



Vagal nerve stimulation for refractory epilepsy: a brief review

Mojdeh Navidhamidi^{1,†}, Nasrin Mehranfard²

ABSTRACT

Vagus nerve stimulation (VNS) is an effective adjunctive treatment for patients with drug-resistance epilepsy. Short and long-term VNS studies demonstrate a seizure-suppressing effect, with a more efficacy in long-term treatment. Precise mechanisms of VNS in attenuating seizure are not fully understood. Clarification of VNS mechanisms of action may improve its clinical efficacy, as it may provide strategies for the development of new therapeutic candidates for patients with refractory epilepsy. It also might be beneficial for identification of optimal stimulation parameters. In this review article, we present some possible mechanisms involved in anti-epileptic effects of VNS.

Keywords

Vagal nerve stimulation, Epilepsy, Locus coeruleus, norepinephrine, serotonin, dopamine

Introduction

Epilepsy is a common neurological disorder and affects about 50 million people worldwide [1]. Most of the patients with epilepsy are successfully controlled with antiepileptic drugs (AEDs), however, approximately 20–40% of patients are refractory to the available AEDs [2]. Vagus nerve stimulation (VNS) is an alternative and promising neurosurgical method for treating patients with refractory epilepsy [3–6]. VNS is a low-risk surgery that involves the placement of a bipolar electrode that is wrapped around the left cervical vagus nerve, with chronic intermittent electrical stimulation of the vagus nerve provided by a remarkably small pulse generator. This pulse generator is implanted subcutaneously in the upper chest and is connected to the electrode. VNS in patients usually is applied at frequency of 20–30 Hz and a stimulation cycle with 30 seconds on, and 5 minutes off. In rats, VNS is applied at frequency of nearly 10–20 Hz, with duration of 0.5–1 ms; and stimulus intensity of 0.2–0.5 mA/mm² of nerve cross-section. Clinical

studies demonstrate more than 50% reduction in seizure frequency in the first year of VNS treatment in 20–40% of patients [7,8]. There are many reports on safety and effectiveness of VNS therapy for children and adults with refractory epilepsy [9–14], but the precise mechanism of action of VNS in suppressing epileptic seizures has not been fully elucidated. The purpose of this review is to focus on the potential mechanisms which may underlie anti-epileptic effect of VNS.

Anti-epileptic effects of VNS

Anticonvulsant effects of VNS have been shown both in human and in experimental models with partial-onset seizures. It reduces or abolishes complex partial seizures in several animal models of epilepsy [15–18]. In penicillin-induced focal interictal spikes, 20 s of VNS reduced the frequency of interictal spike activity by 33% [19]. In children [20] and adult patients with epilepsy [21,22], chronic VNS treatment also decrease interictal epileptiform discharges. In addition to partial seizures, VNS has anticonvulsant effects on generalized seizures

¹School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran

²Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[†]Author for correspondence: Nasrin Mehranfard, Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Ira Iran; Tel. (work): +98-21-66927171; Mob: +989123933646; email: m-navid@tums.ac.ir

of induced by pentylenetetrazol (PTZ) [23-27], mercaptopropionic acid (3-MPA) [28,29], maximal electroshock [29,30] and strychnine [31,32].

VNS treatment in the majority of cases is associated with a clear improvement in seizure activity. Nevertheless, it does not alter or even increases seizure activity in other cases. For example, VNS diminished seizures induced by kainic acid as well as by strychnine and penicillin [16-18] in a number of animals whereas increased seizures in other ones [16,17]. In canine PTZ model of seizure, 1 hour of VNS did not affect seizure threshold [33], while threshold for focal motor seizures in the cortical stimulation model significantly increased following 1 h of VNS [34]. Absence seizures induced by PTZ reduced by VNS whereas absence seizures in genetic absence epilepsy rats from strasbourg (GAERS) did not affect by VNS [4,35,36] and even prolonged the duration of the abnormal EEG pattern at the first day of stimulation. VNS also is ineffective in nearly one-third of patients with epilepsy [6,9,37,38]. This variability of the response to VNS have been suggested to be due to difference in vagus nerve activation, genetic variability in the number of noradrenergic neurons which may be associated with an alteration in NE released levels and synaptic efficacy within noradrenergic neuronal networks [39]. It also might be due to a difference in stimulation parameters. For instance, VNS application with stimulus intensities of that activate mainly myelinated vagal fibers (A and B fibers) has been found to reduce the excitability of more number of cortical neurons than the intensities that recruit nonmyelinated fibers (C fibers) [40]. Stimulus intensity also seems to be an effective feature in NE release (an critical neurotransmitter mediated in therapeutic effects of VNS) from Locus coeruleus (LC) neurons, since stimulation at 0.25 mA had no effect on NE concentrations, while the 0.5 mA stimulation increased NE concentrations significantly in the hippocampus and stimulation at 1.0 mA significantly increased NE concentrations in both the cortex and hippocampus bilaterally [41]. In addition to stimulus intensity, frequency can also affect VNS efficacy for epilepsy. For example, a rat study showed that optimal frequency for inhibition of spike-and-wave seizures is in the range of 130-180 Hz compared to the 30 Hz VNS applied in the clinic [42].

VNS application in different time points might also affect efficacy of the therapy for epilepsy. For

example, Rijkers et al reported that a single three-minute pulse of VNS, 1 min before amygdala kindling and for 2 min later decrease seizure duration, whereas the latency of generalized seizures onset enhanced in a subpopulation of the rats [43]. In another study, application of VNS immediately after the kindling stimulus indicated anti-convulsant effects in rats [5]. Moreover, 1 h of VNS treatment before the kindling in rats induced a delay in generalized seizure activity [44] whereas application of VNS 2 h prior to the kindling stimulus did not affect the kindling rate in rats compared to controls [5]. In another study, VNS when was applied simultaneously with the kindling stimulation, did not affect seizure severity [45]. These findings suggest that VNS could be more effective as treatment is started in very early stages before or after seizure induction.

VNS application in early stages after amygdala kindling has also been found to induce a delay in seizure development, but once animals reached to a more severe stage VNS do not affect seizures [46,47], suggesting VNS can stop seizures progression, but it cannot suppress seizures when they start. Efficacy of VNS for seizure control also increases over time. For instance, follow-up of 26 patients during 5 year revealed a 28% reduction in seizure frequency at 1 year that reached to 72% at 5 years [48].

Anti-epileptic mechanisms of action of VNS

■ Desynchronization

Desynchronization of neuronal activity initially was hypothesized to be a mechanism of action of VNS. The hypothesize arise from several studies that demonstrated VNS can lead to EEG desynchronization [49-53]. Such desynchronization typically illustrates an arousal state and is opposite to EEG neuronal hypersynchronization observed during seizures. Hence, it was assumed that desynchronization of these hyper-synchronized neuronal activities would result in anti-epileptic effects of VNS [54]. VNS has been reported to be both EEG desynchronizing and synchronizing, depending on the stimulation parameters used [50,55,56]. Experiments on cats showed that the effect of VNS on EEG is frequency dependent and is related to the activation of certain fibers at different frequencies [57]. VNS at frequencies more than 70 Hz and intensities above 3 Volts is desynchronizing. Lower frequencies (20 to 50 Hz) and higher voltages were also found to cause EEG desynchronization [58]. Also, VNS at 1 to

15 m/s has been shown to be desynchronizing [51].

Early studies revealed that VNS, in contrast to animals, acutely do not affect EEG in human [59]. However, the evaluation of long term effect of VNS showed an alternating synchronization and desynchronization of EEG, with the desynchronization that progressively become dominant over synchronization [60].

■ VNS effect on alertness and sleep

Chronic VNS has been found to improve alertness in patients with refractory epilepsy [61,62]. Arousal states have been found to considerably increase seizure resistance [63,64]. Indeed, there are evidence that show seizures may occur preferentially during sleep [65-67] with a rarely occurrence during rapid eye movement (REM) sleep. In fact, REM sleep has showed a strong anti-epileptic effect against focal interictal discharges as well as focal and generalized seizures [68]. Chronic VNS has showed an increase in REM sleep [69] and has been associated with an anticonvulsant effect [70].

