# VAGUS NERVE STIMULATION AS A THERAPY FOR TREATMENT RESISTANT DEPRESSION

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**ABSTRACT**

The major depressive disorder (MDD) is a prevalent chronic disease considered one of the most important causes of morbidity and mortality in the world. MDD is predicted to be the leading cause of disability by the year 2020. From all patients with MDD, at least 10% to 20% suffer treatment resistant depression (TDR) since they do not have satisfactory response to current available treatments. This paper summarizes the current pathophysiological hypothesis of depression including the alterations in monoaminergic transmission, the hypothalamic-pituitary-adrenal axis, neuroplasticity and inflammation, and their relation with an adjuvant therapeutic strategy, the vagus nerve stimulation (VNS). This recently accepted therapy consists in the stimulation through implanted electrodes of the left vagus nerve, which is known to be a direct connection between the nervous system and the periphery. This therapy has been accepted for the treatment of epilepsy and depression but is also under study for the treatment of chronic inflammatory disorders. In conclusion, although VNS is nowadays an invasive therapy, it has shown effectiveness in the treatment of mental and inflammatory disorders and promises to be an additional strategy in the treatment of a variety of diseased states.

## HYPOTHESIS

The hypothesis this review tries to verify is whether the vagus nerve stimulation can be a safe and effective therapy to treat depression symptoms.

## OBJECTIVES

The objectives that this review will try to assess are:

- Describe why depression is an important disease to study:
    - Describe the main symptoms of the major depressive disorder.
    - Describe the prevalence of the major depression disorder nowadays.
    - Describe the main treatments for the MDD.
- Describe the main pathophysiological theories of depression:
    - Determine how an alteration of the monoamine synapsis can promote the appearance of depressive symptoms.
    - Explain the relationship between the inflammation and the MDD.
    - Determine the main characteristics of the hypothalamus-pituitary-adrenal axis dysregulation in depressed patients.
    - Explain how an alteration of the neurotrophic factors be related with MDD.
- Review the main characteristics and the molecular mechanism of VNS to treat MDD:
    - Define the main anatomical and physiological characteristics of the vagus nerve.
    - Explain the main characteristics of the vagus nerve stimulation
    - Determine the changes that the VNS can produce in the central system and how this changes are produced
    - Describe how the VNS can regulate the immune system and the inflammation process
    - Determine the relationship between the control of the inflammatory system by VNS and the treatment of depression by this treatment

## 1. INTRODUCTION

The major depressive disorder (MDD) is a high prevalent, chronic, recurrent and disabling biological disorder with high rates of morbidity and mortality (1,2). In fact, mental disorders are considered the leading cause of years lived with disability worldwide, being the 40.5% of this burden uniquely attributable to major depression (3). And it is predicted to be the second leading cause of disability worldwide by the year 2020 (4,5). Disability in depression is characterized as reduced daily activities and high rates of mortality due to suicide and to the increased risk of death due to comorbid medical disorders that co-occur with depression, such as myocardial infraction and stroke (1,2).

MDD is characterized by two or more weeks of depressed mood or decreased interest associated with some symptoms like disturbed sleep, anhedonia, irritability, diminished appetite and libido, psychomotor changes, reduced concentration, excessive guilt and suicidal thoughts or attempts. The cases of MDD are classified in mild, moderate and severe according to the diagnostic and statistical manual of mental disorders where one third of them are classified as severe cases (6).

Nowadays, 322 million people worldwide suffer from MDD at any one time where more than 40 million are located in Europe and 30 million in America (7). Furthermore, the prevalence of the MDD is considered to 1.5 to 3 times higher in females than males and its lifetime risk is up to 17% (6).

Regardless its high socioeconomic impact little is known about the etiology of this disease. It is described that depression disorder can have up to 50% of genetic component thanks to studies based on family aggregation and contrasting monozygotic and dizygotic twins. This heritable component to psychiatric illnesses coupled with environmental factors result in an increased susceptibility to develop depression(8). Moreover, it is known that its neurobiology it is associated with neurochemical and metabolic changes in several brain regions such as prefrontal cortex, anterior cingulate cortex, thalamus, hippocampus, amygdala and basal ganglia(9)(10).

Probably due to the differential etiology of MDD not all the treatments are effectives for different patients. The first line treatments consist on antidepressant drugs, for example serotonin reuptake inhibitors (SSRIs) or monoamine oxidase inhibitors (MAOIs), psychotherapy or a combination of both. If these treatments do not reduce symptoms, second line treatments as electroconvulsive therapy (ECT) may be used (11). The aim of the treatment for this disease is the completely remitting the symptoms and complete restoration of a day-to-day functions. It is also necessary to prevent relapses, which are defined as the return of the current episodes, and recurrences meaning new episodes (12). The world health organization (WHO) has described that more than half of patients with depression are not treated, usually because they are not diagnosed (13).

Although patients that do not respond to a treatment may respond well to another at least 10% to 20% of all depressed patients do not have satisfactory responses to currently available treatments(2). For this reason, a subgroup of depression disorders that do not respond to the standard care have been named “Treatment Resistant Depression” (TRD). TRD patients do not respond in acute phase of the treatment or thee ones that after achieving remission have early relapses (14). 90% of the TRD are patients continue expressing effects of depression symptoms after 2 years of active treatment(2).

This review describes a new therapy approach to treat the treatment resistant depression based on the electro stimulation of the vagus nerve to modulate the brain parts that are responsible of the mood responses.

## 2. PATHOPHYSIOLOGY OF MAJOR DEPRESSIVE DISORDER

The principal aim to define the biological bases of depression is to determine its pathogenic process and to identify predictive biomarkers that could help in both the diagnosis and the follow up the effectiveness of the treatment. The disorder has several pathophysiological mechanisms described which can be understood as an interactive matrix. The four main theories that explain the pathophysiology of depression are the monoaminergic hypothesis, the neurotropic hypothesis, the neuroendocrine hypothesis and the neuroimmune hypothesis.

### 2.1. MONOAMINERGIC HYPOTHESIS OF DEPRESSION

The monoamine hypothesis of depression was the first of the pathophysiological hypothesis of depression described during the 1960 decade by Schildkraut (15). This postulates that depression is caused by an alteration of one or more monoamines in the brain. Monoamines include serotonin (5-HT), norepinephrine (NE) and dopamine (DA) (16).

This hypothesis was originated from different clinical observations. The first antidepressant discovered was imipramine which was initially developed to treat psychiatric disorders but was insufficient. Subsequently, it was shown that this compound had its antidepressant effect by enhancing central 5-HT or NE transmission (17). Also reserpine, which was an antihypertensive drug, is shown to produce depressive symptoms because it depletes monoamine stores (18). These findings together with several studies which described that depressive symptoms could be produced by the pharmacological manipulation of the monoaminergic system led to the formulation of the monoaminergic hypothesis. The hypothesis states that depression is a direct consequence from a reduced availability or functional deficiency of monoaminergic transmitters in some cerebral regions (8). It was supported by the positive outcomes of patients treated with try tricyclic antidepressant drugs (TCAs) and IMAOs, which acutely increase the levels of monoamines in the synapsis (19).

