Comparison of $\Delta$FosB Immunoreactivity Induced by Vagal Nerve Stimulation with That Caused by Pharmacologically Diverse Antidepressants

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ABSTRACT

Vagal nerve stimulation (VNS) has been approved for treatment of refractory depression. However, there have been few, if any, studies directly comparing the effects produced by VNS in animals with those caused by antidepressants, particularly using clinically relevant stimulation parameters in nonanesthetized animals. In this study, $\Delta$FosB immunohistochemistry was used to evaluate different brain regions activated by long-term administration of VNS. Effects of VNS were compared with those caused by sertraline or desipramine (DMI). Double-labeling of $\Delta$FosB and serotonin was used to determine whether serotonergic neurons in the dorsal raphe nucleus (DRN) were activated by long-term VNS. VNS significantly increased $\Delta$FosB staining in the nucleus tractus solitarius (NTS), parabrachial nucleus (PBN), locus ceruleus (LC), and DRN, as well as in many cortical and limbic areas of brain including those involved in mood and cognition. Most, but not all, of these effects were seen also upon long-term treatments of rats with sertraline or DMI. Some areas where VNS increased $\Delta$FosB (e.g., the NTS, PBN, LC, and peripeduncular nucleus) were not affected significantly by either drug. Sertraline was similar to VNS in causing an increase in the DRN whereas DMI did not. Double-labeling of the DRN with $\Delta$FosB and an antibody for serotonin revealed that only a small percentage of $\Delta$FosB staining in the DRN colocalized with serotonergic neurons. The effects of VNS were somewhat more widespread than those caused by the antidepressants. The increases in $\Delta$FosB produced by VNS were either equivalent to and/or more robust than those seen with antidepressants.

Introduction

Vagal nerve stimulation (VNS) was approved by the U.S. Food and Drug Administration for treatment-resistant epilepsy (1997) and for treatment-resistant depression (2005). Clinical data show that both response (27–53%) and remission (15–33%) rates over 12 months were significantly higher in patients who received VNS treatment in addition to medications compared with what was reported in another study with similar patients who received only medications (12% response and 4% remission) (Dunner et al., 2006), and such improvement continues for 24 months (Rosa and Lisanby, 2012).

Despite these promising clinical results, there have been relatively few preclinical studies of the effects produced by VNS in animals, particularly using clinically relevant stimulation parameters in nonanesthetized animals. Most of the previous studies examined only short-term effects of VNS in anesthetized animals (Rutherfurd et al., 1992; Yousfi-Malki and Puizillout, 1994). Anesthesia can change the threshold for activation of different types of fibers in the vagal bundle (Woodbury and Woodbury, 1990). In addition, in most of the earlier studies, stimulation was performed using stimulation parameters that resulted in changes in peripheral autonomic function, which would produce reflexes that could activate brain regions and complicate the interpretation of the results.

We found previously (Furmaga et al., 2011) that VNS given as it was in this study and for a similar time period caused anxiolytic-like and antidepressant-like effects. It was of in-
terest then to examine what regions of brain were activated when VNS caused behavioral effects.

Immunohistochemistry of c-Fos, an immediate early gene product, has become the most widely used functional anatomical mapping tool to identify activation of cells (Kovács, 1998). There are four major members of the Fos protein family: c-Fos, FosB, Fra-1, and Fra-2. FosB has a splice variant termed ΔFosB. These proteins respond to stimuli with different time courses. In general, maximal levels of c-Fos protein occur within 1 to 3 h of stimulus exposure and disappear in 4 to 6 h, whereas ΔFosB shows a more delayed activation but persists longer (McClung et al., 2004). Hence, c-Fos has been suggested to be an indicator of short-term neuronal activation, whereas ΔFosB may reflect longer-term neuroadaptations (McClung et al., 2004).

