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Latest Views on the Mechanisms of Action of Surgically Implanted Cervical Vagal Nerve Stimulation in Epilepsy

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ABSTRACT

Background: Vagus nerve stimulation (VNS) is approved as an adjunctive treatment for drug-resistant epilepsy. Although there is a substantial amount of literature aiming at unraveling the mechanisms of action of VNS in epilepsy, it is still unclear how the cascade of events triggered by VNS leads to its antiepileptic effect.

Objective: In this review, we integrated available peer-reviewed data on the effects of VNS in clinical and experimental research to identify those that are putatively responsible for its therapeutic effect. The topic of transcutaneous VNS will not be covered owing to the current lack of data supporting the differences and commonalities of its mechanisms of action in relation to invasive VNS.

Summary of the Main Findings: There is compelling evidence that the effect is obtained through the stimulation of large-diameter afferent myelinated fibers that project to the solitary tract nucleus, then to the parabrachial nucleus, which in turn alters the activity of the limbic system, thalamus, and cortex. VNS-induced catecholamine release from the locus coeruleus in the brainstem plays a pivotal role. Functional imaging studies tend to point toward a common vagal network that comes into play, made up of the amygdalo-hippocampal regions, left thalamus, and insular cortex.

Conclusions: Even though some crucial pieces are missing, neurochemical, molecular, cellular, and electrophysiological changes occur within the vagal afferent network at three main levels (the brainstem, the limbic system [amygdala and hippocampus], and the cortex). At this final level, VNS notably alters functional connectivity, which is known to be abnormally high within the epileptic zone and was shown to be significantly decreased by VNS in responders. The effect of crucial VNS parameters such as frequency or current amplitude on functional connectivity metrics is of utmost importance and requires further investigation.

Keywords: Epilepsy, functional connectivity, mechanisms of action, vagal nerve stimulation

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INTRODUCTION

James Corning, an American neurologist, was the first to propose vagal nerve stimulation as a treatment for seizures in 1880. At that time, epilepsy was thought to arise from cerebral hyperemia. Corning aimed at decreasing cerebral blood flow by compression of the carotid arteries while attempting to reduce cardiac output through transcutaneous electrical stimulation of the vagus nerve.[1]

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Direct electrical stimulation of the cervical vagus nerve was later shown to reduce strychnine-induced seizures in cats in 1937.[2] It was subsequently shown that stimulation of vagal afferents could modulate cortical activity independently of any cardiovascular alterations.[3–5] The first commercial vagus nerve stimulator for use in humans, the Vagus Nerve Stimulation (VNS) Therapy System® (LivaNova PLC, London, United Kingdom), was approved in the EU in 1994 as an adjunctive therapy for drug-resistant epilepsy. Efficacy has been shown in controlled and numerous open-label studies.[6–8] Cardiac-based seizure detection (CBSD) was later introduced to VNS Therapy Systems. CBSD involves monitoring heart rate to elicit an additional train of stimulation when rapid heart-rate accelerations, often associated with seizures, are detected. The objective is thus to try to abort a seizure or reduce its propagation and thereby improve the clinical results.[9–12]

Similarly to deep brain stimulation (DBS), whose mechanisms of action are still not entirely understood,[13–17] the full mechanisms by which VNS exerts its antiepileptic effect are still elusive. Many mechanisms in humans and in various animal models have been described.[18–22]

When discussing the mechanisms of action of VNS, the comparison with DBS is far from being irrelevant. Indeed, DBS may be regarded as a peculiar extraphysiological situation in which a lead is inserted within the brain to stimulate excitable structures in the vicinity of the electrode. This complicates the situation because different types of neural elements may be affected by the current, such as neuronal cell bodies and myelinated axons of different diameters and origins (afferent, efferent, passing by fibers, dendrites). However, the pulse width (60 μs) speaks for a predominant effect on fibers.[23–25]

In the case of VNS, the situation seems simpler because of the fascicular structure of the nerve itself that implies that only fiber bundles will be affected. However, the different types of fibers making up the trunk of the nerve may be differently organized across individuals and affected differently by the stimulation depending on pulse width, output current, and electrode design, as modeling simulation suggests.[26] The coverage of the nerve by the helical electrode used by the VNS Therapy® indeed affects the current density decay from the edge of the electrodes to the center of the nerve.[27] The current helical design is designed to cover approximately 75% (270°) of its circumference. Regardless of this, the downstream effects toward the brain nuclei are not easy to disentangle.

In this review, the authors, after a short review of key concepts of vagal fascicular and functional anatomy, present a global overview of the existing literature, updated since the seminal paper by Henry et al[18] reviewing successively functional imaging, neurotransmitter release, or electroencephalogram (EEG) studies. Secondly, the authors—based on their own functional connectivity studies in patients with epilepsy—intend to shed light on the putative mechanisms of action of VNS.