Anti-epileptic effect of both alertness and REM sleep has hypothesized to be due to a desynchronized EEG pattern which is associated with a reduction in occurrence of spatial and temporal summation of aberrant depolarizations [68,71]. Since neocortical synchronization is controlled by the activities in cholinergic, norepinephrinergic and serotonergic ascending systems [72], thus VNS effects on EEG desynchronization in REM sleep and alertness might be through modulating these systems. Indeed, cholinergic, serotonergic, noradrenergic and dopaminergic networks originating in the pons and midbrain are considered to be modulators of arousal [73]. An interaction of between epinephrine and ascending vagal fibers in modulating arousing events has also been reported [74]. A role of cholinergic system in VNS-mediated arousal state might be due to activation of nucleus basalis (NB). Because VNS activates NB [75] and NB activation has been shown to promote arousal state and suppress seizure activity [76,77]. The relationship between VNS effects and cholinergic system is also described by the study in which topical application of scopolamine to exposed feline cortex produced seizures whereas physostigmine (an acetylcholinesterase inhibitor) prevented development of seizures [78].

■ Norepinephrine

The LC is considered to be a crucial structure in the seizure-suppressing effects of VNS, since inactivation or bilaterally lesioning of LC prevents VNS attenuating seizures in the maximal electroshock model [30]. The LC is major source of noradrenergic neurons in the brain [79] and sends direct projections to the hippocampus where epileptic seizures in temporal lobe epilepsy (TLE) frequently originate [80]. Acute VNS produces an increase in the LC activity by 24% or 33% above baseline whereas chronic VNS produces substantial activation of the LC neurons [81,82]. VNS-induced hyperactivity of the LC neurons increases extracellular NE levels in the hippocampus [15, 41], amygdala [83] and cortex [41] in rats. NE is considered to play a critical role in effectiveness of VNS therapy [30,84]. In a study in the canine PTZ model of seizure, 1 h of VNS induced a significantly increase in NE levels in the CSF, but did not affect seizure threshold in these animals [33].

NE decreases the activity of hippocampal pyramidal neurons [85,86]. Furthermore, chronic VNS has been reported to increase tonic activation of α 2-adrenoceptors onto hippocampal pyramidal neurons [87] and blockade of these receptors reverses VNS-induced seizure suppression [39]. This suggests a link between enhanced noradrenergic signaling and seizure suppressing effect of VNS.

■ Serotonin

A profound loss of serotonin (5-HT) throughout the brain [88] (except in the striatum) in a rat model of myoclonic epilepsy as well as an enhanced levels of 5-HT in an atypical absence model of AY9944-treated rats [89] has been reported in previous studies, suggesting a disturbed serotonergic transmission in epileptic states. Serotonergic transmission possibly play an important role in seizure suppressing effect of VNS, since destruction of serotonergic neurons in dorsal raphe nucleus (DRN) prevents antiepileptic effects of VNS in PTZ-induced seizures in rats [90]. It has been shown that serotonergic neurons in the DRN are hyper-activated after VNS application [81,91-93]. Indeed, VNS has been found to promptly enhance firing rate of the LC neurons after only 1 h and secondarily increase the firing rate of serotonin neurons after 14 days [81]. The delayed increase in neuronal activity of serotonergic neurons following chronic VNS might be, at least partly, responsible for greater

efficiency VNS over time. The stimulatory effect of VNS on firing rate of serotonergic neurons is NE-dependent and is mediated via an activation of their α_1 -adrenoceptors. Additionally, selective lesioning of the LC suppresses hyperactivity of the serotonin neurons by VNS [92,93]. VNS-induced hyper-activity of the serotonin neurons increases the release of 5-HT in the vicinity of DRN 5-HT cell bodies [81,87]. Hence, alteration in firing activity of serotonin neurons might be due to an increased 5-HT in DRN that change the sensitivity of 5-HT_{1A} somatodendritic autoreceptors on serotonin neurons. A short term increase in 5-HT content of the DRN by antidepressant drugs activates these autoreceptors and decreases their firing activity [94], but long term increased 5-HT levels in DRN by these drugs desensitizes the autoreceptors and restores firing rate of serotonin neurons to normal levels [95]. Surprisingly, sensitivity of the autoreceptors do not affect by chronic VNS [81]. This suggests that other mechanisms might be involved in hyper-activity of DRN serotonin neurons following VNS.

Enhanced firing rate of serotonin neurons would be expected to enhance release of 5-HT in projection areas of the serotonin neurons such as the hippocampus and cortex. VNS, however, does not alter 5-HT levels in the hippocampus [15] and cortex [86].

Chronic VNS has been demonstrated that increases 5-hydroxyindoleacetic acid (5-HIAA; a marker for serotonin activity) levels in cerebral spinal fluid of epileptic patients [96]. VNS-induced hyper-activity of serotonin neurons and elevated levels of 5-HIAA in the CSF despite unaltered 5-HT contents in the hippocampus and cortex might represent increased rates of 5-HT turnover by VNS. A modification in sensitivity of serotonergic terminal autoreceptors (5-HT_{1B}; which regulate the amount of neurotransmitter released) [97] might also be involved in the firing activity changes in DRN serotonin neurons as well as the observed unaltered 5-HT levels in the hippocampus and cortex. Together, these data suggest that VNS might normalize a possible disturbed levels of 5-HT in structures involved in seizure generation, thereby contributing to seizure suppression.

■ Dopamine

The vagus nerve impairment lead to the inhibition of dopamine (DA) in the brain structures [98]. This event suggests that vagus nerve function is necessary for normal action of

dopamine system in the brain. This is supported by study that demonstrates VNS therapy in a rat model of schizophrenia restores aberrant mesolimbic dopamine signaling [99]. In this schizophrenia model, hippocampal hyperactivity was associated with dopaminergic system dysfunction. VNS reduced the hippocampal hyperactivity, decreased hyperactivity of ventral tegmental area (VTA) and normalized DA levels in the hippocampus. Indeed, there is evidence that hyperactivity within the hippocampus lead to aberrant mesolimbic dopamine signaling [99-103]. These findings suggest that a hippocampal hyper-excitability in limbic seizures (which are most often complex partial seizures) might be associated with aberrant dopamine signaling [104] and VNS therapy is likely to exert an anticonvulsant effect through normalizing activity of mesolimbic system. Acute VNS has been shown that do not affect DA levels [105]. While chronic VNS enhances extracellular DA levels in the prefrontal cortex and nucleus accumbens, despite a significant reduced firing activity of VTA neurons [87]. This suggests that an increased extracellular DA, at least in the cerebral cortex, after chronic VNS is not dependent to dopaminergic system activity. Indeed, it has been found that extracellular DA in the cerebral cortex originates not only from dopaminergic terminals but also most often from noradrenergic neurons where DA is co-released with NE [106-108]. An elevated DA levels in the frontal cortex following chronic VNS, despite VTA hypo-activity, might contribute to VNS-improved cognitive performance, since normal cognitive function appear only within a restricted range of dopaminergic and noradrenergic activity [109-114].

■ VNS effect on neuronal excitability

A significantly increased threshold for focal motor seizures induced by cortical electrical stimulation in rats following 1 h of VNS suggests a modulatory effect of VNS on cortical excitability [34]. VNS has been suggested that can modulate neuronal excitability through an activation of muscarinic receptors [115] and increased inhibition of cortical neurons [40,116]. Acute VNS increases the excitatory synaptic transmission at perforant path-granule cells (GCs) synapses and decreases hippocampal GCs excitability [117]. It increases excitatory synaptic transmission in the hippocampal CA3 area through activation of the LC and β -adrenergic receptors since lesions of LC neurons or the β -adrenergic receptor antagonist prevents the

enhancement in synaptic transmission of CA3 neurons by VNS [118]. These data suggest that the increased excitatory synaptic transmission and the reduced GC excitability is likely to contribute to efficacy of VNS treatment for learning deficits and epilepsy especially in limbic epilepsy, respectively.