Several studies have examined mRNA for c-fos or c-Fos protein after antidepressant treatments, with the majority studying short-term effects (Beck, 1995; Fraga et al., 2005; Slattery et al., 2005). Data from these studies have been inconsistent with respect to activation in the dorsal raphe nucleus (DRN), locus ceruleus (LC), hippocampus, and cortical areas of brain. In addition, long-term treatment of rats with either paroxetine (Muigg et al., 2007) or citalopram (Kuipers et al., 2006) did not increase c-Fos in any brain region examined. We measured c-Fos and ΔFosB previously and found that long-term (3 weeks) administration of VNS in conscious Sprague-Dawley rats significantly increased ΔFosB staining in several areas of brain (Cunningham et al., 2008). Because of this and the aforementioned literature, it was of interest to compare the activation patterns of long-term VNS in many more regions of brain than those studied previously with those produced by long-term treatment with antidepressants, primarily focusing on ΔFosB. The antidepressants selected for study, sertraline (SERT) and desipramine (DMI), target serotonin and norepinephrine neurons, respectively. This is the first study to compare directly such activation patterns after long-term treatment with VNS and antidepressants in nonanesthetized rats. In light of its effectiveness in treatment-resistant depression, we speculated that VNS might cause a broader pattern of activation than that seen with antidepressants or the extent of activation in certain areas would be greater than that seen with the drugs.

Materials and Methods

Experiments were performed using adult male Sprague-Dawley rats, weighing 250 to 350 g (Harlan, Houston, TX). Rats were group-housed and maintained in a temperature-controlled environment on a 14:10-h light/dark cycle. Rats had ad libitum access to food and water. Experimental protocols were approved by the institutional animal care and use committee in accordance with the guidelines of the U.S. Public Health Service, American Physiological Society, and Society for Neuroscience.

Vagus nerve electrodes were implanted on the left vagus nerve under aseptic conditions. The surgical procedure was similar to that described by Cunningham et al. (2008) except that the anesthetic was a combination of 75 mg/kg ketamine and 0.5 mg/kg medetomidine. In brief, the coil electrode was placed around the left cervical vagus nerve and carotid sinus ventral to the carotid bifurcation. The bipolar stimulating electrode was configured with the cathode at the proximal lead and the anode at the distal lead to preferentially direct action potential propagation toward the central nervous system by creating an anodal block at the distal lead. The electrodes were connected to a stimulator pack (Cyberonics, Inc., Houston, TX) that was sutured in place in a subcutaneous pouch created on the back of the rat. Rats that received VNS were instrumented with an operational stimulator pack that was programmed by a handheld computer. Controls received a dummy simulator pack that was the same size and weight (48 mm × 33 mm × 7.1 mm; 16 g). Beginning 7 days after surgery, rats received continuous VNS treatment for 14 days. The stimulation paradigm consisted of one burst of 20 Hz, a 250 μs pulse width, and 250 μA current for 30 s every 5 min for 2 weeks. These stimulation parameters are very similar to those used initially in clinical studies (Rush et al., 2005), although parameters may change if patients do not respond. In addition, we found previously that this stimulation protocol does not cause changes in blood pressure, heart rate, respiratory frequency, or locomotor activity in comparison with that measured in rats receiving “dummy” stimulation (Cunningham et al., 2008). However, raising the stimulation current to 500 μA did cause autonomic effects (H. Furmaga, J. T. Cunningham, and A. Frazer, unpublished data). Thirty minutes after the end of continuous VNS for 14 days, rats were perfused, and the brains were removed for subsequent analysis of ΔFosB.

Implantation of Osmotic Minipumps. One day before surgery, osmotic minipumps delivering 5 μl/h (model 2ML2; Durect Corporation, Cupertino, CA) were filled with drug or vehicle, filtered through sterile 0.9-μm nitrocellulose filters (Millipore Corporation, Billerica, MA) using a sterile technique under an air-filtered hood. Drug solution concentrations were determined on the basis of the mean weight of the rats over the 14 days of treatment. Doses were 7.5 mg/kg per day of sertraline or 10 mg/kg per day of desipramine as these have been shown previously to produce serum concentrations of drug in the therapeutic range (Benmansour et al., 1999). Specific surgical details are given in Furmaga et al. (2011). Rats were perfused at the end of the experiment with the minipumps still in place, and the brains were removed for subsequent immunohistochemical analysis.

