

Vagal Nerve Stimulation for Treatment-Resistant Depression

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Abstract Major depressive disorder (MDD) is prevalent. Although standards antidepressants are more effective than placebo, up to 35% of patients do not respond to 4 or more conventional treatments and are considered to have treatment-resistant depression (TRD). Considerable effort has been devoted to trying to find effective treatments for TRD. This review focuses on vagus nerve stimulation (VNS), approved for TRD in 2005 by the Food and Drugs Administration. Stimulation is carried by bipolar electrodes on the left cervical vagus nerve, which are attached to an implanted stimulator generator. The vagus bundle contains about 80% of afferent fibers terminating in the medulla, from which there are projections to many areas of brain, including the limbic forebrain. Various types of brain imaging studies reveal widespread functional effects in brain after either acute or chronic VNS. Although more randomized control trials of VNS need to be carried out before a definitive conclusion can be reached about its efficacy, the results of open studies, carried out over period of 1 to 2 years, show much more efficacy when compared with results from treatment as usual studies. There is an increase in clinical response to VNS between 3 and 12 months, which is quite different from that seen with standard antidepressant treatment of MDD. Preclinically, VNS affects many of the same brain areas, neurotransmitters (serotonin, norepinephrine) and signal transduction mechanisms (brain-derived

neurotrophic factor–tropomyosin receptor kinase B) as those found with traditional antidepressants. Nevertheless, the mechanisms by which VNS benefits patients nonresponsive to conventional antidepressants is unclear, with further research needed to clarify this.

Keywords TRD · VNS · BDNF-TrkB · Monoamines

Both worldwide and in the USA, major depressive disorder (MDD) is quite prevalent. In the USA, the lifetime prevalence is about 30%, with the yearly prevalence being almost 9% [1]. Although antidepressant drugs are effective, their effect is mild to moderate, with selective serotonin reuptake inhibitors having effect sizes for acute response of 0.20 to 0.40; their ability to prevent relapses or recurrences is better, with effect sizes of 0.6 to 0.7 [2]. Another way to state this is that after 2 adequate trials with antidepressants, only about half the patients achieve remission, being defined as a $\geq 50\%$ decrease in a severity rating scale score and a very low final score indicating minimal residual symptoms (see [3]). Remission is now viewed as the “gold standard” for treatment outcome as those with less residual symptoms after treatment for depression subsequently have less depressive symptoms, and better social functioning than those with more residual symptoms [4–6]. Just as importantly, up to 35% of patients with nonpsychotic MDD do not respond to 4 or more conventional treatments [7]. Such patients are considered to have treatment-resistant depression (TRD). Establishment of effective treatments for TRD would be very useful given the large number of patients who have it, their diminished quality of life and health [8, 9], the increased cost associated with it [9], and the overall chronic course of both MDD and TRD [10, 11].

In view of such facts, much attention has been focused on trying to establish treatments that would at least have a

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modicum of effectiveness in such nonresponsive patients, either as standalone or adjunctive treatment. This review covers one such treatment, vagus nerve stimulation (VNS). It begins with a review of the evidence for its effectiveness and concludes with a review of effects it produces on brain function. A variety of neuromodulation techniques in addition to VNS have been tried in TRD, for example deep brain stimulation and repetitive transcranial magnetic stimulation. There are varying levels of evidence for their acute and long-term efficacy, as well as their safety (see [12]). Nevertheless, the Food and Drugs Administration approved VNS for treatment of TRD in 2005 and repetitive transcranial magnetic stimulation in 2008. The Food and Drugs Administration approved VNS as an adjunctive long-term treatment of chronic recurrent depression in patients 18 years of age or older who are experiencing a major depressive episode and have not had an adequate response to 4 or more adequate antidepressant treatments. It is important to note that the approval is for long-term treatment as the data reviewed below show efficacy to improve over months and even years but to be more limited over the first several months.

Interest in this topic has generated a number of review articles mentioned later. Most of these focus on clinical efficacy. Those that reviewed efficacy as well as potential mechanisms of action were published over a decade ago. This review covers both topics. Furthermore, it takes the position as discussed later, that the efficacy of VNS has to be compared with the response over time of patients with TRD not receiving VNS.