Today the antidepressant agents that offer a better therapeutic index and lower side effects, therefore the first line therapy for depression, are the second generation antidepressants such as SSRIs. Those antidepressants have replaced the MAOIs that were classically used as first line pharmacological therapy. Moreover, it frequent the combination of SSRI with other antidepressant drugs to optimize the medication dose and the beneficial effect (20).

Animal models have shown that modifying any of the components of the 5-HT system such as tryptophan hydroxylase, 5-HT transporter, specific 5-HT receptors or their regulation, downstream signals or transcription factors that regulate the 5-HT phenotype, all lead to behavioral changes that mimic depressive disorders (21). However, the serotonin receptors involved in the action of SSRIs remain unknown although selective agonists of serotonin 5-HT4 receptor produce rapid antidepressant effects in rodents (22).

Moreover, it is known that acute increases of synaptic monoamines induced by antidepressant drugs produce secondary neuroplastic changes that occur in a longer timescale and involve transcriptional and translational changes that mediate cellular plasticity (23). Lately, it has been described that the interaction of monoamine receptors modulates the expression of intracellular proteins such as CREB (cAMP response element binding protein) or BDNF (brain-derived neurotrophic factor). Serotoninergic and noradrenergic systems regulate the cyclic adenosine

3'-5'monophosphate cAMP-mediated signal transduction cascade which activates protein kinases (24). This could suggest a direct relationship between the monoaminergic system and neurotrophic factors (18). This findings suggest that increased serotonin levels are necessary for the antidepressant effects, however depletion of serotonin alone might not be enough to cause depressive symptoms (16).

The involvement of norepinephrine in mood regulation is evidenced by the fact that drugs that inhibit NE reuptake or increase NE secretion, such as mirtazapine or tricyclic antidepressant, are effective antidepressants (25). Moreover, dopamine has been shown to have relationship with the HPA axis and neurotoxic effects, as will be discussed below. Also, the mesolimbic pathway which is composed of dopaminergic neurons regulates the reward and motivation pathways and has been related with mood regulation (16).

Although, the monoamine hypothesis is a fact, depression is far from being just a deficiency of central synaptic monoamines. This hypothesis has some limitations as the therapeutic effect of the antidepressant drugs which modulate the monoaminergic system need between 6 or 8 weeks to be produced (17,26). Moreover, these drugs are just efficient in 60-70% of the patients. The hypothesis has evolved over the years including adaptive changes in receptors to explain why the gradual response is produced. However some questions are still not answered, for example why antidepressant drugs are also effective in other disorders as obsessive-compulsive disorder or bulimia (8,23).

## 2.2. NEUROTROPHIC HYPOTHESIS OF DEPRESSION

The requirement for long-term, chronic antidepressant treatments has brought to the hypothesis that alterations in functional and structural plasticity of the neurons are necessary for a therapeutic response (27).

The neurotrophic hypothesis of depression postulates that reduced levels of the neurotrophic factors, being BDNF the most important, produce neural atrophy in regions as hippocampus, amygdala and prefrontal cortex. This neuronal atrophy is observed in depressed patients that do not follow an antidepressant treatment whereas antidepressant treatments exert their therapeutic effects through increased expression of neurotrophic factors in the hippocampus. Therefore, it has been proposed that long-term synaptic plasticity or its modulation might be disturbed in depressed patients (27).

Grey matter reductions have been reported in many brain regions in MDD patients including the hippocampus, prefrontal cortex and amygdala which is correlated with negative changes to both neurons and glia. Hippocampus reductions have been described to be due to a dysregulation of growth factor receptors phosphorylation and expression in depressed patients (28). Also reductions in the densities and sizes of glia, pyramidal and GABAergic neurons have been found in the orbitofrontal cortex and prefrontal dorsolateral cortex (DLPFC) in depressed patients (9). Moreover, decreased levels of interneuron-related synaptic markers have been found in the basolateral and basomedial amygdala(29).

It is described that antidepressant drugs significantly increase the expression of BDNF in the hippocampus (27,30). Moreover, antidepressant and electroconvulsive therapy have been shown to modulate synaptic plasticity and BDNF gene expression in the dentate gyrus and CA1 region of the hippocampus and neostriatum (8,31).

In conclusion, it is known that normal BDNF signaling is both necessary and sufficient for antidepressant drug action (32).

## 2.4. NEUROENDOCRINE HYPOTHESIS OF DEPRESSION

It is known that stress and depression are closely related. This pathophysiological hypothesis of depression was postulated after some studies found an increase of glucocorticoid concentration in serum of depressed patient, fact that suggest a dysfunctional hypothalamic-pituitary-adrenal axis. Therefore, an alteration in HPA axis is associated with depression which can be due to hypersecretion of CRF in the hypothalamus, negative feedback of the HPA axis, enlarged adrenal glands, hypercortisolemia and decreased suppression of cortisol in response to dexamethasone (33). It is known that stress increases serum glucocorticoid concentrations and some depression-like symptoms can be produced by chronic administration of glucocorticoids (23).

Studies of the effects of glucocorticoids in the brain were performed in order to describe the mechanisms that explain the depressive consequences of hypercortisolemia. Increased glucocorticoids modify mainly the medial prefrontal cortex (mPFC), the hippocampus and the amygdala. Moreover, chronic stress decreases the dendritic complexity of pyramidal neurons and increases the transcriptional activity of GABA interneurons in the mPFC (34), decreasing its function which is processing of emotions generated by subcortical regions such as the amygdala. The reduced activity of this area produces and inadequate processing of the negative affect (16). Also, chronic stress produces reduced plasticity and long-term potentiation in CA1 hippocampal neurons that leads to a defective adaptation and learning (35).

Furthermore, it is known that chronic stress leads to an increase of the tyrosine hydroxylase activity which is the enzyme involved in the NE synthesis in the LC. Also stimulates the production and release of NE which leads to an increased secretion of corticotropin-releasing factor (CRF) from the hypothalamus. The CRF release produces the release of ACTH from the pituitary gland which stimulates the adrenal gland to release NE and cortisol (25).

Furthermore, patients with MDD show hypersecretion of cortisol which could be due prolonged hypersecretion of corticotrophin release factor (CRF) and adrenocorticotropic hormone (ACTH). However, data from ACTH is inconsistent. One possible explanation is that hypercortisolism during depression disorders change over time. In acute depression, hypersecretion of CRF stimulates the synthesis and secretion of ACTH in the pituitary gland. Then, ACTH stimulates the synthesis and secretion of cortisol. Therefore, in acute depression there is a hypersecretion of cortisol produced by elevated levels of CRF and ACTH (33).

On the other hand, in chronic depression two different processes take part in order to maintain cortisol levels elevated while ACTH levels are decreased (33). The first process postulates that changes occur in the adrenal gland responsiveness to circulating ACTH, in chronic depression there is adrenal hyper-responsiveness to low levels of this hormone. Therefore, depressed patients produce more cortisol per molecule of ACTH. This process is supported by the fact that the adrenal gland in depressed patients is enlarged (36). The second process describes changes in pituitary responses to hypothalamic CRF. High levels of CRF are associated with low levels of ACTH in chronic depression, whereas in acute depression are associated with high levels of ATCH. It is hypothesized that pituitary corticotropes in hypercortisolemic depressed patients are stimulated by excesive hypothalamic CRF but this stimulatory effect is suppressed by high circulating levels of cortisol which produced a negative feedback loop in both the hypothalamus and the pituitary (33). This hypothesis is supported by the attenuated ACTH response to exogenous CRF in many depressed patients. In hypercortisolemic depressed patients, the pituitary is stimulated by high levels of hypothalamic CRF, but this stimulatory effect is suppressed by high circulating levels of cortisol (37). All this process previously explained is represented in the Figure 1.