For clarity, the authors will not discuss the mechanisms at work for indications other than epilepsy. The topic of transcutaneous VNS will not be covered either in this review owing to the current lack of data supporting the differences and commonalities of its mechanisms of action in relation to invasive VNS.

Anatomical Reminder: The Vagus Nerve and Its Projection

The tenth cranial nerve is the longest cranial nerve[28] and constitutes the main parasympathetic output of the autonomic nervous system. However, recent literature shows more sympathetic fibers in the vagus nerve than previously assumed.[29]

Based on early studies in cats, unmyelinated C-fibers seem to predominate over fast-conducting intermediate and large-caliber myelinated fibers (B- or A-fibers) in the cervical portion of the vagus nerve.[30] Moreover, in cats,[31] the nerve is made up of approximately 80% afferent fibers, mostly unmyelinated, and 20% efferent fibers, predominantly unmyelinated parasympathetic fibers to viscera, with some myelinated fibers to vocal muscles. In mice, pigs, and humans, it has recently been shown that the percentage of myelinated fibers was 54% ± 7% in the cervical vagus nerve compared with the abdominal vagus nerve, which contains mostly unmyelinated fibers. In humans, the myelinated fibers consist predominantly of small-diameter (63%), medium-diameter (33%), and large-diameter fibers (4%). The number of fibers within the cervical vagus nerve is approximately 50,000 (±10,000).[32] As mentioned by Henry et al,[18] the two vagus nerves are asymmetric with regard to cardiac innervation: the left vagus nerve primarily innervates the AV node, whereas the right vagus nerve innervates the SA node. Moreover, the right vagus nerve carries most of the parasympathetic fibers that densely innervate the cardiac atria.

In addition, 80% of the nerve afferent fibers terminate in the nucleus of tractus solitarius (NTS), which represents the main gateway and processing center for information reaching the brainstem through the vagus nerve.[33] Each vagus nerve connects bilaterally on that key center. The afferent fibers of the left vagus nerve synapse on several other nuclei of the dorsal medulla ipsilaterally. The vagus nerve projects to the nucleus ambiguus, the dorsal motor nucleus of the vagus nerve, the area postrema, spinal trigeminal nucleus, and medial reticular formation. In turn, the NTS has a wide range of projections to several key structures in the brainstem such as the noradrenergic locus coeruleus (LC), serotoninergic raphe nucleus (RN), cerebellum, periaqueducal gray matter, and parabrachial nuclei (PBN). The PBN appears as a key node because of its connections to the insula, thalamus, hippocampus, and amygdala. The ascending vago-solitario-parabrachial pathways provide dense innervation to the limbic system. Vagal-LC and vagal-RN interactions may play an important role, given the widespread noradrenergic and serotoninergic dense cortical innervation stemming respectively from those two brainstem nuclei with potential antiseizure effects in rodents.[34–37]

Which Fibers Are Involved, and Which Are the Actual Targets of VNS?

VNS efficacy was shown to be mainly mediated by fast afferent myelinated A- and B-fibers. Indeed, lesioning below the site of VNS electrode in a canine model of epilepsy did not result in loss of efficacy.[38] No loss of seizure-suppressing effects was reported when C-fibers were chemically inactivated by capsaicin.[39] Moreover, efficacious stimulation amplitudes are in the order of magnitude of 1.5 mA (starting from 0.75 mA), which corresponds to an amplitude that lies below the threshold of slow unmyelinated C-fibers. Fiber-type recruitment follows a size principle (large and heavily myelinated A-fiber being recruited first, followed by myelinated medium-sized B, then C). Computational models indicate that output current between 0.75 and 1.75 mA with pulse width of 250 or 500 microseconds will result in predominant activation of the myelinated fibers.[26] Castoro et al[40] studied the excitation properties of the vagus nerve in dogs across a wide range of

stimulation parameters. The rheobase currents of A- and B-fibers were 0.4 mA and 0.7 mA, respectively, and the chronaxie of both types of fibers was 180 microseconds.

Recent evidence derived from experiments conducted in rodents by Chang et al[41] showed a correlation between the type of physiological response and the type of fibers involved. C-fiber activation was, for instance, strongly associated with breathing changes and apnea. It is unlikely that the amplitude required to recruit C-fibers could be attained in humans without also giving rise to significant adverse effects. Their rodent model suggests that quantitative estimation of nerve fiber engagement could prove pertinent to refine VNS therapy by tailoring the desired type of fiber involvement to the indication (predominant C-fiber activation when anti-inflammatory effect is searched for or predominant B-fibers for heart failure).

Further evidence on the fibers activated in humans by VNS is provided by several studies based on intraoperative compound action potentials (CAPs).

Evans et al[42] measured CAPs of the vagus nerve fiber intraoperatively. A-fiber potentials were recordable and activated by very low stimulus currents, A-delta and C-fibers being less reliably elicited, with C-fibers requiring the highest intensities.