Anticonvulsive effect of VNS also might, in part, be via an NO action on neuronal activity. Since, inhibition of NO synthase (NOS) in rats has been shown to reverse the inhibitory effect of VNS on amygdala-evoked responses of neurons in medial prefrontal cortex [119]. This effect is likely to be through an inhibitory action of NO on glutamate release, because pharmacological inhibition of NOS induces interictal discharge activity in somatosensory cortex and the CA1 region of the hippocampus through an interaction between NO and glutamate system [120].

The LC stimulation increases NE release in the cerebral cortex [121] and is associated with the enhancement of cortical neuronal activity [122,123]. The increased cortical neuronal activity has been suggested that result from crosstalk between NE with neurons, astrocytes and arterioles [124-126] which lead to a hyperemic response to activation of the LC-NE pathway. Since there is evidence regarding reduced neuronal activity by VNS treatment in epileptic states, hence VNS might modulate crosstalk between neurons, astrocytes and arterioles through LC-NE pathway so that cortical neurons produce a pronounced increase in the inhibition compared to excitation [40,116].

■ VNS effect on inflammatory responses

There is growing evidence that epilepsy is associated with inflammatory responses and with increased levels of cytokines [127-130]. The effect of VNS on inflammatory responses has been demonstrated in several studies. VNS decreases systemic inflammatory responses through inhibition of the release of tumor necrosis factor (TNF) from macrophages and prevents the development of shock during lethal endotoxaemia in rats [131]. In contrast, vagotomy significantly increases the release of TNF and promotes the development of shock [131]. Anti-inflammatory effect of VNS in this study was mediated via an action of acetylcholine (the principle vagal neurotransmitter) on nicotinic acetylcholine receptor alpha7 subunit of immune cells that inhibits macrophage TNF release [132]. In patients with refractory epilepsy, long term VNS

treatment (6 months) significantly decreases IL-8 induction [133]. VNS also regulates the immune system via alteration in cytokines levels and rebalancing the release of pro-inflammatory and anti-inflammatory cytokines in patients with refractory epilepsy [134].

Activation of anti-inflammatory responses by VNS has been associated with its neuroprotective effects on the hippocampal neurons [135]. This neuroprotective effect of VNS may mediate through an interaction of NE and NOS. Since a decreased level of NE is associated with NOS2 expression within cortical neurons which in turn, lead to neuronal damage and increased inflammatory responses? These responses were reversed by alpha2-adrenergic antagonist [136]. Anti-inflammatory effects of VNS may also be exerted via suppression of NOS2 in glial cells mediated by NE [137]. Since both NOS inhibition and NOS expression are associated with an aggravated seizure state, hence NE might play an essential role in the expression of normal NOS levels in the brain.

■ VNS effect on GABA and glutamate systems

A possible mechanism by which VNS appears to exert anti-epileptic effects might be through a reduction in damage to GABAergic inhibitory neurons within the cerebral cortex and possibly the hippocampal formation [138]. In addition, in patients with partial epilepsy, VNS causes an increase in the inhibitory neurotransmitter levels of GABA in the CSF [96] and normalizes cortical GABA-receptor density [139]. VNS treatment in another study did not alter GABA levels in the hippocampus [15]. These data suggest an enhanced GABA receptor-mediated neuronal inhibition may contribute to the therapeutic efficacy of VNS.

VNS also might affect glutamate system in epileptic states. Because it has been reported that VNS causes a reduction in glutamate levels in the CSF of patients with partial seizures [96]. Since, epilepsy is associated with an excessive increase in glutamate (main excitatory neurotransmitter in CNS) levels; reduced glutamate content in the CSF suggests an anti-epileptic effect of VNS through modulation of glutamate release. Thus, it is possible to hypothesize a neuroprotective role for VNS, probably via reduced glutamate release.

■ Neuronal plasticity

VNS increases expression of fibroblast growth factor and brain derived neurotrophic factor

(BDNF) in the hippocampus and cortex of rats [140,141]. There is evidence that suggest reduced levels of neurotrophic factors, most notably BDNF contribute to the hippocampal atrophy (HA) and memory impairment in Alzheimer disease and depressed patients [142,143]. Furthermore, the HA has been hypothesized to be a common pathogenic mechanism of TLE [143] and side of seizure onset in TLE has been suggested to be correlated with degree of hippocampal atrophy [144]. Hence, an elevated expression of BDNF by VNS therapy might reduce seizure occurrence and improve epilepsy-related disorders (including mood as well as learning and memory impairments) through decreasing hippocampal atrophy (HA is caused due to an increased neuronal damage).

VNS for 2 days is also associated with elevated stem cell proliferation (neurogenesis) in hippocampal dentate gyrus in the adult rat, possibly in part, via an altered noradrenergic activity [145]. Moreover, 3 h of VNS increased the cell proliferation in rat hippocampus that was apparent 24 h and 3 weeks later [140]. However, chronic VNS for 1 month did not change the number of proliferating cells [140].

Hippocampal sclerosis is a frequently pathology in patients with TLE characterized by extensive gliosis with a combination of selective neuronal loss in neuronal loss in hippocampal CA1–4 subregions [146]. A partial loss of excitatory inputs from CA3 to CA1 region due to epilepsy-induced neuronal loss reduces excitatory drive to inhibitory interneurons in stratum lacunosum-moleculare of CA1 which, in turn result in CA1 hyper-excitability [147]. Chronic VNS has been found to induce an increased expression of BDNF in the hippocampal CA3 region [140] which may serve to re- enervate inhibitory interneurons through formation of new neuronal connections [148], and thereby may contribute to anti-seizure effects of VNS. BDNF also induces sprouting of serotonergic fibers in the hippocampus that might be contribute to seizure suppressing effects of VNS [149].

A role of NE and serotonin has been demonstrated both in progenitor proliferation [150,151] and in neuronal sprouting [152, 153], hence VNS-induced neuronal plasticity might be mediated through altered neurotransmission of NE and serotonin.

Possible structures involved in VNS mechanism of action

Identification of the structures activated by VNS might be effective to clarify the antiepileptic mechanism of VNS, possibly via recognition of inhibitory pathways activated by VNS.

■ Thalamus

The thalamocortical network has a capacity to generate both physiological and pathological rhythmical activity, including sleep spindles and spike-wave discharges [154,155]. VNS might modulate activity of this network by disrupting the genesis of pathological rhythmical activity and, thereby control seizure activity. An increased fos (a marker for high neuronal activation) expression has been reported in the thalamic habenular nuclei following VNS [156]. VNS in cat enhances cellular firing rate in the contralateral ventro-postero-medial nuclei of the thalamus [157]. Positron emission testing (PET) studies in human have showed that both high and low levels of VNS increases regional cerebral blood flow (rCBF) in thalami [158-160]. In a study performed on five patients with complex partial seizures, VNS led to activation of the frontal and occipital lobes in all patients. In this study, activation of the thalamus was observed only in the two patients, but with an improvement in seizure control [161]. Indeed, it has been suggested that there may be a relation between thalamic activation and a desirable seizure control. Supporting this idea is a PET study that showed an increase in thalamic blood flow of patients with epilepsy in response to VNS is associated with improvement in seizure control [160].