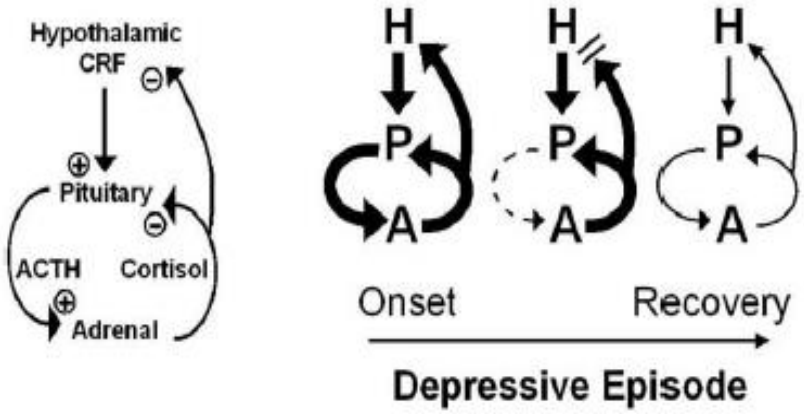


*Fig 1: A) Scheme of the main organs and hormones involved in the HPA axis. B) Scheme of the HPA axis in major depression. The broken line reflect low levels of hormone, the thin lines reflect normal levels of hormones and the thick lines reflect increased levels of hormone. Extracted from Parker.KJ, 2003*

Furthermore, vasopressin seems to be also a regulator of the HPA axis acting synergically with CRF stimulating the secretion of ACTH and other corticoid hormones. In depressed patients an overproduction of vasopressin has been described (8).

The data on the CRF and vasopressin deregulation postulates a profound dysfunction to the endocrine response to stress in MDD. Both hormones are disrupted in both brain and periphery and may co-participate with the monoaminergic stress system (8).

## 2.4. NEUROIMMUNE HYPOTHESIS OF DEPRESSION

The role of cytokines in depression was first proposed by Smith in 1991. He postulated that depression may be associated with an acute-phase inflammatory response (38).

Cytokines, which are humoral mediators of innate and adaptive immunity, are also important modulators for mood. Cytokine receptors within the central nervous system are activated by peripheral and centrally synthesized cytokines (23). Low doses of interleukin 1 (IL-1), the most abundantly expressed cytokine, produce in rodents behavioral effects such as social retraction, decreased exploratory and a reduction of sexual behaviour, a behavior called “sickness behaviour”. This reaction is produced by a release of proinflammatory cytokines such as interferon-α, tumor necrosis factor-α (TNF- α), lL-6 and IL-1β which activate the HPA axis and central monoamine systems (39). It has been stablished that pro-inflammatory cytokines induce not only symptoms of sickness but also true major depressive disorders in physically ill patients (26).

It is known that the brain contains immune cells (macrophages and dendritic cells) located in the choroid plexus and meninges. Brain parenchymal macrophages or microglial cells, respond to inflammatory stimuli by producing pro-inflammatory cytokines and prostaglandins (26).

The brain senses the peripheral innate immune responses by several systems that take action in parallel. First pathway, locally produced pathogen-associated molecular patters (PAMS) and cytokines activate primary afferent nerves, such as the vagal nerve in abdominal or visceral infections and the trigeminal nerve in oro-lingual infections (40). The second pathway is a humoral pathway in which Toll-like receptors (TLRs) are involved. These receptors are located in the circumventricular organs and the choroid plexus on macrophage-like cells and respond to circulating pathogen-associated molecular patters by producing pro-inflammatory cytokines. As circumventricular organs are located outside the blood brain barrier, the cytokines enter the

brain by diffusion (41). Another pathway outlines the access of the pro-inflammatory cytokines, which are overproduced in the systemic circulation, by cytokine transporters in the blood barrier. Finally, the activation of IL-1 receptors, which are located in the perivascular macrophages and endothelial cells of brain venules, by circulating cytokines produces prostaglandin E2 locally. This immune-to-brain communication leads to the production of pro-inflammatory cytokines by microglial cells(42,43).

The relationship between the immune system and depression was supported by the fact that 30% of individuals treated with recombinant interferons (IFN), as a treatment for infections, develop depression as a side effect (summarized at the figure 2) (44). It has been described that interferons affect neurochemical pathways involved in the etiology of depression such as the monoaminergic system, hypothalamic-pituitary-adrenal (HPA) axis, they hypothalamic-pituitary-thyroid axis and the cytokine network (45). The proinflamatory cytokines affect the serotonin metabolism directly or indirectly inducing the enzyme indoleamine 2,3-dioxygenase (IDO) which drives to a depletion of peripheral tryptophan which is a serotonin precursor. Also an increase of tryptophan catabolism has been observed during IFN therapy(46). Therefore, the etiology of IFN-induced depression may lead in part from its anti-serotonergic effects.

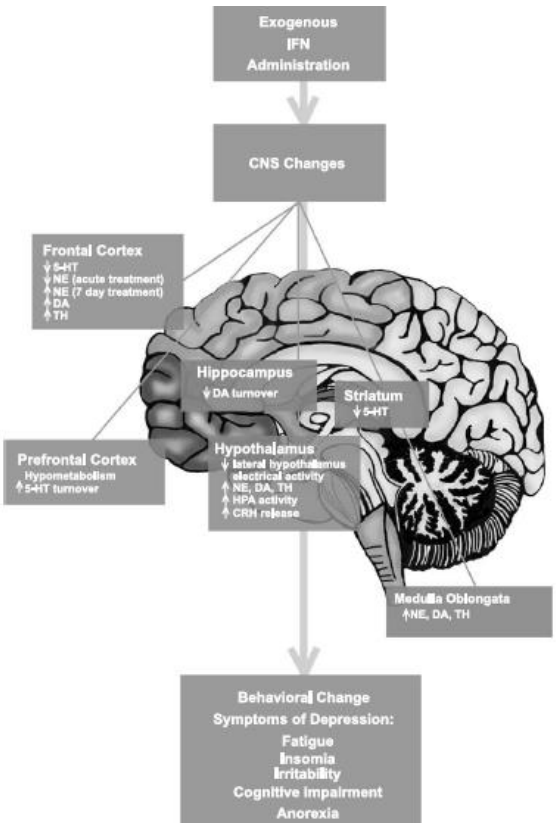


*Figure 2: Central nervous system and behavioral changes associated with IFN administration. IFN treatment produces changes in brain-region specific neurotransmitters that lead to several behavioral changes associated with depression. Extracted from Loftis et al 2004*

Moreover, patients treated with IFN therapy have adrenocorticotropic hormone (ACTH) and cortisol responses significantly higher consistently with the HPA axis alteration in depressed patient(44). Also the use of IFN treatments has been associated with thyroid dysfunction which may be related to depression (47). Several studies demonstrate the existence of a multiple feedback mechanism between serotonergic and hypothalamic-pituitary-thyroid systems(45). It has been described that IFN-α induces also an increase of norepinephrine in plasma and cortisol levels in serum, however, lymphocyte β2-adrenoceptors and epinephrine levels decreased (48). These results are consistent with the reports revealing an increased sympathetic outflow of norepinephrine in major depressive disorders.