Usami et al[43] recorded scalp-evoked potential as a marker of the afferent impulse in clinical vagal nerve stimulation. The short-latency components of the vagus nerve–evoked potential (estimated conduction velocity conduction of 27.4 ± 10.2 m/s) were regarded as directly resulting from the involvement of A-fibers of the nerve.

Vespa et al[44] showed the ability to noninvasively record the laryngeal motor–evoked potentials (LMEPs) induced by VNS that could serve as a biomarker of nerve activation and enable better titration of the parameters. As shown by Chang et al, these data are suggestive of selective involvement of A-α fiber activations, but no significant differences in VNS-induced LMEPs were found between responders and nonresponders.[41]

VNS Therapy and Neurotransmitter and Cytokines Release

Early studies have shown that VNS therapy induces significant alterations of neurotransmitter release. Roosevelt et al[45] found increased extracellular concentrations of noradrenaline (NA) in the rat cortex and hippocampus after vagus nerve stimulation at 1 mA. Interestingly, the increase remained confined to the period of stimulation. No increase of NA release occurred <0.5 mA. Using in vivo microdialysis in rats, Hassert et al[46] also showed that VNS caused a 98% increase of NA.

The amplitude of the P3 component of the event-related potential, a marker of LC activity associated with NA release in the brain, was shown to be significantly increased in responders.[47]

In an animal model of limbic seizures, VNS-induced changes in extracellular hippocampal levels of NA, dopamine, serotonin, and γ-amino butyric acid (GABA) were measured in freely moving rats. A strong positive correlation was found between the noradrenergic and antiseizure effects of VNS.[48] Blockade of hippocampal α(2)-receptors reversed the seizure-suppressing effect of VNS.[49]

Other neurotransmitters such as GABA, glycine, and various amino-acid pools were found to be modified by VNS in the brain.[20,50,51] These studies are of interest for two reasons: first, they were conducted in human patients, and second, they investigated the chronic effect as opposed to studies focusing on acute alterations of neurotransmitter release. Furthermore, it has been postulated that immunologic and antiinflammatory action could play a role in the beneficial effects of VNS. A few studies investigated the changes of proinflammatory cytokines and tryptophane metabolites induced by VNS in peripheral blood.[52–54]

Functional Imaging Studies

Functional imaging studies have shown that VNS induces significant changes in cerebral blood flow. Studies showed regional increase of cerebral blood flow, mainly in the thalamus and the cerebral cortex. Ko et al[55] found, using positron emission tomography (PET) H_2O blood flow imaging, that VNS caused activation of several central areas, including the contralateral thalamus.

Henry et al[56] showed VNS-induced cerebral blood flow increase in bilateral thalami, hypothalami, and inferior cerebellar hemispheres. These alterations were found to occur immediately but also were shown to persist at three months. Functional magnetic resonance imaging (fMRI) studies also have shown bilateral activation, maximal in thalami (left more than right) and insular cortices.[57]

Marrosu et al[58] applied single-photon emission computerized tomography with the benzodiazepine receptor inverse agonist [123I]iomazenil to examine cortical GABA(A) receptor density (GRD) before and one year after VNS implant. VNS therapeutic responses were significantly correlated with the normalization of GRD.

Recently, a resting state–fMRI functional connectivity study on the brainstem-cortical/subcortical structures in eight controls and eight VNS responder patients led to the conclusion that VNS could reorganize the altered functional connectivity (Fc) between the brainstem and insula, precuneus, and cerebellum.[59]

Similarly, in 66 children with epilepsy who had VNS, Yu et al[60] compared metabolic connectivity between responders and nonresponders (≥50% seizure frequency decrease) using preoperative fluorodeoxyglucose PET. Relative changes in glucose metabolism were strongly connected among the areas of the brainstem, cingulate gyrus, cerebellum, bilateral insula, and putamen in patients with ≥50% seizure frequency reduction after VNS. These results support the existence of specific preexisting connectivity patterns in responders vs nonresponders. As suggested by Hachem et al,[61] preoperative connectivity studies not only may serve as biomarkers for selection of patients but also may provide insights as to VNS action mechanisms.[62]

More recent studies aiming at predicting VNS response based on structural and functional connectomic profiling have offered insights. VNS responders showed greater fractional anisotropy in the left thalamocortical, limbic, and association fibers and greater connectivity in a functional network encompassing the left thalamic, insular, and temporal nodes, pointing toward a similar network to that of early functional imaging studies.[63] A similar study from the same group had first shown that thalamocortical intrinsic connectivity could play a key role in the preoperative estimated response to VNS, supporting further the existence of a specific network coming into play and affected by VNS.[64]

Impact on Brain Electrophysiological Signals

EEG Synchronization and Power Spectral Analysis

Early animal studies dating back to the 1960s sought to determine the effect of acute vagal nerve stimulation on the EEG. These studies showed that the effect was highly dependent on stimulation parameters and particularly on the amplitude and frequency[4]: Synchronization (spindle-like activity) could be observed at 1 to 17

Hz, whereas desynchronization seemed to be associated with higher stimulation frequencies (>30 Hz).

