoamine metabolites during long-term antidepressant treatment. *Eur Neuropsychopharmacol* 10:341–349.

Ben-Menachem E, Hamberger A, Hedner T, Hammond EJ, Uthman BM, Slater J, et al (1995): Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. *Epilepsy Res* 20:221–227.

Ben-Menachem E, Manon-Espaillat R, Ristanovic R, Wilder BJ, Stefan H, Mirza W, et al (1994): Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group. *Epilepsia* 35:616–626.

Bohning DE, Lomarev MP, Denslow S, Nahas Z, Shastri A, George MS (2001): Feasibility of vagus nerve stimulation-synchronized blood oxygenation level-dependent functional MRI. *Invest Radiol* 36:470–479.

Bowden CL, Koslow SH, Hanin I, Maas JW, Davis JM, Robins E (1985): Effects of amitriptyline and imipramine on brain amine neurotransmitter metabolites in cerebrospinal fluid. *Clin Pharmacol Ther* 37:316–324.

Carpenter LL, Anderson GM, Siniscalchi JM, Chappell PB, Price LH (2003): Acute changes in cerebrospinal fluid 5-HIAA following oral paroxetine challenge in healthy humans. *Neuropsychopharmacology* 28:339–347.

Carpenter LL, Heninger GR, Malison RT, Tyrka AR, Price LH (2004a): Cerebrospinal fluid interleukin (IL)-6 in unipolar major depression. *J Affect Disord* 79:285–289.

Carpenter LL, Heninger GR, McDougle CJ, Tyrka AR, Epperson CN, Price LH (2002): Cerebrospinal fluid interleukin-6 in obsessive-compulsive disorder and trichotillomania. *Psychiatry Res* 112:257–262.

Carpenter LL, Tyrka AR, McDougle CJ, Malison RT, Owens MJ, Nemeroff CB, et al (2004b): Cerebrospinal fluid corticotropin-releasing factor and perceived early-life stress in depressed patients and healthy control subjects. *Neuropsychopharmacology* 29:777–784.

Chotai J, Adolfsson R (2002): Converging evidence suggests that monoamine neurotransmitter turnover in human adults is associated with their season of birth. *Eur Arch Psychiatry Clin Neurosci* 252:130–134.

Cohen J (1988): *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed. Hillsdale, New Jersey: Lawrence Erlbaum.

Cremniter D, Jamain S, Kollenbach K, Alvarez JC, Lecrubier Y, Gilton A, et al (1999): CSF 5-HIAA levels are lower in impulsive as compared to nonimpulsive violent suicide attempters and control subjects. *Biol Psychiatry* 45:1572–1579.

De Bellis MD, Geracioti TD Jr, Altemus M, Kling MA (1993a): Cerebrospinal fluid monoamine metabolites in fluoxetine-treated patients with major depression and in healthy volunteers. *Biol Psychiatry* 33:636–641.

De Bellis MD, Gold PW, Geracioti TD Jr, Listwak SJ, Kling MA (1993b): Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry* 150:656–657.

Engstrom G, Alling C, Blennow K, Regnell G, Traskman-Bendz L (1999): Reduced cerebrospinal HVA concentrations and HVA/5-HIAA ratios in suicide attempters. Monoamine metabolites in 120 suicide attempters and 47 controls. *Eur Neuropsychopharmacol* 9:399–405.

George MS, Rush AJ, Sackeim HA, Marangell LB (2003): Vagus nerve stimulation (VNS): Utility in neuropsychiatric disorders. *Int J Neuropsychopharmacol* 6:73–83.

Goodwin FK, Jamison KR (1990): Biochemical and pharmacological studies. *Manic-Depressive Illness*. New York: Oxford University Press, 416–502.

Guy W (1976): *ECDEU Assessment Manual*. Rockville, Maryland: National Institute of Mental Health.

Hamilton M (1960): A rating scale for depression. *J Neurol Neurosurg Psychiatry* 23:56–62.

Handforth A, DeGiorgio CM, Schachter SC, Uthman BM, Naritoku DK, Tecoma ES, et al (1998): Vagus nerve stimulation therapy for partial-onset seizures: A randomized active-control trial. *Neurology* 51:48–55.

Henry TR, Bakay RA, Votaw JR, Pennell PB, Epstein CM, Faber TL, et al (1998): Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation. *Epilepsia* 39:983–990.

Hsiao JK, Agren H, Bartko JJ, Rudorfer MV, Linnoila M, Potter WZ (1987): Monoamine neurotransmitter interactions and the prediction of antidepressant response. *Arch Gen Psychiatry* 44:1078–1083.

Kaplan JR, Manuck SB, Fontenot MB, Mann JJ (2002): Central nervous system monoamine correlates of social dominance in cynomolgus monkeys (*Macaca fascicularis*). *Neuropsychopharmacology* 26:431–443.

Krahl SE, Clark KB, Smith DC, Browning RA (1998): Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia* 39:709–714.

Leckman JF, Goodman WK, Anderson GM, Riddle MA, Chappell PB, McSwiggan-Hardin MT, et al (1995): Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology* 12:73–86.

Little JT, Ketter TA, Mathe AA, Frye MA, Luckenbaugh D, Post RM (1999): Venlafaxine but not bupropion decreases cerebrospinal fluid 5-hydroxyindoleacetic acid in unipolar depression. *Biol Psychiatry* 45:285–289.

Lomarev M, Denslow S, Nahas Z, Chae JH, George MS, Bohning DE (2002): Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects. *J Psychiatr Res* 36:219–227.

Marangell LB, Rush AJ, George MS, Sackeim HA, Johnson CR, Husain MM, et al (2002): Vagus nerve stimulation (VNS) for major depressive episodes: One year outcomes. *Biol Psychiatry* 51:280–287.