In conclusion, these facts suggest that inflammation and immune activation might have an important role in the pathophysiology of depression and a subset of depression cases can be associated with autoimmune diseases such as rheumatoid arthritis.

## 3. VAGUS NERVE STIMULATION

A strategy that has recently received much attention is the vagus nerve stimulation VNS. It consist in the electrical stimulation of the left vagus nerve in order to modify the activities of

some brain areas involved in specific nervous disorders like epilepsy or depression. Nowadays, the application of this type of therapy to control some alteration of peripheral systems is being studied, like the modulation of the immune system to treat chronic inflammatory disorders.

### 3.1 VAGUS NERVE

The vagus nerve is the 10th and longest cranial nerve of the human body (49). It contains approximately 100.000 axons that form 80% afferent sensory fibers and 20% efferent motor fibers, therefore it's considered a mixed nerve(50).

The efferent (motor) fibers of the vagus nerve are parasympathetic and highly myelinated motor fibers; therefore, the vagus nerve controls and regulates the autonomic tone of the organs it innervates(1). It arises from the medulla and has many rootlets between the inferior olive and the inferior cerebellar peduncle. The rootlets form a single trunk that cross the subarachnoid space and the jugular foramen leaving the skull. In the jugular foramen, the vagus nerve makes a small enlargement named jugular or superior ganglion and underneath the jugular foramen the nerve expands forming its nodose or inferior ganglion (51). After exiting the skull the vagus nerve descends in the neck inside the carotid sheath forming the vasculo-nervous axis of the neck together with the internal carotid artery and the internal jugular vein. At the level of the nodose ganglion it gives off many branches that will contribute to form the pharyngeal plexus, cardiac plexus and the superior laryngeal nerve. The vagus nerve continues at the root of the neck in different ways depending if it is the right or left side. On the right side it will form the inferior laryngeal nerve, the recurrent nerve and contribute to form the esophageal plexus. Furthermore, on the left side it will form the left recurrent laryngeal nerve and will contribute to form the cardiac and pulmonary plexus. Finally right and left vagus nerve join together in the abdomen forming the celiac plexus and the lineal plexus (52). Moreover, the cardiovascular efferent fibers regulate the heart rate and blood pressure, however there are differences between the right and left vagus nerve. The right vagus nerve innervates the sinoatrial node that controls the pace of the heart while the left one innervates the atrioventricular node regulating the force of contraction (50).

The afferent fibers of the vagus nerve bring sensory information from the head, neck abdomen and thorax to the dorsal medullar complex, more precisely to the solitary nucleus (NTS). The afferent cell bodies are located in the nodose ganglion which is located anterior to the internal jugular vein just inferior to the jugular foramen and transfer the information to the NTS. Direct and indirect connections are widespread from the NTS over other brain areas. There are 3 main pathways though which the incoming information can be relayed: an autonomic feedback loop, direct projections to the reticular formation in the medulla and ascending projections to the forebrain through the parabrachial nucleus (PBN) and the locus coeruleus (LC) (12,53).

The NTS transfers information via direct projections to the PBN, the cerebellum, the raphe nuclei, the periaqueductal gray (PAG) and the locus coeruleus. Moreover, some secondary projections are made to some limbic, paralimbic and cortical region. The PBN transmits the information coming from the NTS to the insular orbitofrontal and prefrontal cortices mainly but also to some other forebrain structures (1).

The projections from the NTS to the locus coeruleus and the raphe nuclei, which are the main brain regions containing the noradrenergic and serotonergic neurons respectively, makes them key for the treatment of depression with vagus nerve stimulation(1).

The PBN and LC, which are located adjacent, send direct connections to every level of the forebrain, the hypothalamus and several thalamic regions that regulate the orbitofrontal and prefrontal cortex and the insula. The PBN/LC have direct connections to the amygdala and bed nucleus of the stria terminalis, which are implicated in mood and emotion recognition. Therefore, these PBN/LC connections can be key to control mood regulation disorders (53).

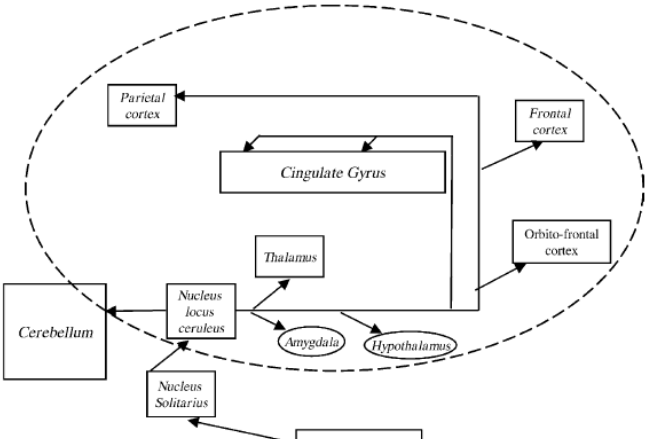


*Figure 3: Schematic representation of neural links of the vagus nerve to cortical and subcortical structures in the human brain. Extracted from Barnes et al, 2003.*

In 2003 Barnes et al provided a map of the main nervous system areas of influence of the vagus nerve shown in the figure 3 (54).

The vagus nerve is composed by three different types of fibers based on their diameter and physical properties which are named A, B and C, where the C are the most abundant. The characteristics of each fiber are reviewed, described and summarized in the table 1 adapted from Groves et al in 2005 and Ruffoli et al in 2011 (50,52).

| | FIBRE | | |
|---|---|---|---|
| | A | B | C |
| Fibre diameter (mm) | 5-20 | <3 | 0.4-2 |
| Gross anatomical | Large | Small | Small |
| Main function | Fast pain, temperature, touch, Muscle tone | Vasomotor, Visceromotor | Vasomotor, Visceromotor, Slow pain, temperature, touch |
| Myelinated | ✓ | ✓ | ✗ |
| Threshold (mA) | 0.02-0.2 | 0.04-0.6 | 2.0+ |
| Conductance velocity (ms) | 30-90 | 10-20 | 0.3-6 |
| Information conveyed | Mechano sensitive cardio-pulmonary | Mechano sensitive cardio-pulmonary | Cardio-pulmonary chemore-fletxes |
| Recruitment order | 1st | 2nd | 3rd |
| Effect of VNS on EEG | Synchronisation | Syncronisation | Desyncronisation |

*Table 1: table adapted from Ruffoli et al, 2011 and Groves et al 2005*

In conclusion, the vagus nerve is one of the most important nerves of the human body because it is the direct connection between the central nervous system and a number of relevant peripheral organs. The study of its anatomy has allowed to create the VNS.

### 3.2 BASICS OF VAGUS NERVE STIMULATION

The vagus nerve stimulation (VNS) is a therapy that consists on the stimulation of the left cervical vagus nerve using a commercial device called the neurocibernetic prosthesis (NCP) which is surgically implanted as shown in the figure 4. The NCP system was developed by

Cyberonics and first approved as an adjunct treatment to pharmacotherapy for epilepsy in Europe in 1994 and by the FDA in 1997(11).