Chase et al[5] reported that much higher frequency and voltage gave rise to synchronization (100 Hz or 200 Hz), whereas 25 Hz resulted in desynchronization. Salinsky et al[65] analyzed the effect of acute VNS on awake EEG rhythms in humans. They found no effects on spectral analysis, even in patients showing apparent response to VNS.

Marrosu et al[66] investigated the chronic effect of VNS on EEG rhythms. These authors compared the power spectrum before and one year after VNS implant and found no significant changes for the delta, theta, alpha, and beta bands but an increase in power in the gamma band. They also observed a significant decrease in synchronization in theta frequencies and an increase in gamma band and interhemispheric synchronization.

Ernst et al[67] also reported that acute VNS stimulation resulted in desynchronization in theta bands in ECoGs recorded in patients with dual (VNS and responsive neurostimulation) neurostimulators.

Brádzil et al[68] studied EEG reactivity to standard stimuli (photic, hyperpnea) to predict individual response to VNS. Power spectral analysis revealed significant differences in EEG reactivity between responders and nonresponders; in particular, the dynamics of alpha and gamma activity was shown to be strongly associated with VNS efficacy.

Spikes and Interictal Epileptiform Activity

Irrespective of spectral analysis, a decrease in spike frequency could be shown with VNS in several studies. Hallböök et al[69] reported that VNS reduced interictal epileptiform discharges (IEDs), especially during sleep (rapid eye movement, delta-sleep) and the number of recorded EEG seizures. In addition, Zanchetti et al[3] observed a reduction of spindles and suppression of spiking activity after afferent vagal stimulation on the EEG of a cat. Koo[70] investigated EEG changes in patients at baseline and three, six, and 12 months postoperatively. VNS was shown to induce EEG changes in the form of clustering of epileptiform activity followed by progressively increased periods of spike-free intervals. Kuba et al[71,72] studied the effect of both acute and chronic vagal nerve stimulation on IEDs. The authors compared the rate and duration of IEDs on EEG at baseline and at five-year follow-up visit in 32 patients and found both the rate and duration to decrease significantly over time, especially in responders. Wang et al[73] showed on serial EEGs a progressive decrease in the number of IEDs with time.

The effect of VNS on interictal epileptiform activity also was recorded with intracranial recordings using one hippocampal depth electrode in a patient with intractable seizures. Spike frequencies and the occurrence of epileptiform sharp waves were compared before and during VNS using a 5- and a 30-Hz stimulus. Stimulation at 30 Hz produced a significant decrease in the occurrence of epileptiform sharp waves, whereas 5-Hz stimulation was associated with a significant increase in epileptiform sharp waves, providing additional evidence as to the decisive role of the stimulating frequency.[74]

Cortical Excitability

In addition, VNS was shown to modulate cortical excitability in a rat model of motor cortex excitability. De Herdt et al[75] observed an overall significant increase of the motor seizure threshold after one hour of VNS compared with baseline. Another study[76] from the same group showed that output current intensities as low as 0.25 mA were sufficient to decrease cortical excitability in a protocol of stimulation of five one-hour periods for four days. Higher output intensities (0.5–1 mA) did not result in further increase of cortical excitability thresholds. These data are of great interest because they clearly show the effect of VNS upon cortical excitability and highlight the absence of a linear correlation between the amplitude of the output current and the decrease in excitability. These results are consistent with our own findings (described later).

The question of the effect of output current was addressed by Bunch et al[77] on the basis of the retrospective analysis of a multicenter randomized trial of three unique paradigms of VNS. It was shown that in 61 patients, the output current, ranging from 0.25 to 1.5 mA, was not a major determinant of the acute response to VNS. As found by other studies, many patients acutely respond to low current (<1 mA), and only a few showed further improvement through a higher output current.[77,78]

Resting State MEG Connectivity Analysis

On the basis of a resting state magnetoencephalography (MEG) connectivity analysis, Babajani-Feremi et al[79] found differences between VNS responders and nonresponders. They observed that the modularity and transitivity in VNS responders were significantly larger and smaller, respectively, than those observed in VNS nonresponders. Despite the impressive ability of their model to properly classify the patients between controls (prediction accuracy of 87%), VNS responders, and nonresponders, identification of the characteristics of the network of patients likely to respond to VNS does not provide clear insights into how VNS affects the involved networks in responders.