The Vagus Nerve and VNS

There is both an anatomical and functional rationale for stimulating the cervical vagus nerve to treat diseases of the brain. Since 1937, the vagus has been known to be mixed nerve with about 80% of its fibers carrying sensory afferent information to the brain and having about 20% efferent motor fibers [13]. Early evidence for the functionality of its afferent projection was the observation that its stimulation caused electroencephalography changes [14]. Its name comes from the Latin word for “wandering” owing to its having such a course along the esophagus and arteries to innervate numerous peripheral organs and structures (see [15]). Its visceromotor efferent component originates in the dorsal motor nucleus of the medulla, whereas the initial termination point of its afferent fibers in brain is primarily the nucleus tractus solitarius (NTS), also in the medulla. Neurotransmitters used by vagal afferents include peptides such as substance P and calcitonin gene-related peptide and the excitatory amino acid transmitter L-glutamate.

The major outputs from the NTS can be characterized as 1) local projections to medullary motor nuclei; 2) projections to

the midbrain including the locus coeruleus (LC), dorsal raphe nucleus (DRN) and other brainstem interneurons in the reticular formation and to the parabrachial nucleus (PBN); and 3) projections to forebrain sites such as the hypothalamus, amygdala, bed nucleus of the stria terminalis (BNST), and insular cortex (see [15–17]). And of course, there are projections from nuclei such as the LC and DRN to limbic forebrain areas. Thus, areas in brain long thought to be involved in behaviors relevant for depression are innervated, either directly or indirectly, from projections of afferent vagal fibers terminating in the NTS.

The VNS procedure involves implantation of a stimulation generator connected to bipolar electrodes that are placed around the left vagus nerve. The rationale for stimulating the left vagus nerve for TRD (or epilepsy) is that it innervates the AV node of the heart so as to have less of an effect on heart rate than the right vagus, which innervates the SA node. Stimulation of each vagus nerve produces effects on heart rate consistent with such innervation [18, 19]. Further, the bipolar stimulating electrode is configured with the cathode at the proximal lead and the anode at the distal lead so as to direct action potential propagation to the central nervous system by creating an anodal block at the distal lead. This procedure is carried out as day surgery with local or general anesthesia. In the USA, such surgery is usually done by a neurosurgeon. The stimulation device is activated telemetrically by a wand connected to a hand-held computer. Stimulation parameters used include current (mA), frequency (Hz), pulse width (μ s), and duty cycle (the duration that stimulation is on or off). Such parameters reflect the “dose” of VNS. Stimulation is usually started with a low current, 0.25 to 0.75 mA, which can be increased gradually. Frequencies on the order of 20 to 30 Hz are used clinically as frequencies of 50 Hz or higher can damage the vagus [20]. A pulse width of 250 μ s and a duty cycle of stimulation for 30 s on/300 s off is often used.

Clinical Studies

As reported in Penry and Dean [21], VNS was used originally from 1988 to 1989 for inpatients with treatment-resistant epilepsy. In 2000, Elger et al. [22] were the first to note improvement of mood in patients with epilepsy that was independent of their seizure attenuation. Since 2005, almost 3000 clinical and preclinical papers dealing with VNS have been published, with many concerning its use in epilepsy or depression.

Given the importance of “dose” in producing a beneficial therapeutic response, it is somewhat surprising that the only prospective study examining dose of VNS for therapeutic outcome in TRD occurred quite recently, in 2013. Aaronson et al. [23] compared the response of patients with TRD to adjunctive VNS over 22 or 50 weeks and used 3 different “doses”. The frequency and duty cycle were the same across the 3