■ Cerebellum

There is evidence that show cerebellum is involved in modulating seizures [162,163]. Several studies have reported an effect of VNS on the cerebellum. In a study, VNS caused a significant increase in rCBF of ipsilateral cerebellum in epileptic patients [164]. In another study in rabbits, VNS produced field potentials in the ipsilateral and contralateral nodulus and uvula of the cerebellar vermis [165]. A beneficial role of cerebellar vermis in reducing seizure activity is supported by the study that demonstrated cerebellar vermis stimulation in anesthetized cats decrease focal penicillin-induced seizures [166]. Since the LC bilaterally projects to the cerebellum [167,168], the cerebellar effects of VNS are likely mediated by the LC activation.

■ Nucleus solitaries

Anticonvulsant effect of VNS also might be exerted through nucleus of the solitary tract (NTS). NTS receives afferents from vagus nerve which, in turn, send projections to many regions, including the LC, DRN, thalamus, amygdala, forebrain, and through medullary reticular formation to other cortical regions [58, 169-173].

Chronic VNS significantly increases staining of Δ FosB bilaterally in tractus solitaries [174,175]. VNS in rats has been found to cause inhibition of the NTS correlated with reduction of glucose metabolism in this structure [176]. A hypo-activity role of NTS in suppressing seizure activity is supported by data obtained from the study that show the administration of GABA agonist or glutamate antagonist (for enhancing GABA transmission or inhibiting glutamate transmission, respectively) in the mediocaudal nucleus solitarius lead to seizure suppression [177].

■ Cortex

Several studies have shown VNS effects on the cerebral cortex. In a study performed on five patients with complex partial seizures, VNS led to activation of the frontal and occipital lobes in all patients [161]. In another study in rats, VNS caused a reduced glucose metabolism in frontal and parietal cortices, suggesting an inhibition in these structures [176]. In dogs, acute microburst VNS (a novel stimulation paradigm) produced a significant hypoperfusion in the left frontal lobe and in the right parietal lobe [178]. The LC provides the majority of noradrenergic innervation to the cortex. The LC projections to the cerebral cortex are predominantly ipsilateral [167, 168]. Hence, VNS possibly modulates cortical activity and blood flow through LC projections to this area.

■ Limbic structures

Limbic structures are known to be involved early in the propagation of abnormal neuronal activity through the epileptic network underlying complex partial seizures. These structures include hippocampus, amygdala, hypothalamus, cingulate gyrus, parahippocampal gyrus and septal area [179]. VNS has been found to affect most limbic structures. For instance, VNS increases fos expression in amygdala and cingulate gyrus [156]. It also causes a significant change in blood flow of cingulate gyrus [180]. PET studies in human have showed that both high and low levels of VNS increases cerebral

blood flow in hypothalamic and insular cortices [158,159]. Reduced glucose metabolism in the hypothalamus has also been reported by VNS therapy in rats [176], suggesting an inhibition of this structure. Another study, demonstrated a significant hypo-perfusion in the right and left parahippocampal gyrus [181]. A reduction in blood flow of the hippocampal region has also been described in several studies [160,182, 183]. A hypo-perfusion state is linked to decreased neuronal activity and functional inhibition [184,185] and may reflect a suppression of seizure propagation.

Conclusion

Although the precise mechanisms of VNS in suppressing seizure are not fully understood, it is thought to involve vagal afferents to the NTS, which in turn, projects to the LC. Both the structures have widespread projections to the brain, thereby modulating neuronal systems involved in seizure generation and propagation. Multiple processes work together to be mediated anti-epileptic effects of VNS. These include increased activity of the LC neurons with an elevated NE release in the hippocampus and cortex as well as amygdala, the hypo-activity of VTA neurons with a possible normalization of DA levels in the hippocampus, the hyper-activity of DRN serotonin neurons with an increased level of 5-HT in DRN and a possible normalization of 5-HT levels in other brain structures, the activation of anti-inflammatory and neuroprotective responses, activation of adrenergic signaling pathway, VNS-modulatory effects on other neurotransmitter systems such as cholinergic, GABAergic, glutamatergic and NOergic. These effects are dependent to the activation of LC-NE pathway.

It seems that VNS could be more effective as treatment starts at early stages before or after seizure onset. It thus may important to determine the preventative role of VNS in human epileptogenesis when the treatment begins at the early stages of disease. Surprisingly, a retrospective study has recently showed that outcome of drug therapy is comparable to VNS therapy [186]. Although further studies are needed to determine optimal stimulation parameters, there are two convincing explanations for more advantage of VNS therapy than available AEDs used. First, VNS treatment is effective in approximately more than 50% patients with refractory epilepsy which do not

respond to AEDs. Second, epilepsy-related long-term morbidities including enhanced incidence of depression as well as learning and memory impairment may progress despite sufficient

seizure control with current AEDs. VNS therapy might ease the large burden of these epilepsy comorbidities through an increased expression of neurotrophic factors and neurogenesis.