Brain Functional Connectivity Studies

Functional connectivity refers to the statistical link that can exist between the activities recorded from distinct brain structures, reflecting links between underlying neuronal populations. Functional connectivity at the macroscopic scale can be measured by EEG (scalp or intracranial), MEG, and fMRI (the latter being an indirect marker of neural activity through the hemodynamic response).[80] Connectivity measures across several channels lead to potentially very complex matrices (that can, in addition, evolve with time). It is thus interesting to summarize these data using methods derived from graph theory to describe the general organization of brain networks and their efficiency in transmitting information.[81]

Fraschini et al[82] showed that VNS-induced global decrease of functional connectivity in the gamma band was significantly higher in responders than in patients who failed to show improvement after VNS. The analysis was based on the phase lag index (PLI),[83] which allows the study of the global functional connectivity among the EEG sensors and reduces the effect of volume conduction. Subsequent studies have extensively investigated the effect of chronic VNS on EEG functional connectivity, including the one by Bodin et al[84] from our group looking at the alterations of connectivity between ON and OFF stimulation periods. In 19 patients with chronic VNS, responders to VNS (R) had reduced interictal functional connectivity on scalp EEG. R had a lower global level of functional connectivity (EEG broadband) than nonresponders ($p < 0.0001$). In addition, ON periods of stimulation were characterized by lower values of Fc than OFF periods.[84]

We replicated this study with intracerebral recordings (depth electrodes) on five adult patients who simultaneously had stereoelectroencephalography (SEEG) and a VNS implant.[85] Interdependencies between bipolar SEEG channels were estimated by

nonlinear regression analysis (h^2 index) and compared between ON and OFF periods of stimulation. All these patients but one had no benefit from VNS. The only responder had >50% reduction of seizure on VNS but was still very disabled by his seizures. Fc analysis revealed an increase of functional connectivity values during ON periods for four patients and decreased values for the R patient. These results were consistent with the hypothesis that the therapeutic effect may be related to the VNS-induced decrease in functional connectivity. Furthermore, and most importantly, this study analyzed several combinations of stimulation parameters. Surprisingly, Fc tended to vary in a nonlinear fashion with different settings of stimulation. This implies that setting the parameters of stimulation is complex and merely increasing the intensity or frequency of stimulation may prove irrelevant to decrease the synchrony and optimize the therapeutic effect.

More recently, Sangare et al[86] replicated these results in 35 patients with epilepsy. The synchronization in scalp-EEG time series was compared between ON and OFF periods of stimulation, using average PLI in sensor space and phase-locking value between ten sources. For responder patients, PLI during ON periods was significantly lower than that during OFF periods in several EEG subband frequencies (delta, theta, and beta). For nonresponders, there were no significant differences. The correlation between VNS-induced interictal EEG time-series decrease in functional connectivity and decrease in seizure frequency suggested that the therapeutic effect of VNS may be related to changes in interictal functional connectivity. A recent study[87] investigated the effect of VNS on Fc during two states of vigilance (wakefulness vs stage-N2 sleep). Weighted PLI was computed as a connectivity measure of synchronization for VNS OFF and ON conditions in 24 patients. In responders, stronger VNS-induced theta desynchronization ($p < 0.05$) was found in sleep but not during wakefulness. There also is some evidence showing that chronic VNS influences network measures. Fraschini et al[82] reported significant effects of long-term VNS on Minimum Spanning Tree in responders with a more integrated/efficient global network, results that were, however, not replicated by Sangare et al,[86] and Vespa et al[87] showed a stronger decrease of global efficiency during sleep in responders than in nonresponders.

Interictal connectivity is profoundly modified in patients with epilepsy.[88] SEEG and EEG/MEG connectivity studies revealed an increased connectivity within the epileptogenic zone (EZ), whereas the other zones tended to show decreased connectivity.[89] Reducing the hyperconnectivity in the EZ could be a pivotal mechanisms of VNS therapy in focal epilepsies. In generalized epilepsy, a more widespread effect on Fc is probably at work along with modulation of the noradrenergic drive.

In addition, the effect of VNS stimulation on functional connectivity during seizure has been investigated. Focal seizures are associated with an increase in functional connectivity affecting the EZ network and also distant regions (propagation networks) (Bartolomei et al[88]). Ravan et al[90] evaluated whether the automated delivery of VNS at seizure onset could reduce the severity of seizures as reflected by EEG spatial synchronization—a measure of seizure propagation. Acutely stimulated seizures displayed reduced ictal spread, indicating that when delivered within the appropriate time frame, VNS may decrease spatial synchronization (extent of hypersynchrony) in addition to temporal synchronization.

In another study by the same authors,[91] it is reported that automatic delivery of VNS therapy acutely reduces ictal spatial synchronization (EEG-based quantitative feature) in patients who later responded to VNS in long-term follow-up, indicating a susceptibility of the same network to VNS to be responsible for both acute seizure termination and also long-term seizure prevention. These findings show that the timing of stimulation also is pivotal in the equation.

Table 1. Main VNS Studies in Epilepsy With Special Attention to the Stimulation Parameters.