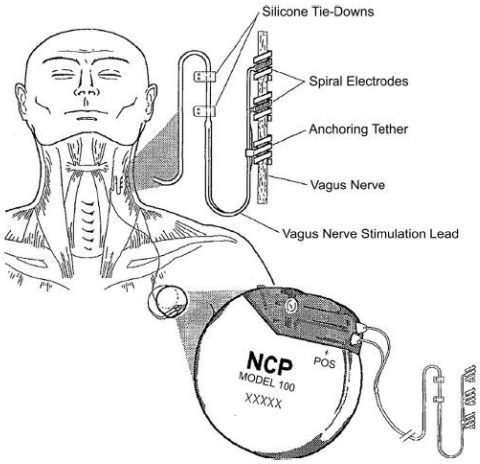


*Figure 4: Representation of the location of the neurocibernetic prothesis generation and the lead in the neck. Extracted from Milby et al, 2011.*

Some anecdotal clinical observations in epilepsy patients led to some pilot studies that assessed the VNS effects on mood in epilepsy patients treated with this therapy (11). In 2000, Harden et al did a pilot prospective study that consisted on assessing if there is a quantifiable effect on mood in VNS treated patients. They compared a group of patients treated with VNS against seizure effects with a group treated with pharmacological anti-seizure treatments (n=20). The data obtained suggest that VNS may be associated with improved mood in epilepsy patients, which was demonstrated by the significant decrease in all mood scare scores across time. Moreover, it was described that the antidepressant effect was independent of the anti-seizure effect (55).

The same finding was independently reported the same year by Elger et al. They did a multicenter study where the effectiveness of VNS for drug-resistant partial-onset seizures was evaluated. They estimated, in 11 patients randomly assigned to low or high stimulation, mood changes during VNS therapy at 3 and 6 months after the implantation. There were differences in the mood between the baseline and the 3-month follow-up in both groups of patients since the depression rating scale (MADRS) significantly decreased. However, no differences between both groups were found in the 6-month follow-up. In conclusion, they showed VNS-sustained mood improvements in patients with epilepsy and these effects seemed to be independent of the anti-seizure effects (56).

Considering the previous information two pilot studies were carried out treating TRD patients with VNS. The first trial was a multicenter study conducted by Rush AJ et al in which the safety and efficacy of VNS in treating patients with TRD and bipolar disorders were assessed. They determined the timing of antidepressant effects, the safety and the tolerability of the treatment in a group of 45 patients, 30 of them treated with NCP implants. The efficacy of this therapy in treatment-resistant depressed patients was shown since antidepressant responses were found between 1 and 10 weeks after the initiation of stimulation (response rate of 40% and full recovery remission rate of 17%)(12).

The second pilot study was carried out by Shackheim et al in 2001. They combined the previous study cohort with 30 more TDR unipolar and bipolar patients making a total sample of 60 individuals. The response rate they obtained was the 30% and the remission rate 15%; therefore, results were less promising than in the previous study(57).

Futhermore, both pilot studies showed that patients who had never received electroconvulsive therapy (ECT) were found to be more likely to respond to VNS. Therefore, ECT exposure may be a possible predictor of non-response to VNS(49,57).

Subsequenly, more controlled clinical trials that assess the outcomes of the VNS treatment for a longer time in TRD patients were reported (58,59). In addition studies that assess the optimal stimulation parameters of the VNS in depressed patients have been conducted(60).

In light of the above, the FDA approved in 2005 VNS for patients with unipolar or bipolar depression that failed to respond to four or more antidepressant treatments. FDA demanded a contrasted one-year outcome comparison between TRD patients with VNS and TRD patients treated with the treatment as usual (TAU) (11). This study was performed in 2005 by George M.S et al with 250 patients either treated with VNS +TAU or treated only with TAU. It was shown that there was a significant difference on the IDR-SR30 (inventory of depressive symptoms) scores over 12 months between both groups, being the VNS + TAU scores lower as shown in the figure 5 (61).

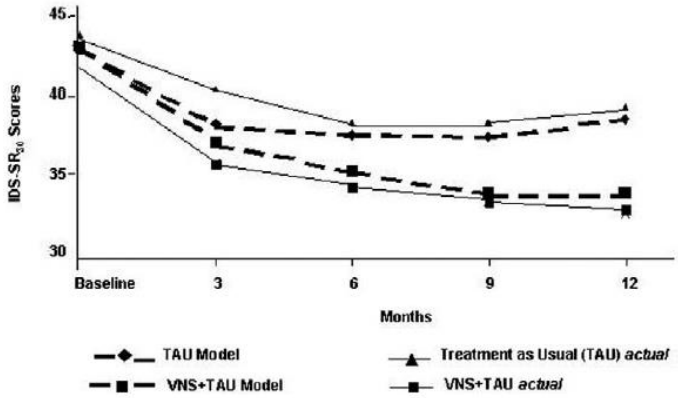


*Figure 5: Results of repeated-measures linear regression model of the IDR-SR30 (inventory of depressive symptoms) in group of patients treated with TAU (treatment as usual) compared with a group treated with TAU+VNS. Extracted from George MS et al 2005*

All these clinical findings have led to the application of this therapy as a co-adjuvant of pharmacological and psychological therapy for depression. Moreover, researchers have been trying to answer how this improvement of depressive symptoms is produced after the stimulation of the vagus nerve. In the following section the molecular mechanisms known that are consequence of the VNS and could explain the mood improvement produced are reviewed.

### 3.3. VNS CENTRAL ACTION

Effects of VNS in brain blood flow and brain glucose metabolism

After the finding that mood enhances after vagus nerve stimulation some imaging studies have been performed.

Several studies that performed functional MRI (fMRI) in depressed patients have reported VNS increased blood oxygenation level-dependent (BOLD) activity in specific brain regions bilaterally. These regions are orbitofrontal and parieto-occipital cortex, left temporal cortex, the hypothalamus and the left amygdala. Moreover, an overall increase in the brain activity has been described. However, no BOLD changes were reported in the thalamus (62).

Moreover, Nahas et al performed a serial-intervaled VNS/ functional MRI study with 9 MDD patients. Their results showed significant BOLD increases during VNS in the bilateral superior

temporal gyrus and left somatosensory cortex and significant BOLD decreases in the left middle gyrus, left ventromedial frontal lobe, right cerebellum and midbrain. These results are consistent with the known vagus afferent projections. In addition, they performed a multiple regression model with BOLD signal as the dependent variable. The results suggested that BOLD right insula deactivation and ventro-medial prefrontal cortex activation correlates with antidepressant response to VNS therapy (63).

Cornway et al used PET to identify changes in regional cerebral blood flow (rCBF) in response to the VNS in four patients with TRD. They found VNS-induced increases in rCBF in the bilateral orbitofrontal cortex, bilateral anterior cingulate cortex, right superior, medial frontal cortex, cerebellum and right temporal lobe. In contrast, decreased rCBF were found in bilateral temporal cortex and right parietal area (64). These findings were consistent with Zobel et al who described increased rCBF in the middle frontal gyrus and decreased in multiple limbic and paralimbic structures such as the amygala, the left hippocampus, cingulate gyrus, the right thalamus and the brain stem (65).