Immunohistochemistry of ΔFosB. The staining procedure was similar to that described by Cunningham et al. (2008). In brief, rats were anesthetized with a cocktail of 75 mg/kg ketamine and 0.5 mg/kg medetomidine and perfused with 0.1 M phosphate-buffered saline (PBS) followed by 500 ml of 4% paraformaldehyde in PBS. Brains were removed and placed in PBS with 30% sucrose for 4 days. Three serial sets of 40-μm coronal sections were cut in a cryostat and placed in cryoprotectant and stored at −20°C until processed for ΔFosB immunohistochemistry.

Sets of serial sections were stained for FosB [goat anti-FosB (102); Santa Cruz Biotechnology, Inc., Santa Cruz, CA]. The primary antibody used in this study does not discriminate between FosB and its splice variant ΔFosB. However, because of the fact that ΔFosB accumulates with long-term stimulation as a result of its long half-life, particularly the 37-kDa isoform (McClung et al., 2004), the antibody chosen for this study can specifically detect this isoform. For this reason, we refer to long-term stimulation increasing ΔFosB levels although a contribution from FosB cannot be excluded. To assess ΔFosB, sections were incubated with FosB antibody (1:5000) for 72 h at 4°C. The sections were then incubated with Alexa Fluor 488 donkey anti-goat IgG (1:1000; Molecular Probes, Carlsbad, CA). Some sets of sections were double-labeled for ΔFosB and serotonin (1:300; Abcam, Cambridge, MA). Sections were then incubated with Alexa Fluor 488 donkey anti-goat IgG (1:1000) for FosB and Alexa Fluor 546 donkey anti-mouse IgG (1:1000) for serotonin at room temperature for 4 h. The sections were washed in PBS and mounted on gelatin-coated slides and coverslipped using ProLong Gold Anti-fade Reagent (Invitrogen, Carlsbad, CA). No immunoreactivity was detected with controls that were incubated with either primary or secondary antibody alone.

The number of ΔFosB-positive cells per section in selected brain regions was quantified by observers blind to the experimental conditions as described previously (Cunningham et al., 2008). More areas were analyzed than in our previous study and included the nucleus tractus solitaries (NTS), LC, DRN, parabrachial nucleus (PBN), basolateral (BLA) and central (CeA) nuclei of the amygdala,
hippocampus, frontal cortex, cingulate cortex, nucleus accumbens (NAc), striatum, bed nucleus stria terminalis (BNST), substantia nigra (SN) or ventral tegmental area (VTA), and peripendular area. The areas were defined on the basis of the stereotactic atlas of Paxinos and Watson (1986). Areas in which cell counts were quantified are shown with red boxes over representative atlas schematic diagrams (Fig. 1). Sections were examined by fluorescence microscopy. For quantitative analysis, at least four representative sections from each brain region were imaged. Digital images were collected using an Olympus BX40 microscope equipped with a DP27 Olympus camera connected to a Pentium computer running imaging software.

**Single-Labeling In Situ Hybridization for c-Fos mRNA.** The labeling procedure was similar to that described by Liu et al. (2007). Antisense 35S-labeled cRNA probes for rat c-Fos were generated with labeling procedure was similar to that described by Liu et al. (2007). Brain sections were acetylated in 0.1 M formic acid for 1 h, and rinsed in 2X SSC (0.15 M NaCl and 0.01 M sodium citrate, pH 7.2). Brain sections were acetylated in 0.1 M formic acid for 1 h, and rinsed in 2X SSC (0.15 M NaCl and 0.01 M sodium citrate, pH 7.2). Brain sections were acetylated in 0.1 M formaldehyde for 1 h and rinsed in 2X SSC (0.15 M NaCl and 0.01 M sodium citrate, pH 7.2). Sections were hybridized with 70 μl of the diluted probes and placed in plastic trays moistened with 50% formamide at 55°C overnight. On the following day, coverslips were lifted with 2X SSC, and slides were rinsed three times for 5 min each in 2X SSC and distilled water, and dehydrated through a graded series of alcohol.