Martensson B, Nyberg S, Toresson G, Brodin E, Bertilsson L (1989): Fluoxetine treatment of depression. Clinical effects, drug concentrations and monoamine metabolites and N-terminally extended substance P in cerebrospinal fluid. *Acta Psychiatr Scand* 79:586–596.

Morris GL 3rd , Mueller WM (1999): Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. *Neurology* 53:1731–1735.

Naritoku DK, Terry WJ, Helfert RH (1995): Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Res* 22:53–62.

Post RM, Goodwin FK (1978): Approaches to brain amines in psychiatric patients: A reevaluation of cerebrospinal fluid studies. In: Iversen LL, Iversen SD, Snyder SH, editors. *Handbook of Psychopharmacology, Volume 13: Biology of Mood and Antianxiety Drugs*. New York: Plenum Press, 147–185.

Potter WZ, Scheinin M, Golden RN, Rudorfer MV, Cowdry RW, Calil HM, et al (1985): Selective antidepressants and cerebrospinal fluid. Lack of specificity on norepinephrine and serotonin metabolites. *Arch Gen Psychiatry* 42:1171–1177.

Risby ED, Hsiao JK, Sunderland T, Agren H, Rudorfer MV, Potter WZ (1987): The effects of antidepressants on the cerebrospinal fluid homovanillic acid/5-hydroxyindoleacetic acid ratio. *Clin Pharmacol Ther* 42:547–554.

Roy A, Berman J, Williams R, Kuhn C, Gonzalez B (2002): Higher levels of CSF homovanillic acid in recently abstinent cocaine-dependent patients. *Am J Psychiatry* 159:1053–1055.

Rudorfer MV, Manji HK, Potter WZ (1992): Monoaminergic actions of ECT. *Clin Neuropharmacol* 15(suppl 1 pt A):677A–678A.

Rudorfer MV, Risby ED, Hsiao JK, Linnoila M, Potter WZ (1988a): Disparate biochemical actions of electroconvulsive therapy and antidepressant drugs. *Convuls Ther* 4:133–140.

Rudorfer MV, Risby ED, Hsiao JK, Linnoila M, Potter WZ (1988b): ECT alters human monoamines in a different manner from that of antidepressant drugs. *Psychopharmacol Bull* 24:396–399.

Rush AJ, George MS, Sackeim HA, Marangell LB, Husain MM, Giller C, et al (2000): Vagus nerve stimulation (VNS) for treatment-resistant depressions: A multicenter study. *Biol Psychiatry* 47:276–286.

Sackeim HA, Keilp JG, Rush AJ, George MS, Marangell LB, Dormer JS, et al (2001a): The effects of vagus nerve stimulation on cognitive performance in patients with treatment-resistant depression. *Neuropsychiatry Neuropsychol Behav Neurol* 14:53–62.

Sackeim HA, Rush AJ, George MS, Marangell LB, Husain MM, Nahas Z, et al (2001b): Vagus nerve stimulation (VNS) for treatment-resistant depression: Efficacy, side effects, and predictors of outcome. *Neuropsychopharmacology* 25:713–728.

Salinsky MC, Uthman BM, Ristanovic RK, Wernicke JF, Tarver WB (1996): Vagus nerve stimulation for the treatment of medically intractable seizures. Results of a 1-year open-extension trial. Vagus Nerve Stimulation Study Group. *Arch Neurol* 53:1176–1180.

Schachter SC (2002): Vagus nerve stimulation therapy summary: Five years after FDA approval. *Neurology* 59:S15–S20.

Scheinin M (1985): Monoamine metabolites in human cerebrospinal fluid: Indicators of neuronal activity? *Med Biol* 63:1–17.

Sheline Y, Bardgett ME, Csernansky JG (1997): Correlated reductions in cerebrospinal fluid 5-HIAA and MHPG concentrations after treatment with selective serotonin reuptake inhibitors. *J Clin Psychopharmacol* 17:11–14.

Sher L, Oquendo MA, Li S, Huang YY, Grunebaum MF, Burke AK, et al (2003): Lower CSF homovanillic acid levels in depressed patients with a history of alcoholism. *Neuropsychopharmacology* 28:1712–1719.

Shimamoto H, Takasaki K, Shigemori M, Imaizumi T, Ayabe M, Shoji H (2001): Therapeutic effect and mechanism of repetitive transcranial magnetic stimulation in Parkinson's disease. *J Neurol* 248(suppl 3): III48–I52.

Soderstrom H, Blennow K, Sjodin AK, Forsman A (2003): New evidence for an association between the CSF HVA: 5-HIAA ratio and psychopathic traits. *J Neurol Neurosurg Psychiatry* 74:918–921.

Struys EA, Guerand WS, ten Brink HJ, Jakobs C (1999): Combined method for the determination of gamma-aminobutyric acid and beta-alanine in cerebrospinal fluid by stable isotope dilution mass spectrometry. *J Chromatogr B Biomed Sci Appl* 732:245–249.

Ter Horst GJ, Streetland C (1994): Ascending projections of the solitary tract nucleus. In: Robin I, Barraco A, editors. *Nucleus of the Solitary Tract*. London: CRC Press.

Vonck K, Van Laere K, Dedeurwaerdere S, Caemaert J, De Reuck J, Boon P (2001): The mechanism of action of vagus nerve stimulation for refractory epilepsy: The current status. *J Clin Neurophysiol* 18:394–401.

Walker BR, Easton A, Gale K (1999): Regulation of limbic motor seizures by GABA and glutamate transmission in nucleus tractus solitarius. *Epilepsia* 40:1051–1057.