References

- Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N. Engl. J. Med* 342(0), 314-319 (2000).
- French JA. Refractory epilepsy: clinical overview. *Epilepsia* 48(1), 3-7 (2007).
- Boon P, Vonck K, Reuck J De, et al. Vagus nerve stimulation for refractory epilepsy. *Seizure. Eur. J. Epilepsy* 10(6), 448-55 (2001).
- Dedeurwaerdere S, Vonck K, Hese P Van, et al. The acute and chronic effect of vagus nerve stimulation in genetic absence epilepsy rats from Strasbourg (GAERS). *Epilepsia* 46(5), 94-97 (2005).
- Dedeurwaerdere S, Gilby K, Vonck K, et al. Vagus nerve stimulation does not affect spatial memory in fast rats, but has both anti-convulsive and proconvulsive effects on amygdala-kindled seizures. *Neuroscience* 140(1), 1443-1445 (2006).
- Vonck K, Thadani VY, Gilbert K, et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. *J. Clin. Neurophysiol* 21(4), 283-289 (2004).
- Handforth A, DeGiorgio CM, Schachter SC, et al. Vagus nerve stimulation therapy for partial-onset seizures: A randomized active-control trial. *Neurology* 51(1), 48-55 (1998).
- The Vagus Nerve Stimulation Study Group. A randomized controlled trial of chronic vagus nerve stimulation for treatment of medically intractable seizures. *Neurology* 45(2), 224-230 (1995).
- Herd V De, Boon P, Ceulemans B, et al. Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. *Eur. J. Paediatr. Neurol* 11(5), 261-269 (2007).
- DeGiorgio CM, Schachter SC, Handforth A, et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. *Epilepsia* 41(9), 1195-1200 (2000).
- Murphy JV, Torkelson R, Dowler I, et al. Vagal nerve stimulation in refractory epilepsy: the first 100 patients receiving vagal nerve stimulation at a pediatric epilepsy center. *Arch. Pediatr. Adolesc. Med* 157(6), 560-564 (2003).
- Benifla M, Rutka JT, Logan W, et al. Vagal nerve stimulation for refractory epilepsy in children: indications and experience at the hospital for sick children. *Childs. Nerv. Syst* 22(8), 1018-1026 (2006).
- Rychlicki F, Zamponi N, Trignani R, et al. Vagus nerve stimulation: clinical experience in drug-resistant pediatric epileptic patients. *Seizure* 15(7), 483-490 (2006).
- Elliott RE, Morsi A, Kalhorn SP, et al. Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: long-term outcomes and predictors of response. *Epilepsy. Behav* 20(1), 57-63 (2011).
- Meurs A, Clinckers R, Raedt R, et al. Vagus nerve stimulation suppresses pilocarpine induced limbic seizures and increases hippocampal extracellular noradrenalin concentration. *Epilepsia* 49(1), 350 (2008).
- Stoica I, Tudor I. Effects of vagus efferents on strychninic focus of coronal gyrus. *Rev. Roum. Neurolog* 4(1), 287-295 (1967).
- Stoica I, Tudor I. Vagal trunk stimulation influences on epileptic spiking focus activity. *Rev. Roum. Neurolog* 5(1), 203-210 (1968).
- Zanchetti A, Wang SC, Moruzzi G. Effect of afferent vagal stimulation on the electroencephalogram of the cat in cerebral isolation. *Boll. Soc. Ital. Biol. Sper* 28(4), 627-628 (1952).
- McLachlan RS. Suppression of interictal spikes and seizures by stimulation of the vagus nerve. *Epilepsia* 34(1), 918-923 (1993).
- Hallbook T, Lundgren J, Blennow G, et al. Long term effects on epileptiform activity with vagus nerve stimulation in children. *Seizure* 14(8), 527-533 (2005).
- Kuba R, Nesvadba D, Brazdil M, et al. Effect of chronic vagal nerve stimulation on interictal epileptiform discharges. *Seizure* 19(6), 352-355 (2010).
- Wang H, Chen X, Lin Z, et al. Long-term effect of vagus nerve stimulation on interictal epileptiform discharges in refractory epilepsy. *J. Neurol. Sci* 284(1-2), 96-102 (2009).
- Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibers does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats. *Epilepsia* 42(5), 586-589 (2001).
- Krahl SE, Senanayake SS, Handforth A. Right-sided vagus nerve stimulation reduces generalized seizure severity in rats as effectively as left-sided. *Epilepsy. Res* 56(1), 1-4 (2003).
- Sahin D, Ilbay G, Imal M, et al. Vagus nerve stimulation suppresses generalized seizure activity and seizure-triggered postictal cardiac rhythm changes in rats. *Physiol. Res* 58(3), 345-350 (2009).
- Zhang JL, Zhang SP, Zhang HQ. Antiepileptic effect of electroacupuncture vs. vagus nerve stimulation in the rat thalamus. *Neurosci. Lett* 441(2), 183-187 (2008a).
- Zhang JL, Zhang SP, Zhang HQ. Antiepileptic effects of electroacupuncture vs vagus nerve stimulation on cortical epileptiform activities. *J. Neurol. Sci* 270(1-2), 114-121 (2008b).
- Sunderam S, Osorio I, Watkins III JF, et al. Vagal and sciatic nerve stimulation have complex, time-dependent effects on chemically-induced seizures: a controlled study. *Brain. Res* 918(0), 60-66 (2001).
- Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. *Epilepsia* 31(2), S7-S19 (1990).
- Krahl SE, Clark KB, Smith DC, et al. Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia* 39(7), 709-714 (1998).
- Zabara J. Peripheral control of hypersynchronous discharge in epilepsy. *Electroencephalogr. Clin. Neurophysiol* 61(1), S162 (1985).
- Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation. *Epilepsia* 33(6), 1005-1012 (1992).
- Martle V, Raedt R, Waelbers T, et al. The effect of vagus nerve stimulation on CSF monoamines and the PTZ seizure threshold in dogs. *Brain. Stimul* 8(1), 1-6 (2015).
- Herd V De, Waele J De, Raedt R, et al. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures. *Acta. Neurol. Scand* 121(4), 271-276 (2010).
- Dedeurwaerdere S, Vonck K, Claeys P, et al. Acute vagus nerve stimulation does not suppress spike and wave discharges in genetic absence epilepsy rats from Strasbourg. *Epilepsy. Res* 59(2-3), 191-198 (2004).
- Dedeurwaerdere S, Cornelissen B, Laere K Van, et al. Small animal positron emission tomography during vagus nerve stimulation in rats: a pilot study. *Epilepsy. Res* 67(3), 133-141 (2005a).
- Ben-Menachem E, Manon-Espaillet R, Ristanovic R, et al. Vagus nerve stimulation for treatment of partial seizures: I. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group. *Epilepsia* 35(3), 616-626 (1994).
- Handforth A, DeGiorgio CM, Schachter SC, et al. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. *Neurology* 51(1), 48-55 (1998).