**Results**

Administration of VNS for 14 days to conscious Sprague-Dawley rats significantly increased ΔFosB staining in the NTS [F(4,19) = 195.6, P < 0.0001] and regions that receive both direct and indirect projections from it such as the PBN [F(4,19) = 69.87, P < 0.0001], LC [F(4,19) = 49.58, P < 0.0001], and DRN [F(4,19) = 32.56, P < 0.0001] (Fig. 2). Because the NTS and PBN are not traditional targets of monoamine-based antidepressants, it was not unexpected that they were not affected significantly by either long-term administration of sertraline or DMI (Fig. 2). Sertraline significantly increased ΔFosB staining in the DRN, although the effect was not as robust as in the NTS. The patterns of activation were similar between VNS and sertraline within the DRN, with ΔFosB staining mainly localized to the lateral wings and little activation in the ventromedial subnucleus. In contrast, noradrenergic neurons in the LC were not activated either by DMI or sertraline (Fig. 2).

Because essentially all the cells in the LC are noradrenergic (Swanson, 1976), it can be assumed that noradrenergic cells are activated by VNS in the LC. However, only approximately 40% of the cells in the DRN are serotonergic (Nano-
double-labeling immunohistochemistry was performed in the DRN to see whether the cells activated, i.e., exhibiting an increase in ΔFosB, were serotonergic. To do this, the cells in the DRN were labeled with an antibody for serotonin. As shown in Fig. 3, long-term VNS significantly increased ΔFosB staining in the DRN but only a very small percentage of cells labeled with ΔFosB colocalized with serotonergic cells.

Because dopaminergic cell bodies are not traditional targets for antidepressant drugs, it was of interest to see whether VNS would cause activation in the SN or VTA. Neither VNS nor the drugs did this (data not shown). Of interest, on the sections containing the SN and VTA, it was observed that VNS produced staining in the peripeduncular nucleus (F(4,19) = 44.65, P < 0.0001), but it was subsequently found that the drug treatments did not do this (Fig. 4).

VNS induced widespread increases in ΔFosB in many cortical and limbic areas of brain including regions involved in mood and cognition. Most, but not all, of these effects were seen also upon repeated treatments of rats with sertraline or DMI. Neither drug treatment increased ΔFosB in the peripeduncular nucleus, whereas VNS did. In general, the increases produced by VNS were either equivalent to or more robust than those caused with antidepressants.

A comparison of the areas activated by long-term treatment with VNS, sertraline, or DMI is presented in Table 1. VNS caused widespread activation with only four areas, among 15 examined, not showing activation, namely the CeA, SN, VTA, and striatum. The two drug treatments did not activate these areas either (Table 1). Neither sertraline nor DMI elevated ΔFosB in the NTS, PBN, LC, and peripeduncular area, whereas sertraline elevated ΔFosB in the DRN and desipramine did not.

As stated previously, it is widely accepted that the maximal levels of c-Fos protein occur within 1 to 3 h of stimulus exposure and disappear in 4 to 6 h, whereas ΔFosB shows a more delayed activation that persists longer. Hence, increases in c-Fos have been suggested to be an indicator of short-term neuronal activation, whereas ΔFosB reflects long-term activation (McClung et al., 2004). Of interest, we observed increases in c-Fos after long-term VNS or antidepressant drug administration in the same brain regions as seen with ΔFosB (data not shown). Because of this somewhat surprising result, quantitative analysis of mRNA levels for c-fos using in situ hybridization was performed in rats given VNS. Expression of c-Fos increased in the DRN with VNS treatment, with the increase present throughout the nucleus (Fig. 7). This result implies that the increases in c-Fos measured by immunohistochemistry reflected increases in c-Fos.