groups, whereas stimulation in the “low” group was 0.25 mA current with a 130 μ s pulse width. For the “medium” group, the parameters were 0.5 to 1.0 mA and 250 μ s, whereas it was 1.25 to 1.50 mA and 250 μ s for the “high” group. This study was a double-blind, randomized comparison of the effect of the different doses of VNS, but there was no control group (sham stimulation). The high dose was associated with less tolerability although 70% to 75% of patients in the high group reached their assigned dose. Similar efficacy was found in the 3 dose groups, that is, differences in outcome measures were not statistically significant. However, after 22 weeks of treatment, 10% to 20% of patients in the low group responded (depending on the scale used for efficacy) whereas 19% to 31% of patients did so in the high group. Consistent with data described below, behavioral improvement continued over time in that 25% of the patients who had not responded at 22 weeks did so at 50 weeks. Importantly, response was sustained up to 50 weeks in those who responded at 22 weeks, especially those receiving medium or high doses. Muller et al. [24] carried out a retrospective analysis of the effectiveness of 2 different doses of VNS in 2 groups of patients: 1) a low-strength/high-frequency (≤ 1.5 mA, 20 Hz) group *versus* a high-strength/low-frequency (> 1.5 mA, 15 Hz) group. Change in the Hamilton Rating Scale for Depression (HAM-D) at 6-month intervals was the outcome measure. Better outcome was achieved with the low-strength/high-frequency group. Further prospective studies are clearly needed as there is evidence that VNS does have frequency-dependent effects in patients, as shown by results using functional magnetic resonance imaging (fMRI) [25].

In addition to original studies of the efficacy of VNS in TRD, there have been quite a few reviews of this topic [16, 26–34]. And, not surprisingly, the overall conclusion is that more research, particularly randomized control trials (RCTs), is needed before efficacy can be established conclusively. This is so, but our opinion is that the evidence for efficacy is quite substantial in that its effect has to be compared with the response over time of depressed patients resistant to treatment not receiving neurostimulation therapies such as VNS. And that response is quite modest. Dunner et al. [35] reported a prospective study of outcomes of 124 patients with TRD (15 of whom were bipolar) treated with standard care [i.e., treatment as usual (TAU)] over 2 years. After 12 months, the response rate was about 12% and it was 18.4% after 24 months. Remission rates were 3.6% and 7.8%, respectively. And neither response nor remission was well-maintained over time with TAU. The emphasis on RCTs with VNS is likely based, in part, on the high placebo response rates in clinical trials of antidepressants. In such short-term trials, placebo response rates of almost 40% are usual [36]. Although placebo responses may be maintained for 12 weeks after stopping the clinical trial [37], maintaining recurrent depressives on placebo over 6 to 12 months is much less effective than maintaining

them on drug [38]. Further, the current poor response of patients with TRD to TAU is noteworthy in light of the increase in placebo response rates since the 1980s to 1990s in nontreatment-resistant patients [39]. Thus, even though there is clearly a need for more long-term RCTs with VNS, the data reviewed below on patients with TRD should be considered in this context.

Based on results of earlier open trials of the short-term (10 weeks) efficacy of VNS in patients with TRD that showed response rates of 30% to 40% [40, 41], an RCT of 10 weeks of treatment with VNS *versus* sham stimulation was carried out [42]. On the primary HAM-D response measure, VNS produced a response rate of 15.2% *versus* 10.0% in the sham group—this was not a significant difference. However, differences in response rates with a secondary outcome measure were significant. To date, this is the only RCT to employ sham stimulation as a comparison to VNS. Attention has been focused appropriately on this negative result and the fact that there is considerable variability in effectiveness among the studies [30]. And longer-term studies (e.g., 2 years) with VNS (reviewed below) can be influenced by the natural course of MDD. For example, Nahas et al. [43] followed the 59 patients in the original open 10-week study of Sackeim et al. [41] for 2 years. Response rate increased over time from 31% at 3 months to 42% to 44% at 12 to 24 months. Remission rates were about 25% after 1 to 2 years. And for those that responded at 3 months, 50% to 75% remained well at 12 to 24 months. Subsequently, with a larger cohort of 205 patients, of those who responded to VNS at 3 months, 76.7% maintained response at 24 months and for those who did not respond until 12 months, 65% were still responders at 24 months [44]. For patients with TRD, these response and remission rates are considerably higher than those achieved over a similar time with TAU [35] such that the natural course of treatment-resistant patients is different from those receiving treatment who are not resistant, where recovery rates of 60% to 85% are seen over 2 years [45, 46].

George et al. [47] attempted to put such results from this open trial into perspective by analyzing data from those treated with VNS for 1 year in open studies with results from comparable patients receiving TAU as reported by Dunner et al. [35]. Although there was not *a priori* randomization, the patient characteristics seem comparable with similar inclusion and exclusion criteria and demographics, with the studies being carried out over a similar time period. Also, 12 of the 13 sites for the TAU study participated in the VNS + TAU study with 9 additional sites only participating in the VNS + TAU study. Irrespective of the method of data analysis, the addition of VNS to TAU produced a greater reduction of depressive symptoms with the effect of VNS becoming greater the longer the treatment. Importantly, the effect of VNS was sustainable in that about 55% of those who responded in the VNS group

were also responders at 12 months, whereas this percentage was only 11.5% in the TAU group.