Study	Parameters
Hammond et al[20]	1.25–3 mA, 10–30 Hz (1 patient at 2 Hz), 500 μs
Evans et al[42]	0.25–3 mA, 1–5 Hz, 16 stimuli during surgery
Usami et al[43]	0.25–1 mA, 30 Hz, 130–500 μs
Vespa et al[44]	0.25–1 mA, 20 Hz, 250 μs
Bouckaert et al[92]	>0.25 up to tolerability, 30 Hz, 130–500 μs, 7 s ON–18 s OFF
De Taeye et al[47]	0.75–3 mA, 25–30 Hz, 250–500 μs
Ben-Menachem et al, 1995[51]	High stim = 1.25–3 mA, 30 s ON–5 min OFF Low stim = 1.25–3 mA, 3 s ON–90 min OFF
Aalbers et al[52]	High stim = 0.25 mA, 30 Hz, 500 μs, 30 s ON–5 min OFF Low stim = 0.25 mA, 1 Hz, 100 μs, 14 s ON–1 min OFF
Klinkenberg et al[54]	High stim = <2.75 mA, 30 Hz, 500 μs, 30 s ON–5 min OFF Low stim = <2.75 mA, 1 Hz, 100 μs, 14 s ON–60 min OFF
Ko et al[55]	2 mA, 30 Hz, 60 s during PET scan
Henry et al[56]	High stim = 0.25–2.5 mA, 30 Hz, 500 μs, 30 s ON–5 min OFF Low stim = 0.25–1.25 mA, 1 Hz, 130 μs, 30 s ON–180 min OFF
Narayanan et al[57]	0.5–2.0 mA, 30 Hz, 30 s ON and 30 s OFF
Marrosu et al, 2013[58]	1.75–2 mA, 30 Hz, 500 μs, 30 s ON–5 min OFF
Zhu et al[59]	1.5 mA, 30 Hz, 250 μs, 30 s ON–5 min OFF
Yu et al[60]	2–2.5 mA, 30 s ON–5 min OFF
Hallböök et al[69]	1.5 mA, 30 Hz, 500 μs, 30 s ON–5 min OFF
Koo et al[93]	Increase of 0.25 mA every 2 wk up to patient tolerance, 20–30 Hz, 500 μs, 30 s ON–5 min OFF
Kuba et al[71]	20–30 Hz, 500 μs, 30 s ON–5 min OFF or 21 s ON–3 min OFF
Kuba et al[72]	20–30 Hz, 500 μs, 21 s ON–3 min OFF or 21 s ON–1.8 min OFF
Wang et al[73]	0.75–1.75 mA, 20–30 Hz, 250–500 μs, 30 s ON–5 min OFF
Olejniczak et al[74] (case report)	2.75 mA, 30 Hz, 500 μs, 30 s ON–1.1 min OFF
Bunch et al[77]	Group A: <1.5 mA, 20 Hz, 500 μs, 7 s ON–18 s OFF Group B: <1.5 mA, 20 Hz, 250 μs, 30 s ON–30 s OFF Group C: <1.5 mA, 30 Hz, 500 μs, 30 s ON–3 min OFF
Fraschini et al[82]	30 Hz, 30 s ON–5 min OFF
Bodin et al[84]	0.5–2.5 mA, 30 Hz, 500 μs
Bartolomei et al[85]	1.5–2 mA, 30 Hz, 30 s ON–500 s OFF
Ravan et al[90]	>0.5 mA

Stim, stimulation.