39. Raedt R, Clinckers R, Mollet L, *et al.* Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J. Neurochem* 117(3), 461-469 (2011).
40. Zagon A, Kemeny AA. Slow Hyperpolarization in Cortical Neurons: A Possible Mechanism Behind Vagus Nerve Stimulation Therapy for Refractory Epilepsy. *Epilepsia* 41(11), 1382-1389 (2000).
41. Roosevelt RW, Smith DC, Clough RW, *et al.* Increased Extracellular Concentrations of Norepinephrine in Cortex and Hippocampus Following Vagus Nerve Stimulation in the Rat. *Brain. Res* 1119(1), 124-132 (2006).
42. Jiao, Harreby J, Sevcencu C, *et al.* Optimal Vagus Nerve Stimulation Frequency for Suppression of Spike-and-Wave Seizures in Rats. *Artif. Organs* 40(1), E120-7 (2016).
43. Rijkers K, Aalbers M, Hoogland G, *et al.* Acute seizure-suppressing effect of vagus nerve stimulation in the amygdala kindled rat. *Brain. Res* 1319(1), 155-163 (2010).
44. Naritoku DK, Mikels JA. Vagus Nerve Stimulation (VNS) is antiepileptogenic in the Electrical Kindling Model. *Epilepsia* 38(1), 220 (1997).
45. Raedt R, Waterschoot L, Wyckhuys T, *et al.* Effect of acute and long-term vagus nerve stimulation in fully kindled rats. *Epilepsia* 50(1), 93 (2009).
46. Magdaleno-Madrigal V, Valdes-Cruz A, Martinez-Vargas D, *et al.* Effect of vagus nerve stimulation on later stages of amygdaloid kindling in freely-moving cats. *FENS. Abstr* 2 A124.118 (2004).
47. Magdaleno-Madrigal VM, Valdes-Cruz A, Martinez-Vargas D, *et al.* Effect of vagus nerve stimulation on electrical kindling in different stages of seizure severity in freely moving cats. *Epilepsy. Res* 108(1), 81-89 (2014).
48. Spanaki MV, Allen LS, Mueller WM, *et al.* Vagus nerve stimulation therapy: 5-year or greater outcome at a university-based epilepsy center. *Seizure* 13(8), 587-590 (2004).
49. Zanchetti A, Wang SC, Moruzzi G. The effect of vagal afferent stimulation on the EEG pattern of the cat. *Electroencephalogr. Clin. Neurophysiol* 4(3), 357-361 (1952).
50. Chase MH, Sterman MB, Iemmett CDC. Cortical and subcortical patterns of response to afferent vagal stimulation. *Exp. Neurol* 16(1), 36-49 (1966).
51. Chase MH, Nakamura Y, Iemmett CDC, *et al.* Afferent vagal stimulation: neurographic correlates of induced EEG synchronization and desynchronization. *Brain. Res* 5(2), 236-249 (1967).
52. Chase MH, Nakamura Y. EEG response to afferent abdominal vagal stimulation. *Electroencephalogr. Clin. Neurophysiol* 24(4), 396 (1968).
53. Sasaki H, Coffey P, Villegas-Perez MP, *et al.* Light induced EEG desynchronization and behavioral arousal in rats with restored retinocollicular projection by peripheral nerve graft. *Neurosci. Lett* 218(1), 45-48 (1996).
54. Zabara J. Time course of seizure control to brief repetitive stimuli. *Epilepsia* 26(1), 518 (1985).
55. Balzamo E, Jammes Y. Vagal afferents and EEG rhythms in the SI area in anesthetized cats: similarities between responses to electrical and chemical (phenyldiuganide) stimulations. *Arch. Int. Physiol. Biochim* 97(6), 483-492 (1989).
56. Magnes J, Moruzzi G, Pompeiano O. Synchronization of the EEG produced by low frequency electrical stimulation of the region of the solitary tract. *Arch. Ital. Biol* 99(1), 33-67 (1961).
57. Rutecki P. Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus nerve stimulation. *Epilepsia* 31(2), S1-S6 (1990).
58. Shi C, Flanagan SR, Samadani U. Vagus Nerve Stimulation to Augment Recovery from Severe Traumatic Brain Injury Impeding Consciousness: A Prospective Pilot Clinical Trial. *Neurol. Res* 35(3), 263-276 (2013).
59. Hammond EJ, Uthman BM, Reid SA, *et al.* Vagus nerve stimulation in humans: neurophysiological studies and electrophysiological monitoring. *Epilepsia* 31(2), S51-S59 (1990).
60. Koo B. EEG changes with vagus nerve stimulation. *J. Clin. Neurophysiol* 18(5), 434-441 (2001).
61. Rizzo P, Beelke M, Carli F De, *et al.* Chronic vagus nerve stimulation improves alertness and reduces rapid eye movement sleep in patients affected by refractory epilepsy. *Sleep* 26(5), 607-11 (2003).
62. Malow BA, Edwards J, Marzec M, *et al.* Vagus nerve stimulation reduces daytime sleepiness in epilepsy patients. *Neurology* 57(5), 879-84 (2001).
63. Kotagal P, Yardi N. The relationship between sleep and epilepsy. *Semin. Pediatr. Neurol* 15(2), 42-49 (2008).
64. Beenhakker MP, Huguenard JR. Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy. *Neuron* 62(5), 612-632 (2009).
65. Billiard M. Epilepsies and the sleep-wake cycle. *Sleep. Epilepsy* 269-286 (1982).
66. Passouant P. Historical aspects of sleep and epilepsy. *Epilepsy. Res* 2(1), 19-22 (1991).
67. Touchon J, Baldy-Moulinier M, Billiard M, *et al.* Sleep organization and epilepsy. *Epilepsy. Res* 1(1), 73-81 (1991).
68. Ng M, Pavlova M. Why are seizures rare in rapid eye movement sleep? Review of the frequency of seizures in different sleep stages. *Epilepsy. Res. Treatm* 932790: 2013 (2013).
69. Valdes-Cruz A, Magdaleno-Madrigal VM, Martinez-Vargas D, *et al.* Long-term changes in sleep and electroencephalographic activity by chronic vagus nerve stimulation in cats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32(3), 828-834 (2008).
70. Fernandez-Guardiola A, Martinez A, Valdes-Cruz A, *et al.* Vagus nerve prolonged stimulation in cats: effects on epileptogenesis (amygdala electrical kindling): behavioral and electrographic changes. *Epilepsia* 40(7), 822-829 (1999).
71. Shouse MN, Scordato JC, Farber PR. Sleep and arousal mechanisms in experimental epilepsy: epileptic components of NREM and antiepileptic components of REM sleep. *Ment. Retard. Dev. Disabil. Res. Rev.* 10(2), 117-21 (2004).
72. Timofeev I, Bazhenov M, Seigneur J, *et al.* Neuronal Synchronization and Thalamocortical Rhythms in Sleep, Wake and Epilepsy (2012).
73. Ma S, Gundlach AL. Ascending Control of Arousal and Motivation: Role of Nucleus Incertus and its Peptide Neuromodulators in Behavioural Responses to Stress. *J. Neuroendocrinol* 27(6), 457-467 (2015).
74. Chen CC, Williams CL. Interactions between epinephrine, ascending vagal fibers, and central noradrenergic systems in modulating memory for emotionally arousing events. *Front. Behav. Neurosci* 6: 35 (2012).
75. Detari L, Juhasz G, Kukorelli T. Effect of stimulation of vagal and radial nerves on neuronal activity in the basal forebrain area of anaesthetized cats. *Acta. Physiol. Hung* 61(3), 147-154 (1983).
76. Metherate R, Cox CL, Ashe JH. Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci* 12(12), 4701-4711 (1992).
77. Berdiev RK, Chepurinov SA, Veening JG, *et al.* The role of the nucleus basalis of Meynert and reticular thalamic nucleus in pathogenesis of genetically determined absence epilepsy in rats: a lesion study. *Brain. Res* 1185(1), 266-274 (2007).
78. Tan U, Senyuva F, Marangoz C. Electroencephalographic effects of topically applied scopolamine. *Epilepsia* 19(3), 223-232 (1978).
79. Henry TR. Therapeutic mechanisms of vagus nerve stimulation. *Neurology* 59(6), S3-S14 (2002).
80. Castle M, Comoli E, Loewy AD. Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience* 134(2), 657-669 (2005).
81. Dorr AE, Debonnel G. Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *J. Pharmacol. Exp. Ther* 318(2), 890-898 (2006).
82. Groves DA, Bowman EM, Brown VJ. Recordings from the rat locus coeruleus during acute vagal nerve stimulation in the anaesthetised rat. *Neurosci. Lett* 379(3), 174-179 (2005).