In addition, recent studies performed by Conway et al described that VNS induces significant rCBF increases in the right dorsal anterior cingulate, left posterior limb of the medial putamen, right superior temporal gyrus and left cerebellar body. In contrast, significant rCBF decreases were found in the lateral orbitofrontal cortex and the left inferior temporal lobe (66).

Furthermore, a study that used SPECT to investigate VNS effects in depression was performed in 2011 and the results showed that patients who respond to VNS showed an increase of rCBF in the left dorsolateral and ventrolateral prefrontal cortex and decreased rCBF in the right posterior cingulate area, the lingual gyrus and the left insula(67). These results agree with previous results. Therefore, the modulation of the activity in this regions could be associated with antidepressant efficacy of VNS.

In addition, the relationship between brain glucose metabolism and VNS therapy has been assessed. A study performed in 2013 described that subjects who responded to VNS for TRD at 12 months had a significant mean regional cerebral glucose metabolic rate in the right dorsolateral prefrontal cortex. However, it was shown that the decrease in this area was produced at 3 months of VNS therapy and it returned to the baseline at 12 months. Moreover, this study showed a clear hemispheric dichotomy pattern in glucose brain metabolism being decreased on the right hemisphere and increased in the left one. The same study also showed a higher metabolism in the ventral tegmental area which contains dopaminergic cell bodies (68). Controversally, a previous study performed by Pardo et al described a decrease of the glucose metabolism in the ventromedial prefrontal cortex. However, this finding does not agree with other findings as the increasement of the blood flow in this region described previously(69).

Incorporating all the information above, there may be an agreement about the anatomic structures altered by the VNS. The VNS is associated with an increase of rCBF and glucose metabolism in the prefrontal/frontal regions and a decrease in limbic regions. This pattern agrees with Mayberg's depression model which propose an imbalance of limbic-frontal circuitry due to a hypomethabolism in the prefrontal brain region and a hypermethabolism in the limbic region (70).

VNS consequences in monoaminergic neurotransmission

In order to assess the molecular mechanism that explains the antidepressant effect of VNS, researchers have tried to relate molecular changes during the treatment with the already known pathophysiological hypothesis of depression. As the most accepted hypothesis is the

monoaminergic hypothesis, changes in these neurotransmitters after VNS have been determined.

Several studies have shown that VNS increases extracellular NE concentrations in structures receiving noradrenergic innervations exclusively from the LC, such as amygdala, the hippocampus or the cortex (71). These findings were supported by a study in which the rat LC was recorded during vagal nerve stimulation which showed that VNS produces a long lasting increase of NE in the basolateral amygdala. It was demonstrated that the increase of NE is consequence of an increased firing of LC neurons which project directly to the amygdala (72). The enhancement of the firing rate of NE neurons is normally controlled by α2-autoceptors (73). However, it has been described that this activation may be produced through an activation of their excitatory α1 autoceptors (52).

Moreover, 5-HT CSF levels have been determined to increase after a VNS in epileptic patients (74). First it was hypothesized that the VNS mechanism to increase 5-HT would be the same as the SSRI (selective serotonin reuptake inhibitors) which increase the occupancy of $5HT_{1A}$ autoceptors and decrease the firing rate of 5-HT neurons located in the dorsal raphe nucleus (DRN). However, the VNS has been shown to increase the 5-HT neurons firing rate (1). Normally, the increase in 5-HT firing rate is associated with increased endogenous 5-HT release which should activate the somatodendritic autoceptors. This activation should hyperpolarize the cells though G-proteins and decrease 5-HT firing rate. However, despite the significantly increased DRN 5-HT firing rate no changes in the $5\text{-}HT_{1A}$ receptor were observed. Therefore a possible explanation is that VNS increases the release of 5-HT in terminal regions but not in the vicinity of the cell bodies (73).

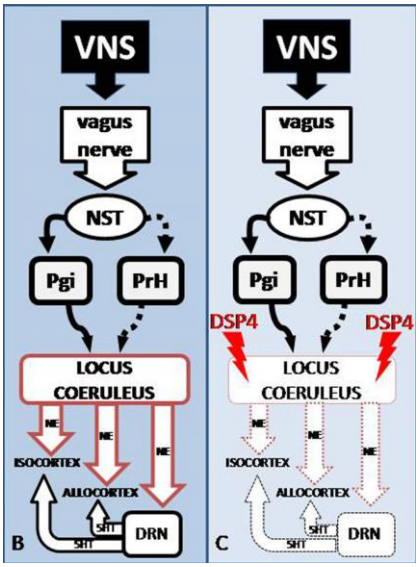


*FIG 6: The vagus nerve stimulation (VNS) increases the firing rate of NE neurons of the LC and indirectly of 5-HT neurons of the DRN. The excitatory action on 5-HT neurons is prevented by lesion of the NE neurons. (Extracted from Ruffoli et al, 2011)*

Animal studies have shown an increase of short-term firing activity in the NE neurons while 5-HT neurons increase their firing rate after 90 days of VNS (75,76). This leads to the hypothesis that the stimulatory effect of VNS on 5-HT neuronal firing is indirect and likely mediated by the activation of the NE system. This finding was supported by the prevention of the increasing 5-HT neuron firing after a selective lesion in the LC (75,76).

In conclusion, it has been determined that VNS facilitates the excitatory pathway from the NTS to the LC, via the paragigantocellularis nucleus (PGI) more than the inhibitory one, via the nucleus hypoglossi (PrH) which will activate the NE neurons. This will indirectly activate the 5-HT neurons in the DRN. This process, summarized in the figure 6, will increase both 5-HT and NE concentrations in the brain (52). These findings are consistent with the fact that antidepressant behavioral effects of VNS are not seen in rats with lesions of serotonergic or noradrenergic neurons (77).

Furthermore, as dopamine (DA) has been known to be implicated in the pathophylogy of depression several studies have determined the changes of this neurotransmitter after VNS. In the examination of CSF an increment in the concentration of homovanillic acid (HVA) after active VNS 10-week treatment period was assessed. Since HVA is a known metabolite of dopamine, these changes in CSF HVA were observed as a reflection of the synaptic dopamine concentrations. Therefore, it was concluded that dopamine turnover is augmented during VNS

treatment. Researchers hypothesize that VNS influences dopamine by activating extrapyramidal motor systems (78). Moreover, in later studies an increase of extracellular DA levels in the prefrontal cortex (PFC) and the nucleus accumbens (NAc) was determined but decreased firing rate of the ventral tegmental area (VTA) DA neurons was found. Therefore, other mechanism may be involved in an increase of extracellular DA (79). A possible explanation for the incremented extracellular DA levels could be a change in terminal DA $D_2$ autoceptors sensitivity. Actually, an in vitro electrophysiological study demonstrated the ability of terminal $D_2$ autoceptor, which normally inhibits DA release, to decrease their effectiveness (80). Thus, it would be appropriate to determine the $D_2$ receptor function following a chronic VNS (79).

Consequences of the VNS in the hypothalamic-pituitary- adrenal (HPA) axis

As it has been previously explained, there are considerable evidences to consider that the overdrive of the HPA axis is a characteristic of the MDD pathophysiology. This overdrive results from corticotrophin-releasing hormone (CRH) hypersecretion, probably as consequence to a dysfunctional regulatory feedback of glucocorticoid inhibition in the hippocampus (81).