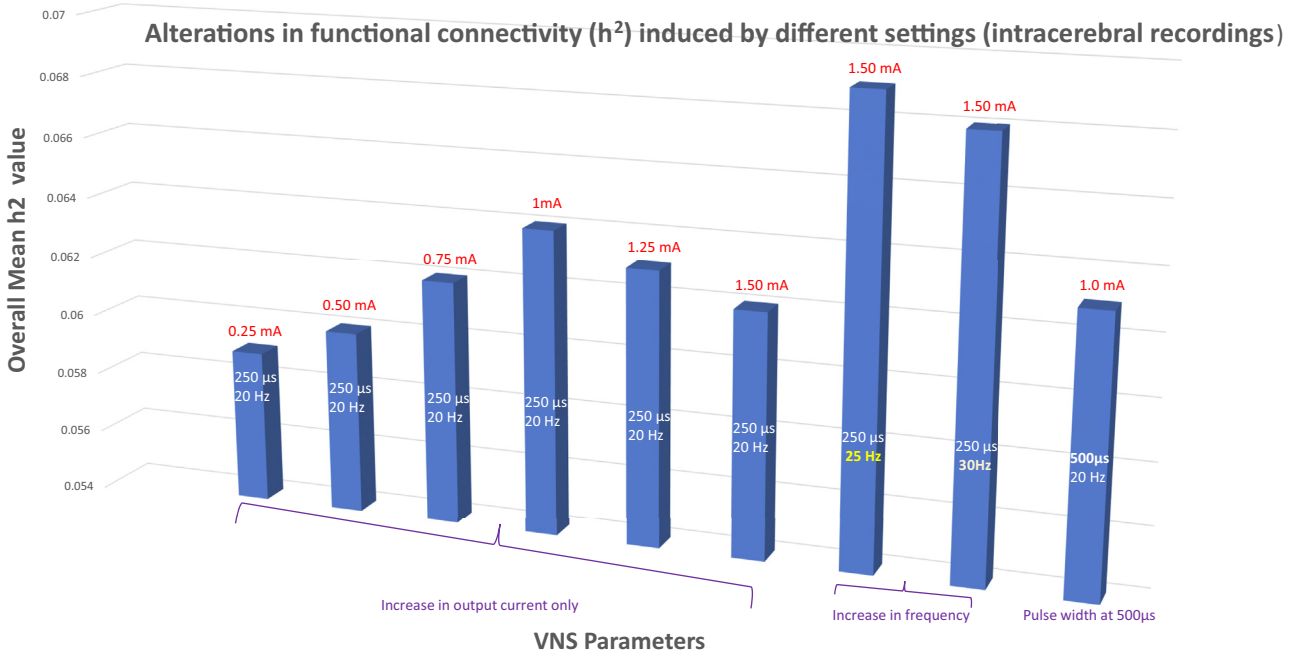

Figure 1. Alterations in functional connectivity (h^2) under different VNS parameter settings. The figure shows the nonlinear relationship between functional connectivity index (here h^2 derived from intracerebral recordings) and VNS parameters. Increasing the stimulation amplitude from 0.25 to 1 mA gives rise to an increase in Fc, whereas a further increase from 1 to 1.5 mA leads to its decrease. Changing the frequency from 30 to 20 Hz or the pulse width from 250 to 500 ms decreases the Fc. Data derived from Bartolomei et al[85] globally show a high variability across patients and plead for an individualized analysis of connectivity indexes to optimize VNS parameters in each patient. [Color figure can be viewed at www.neuromodulationjournal.org]

DISCUSSION

Despite the large number of studies, crucial puzzle pieces are missing to fully understand the antiseizure effect of VNS. The means by which the information is processed, filtered, and relayed from one input nucleus to the successive output structures within the so-called vagal afferent network[61] is obviously highly complex. A linear model of successive bottom-up transmission of information (excitation/inhibition) may be too crude to reflect the complexity of the effect of stimulation delivered at 20 to 30 Hz and may turn out to be a misconception of the dynamic modulation induced by stimulation.

The effect on the cortex itself viewed as a final target can be studied regardless of the preceding steps occurring within the "black box." A clear understanding of the correlation between the parameters of stimulation and functional connectivity and spatial and temporal synchronization still needs to be established.

As shown with the nonlinear relationship between parameters and functional connectivity, there is probably no such thing as one single mechanism but several, depending on parameters of stimulation and duty cycle, which significantly vary across studies (Table 1), and certainly depending on the dynamic state of the brain when the stimulation reaches the target neurons.

Individual variability plays a role as well. The variability of the vagus nerve itself determines the activation profile of fibers throughout the nerve.[26] As for duty cycle, it was shown in freely moving rats that VNS applied with a rapid cycle (7 seconds on, 18 seconds off) was more robust and had a greater effect on hippocampal EEG than the standard cycle (30 seconds on, 300 seconds off).[94]

Other parameters, such as the pattern of stimulation itself[95] and the timing of stimulation in relation to seizure onset, also play a key role. As shown by Contreras et al,[96] neocortical activation is determined not only by the dynamic character of the input but also by the intrinsic dynamics of the cortical circuitry. The epileptic brain may not require the same stimulation all the time. Switching parameters gives rise to different and even opposite effects, as shown by the early EEG studies with frequency. Merely increasing the output current may not always be the right way to optimize the effect. Increasing from 0.25 to 1 mA gives rise to an increase in Fc, whereas a further increase from 1 to 1.5 mA leads to its decrease (Fig. 1). Indeed, excessive output current may prove deleterious. One hypothesis could be that response to VNS obeys an inverted U shape curve. This was reported in some studies on VNS and central plasticity[97] and hippocampal progenitor proliferation[98] and in a more recent study on parameters associated with clinical response in epilepsy.[99]

The nonlinear relationship between the alteration of parameters and functional connectivity not only highlights the tremendous complexity of the issue but also should prompt an analysis of the effect of each parameter individually and in a controlled way. An ongoing multicentric randomized controlled study called OPSTIM-VAG is under way in France (study registration number: ISD RCB: 2020-A02657-32/SI: 20.10.26.53022). In the treatment group, the settings of parameters are determined on the basis of the PLI-based functional connectivity values. Furthermore, the effect may vary among patients and epilepsy type. With the advent of personalized medicine, a biomarker of functional connectivity would be very useful to set up the parameters on the basis of an objective and reproducible factor. It would be a smart way to shorten the trial-and-error period and may allow us to quickly