83. Hassert DL, Miyashita T, Williams CL. The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behav. Neurosci* 118(1), 79-88 (2004).
84. Giorgi FS, Pizzanelli C, Biagioni F, *et al.* The role of norepinephrine in epilepsy: from the bench to the bedside. *Neurosci. Biobehav. Rev* 28(5), 507-524 (2004).
85. Curet O, Montigny C de. Electrophysiological characterization of adrenoceptors in the rat dorsal hippocampus: I. Receptors mediating the effect of microiontophoretically applied norepinephrine. *Brain. Res* 475(1), 35-46 (1988a).
86. Curet O, Montigny C de. Electrophysiological characterization of adrenoceptors in the rat dorsal hippocampus: II. Receptors mediating the effect of synaptically released norepinephrine. *Brain. Res* 475(1), 47-57 (1988b).
87. Manta S, Mansari M El, Debonnel G, *et al.* Electrophysiological and neurochemical effects of long-term vagus nerve stimulation on the rat monoaminergic systems. *Int. J. Neuropsychopharmacol* 16(2), 459-470 (2013).
88. Welsh JP, Placantonakis DG, Warsetsky SI, *et al.* The serotonin hypothesis of myoclonus from the perspective of neuronal rhythmicity. *Adv. Neurol* 89(1), 307-329 (2002).
89. Bercovici E, Cortez MA, Wang X, *et al.* Serotonin depletion attenuates AY-9944-mediated atypical absence seizures. *Epilepsia* 47(2), 240-246 (2006).
90. Browning RA, Clark KB, Naritoku DK, *et al.* Loss of anticonvulsant effect of vagus nerve stimulation in the pentylenetetrazol seizure model following treatment with 6-hydroxydopamine or 5,7-dihydroxytryptamine. *Soc. Neurosci. Abstr* 23(1), 2424 (1997).
91. Manta S, Dong J, Debonnel G, *et al.* Vagus nerve stimulation: effects on noradrenergic neuronal firing and serotonin transmission in the rat brain. *Eur. Neuropsychopharmacol* 17(1), S368-S369 (2007).
92. Manta S, Dong J, Debonnel G, *et al.* Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J. Psychiatry. Neurosci* 34(4), 272-280 (2009a).
93. Manta S, Dong J, Debonnel G, *et al.* Optimization of vagus nerve stimulation parameters using the firing activity of serotonin neurons in the rat dorsal raphe. *Eur. Neuropsychopharmacol* 19(4), 250-255 (2009b).
94. Blier P, Piñeyro G, Mansari M, *et al.* Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann. N Y Acad. Sci* 861(1), 204-16 (1998).
95. Blier P. The pharmacology of putative early-onset antidepressant strategies. *Eur. Neuropsychopharmacol* 13(2), 57-66 (2003).
96. Ben-Menachem E, Hamberger A, Hedner T, *et al.* Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. *Epilepsy. Res* 20(3), 221-227 (1995).
97. McDevitt RA, Neumaier JF. Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J. Chem. Neuroanat* 41(4), 234-246 (2011).
98. Ziomber A, Thor P, Krygowska-Wajs A, *et al.* Chronic impairment of the vagus nerve function leads to inhibition of dopamine but not serotonin neurons in rat brain structures. *Pharmacol. Rep* 64(6), 1359-1367 (2012).
99. Perez SM, Carreno FR, Frazer A, *et al.* Vagal Nerve Stimulation Reverses Aberrant Dopamine System Function in the Methylazoxymethanol Acetate Rodent Model of Schizophrenia. *The Journal of Neuroscience* 34(28), 9261-9267 (2014).
100. Lodge DJ, Grace AA. Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends. Pharmacol. Sci* 32(9), 507-513 (2011).
101. Heckers S. Neuroimaging studies of the hippocampus in schizophrenia. *Hippocampus* 11(5), 520-528 (2001).
102. Schobel SA, Kelly MA, Corcoran CM, *et al.* Anterior hippocampal and orbitofrontal cortical structural brain abnormalities in association with cognitive deficits in schizophrenia. *Schizophr. Res* 114(1-3), 110-118 (2009).
103. Schobel SA, Lewandowski NM, Corcoran CM, *et al.* Small, Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders. *Arch. Gen. Psychiatry* 66(1), 938-946 (2009).
104. Lodge DJ, Grace AA. Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. *J. Neurosci* 27(42), 11424-11430 (2007).
105. Mollet L, Clinckers R, Raedt R, *et al.* Anti-epileptic effects of vagus nerve stimulation in the focal pilocarpine model. *Acta. Physiologica* 195(1), 10 (2009).
106. Devoto P, Flore G. On the Origin of Cortical Dopamine: Is it a Co-Transmitter in Noradrenergic Neurons. *Curr. Neuropharmacol* 4(2), 115-125 (2006).
107. Devoto P, Flore G, Pani L, *et al.* Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol. Psychiatry* 6(6), 657-64 (2001).
108. Devoto P, Flore G, Vacca G, *et al.* Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. *Psychopharmacology (Berl)* 167(1), 79-84 (2003).
109. Arnsten AFT. Catecholamine regulation of the prefrontal cortex. *J. Psychopharm* 11(2), 151-162 (1997).
110. Arnsten AFT. Adrenergic targets for the treatment of cognitive deficit in schizophrenia. *Psychopharmacology (Berl)* 174(1), 25-31 (2004).
111. Granon S, Passetti F, Thomas KL, *et al.* Robbins, Enhanced and impaired attentional performance after infusion of D₁ dopaminergic receptor agents into rat prefrontal cortex. *J. Neurosci* 20(1), 1208-1215 (2000).
112. Mattay VS, Goldberg TE, Fera F, *et al.* Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc. Nat. Acad. Sci. USA* 100(10), 6186-6191 (2003).
113. Murphy BL, Arnsten AF, Goldman-Rakic PS, *et al.* Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc. Natl. Acad. Sci. USA* 93(3), 1325-1329 (1996).
114. Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D₁ receptors in prefrontal cortex. *Nature* 376(6541), 572-575 (1995).
115. Nichols JA, Nichols AR, Smirnakis SM, *et al.* Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. *Neuroscience* 189(1), 207-214 (2011).
116. Lazzaro V Di, Oliviero A, Pilato F, *et al.* Effects of vagus nerve stimulation on cortical excitability in epileptic patients. *Neurology* 62(12), 2310-2312 (2004).
117. Ura H, Sugaya Y, Ohata H, *et al.* Vagus nerve stimulation induced long-lasting enhancement of synaptic transmission and decreased granule cell discharge in the hippocampal dentate gyrus of urethane-anesthetized rats. *Brain. Res* 1492(1), 63-71 (2013).
118. Shen H, Fuchino Y, Miyamoto D, *et al.* Vagus nerve stimulation enhances perforant path-CA3 synaptic transmission via the activation of β -adrenergic receptors and the locus coeruleus. *Int. J. Neuropsychopharmacol* 15(4), 523-530 (2012).
119. Lyubashina O, Pantelev S. Effects of cervical vagus nerve stimulation on amygdala-evoked responses of the medial prefrontal cortex neurons in rat. *Neurosci. Res* 65(1), 122-125 (2009).
120. Ferraro G, Montalbano ME, Grutta V La. Nitric oxide and glutamate interaction in the control of cortical and hippocampal excitability. *Epilepsia* 40(7), 830-836 (1999).

121. Florin-Lechner SM, Druhan JP, Aston-Jones G, *et al.* Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. *Brain. Res* 742(1-2), 89-97 (1996).
122. Berridge CW, Foote SL. Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. *J. Neurosci* 11(10), 3135-3145 (1991).
123. Carter ME, Yizhar O, Chikahisa S, *et al.* Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci* 13(12), 1526-1533 (2010).
124. Filosa JA, Bonev AD, Straub SV, *et al.* Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat. Neurosci* 9(11), 1397-1403 (2006).
125. Cauli B, Hamel E. Revisiting the role of neurons in neurovascular coupling. *Front. Neuroenergetics* 2(1), 9 (2010).
126. Dunn KM, Nelson MT. Potassium channels and neurovascular coupling. *Circ. J* 74(4), 608-616 (2010).
127. Aronica E, Crino PB. Inflammation in epilepsy: clinical observations. *Epilepsia* 52(3), 26-32 (2011).
128. Choi J, Nordli DR Jr, Alden TD, *et al.* Cellular injury and neuroinflammation in children with chronic intractable epilepsy. *J. Neuroinflammation* 6(1), 38(2009).
129. Ravizza T, Boer K, Redeker S, *et al.* The IL-1beta system in epilepsy-associated malformations of cortical development. *Neurobiol. Dis* 24(1), 128-143 (2006).
130. Ravizza T, Gagliardi B, Noe F, *et al.* Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol. Dis* 29(1), 142-160 (2008).
131. Borovikova LV, Ivanova S, Zhang M, *et al.* Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405(6785), 458-462 (2000).
132. Wang H, Yu M, Ochani M, *et al.* Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421(6921), 384-388 (2003).
133. Herdt V De, Bogaert S, Bracke KR, *et al.* Effects of vagus nerve stimulation on pro- and anti-inflammatory cytokine induction in patients with refractory epilepsy. *Journal of neuroimmunology* 214(1-2), 104-108 (2009).
134. Majoie HJ, Rijkers K, Berfelo MW, *et al.* Vagus nerve stimulation in refractory epilepsy: effects on pro- and anti-inflammatory cytokines in peripheral blood. *Neuroimmunomodulation* 18(1), 52-56 (2011).
135. Chen N, Zhang H, Gao XD, *et al.* Neuroprotective effects of vagus nerve stimulation on hippocampal neurons in intractable epilepsy. *Med. Hypotheses* 81(6), 1066-1068 (2013).
136. Kalinin S, Polak PE, Madrigal JL, *et al.* Beta-amyloid-dependent expression of NOS2 in neurons: prevention by an alpha2-adrenergic antagonist. *Antioxid. Redox. Signal* 8(5-6), 873-883 (2006).
137. Madrigal JL, Russo C Dello, Gavriyuk V, *et al.* Effects of noradrenaline on neuronal NOS2 expression and viability. *Antioxid. Redox. Signal* 8(5-6), 885-92 (2006).
138. Neese SL, Sherill LK, Tan AA, *et al.* Vagus nerve stimulation may protect GABAergic neurons following traumatic brain injury in rats: An immunocytochemical study. *Brain. Res* 1128(1), 157-163 (2007).
139. Marrosu F, Serra A, Maleci A, *et al.* Correlation between GABA(A) receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. *Epilepsy. Res* 55(1-2), 59-70 (2003).
140. Biggio F, Gorini G, Utzeri C, *et al.* Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. *Int. J. Neuropsychopharmacol* 12(9), 1209-1221 (2009).
141. Follsea P, Biggio F, Gorini G, *et al.* Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain. Res* 1179(1), 28-34 (2007).
142. Lim YY, Villemagne VL, Laws SM, *et al.* Effect of BDNF Val66Met on Memory Decline and Hippocampal Atrophy in Prodromal Alzheimer's Disease: A Preliminary Study. *PLoS. One* 9(1), e86498 (2014).
143. Kanner AM. Hippocampal Atrophy: Another Common Pathogenic Mechanism of Depressive Disorders and Epilepsy. *Epilepsy. Curr* 11(5), 149-150 (2011).
144. Garcia-Finana M, Denby CE, Keller SS, *et al.* Degree of hippocampal atrophy is related to side of seizure onset in temporal lobe epilepsy. *AJNR. Am. J. Neuroradiol* 27(5), 1046-52 (2006).
145. Revesz D, Tjernstrom M, Ben-Menachem E, *et al.* Effects of vagus nerve stimulation on rat hippocampal progenitor proliferation. *Exp. Neurol* 214(2), 259-265 (2008).
146. Thom M. Review: Hippocampal sclerosis in epilepsy: a neuropathology review. *Neuropathol. Appl. Neurobiol* 40(5), 520-543 (2014).
147. Morin F, Beaulieu C, Caille JC La. Cell-specific alterations in synaptic properties of hippocampal CA1 interneurons after kainate treatment. *J. Neurophysiol* 80(6), 2836-2847 (1998).
148. Lipsky RH, Marini AM. Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann. N Y Acad. Sci* 1122(1), 130-143 (2007).
149. Mamounas LA, Altar CA, Blue ME, *et al.* BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. *J. Neurosci* 20(2), 771-782 (2000).
150. Brezun JM, Daszuta A. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* 89(4), 999-1002 (1999).
151. Kulkarni VA, Jha S, Vaidya VA. Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. *Eur. J. Neurosci* 16(10), 2008-2012 (2002).
152. Laifenfeld D, Klein E, Ben-Shachar D. Norepinephrine alters the expression of genes involved in neuronal sprouting and differentiation, of granule cell progenitors in the adult rat hippocampus. *J. Neurochem* 83(5), 1054-1064 (2002).
153. Bagdy G, Kecskemeti V, Riba P, *et al.* Serotonin and epilepsy. *J. Neurochem* 100(4), 857-73 (2007).
154. Blumenfeld H, McCormick DA. Corticothalamic Inputs Control the Pattern of Activity Generated in Thalamocortical Networks. *J. Neurosci* 20(13), 5153-5162 (2000).
155. Bal T, Debay D, Destexhe A. Cortical Feedback Controls the Frequency and Synchrony of Oscillations in the Visual Thalamus. *J. Neurosci* 20(19), 7478-7488 (2000).
156. Naritoku DK, Terry WJ, Helfert RH. Regional introduction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy. Res* 22(1), 53-62 (1995).
157. Saleh TM, Cecehtto DF. Peptides in the parabrachial nucleus modulate visceral input to the thalamus. *Am. J. Physiol* 264(4-2), R668-R675 (1993).
158. Henry TR, Bakay RA, Pennell PB, *et al.* Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation. *Epilepsia* 45(9), 1064-1070 (2004).
159. Henry TR, Bakay RA, Votaw JR, *et al.* Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation. *Epilepsia* 39(9), 983-990 (1998).
160. Henry TR, Votaw JR, Pennell PB, *et al.* Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. *Neurology* 52(6), 1166-1173 (1999).
161. Liu WC, Mosier K, Kalnin AJ, *et al.* BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. *J. Neurol. Neurosurg. Psychiatry* 74(6), 811-813 (2003).
162. Krauss GL, Fisher RS. Cerebellar and thalamic stimulation for epilepsy. *Adv. Neurol* 63(1), 231-245 (1993).