In order to determine whether VNS exerts any action in the HPA axis, several studies have been performed. It has been studied the corticotropin-release hormone (CRH) challenge test response in patients with chronic MDD before and after 3 month of treatment with VNS. The outcome of these patients was compared with the outcome of a control group composed by healthy patients. The results of this study showed a significant reduction in depression scores in the patients treated with VNS. Moreover, CRH/ACTH responses before VNS implantation were significantly higher in the patients group than in the control group and were reduced to normal after 3 months of VNS treatment. Furthermore, the mean concentration of cortisol in serum after administration of CRH was increased in the patient's group before treatment compared to post-treatment concentration (82).

Consistently with the previous findings, De Herdt et al postulated that 1h of high frequency VNS significantly increased serum corticosterone levels in rat models compared to sham stimulation (83).

Moreover, other animal studies have postulated that after chronic VNS treatment, plasmatic ACTH level has a quicker recovery in response to an acute stressor. Moreover, it has been described that VNS does not affect basal plasma ACTH concentration; meaning that VNS does not disturb HPA axis in normal conditions. The effects of VNS are only evident when the sympathovagal balance is disturbed, meaning a greater feedback after a stressor (84).

In conclusion, all those findings suggest that VNS activates the HPA axis normalizing the feedback loop and decreasing the levels of CRH hormone in MDD patients. One possible mechanism of action that would explain the endocrine normalization after VNS treatment would be that the electrical stimuli worked as a stimulatory effect transmitted from the vagus nerve till the NTS. The NTS has anatomical connections with the corticotrophin releasing hormone cells located in the paraventricular nucleus of the hypothalamus that will become active releasing ACTH and corticosterone. This hypothesis is supported by the fact that VNS induces and increases the expression of CRF mRNA in the hypothalamus (82).

Another possible mechanism of action for the effects previously described would be related with the amygdala. It has been described that VNS decreases the amygdala activity and some evidences suggest that a decrease in the amygdala activity facilitates the down regulation of HPA axis in TRD patients (85).

Effects of VNS in neurogenesis and neurorophins

The neurogenetic and neurotrophic hypothesis of depression is based on the findings that there is a decrease in adult neurogenesis in the hippocampus that can be reversed with antidepressant therapy (27). It is hypothesized that those changes could be produced by an alteration in the levels of neurotrophic factors such as BDNF in the hippocampus. In order to support this theory, changes in the hippocampal volume have been observed in patients with depression as well as changes in some aspects of hippocampal function (72).

Some animal studies have assessed the morphological changes in the hippocampus after VNS treatment. Biggio et al demonstrated that an increase in the number of proliferating cells occur in acute VNS proliferating rats (86). Moreover, acute VNS induces an immediate increase in proliferating cells, which are BdrU+, in the dentate gyrus but do not affect to the progenitor cell survival (87). That finding is supported by the fact that the decrease in differentiated $BdrU^+$ neurons induced by bulbectomy is prevented by VNS (88). Unlike positive results with acute VNS, acute treatment with antidepressant drugs (AD) does not increase neurogenesis while acute ECT does (89).

In addition, chronic VNS is known to enhance the expression of DCX, which is a microtubule-associated protein considered a biomarker for neurogenesis, and BDNF in the rat brain. These facts are supported by the increasing of the $BDNF^+$ cells and BDNF signal in the hippocampus in an immunostaining test. However, Chronic VNS does not alter the number of $BdrU^+$ or $DCX^+$ neurons and also the effects of chronic VNS in the hippocampus were not associated with behavioral tests (86).

Biggio et al also described that and increase in the complexity of dendritic arborisation of $DCX^+$ neurons. Acute VNS increases the complexity of dendrites by increasing the number of intersections at distances between 60 and 80 μm from the soma. On the other hand, the number of intersections at distances between 100 and 170 μm from the soma was greater in rats treated with chronic VNS. Moreover, in both VNS treatments the length of the dendrites that project in the molecular layer of the hippocampus was greater (86).

All those findings suggest that chronic VNS may play an important role in consolidating the changes in neuronal connection by promoting the survival and tropism of new cells generated in early phases of stimulation (89).

Furthermore, it has been described that VNS enhances the excitatory synaptic transmission in the hippocampal CA3 (90) and reduces granular cells excitability by reducing its action potential discharge. Therefore, VNS probably improves the hippocampus-dependent behavioral responses to AD through facilitation of adult neurogenesis of dentate granular cells (91). VNS-induced synaptic enhanced in the dentate gyrus has been postulated to be caused by an increase of glutamate release from presynaptic terminals by β-adrenergic receptors activation (90,91).

In view of the above, it was postulated that VNS may modify gene expression of growth factors by altering the intensity of trans-synaptic neurotransmission, the electric activity of discrete neuronal circuits and thee firing rate of specific brain stem nuclei (92).

One of the most studied growth factors is the BDNF, it is known that VNS increases its mRNA in the hippocampus and cerebral cortex (86). This is consistent with the fact that the increase in expression of BDNF and its receptor tropomyosin receptor kinase B (TrkB) may block or reverse the neuronal loss associated with depression (89).

When studying the TrkB, it was demonstrated that both VNS and AD activate the receptor phosphorylating it in 2 tyrosine residues, Y701 and Y816. In contrast VNS phosphorylates an extra tyrosine in TrkB Y515 while AD do not (77). Y705 is an autophosphorylation site whereas Y816 and Y515 interact with PLCγ1 and MAPK/PI3K signaling pathways respectively when phosphorylated, as shown in the figure 7 (93). Consistent with this phosphorylation sites, increased phosphorylation of PLCγ1 is also detected following acute administration of VNS and after a chronic treatment with antidepressants in mice. Moreover, chronic administration of VNS causes also phosphorylation of signaling molecules downstream such as AKT, ERK and P70S6 (94).

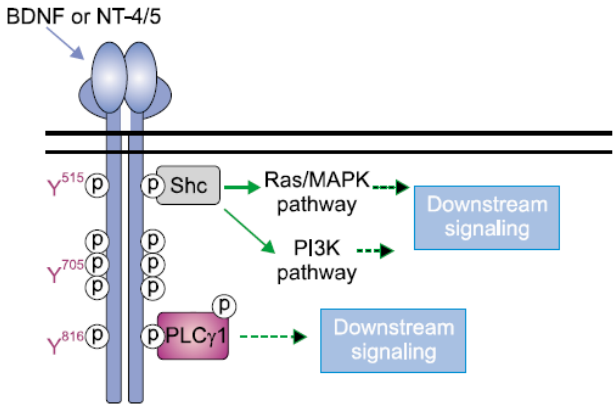


*Figure 7: Shceme of TrkB signaling.This receptor has 3 phosphorilation sites Y515, Y705 and Y816. Y705 is the autophosphorilation site and Y515 and Y816 phosphorilated activate different downstream signaling cascades. Extracted from Shah A 2014*

Recently, it was demonstrated that an intracerbroventricular pretreatment with K252a, which is an inhibitor of the tyrosine kinase activity, blocks the phosphorylation caused by in all three tyrosines (77). These finding is supported by the results of a complementary study which showed that pretreatment with K252a blocks the anxiolytic-like and antidepressant-line effects on VNS in the forced swim test in rats. However, those results did not occur if rats were treated with desipramine, a SSRI (95).Those results demonstrate that the activation of TrkB by BDNF may be necessary for their antidepressant effect (89).