Discussion

VNS significantly increased ΔFosB staining in the NTS and regions that receive either direct and/or indirect projections from it such as the PBN, LC, and DRN (Peyron et al., 1996; Berthoud and Neuhuber, 2000). In addition, VNS induced widespread increases in ΔFosB in many cortical and limbic areas of brain including regions involved in mood and cognition. Most, but not all, of these effects were seen also upon repeated treatments of rats with sertraline or DMI. Neither drug treatment caused an increase in ΔFosB in the LC, whereas sertraline was similar to VNS in causing an increase in the DRN. In addition, neither drug treatment increased ΔFosB in the peripeduncular nucleus, whereas VNS did. In general, the increases produced by VNS were either equivalent to or more robust than those caused with antidepressants.

There is an anatomical rationale for VNS having effects on many cortical and limbic areas of brain involved in mood and cognition. Approximately 80% of vagal fibers carry afferent sensory information to the central nervous system (Foley and...
DuBois, 1937), with the initial projection area in brain being
the NTS (Kalia and Sullivan, 1982). One set of projections
from the NTS contains ascending projections from the NTS to
the midbrain, hypothalamic, and cortical regions involved in
central autonomic control; included in this ascending system
are direct/and or indirect projections to the LC and DRN
(Peyron et al., 1996; Van Bockstaele et al., 1999; Berthoud
and Neuhuber, 2000) containing cell bodies for noradrenergic
or serotonergic neurons, respectively, and it is well estab-
lished that such neurons are targets for antidepressants. The
NTS also sends projections throughout the brain involving
areas thought to be involved in mood and emotion (Berthoud
and Neuhuber, 2000).

The stimulation parameters used here are similar to those
used initially in clinical studies (e.g., Rush et al., 2005). In
addition, when VNS was given to rats using these stimula-
tion parameters for time periods similar to those in the
current study, both anxiolytic-like and antidepressant-like
effects were observed (Furmaga et al., 2011). Thus, effects
seen in this study are probably occurring in rats when VNS
is effective behaviorally. If ΔFosB, then, can be used as an
index of long-term cellular activation, some of the brain re-
gions demonstrated to be activated in this study may be
involved in the behavioral effects of VNS.

In agreement with our previous study (Cunningham et al.,
2008), repeated VNS administration significantly increased
ΔFosB staining in the NTS, PBN, LC, and DRN. Moreover,
the present study revealed that VNS induced widespread
ΔFosB activation in many cortical and limbic areas such as
the frontal cortex, cingulate cortex, hippocampus, BNST, and
NAc. Results of imaging studies in patients with epilepsy or
depression who are treated with VNS also show widespread
effects on subcortical and cortical regions, with short-term
VNS producing increases in blood flow in the hypothalamus,
thalamus, and insular cortex but decreases in the hippocam-
pus and posterior cingulate gyrus (Chae et al., 2003). Long-
term VNS produced both increased (Kosel et al., 2011) and
decreased (Nahas et al., 2007) changes in blood flow in cor-
tical regions although subcortical regions were activated
(Henry et al., 2004). Inconsistent results were also obtained
in the amygdala (Zobel et al., 2005; Conway et al., 2006).
VNS causes short-term limbic hyperperfusion and long-term
thalamic hypoperfusion in patients with refractory epilepsy,
and these changes correlate with clinical efficacy (Vonck et
al., 2008).