Results similar to these from the USA were found in an open-label study enrolling patients with TRD from 6 European countries. Seventy patients were evaluated at 3 months with 60 at 12 months [48] and 49 at 24 months [49]. In the initial report [46], response rates of 37% and 53% were found after 3 and 12 months, respectively, whereas remission rates at these times were 13% and 33%, respectively. In the follow-up 2-year study [47], 53% fulfilled the response criterion and 39% did so for the remission criterion [49]. Furthermore, a reanalysis of this study was carried out by Christmas et al. [50] in which only patients who had failed 4 or more antidepressant trials were included. Here, a response rate of 35.7% was found after 12 months, somewhat lower than that found in the entire patient sample. Once again, initial response was reasonably well-maintained over time with almost 40% of those responding at 3 months still responding at 12 and 24 months. These studies, then, demonstrate response to VNS increasing over months with response being reasonably well maintained in these difficult-to-treat depressed patients.

Very recently, Aaronson et al. [51] published a 5-year follow-up to their previous study comparing the effects of VNS or TAU in patients with TRD carried out for 50 weeks. This study has the longest duration of treatment so far. Consistent with their earlier results, the group receiving adjunctive VNS had better clinical outcomes than the group receiving TAU, with those receiving VNS having a significantly higher 5-year cumulative response rate (67.6% vs 40.9%) and a significantly higher remission rate (cumulative first-time remitters, 43.3% vs 25.7%) than those getting TAU.

A recent review by Cimpianu et al. [28] systematically reviewed the evidence for the efficacy of VNS in TRD, as well as other psychiatric disorders. For the other disorders, there were scant data and no conclusions could be made. The interested reader is directed to this comprehensive review, particularly Table 1, which gives details about the 33 studies included in their quantitative analysis, with 24 using standard VNS for TRD [as opposed to transcutaneous VNS (see below)]. As mentioned, the evidence from these in general nonrandomized open studies carried out over long time periods indicates benefit from VNS. This view is supported by a meta-analysis of 6 outpatient multicenter clinical trials of VNS + TAU or TAU alone, all of which were sponsored by Cyberonics, Inc., the manufacturer of the stimulation device. This analysis found that adjunctive VNS therapy was associated with greater response and remission than TAU alone, at time periods from 12 to 96 weeks [26]. Retrospective observational studies also support this view such as one involving Medicare patients [52] where patients with TRD receiving VNS had lower yearly medical costs postimplantation than those receiving TAU, and reduced annual mortality rates.

Nevertheless, it is wise to be cautious in the absence of more RCTs of the effectiveness of VNS in TRD before reaching definitive conclusions. This is likely why some national guidelines recommend VNS for TRD, whereas others are more circumspect (see [28]). But patients with TRD need help today. Given that VNS is generally well tolerated [12, 16, 49], together with the negative consequences of TRD, it should definitely be considered in the armamentarium of treatments for TRD.

A relatively new development may aid in its use for TRD. It has now been established that in humans the auricular branch of the vagus nerve is close to the surface in the ear, particularly the middle-third and lower-third area of the medial surface of the auricle [53]. This observation led to the use of ear clips to stimulate the vagus at this site. Furthermore, other auricular areas do not receive vagal innervation, such as the superior scapha, and this permits sham stimulation to be carried out, as well as actual stimulation. This noninvasive stimulation of the vagus nerve is referred to as transcutaneous VNS (tVNS). Such stimulation produces sensory evoked potentials recorded from the scalp [54], which provides a rationale that this technique could be used to affect brain function. Now, several studies have shown that short-term VNS generates fMRI blood oxygen level-dependent signal activations in limbic and brainstem areas [55, 56]. Furthermore, tVNS does cause changes in functional connectivity in brain in depressed patients, as measured using fMRI [57, 58]. In these latter studies, stimulation was carried out for 30 min twice each day for 5 days each week over 4 weeks. So as with the invasive VNS procedure, there is both an anatomic and functional rationale for studying effects of auricular stimulation of the vagus nerve for different types of brain disorders.