163. Krauss GL, Koubeissi MZ. Cerebellar and thalamic stimulation treatment for epilepsy. *Acta. Neurochir. Suppl* 97(1), 347-56 (2007).
164. Ko D, Heck C, Grafton S, *et al.* Vagus Nerve Stimulation Activates Central Nervous System Structures in Epileptic Patients During PET H2 150 Blood Flow Imaging. *Neurosurgery* 39(2), 426-30 (1996).
165. Nisimaru N, Katayama S. Projection of cardiovascular afferents to the lateral nodulus-uvula of the cerebellum in rabbits. *Neurosci Res.* 21(4), 343-350 (1995).
166. Hutton JT, Frost JD Jr, Foster J. The influence of the cerebellum in cat penicillin epilepsy. *Epilepsia* 13(3), 401-408 (1972).
167. Loughlin SE, Foote KL, Fallon JH. Locus coeruleus projections to cortex: topography, morphology and collateralization. *Brain. Res. Bull* 9(1-6), 287-294 (1982).
168. Loughlin SE, Foote SL, Grzanna R. Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. *Neuroscience* 18(2), 307-319 (1986).
169. Bockstaele EJ Van, Peoples J, Telegan P. Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway. *J. Comp. Neurol* 412(3), 410-428 (1999).
170. Groves DA, Brown VJ. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neurosci. Biobehav. Rev* 29(3), 493-500 (2005).
171. Nemeroff CB, Mayberg HS, Krahl SE, *et al.* VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology* 31(7), 1345-1355 (2006).
172. Vonck K, Laere K Van, Dedeurwaerdere S, *et al.* The mechanism of action of vagus nerve stimulation for refractory epilepsy: the current status. *J. Clin. Neurophysiol* 18(5), 394-401 (2001).
173. Fornai F, Ruffoli R, Giorgi FS, *et al.* The role of locus coeruleus in the antiepileptic activity induced by vagus nerve stimulation. *Eur. J. Neurosci* 33(12), 2169-2178 (2011).
174. Cunningham JT, Mifflin SW, Gould GG, *et al.* Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. *Neuropsychopharmacology* 33(8), 1884-1895 (2008).
175. Furmaga H, Sadhu M, Frazer A. Comparison of DeltaFosB immunoreactivity induced by vagal nerve stimulation with that caused by pharmacologically diverse antidepressants. *J. Pharmacol. Exp. Ther* 341(2), 317-325 (2012).
176. Terry WJ, Takaya M, Varitoku DK. Regional changes in brain glucose metabolism in rats following anticonvulsant stimulation of the vagus nerve. *Epilepsia* 37(1), 117 (1996).
177. Walker BR, Easton A, Gale K. Regulation of limbic motor seizures by GABA and glutamate transmission in the nucleus tractus solitaries. *Epilepsia* 40(8), 1051-1057 (1999).
178. Martle V, Peremans K, Raedt R, *et al.* Regional brain perfusion changes during standard and microburst vagus nerve stimulation in dogs. *Epilepsy Res* 108(4), 616-622 (2014).
179. Rajmohan V, Mohandas E. The limbic system. *Indian. J. Psychiatry* 49(2), 132-139 (2007).
180. Garnett ES, Nahmias C, Scheffel A, *et al.* Regional cerebral blood flow in man manipulated by direct vagal stimulation. *Pacing. Clin. Electrophysiol* 15(10-2), 1579-80 (1992).
181. Vonck K, Herdt V De, Bosman T, *et al.* Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. *Seizure* 17(8), 699-706 (2008).
182. Vonck K, Boon P, Laere K Van, *et al.* Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. *Epilepsia* 41(5), 601-609 (2000).
183. Barnes A, Duncan R, Chisholm JA, *et al.* Investigation into the mechanisms of vagus nerve stimulation for the treatment of intractable epilepsy, using 99mTc-HMPAO SPECT brain images. *Eur. J. Nucl. Med. Mol. Imaging* 30(2), 301-305 (2002).
184. Shmuel A, Augath M, Oeltermann A, *et al.* Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1. *Nat. Neurosci* 9(1), 569-577 (2006).
185. Schafer K, Blankenburg F, Kupers R, *et al.* Negative BOLD signal changes in ipsilateral primary somatosensory cortex are associated with perfusion decreases and behavioral evidence for functional inhibition. *Neuroimage* 59(4), 3119-3127 (2012).
186. Hoppe C, Wagner L, Hoffmann JM, *et al.* Comprehensive long-term outcome of best drug treatment with or without add-on vagus nerve stimulation for epilepsy: A retrospective matched pairs case-control study. *Seizure* 22(2), 109-115 (2013).