Data from previous studies that examined mRNA for c-fos
or c-Fos protein after short-term antidepressant administra-
tion have been inconsistent with respect to the LC, DRN, and
many cortical areas. Somewhat surprisingly, administration of fluoxetine (Fraga et al., 2005; Slattery et al., 2005) but not other types of antidepressants, with one exception (Kovács, 1998), was found to increase c-Fos in the LC. In general, increases have not been reported in the DRN although Fraga et al. (2005) found an increase after short-term administration of fluoxetine. Most studies show increases in c-fos or c-Fos in the CeA and BNST after short-term administration of different classes of antidepressants (Beck, 1995; Fraga et al., 2005; Slattery et al., 2005; Bechtholt et al., 2008). Long-term treatment of rats with either paroxetine (Muigg et al., 2007) or citalopram (Kuipers et al., 2006) did not increase c-Fos in any brain region examined, including the CeA. In contrast, Bechtholt et al. (2008) showed that long-term fluoxetine increased c-Fos in multiple brain areas including the BNST, cingulate cortex, anterior NAc, and hippocampus and long-term mirtazapine treatment increased c-Fos in the CeA and dentate gyrus (Gerrits et al., 2006).

There are very few studies that examined the effects of antidepressant drug treatments on ΔFosB. In a recent study, Vialou et al. (2010) showed that long-term fluoxetine treatment produced an accumulation of ΔFosB in the NAc shell. In addition, they showed that virus-mediated overexpression of ΔFosB in the rat NAc produced a significant antidepressant-like effect in the forced swim test.

Comparing such results with those seen after long-term sertraline and DMI in the present study reveals both similarities and differences. This is probably because most of the previous work involved short-term antidepressant treatment and measured c-Fos, whereas we report on ΔFosB. However, although the data are not shown, we measured c-Fos as well and found results with it that were comparable to those found with ΔFosB. Of interest, we found that sertraline increased c-Fos staining in the DRN, whereas this was not seen with or seen inconsistently with short-term antidepressant treatments other than fluoxetine (Fraga et al., 2005). Even though ΔFosB staining increased in the DRN, this was not primarily in serotonergic soma (Fig. 4). In agreement with most previous studies, we found that neither drug treatment caused an increase in ΔFosB in the LC. This finding is not surprising because activation of α2-autoreceptors in the LC persists in restraining norepinephrine neurotransmission in the face of tonically elevated basal norepinephrine levels after long-term reuptake blockade (Garcia et al., 2004).
increase in ΔFosB staining in the LC after VNS stimulation is probably a consequence of efferents from the NTS, the initial projection area of vagal afferents in brain, innervating noradrenergic dendrites of LC neurons with synaptic contacts characteristic of both excitatory- and inhibitory-type transmitters (Van Bockstaele et al., 1999).

Similar to the results found with long-term treatment of rats with paroxetine (Muigg et al., 2007) and citalopram (Kuipers et al., 2006), none of the treatments in this study increased ΔFosB in the CeA. Vialou et al. (2010) hypothesized that ΔFosB induction in NAc is required for the antidepressant action of fluoxetine. Whether this is true of sertraline and DMI remains to be seen although our data show that they, as well as VNS, also increase ΔFosB in the NAc.

Long-term VNS and antidepressants increased ΔFosB in many cortical areas including the frontal cortex, cingulate cortex, and NAc. As mentioned, antidepressant drug-induced increases in these areas have not consistently been observed by others. To the best of our knowledge, though, we are the first to examine this upon long-term administration of antidepressants administered using osmotic minipumps to obtain steady-state plasma concentrations of drugs in the therapeutic range. VNS increased ΔFosB in the BLA, although it did not in the CeA. The absence of an effect in the CeA is

TABLE 1

Summary data for the effects of long-term VNS, SERT, or DMI on ΔFosB staining in the rat brain

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Hip, hippocampus; ST, striatum; Fr, frontal cortex; Cg, cingulate cortex; PP, peripeduncular nucleus.

+, significant increases in ΔFosB staining; ++, increase induced by VNS is significantly more than those of antidepressants; 0, no significant changes.
different from results reported by others studying effects of antidepressants, as mentioned above. However, we also did not see any effect of antidepressants on ΔFosB in the CeA. This difference could be due to the anesthetic used in the previous studies. It is well known that c-Fos expression can be greatly affected by anesthesia. For example, barbiturates may interfere with c-Fos expression, whereas other anesthetics such as urethane and chloralose cause a high level of baseline expression (Miura et al., 1994; Dampney et al., 1995; Rocha and Herbert, 1997).