Two studies have assessed the efficacy of tVNS in patients with MDD; there is no indication that these patients were treatment resistant. In the first study [59], 37 patients received stimulation for 15 min either once or twice a day, 5 days weekly, for a rather short time interval (2 weeks). Stimulation parameters were adjusted such that the intensity was just below the threshold of perception, that is, when the stimulus was just noticeable. Sham stimulation consisted of using similar electrodes but having no current applied. Effectiveness was evaluated using both the patient-rated Beck Depression Inventory (BDI) and the physician-completed HAMD. In comparison with the effect of sham stimulation, significant improvement in depressive symptomatology was found using the BDI. However, this was not so for the HAMD, possibly because the sham stimulation had a greater effect on the HAMD than the BDI. Rong et al. [60] studied a total of 160 patients, with 91 receiving only tVNS for 12 weeks, whereas 69 initially received sham stimulation for 4 weeks followed by 8 weeks of tVNS. In this study, sham stimulation did consist of actual stimulation but at a place on the ear not receiving a distribution from the vagus nerve. Stimulation occurred for 30 min twice each day for 5 days

weekly, with the stimulation occurring at home. Stimulation intensity was adjusted to the highest point that the patients could tolerate. After 4 weeks of treatment, tVNS reduced HAMD scores significantly more than the reduction of those receiving sham stimulation and 27% of the patients were judged as responders after 4 weeks of tVNS, whereas no patient achieved response with sham stimulation. Further improvement continued at 8 and 12 weeks.

These preliminary results are promising, but obviously much more research needs to be carried out, not the least of which would be studies to determine the optimal frequency and duration to administer tVNS and whether it would be effective in patients with TRD.

Preclinical Studies

Before reviewing preclinical studies trying to ascertain the mechanism(s) of action of VNS, a brief overview of functional brain-imaging studies in humans treated with it is relevant as such research is helping to understand brain regions and circuits modulated by VNS either acutely or long term. A limitation of such studies, and indeed all clinical studies involving VNS, as mentioned later, is the fact that the study population remains on their drug regimen, which may involve multiple drugs, and can change during the course of the study. Blood cerebral flow, fMRI, and blood oxygen level-dependent fMRI reveal that acute VNS somewhat consistently causes changes in orbitofrontal, anterior cingulate, dorsolateral, prefrontal, and insular cortices, as well as the striatum, cerebellum, and brainstem [25, 61–64]. With regard to the chronic effects of VNS on brain regions, changes have been noted in the thalamus, prefrontal cortex (PFC), limbic system, hypothalamus, medulla, and cerebellum [62, 65–67], although there is some inconsistency with regard to the direction of the change caused by VNS.

As for tVNS in humans, Kraus et al. [56] studied healthy volunteers with electrical stimulation of the nerves in the left outer auditory canal. The brain areas activated by tVNS are consistent with those found with conventional VNS. In the same study, a control group receiving stimulation of the ear lobe instead of the left outer auditory canal showed no effect on limbic areas. Further, in patients with depression, the pattern of brain areas modulated by tVNS is also consistent with that obtained by conventional VNS [57, 68]. Recently, 4 weeks of VNS was found to alter the resting state functional connectivity between the right amygdala and left dorsolateral PFC as well as to enhance activation of the left insula, with such changes associated with improvement in depressive symptomatology [58, 69]. A more recent study using fMRI aimed to optimize activation of brain areas by tVNS by comparing 4 stimulation locations in the ear: the inner tragus, inferoposterior wall of the ear canal, cymba conchae, and

earlobe (sham). Only stimulation of the cymba conchae produced a significantly stronger activation in both the NTS and LC than did the sham stimulation [70]. There have not yet been any studies evaluating such activation in improving depressive symptomatology.