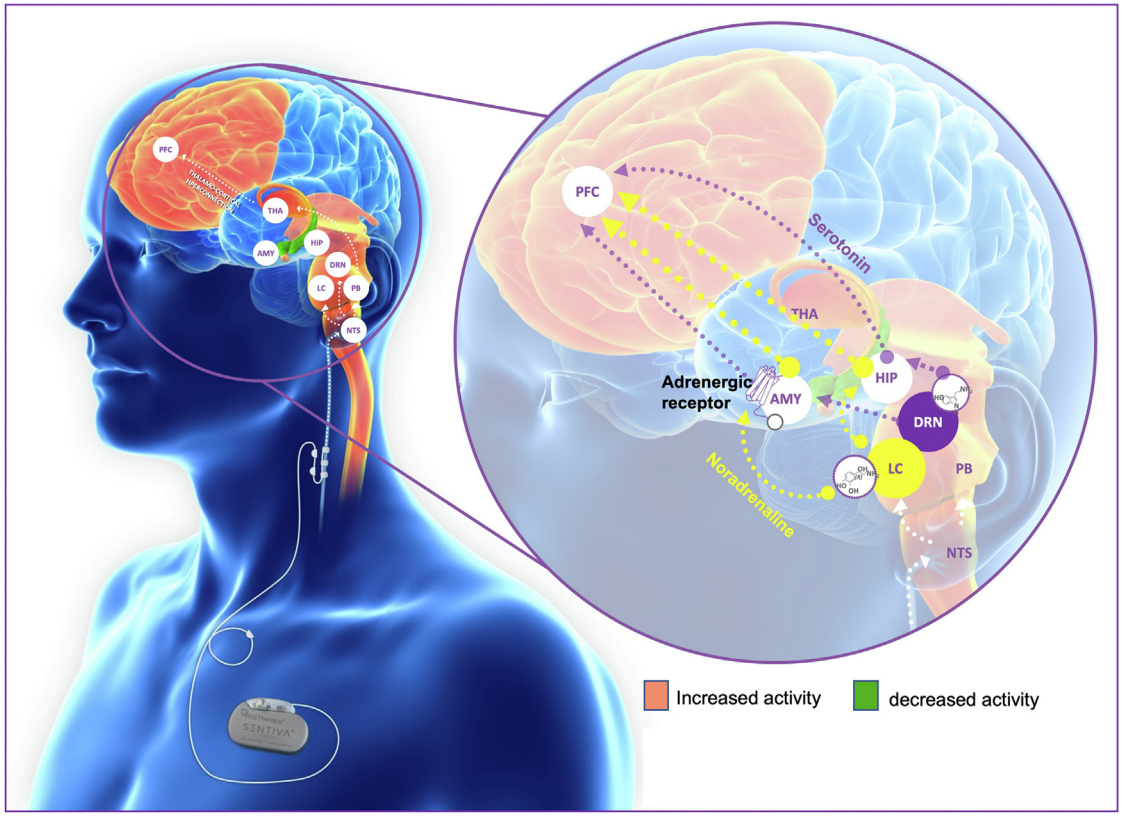

Figure 2. Main structures involved in the afferent vagal network. [Color figure can be viewed at www.neuromodulationjournal.org]

identify nonresponders and provide guidance for these patients toward other strategies, such as DBS with innovative targets. Even though many studies clearly show that VNS reduces both functional connectivity (global interictal synchronization) and spatial synchronization, it is still unclear how this effect is obtained. It is likely that the desynchronizing effect of VNS derives from the alteration of the activity of brainstem nuclei such as the NTS that in relay strongly modifies the activity of the left thalamus, which then has widespread connections to many cortical areas (Fig. 2). Bilateral VNS, although still unexplored, could amplify the effect of VNS on functional connectivity by bringing into play the right-sided vagal network, in addition to the left.

CONCLUSIONS

The modulation of the vagal afferent network through stimulation by an implanted VNS device triggers a cascade of neurochemical and electrophysiological events that arise at the brainstem, then reach the limbic system and eventually the cortex. Increasing evidence obtained from surface EEG and depth recordings has shown that in responders, VNS is associated with a reduction in functional connectivity. Thus, it can be concluded that VNS can counteract specific epileptic networks, reducing their abnormally high connectivity through modulation of the vagal afferent network. The dismantlement of the individual role of each of the different parameters of VNS on functional connectivity metrics will undoubtedly be of the utmost importance to advance further.

Authorship Statements

Romain Carron wrote the original draft of the manuscript and edited its different versions. Paolo Roncon edited the document and designed Figure 2. Stanislas Lagarde, Maxine Dibue, Marc Zanello, and Fabrice Bartolomei reviewed and edited the different versions of the manuscript.

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COMMENT

The submitted article thoroughly reviews the literature of basic experimental research for VNS in epilepsy. The authors carefully guide the reader from the first results of VNS to anatomy, cellular, and neurotransmitter research to functional magnetic resonance imaging and electroencephalogram studies. Therefore, the review provides a good overview on actual findings and opens perspectives for future studies.

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