Little, if any, overlap was found between the cells showing ΔFosB and those staining for 5-HT in the DRN. This is unexpected because Dorr and Debonnel (2006) showed that repeated administration of VNS to nonanesthetized rats, using stimulation parameters identical to ours, raised the firing rate of noradrenergic neurons in the LC and serotonergic neurons in the DRN. This discrepancy could be due to limitations of using Fos proteins as markers for neuronal activation. Neurons may differ in their capacity to produce Fos, the time course of Fos induction and decay varies with different inducing stimuli, and some brain regions do not express Fos regardless of stimuli (Dampney and Horiuchi, 2003). Herdegen et al. (1991) reported that the onset of Fos production in somatic motor neurons is considerably delayed (by several hours) compared with that in most other neurons (typically 30–60 min). The temporal pattern of activation is of great significance given that Dorr and Debonnel (2006) showed that stimulation of the vagus nerve for as little as 1 h produced increases in the firing rate of noradrenergic neurons, whereas it took 14 days for the firing rate of serotonergic neurons to increase. Moreover, Dragunow et al. (1989) reported that high-frequency stimulation protocols that produced good long-term potentiation do not lead to c-Fos induction. Hence, the absence of ΔFosB in serotonergic neurons in the DRN after long-term VNS does not necessarily mean that they were not activated, especially in light of the data of Dorr and Debonnel (2006).

As shown in Fig. 4, the majority of VNS-induced ΔFosB expression in the DRN was observed in its lateral margins and comprised an area corresponding to the ventrolateral periaqueductal gray (vLPAG). This distinctive pattern of ΔFosB induction is interesting because the vLPAG has been characterized previously as an important neural substrate for passive coping responses (Bandler and Shipley, 1994). Berton et al. (2007) show that the strongest ΔFosB induction in the vLPAG was observed in animals that were most resilient to behavioral despair. They hypothesized that expression of ΔFosB is part of an adaptive mechanism that promotes resilience to stress by inhibiting the stress-induced activation of substance P neurotransmission to the forebrain. This is in good agreement with our behavioral data showing that repeated administration of VNS produces anxiolytic-like and antidepressant-like effects in the novelty suppressed feeding test and the forced swim test, respectively (Furmaga et al., 2011).

The in situ hybridization signal for c-Fos occurs throughout the DRN, whereas ΔFosB immunohistochemistry was localized primarily to the lateral wings. The most likely reason for this discrepancy is that the increase in these two immediate early genes is occurring in different cells or at least the increase in ΔFosB is occurring in only a subset of cells in which c-Fos is elevated. As mentioned previously, these proteins respond to stimuli with different time courses with c-Fos increasing more rapidly than ΔFosB (McClung et al., 2004). Consistent with this, we found previously that 2 h of VNS increased c-Fos in many brain areas, whereas ΔFosB was not increased in any region at this time (Cunningham et al., 2008).

In conclusion, the present study identified potential sites in the brain activated by VNS. In general, the effects of VNS were somewhat more widespread than those caused by the antidepressants, and in some areas, the extent of activation was greater with VNS than with the drugs. Whether such differences in effects between VNS and antidepressants contribute to the utility of VNS in treatment-resistant depression remains to be determined.

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Authorship Contributions

Participated in research design: Furmaga and Frazer.
Conducted experiments: Furmaga and Sadhu.
Contributed new reagents or analytic tools: Furmaga and Sadhu.
Performed data analysis: Furmaga and Frazer.
Wrote or contributed to the writing of the manuscript: Furmaga, Sadhu, and Frazer.

References

Chae JH, Nahas Z, Lomarev M, Denslow S, Lorberbaum JP, Bohning DE, and


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