Brain Areas Activated by VNS

Studies with animals are important to elucidate the mechanisms by which VNS produces its effects without confounds of having other drugs on board (as is the case in the clinic), as well as to optimize therapeutic stimulation parameters to increase further the number of patients with TRD achieving response and remission following VNS treatment. Acute, as well as chronic (sustained), activation of brain circuits following VNS treatment have been studied by conventional immunohistochemistry for c-fos or Δ FosB respectively [71–73]. Both c-fos and Δ FosB are expressed following neuronal activation. On the one hand, c-fos expression peaks within 1 to 3 h, being used as a marker of acute neuronal activation [74]. On the other hand, Δ FosB shows a time lag for its expression, but persists longer, so it is used as an indicator of sustained neuronal activation [75, 76].

Using nonanesthetized rats and stimulation parameters that do not cause cardiovascular activation, Cunningham et al. [71] reported that acute VNS treatment (2 h) induces c-fos expression in the NTS, paraventricular nucleus of the hypothalamus, PBN, BNST, and LC, but not in the cingulate cortex or DRN. VNS treatment for 2 weeks revealed significant increases in Δ FosB immunoreactivity in NTS, PBN, LC, peripeduncular nucleus, frontal cortex, cingulate cortex, hippocampus, basolateral amygdala, nucleus accumbens, BNST, and now the DRN [71, 72]. Results such as these show that VNS induces a rather complex pattern of activation of brain circuits implicated not exclusively in regulation of mood. That could perhaps expand the indications for the use of VNS (see [28]). In fact, some other clinical trials show some promise for the use of VNS for neurological diseases [77–79], ischemic stroke [80, 81], drug-seeking behavior [82], and trigeminal allodynia [83], amongst others.

Given the role of the vagus nerve in modulating the inflammatory reflex (see [84]), there has been a developing hypothesis that another putative mechanism by which VNS exerts its therapeutic effects in treating depression could be due to its role in decreasing proinflammatory cytokine synthesis [85, 86]. It is known that higher levels of proinflammatory cytokines are often measured in the serum or cerebrospinal fluid of depressed patients [87–89]. A small clinical trial [86] in patients with TRD showed that before VNS treatment such patients exhibited high levels of some proinflammatory cytokines. Three months following VNS treatment, an increased circulating level of anti-inflammatory cytokines was

measured in the same patients, corroborating the hypothesis that VNS modulates the immune system [86]. More recently another clinical trial showed that VNS inhibited proinflammatory cytokine production [90]; however, this study was not done in patients with TRD, but rather in patients with rheumatoid arthritis that had the disease attenuated by VNS [90]. More studies evaluating the role of VNS in reducing inflammation in patients with TRD are needed.

Effects on Biogenic Amine systems

It has long been hypothesized that the mechanism of action of conventional antidepressants is associated with enhancement in neurotransmission within the serotonergic and/or noradrenergic systems [91–95]. So, electrophysiological recordings from noradrenergic neurons in the LC or serotonergic neurons in the DRN were performed to study, selectively, the activation of these cell types following acute and chronic VNS treatments. These were followed by microdialysis studies to measure extracellular norepinephrine (NE) and serotonin (5-HT) in different brain areas upon acute and chronic VNS treatment. Consistent with the immunohistochemical studies, electrophysiological recordings revealed that VNS acutely increases spontaneous firing activity in noradrenergic neurons in the LC [96], but not in serotonergic neurons in the DRN. This result is consistent with a recent brain-imaging study using positron emission tomography and the selective α_2 adrenergic receptor antagonist [^{11}C] yohimbine in anesthetized minipigs that showed that acute VNS decreased α_2 adrenergic receptor binding in limbic, thalamic, and cortical brain regions [97]. Only chronic (2 weeks) VNS treatment was able to enhance the firing rate of serotonergic neurons, and noradrenergic neurons in the LC remained activated by chronic treatment [96]. Lesion studies revealed that the chronic activation of serotonergic neurons in the DRN is downstream of VNS-induced activation of noradrenergic neurons in the LC. Moreover, the increased activity of the DRN neurons is mediated through activation of postsynaptic α_1 adrenoreceptors [96].

As shown by microdialysis, VNS rapidly increases NE levels in the cortex, medial PFC, amygdala, and hippocampus [98–100]. This is consistent with the rapid activation of NE neurons in the LC, which is upstream of these terminal regions. VNS-induced increases in extracellular NE in the PFC and hippocampus was also reported following chronic treatment [101]. Chronic, but not acute, VNS treatment was also found to cause an increase in 5-HT levels in the DRN, but not in the hippocampus [101].

The effect of VNS on the dopaminergic system shows some peculiarity. Manta et al. [101] found that in spite of dopamine (DA) cells in the ventral tegmental area (VTA) decreasing their firing rate in response to 2 weeks of VNS, extracellular DA levels in the PFC and nucleus accumbens were

increased. Perez et al. [102] found that 2 weeks of VNS treatment in freely moving rats did not affect the number of spontaneously active DA cells in the VTA, nor their firing rate or burst firing [102]. By contrast, in the same study Perez et al. found that in a model where VTA cells were spontaneously active, namely the MAM model of schizophrenia [103, 104], chronic VNS normalized the activity within the VTA cells [102]. This might imply some utility of VNS in the treatment of schizophrenia. To date, there has only been one study such as this, using tVNS [105] of the outer ear canal. In this 26-week study, there was no significant improvement in schizophrenia symptomatology with tVNS *versus* sham tVNS.

Preclinical Behavioral Effects of VNS

The forced swim test (FST) has predictive validity for drugs or therapies that significantly improve depressive symptomatology in humans [106], whereas the NSFT is used to screen anxiolytic-like compounds or therapies [107]. Both acute and chronic VNS treatments decrease the time animals spend immobile in the FST, which is consistent with an antidepressant-like effect of VNS [71, 108, 109]. Chronic, but not acute treatment with VNS has an anxiolytic-like effect [108]. Lesioning either serotonergic or noradrenergic systems completely abolished the VNS-induced antidepressant and anxiolytic-like effects [108]. In the kainic acid rat model for temporal lobe epilepsy, Grimmonprez et al. [110] found a decrease in saccharin preference, quite often associated with the human equivalent of anhedonia, a core symptom of depression. Such a decrease in saccharin preference was reversed upon VNS for 2 weeks. Such results support its clinical efficacy in patients with anhedonia.

Effect on Neurogenesis and Neurotrophins

The neurogenesis theory of depression is based on the findings that there is a stress-induced decrease in adult neurogenesis in the hippocampus, and that treatment with antidepressants reverse such deficit in neurogenesis [111–113]. Biggio et al. [114] examined the effects of acute (3 h) and chronic (1 month) VNS upon cell proliferation and found that the number of BrdU-positive cells in the dentate gyrus was significantly increased 24 h and 3 weeks after treatment. Another study by Revesz et al. [115] also showed that acute (48 h) VNS increased cell proliferation in the hippocampus. Another study looking at the effects of chronic VNS on hippocampal neurogenesis in an animal model of depression, namely bulbectomy, revealed that the bulbectomy-induced decrease in neuronally differentiated BrdU-positive cells

within the dentate gyrus was prevented by VNS but not by sham stimulation [116].

Unlike positive results with acute VNS, acute treatment with traditional ADs does not increase neurogenesis; however, acute ECT, also used for TRD, does [117]. Antidepressants typically take 2 weeks to promote an enhancement in proliferation, but the outcome is rather variable [118–121]. Doublecortin (DCX) is a microtubule-associated protein used as a marker for neurogenesis, as it is expressed by neuronal precursor, as well as immature neurons, for about 2 weeks. Once they differentiate into mature neurons, they no longer express DCX [122, 123]. Both, acute and chronic VNS were associated with significantly higher expression of DCX in the dentate gyrus for up to 3 weeks after discontinuation of treatment [114]. Similar results were found after chronic treatment with fluoxetine [124]. This topic has been reviewed recently [125].

As with the neurogenesis theory, the neurotrophic theory of depression is based on findings that neurotrophic factor expression in brain circuits associated with mood regulation is inversely proportional to the effects of stress and antidepressants. One such neurotrophic factor widely studied in this framework is brain-derived neurotrophic factor (BDNF), along with its receptor, namely tropomyosin receptor kinase B (TrkB). From a functional perspective, the neurotrophic hypothesis is linked to the neurogenesis hypothesis as the increase in expression of neurotrophins with antidepressant treatment may block or reverse the neuronal loss associated with depression [126]. Chronic treatment (21 days) with classical antidepressants belonging to different classes increases expression of mRNAs for BDNF and *trkB* in the hippocampus (CA1, CA3, and dentate gyrus) [127, 128]. Acute (3 h) VNS treatment increases mRNA for BDNF in the hippocampus and cortex [98].

As reviewed by Shah et al. [33], drawing conclusions based on changes in mRNA for BDNF or protein levels can be complicated owing to factors such as translation, proteolytic cleavage, and release that are not accounted for. Hence, analyzing activation of its receptor, TrkB, may provide additional clues about the effect of antidepressant treatment on BDNF function. We have shown that both acute (2 h) and chronic (14 days) VNS activates the TrkB receptor, as shown by its causing phosphorylation at 3 tyrosine residues (Y705, Y816, and Y515) on TrkB [129]. Intraventricular pretreatment with a scavenger compound for TrkB, namely TrkB-Fc, which is a homodimer containing the extracellular ligand-binding domains of TrkB linked to human IgG1, prevented the acute VNS-induced increase in TrkB phosphorylation [130]. This suggests that VNS-induced activation of TrkB requires ligand to bind to it [130]. In contrast to the effects of VNS on the phosphorylation of TrkB, acute and chronic antidepressant treatments only cause phosphorylation at Y705 and Y816 but not at Y515 [129, 131, 132]. Y705 is the

autophosphorylation site, whereas Y816 and Y515 are linked with the phospholipase C gamma 1 (PLC γ 1) and MAPK/PI3K signaling pathways, respectively. Consistent with the phosphorylation of these tyrosine residues, acute VNS, as well as antidepressants, cause phosphorylation of PLC γ 1 [133]; however, this is not maintained with chronic treatments. Only acute and chronic VNS, but not antidepressants, cause phosphorylation of ERK1/2 and Akt that are downstream of Y515 [129, 133]. A recent study by Shah et al. [130] show that intracerebroventricular pretreatment with K252a, an inhibitor of receptor tyrosine kinases, blocks the anxiolytic-like effect of VNS (2 weeks) in the NSFT. Intracerebroventricular pretreatment with K252a also blocked the acute effects of VNS in the FST. Such results indicate that the activation of TrkB by BDNF may be necessary for these behavioral effects of VNS.

As is so clinically, effects of noninvasive tVNS have not yet been intensively studied preclinically. In the Zucker diabetic fatty rat (fa/fa), in addition to the metabolic disarrangements a depressive-like phenotype is observed as seen by an increase in time spent immobile in the FST. Chronic (4 weeks), 30 min daily tVNS ameliorated the depressive-like phenotype [134]. In this study, 2 opposite magnetic electrodes were placed in the right auricular concha regions, inside and out for the cathode and anode, respectively. Finally, although not tVNS, it has been found that electroacupuncture of the auricular concha region, which is densely innervated by nerve endings from the vagus nerve, abolished the behavioral and neurochemical deficits caused by the unpredictable chronic mild stress paradigm [135]. Future studies regarding tVNS or electroacupuncture of the auricular concha regions in rodents need to be performed to evaluate which brain areas are being activated by such paradigms and the effects they produce in such areas, as has now been done for VNS.

In conclusion, VNS has been found to affect many of the same brain areas, neurotransmitters, and signal transduction mechanisms as altered by conventional antidepressants. More often than not, similarities have been found between preclinical effects of VNS and traditional antidepressants, although there are some exceptions [117, 133]. In spite of this, it is not clear what VNS is doing to allow it to be effective in patients when traditional antidepressants are not.

Further, the time course of clinical response to VNS with considerable improvement being noted between 3 to 12 months is quite different from the time course of drug-induced improvement of MDD, where optimal improvement often occurs in 8 to 12 weeks. This difference in the time course of clinical response might indicate some fundamental difference in the mechanisms of action of VNS, that is, indicating time-dependent effects not caused by traditional antidepressants. Another complexity in understanding effects of VNS is the absence of validated, widely used models for TRD, although some have been proposed [136–140]. Also,

animal studies are not carried out on the time scale where VNS is effective. Finally, animal studies have used VNS in the absence of other drugs on board. Yet this is not how VNS is used clinically where, as mentioned, it is added to TAU, which might involve multiple drugs and changing them. Whether there is some interaction between VNS and the effects of such drugs, in particular antidepressants, needs further study.

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Required Author Forms [Disclosure forms](#) provided by the authors are available with the online version of this article.

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