In order to determine whether the activation of TrkB caused by VNS or desipramine is ligand dependent, Shah et al made a molecular scavenger for ligands of TrkB named TrkB-FC. Intraventricular administration of this molecule blocked the acute activation of this receptor induced by either treatment, indicating that the activation is ligand dependent (95). However, previous studies have shown that BDNF induces TrkB phosphorylation at Y816 and Y515. Therefore as AD do not phosphorylate Y515 may not have a ligand-dependent antidepressant action (96). In conclusion, as it has been demonstrated that desipramine requires the ligand to activate TrkB, the reason for the lack of phosphorylation in Y515 remains unclear (89).

Finally, a relationship between TrkB and neurogenesis and antidepressant efficacy has been described. Li et al showed that the reduction of TrkB in hippocampal neural progenitor cells of the dentate gyrus caused a defective proliferation and neurogenesis of these cells. It also produced mice insensitivity to chronic AD treatment. In contrast, mice lacking TrkB only in differentiated dentate gyrus neurons showed normal neurogenesis and responded normally to chronic AD (97).

### 3.4. VNS PERIPHERAL ACTION: MODULATION OF THE INFLAMATORY SYSTEM

All the information above has described the use of VNS to stimulate brain and treat depression. However, it has been described that the VNS can also have some effects in modulating the peripheral inflammatory system. This property may be used to treat some chronic inflammatory diseases such as rheumatoid arthritis or Crohn's disease or diseases in which inflammation has a key role in their pathophysiology.

In many of chronic inflammatory diseases an imbalance in the autonomic nervous system has been observed (98). Some studies have shown that both SNS and SNPS can sense inflammation and influence development and severity of inflammation (99).

Furthermore, some studies have shown that efferent vagus nerve plays a key role in suppression of inflammation in some animal models. In 2000 Boronikova et al discovered that the central nervous system through the vagus nerve may modulate the level of circulating tumor necrosis factor (TNF-α) induced by endotoxin (100). A mechanism in which the efferent fibers of the vagus nerve can control the levels of pro-inflammatory cytokines has been postulated termed the cholinergic anti-inflammatory pathway (101). This pathway was postulated to be mediated by a binding of acetylcholine (Ach) on the nicotinic acetylcholine receptor type 7 (α7nAChR) (102,103).

Some animal studies showed that the electrical stimulation of the vagus nerve and the splenic nerve inhibits lipopolysaccharidie (LPS)-induced TNF release in mice. Therefore, it was postulate that VNS inhibits inflammation by activating the noradrenergic splenic nerve (104). In agreement with that, Ballina et al postulated that the vagus nerve may connect in the coeliac ganglion leading to a NE production in the proximity of CHART+ T cells. This NE production activates the β- adrenergic receptors of the T-cells promoting a release of acetylcholine in the spleen and other tissues. Moreover, it is known that Ach is the ligand of α7nAChR located in monocytes, macrophages and stroma cells (105). The ligand binding inhibits the nuclear translocation of the NF-κB and inhibits the inflammasome activation in the macrophages which are activated by exposure of some proinflammatory factors such as lipopolysaccharidie (LPS) (106). Therefore, the ligand-receptor binding produces a suppression of proinflammatory cytokine production and inflammation (107). All this process is summarized in the figure 8.

There are several findings that support the hypothesis that the stimulation of the cholinergic anti-inflammatory pathway can be useful to treat some inflammatory diseases. It was shown that the stimulation of α7nAChR with nicotine resulted in reduced clinical arthritis scores and decreased expression of BDNF (108). Consistent with that, α7nAChR-KO mice had higher clinical arthritis scores and higher levels of TNF and monocyte chemoattractant protein 1 (109). Moreover, it has been shown that the stimulation of this process attenuates experimental arthritis severity in rats (110).

In conclusion, as a relative insufficiency of vagal activity has been implicated in the pathophysiology of inflammatory diseases (111), the VNS may be a promising therapy to treat them. Some clinical data has supported the use of this therapy. It has been shown that patients with rheumatoid arthritis present a lower activity of the vagus nerve than controls (112). Moreover, serum high mobility group box one (HMGB1) which is a biomarker from inflammation and vagus nerve activity show a significant inverse association. Furthermore, it has been shown that VNS in epilepsy patients inhibits peripheral blood production of TNF, IL-1β and IL-6 (113). Also, VNS in rheumatoid arthritis patients significantly inhibited TNF production after 84 days and the severity of the disease improved significantly. Lately, the effectiveness of VNS has also been demonstrated in Crohn's disease (110,114).

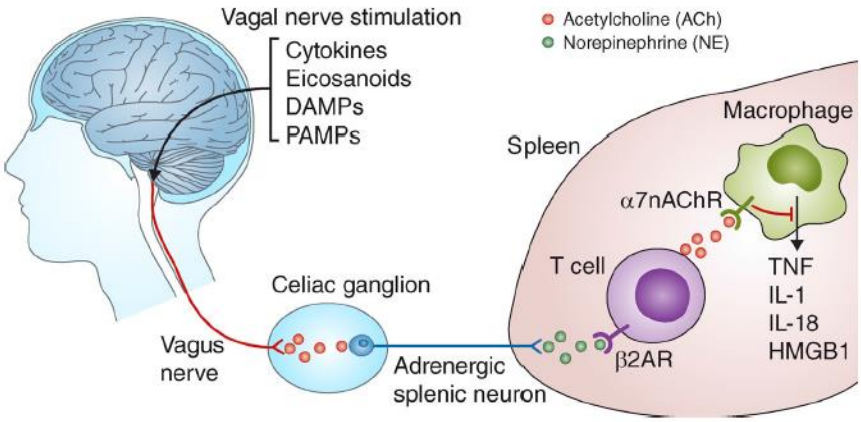


*Figure 8: The cholinergic antiinflamatory pathway. The efferent pathway of the vagal nerve activated by inflammatory signals inhibits the cytokine production in the periphery. Extracted from Anderson U et al. 2012*

### 3.5. RELATIONSHIP BETWEEN CENTRAL NERVOUS SYSTEM AND PERIPHERAL SYSTEMS THROUGH THE VAGUS NERVE

As it has been already mentioned, inflammation and immune activation have an important role in MDD. Therefore, it is clear that the peripheral immune system and the central nervous system are connected. Since VNS is a therapy that modulates inflammation and central systems like HPA axis, the hypothesis that the vagus nerve is the connection between immune and central nervous system has been postulated.

The nervous system which receives information from the immune system can suppress inflammation (101). It has been postulated that the vagus nerve has anti-inflammatory effect through its afferent fibers which activate the HA axis to release corticosteroids. The stimulation of the toll like receptors (TLR) located in the immune cells by pathogen-associated molecular patterns increase the pro-inflammatory cytokines which communicate with the brain (26). The cytokines stimulate the afferent fibers of the vagus nerve through receptors on dendritic immune cells located in the VN expressing IL-1β receptors. Therefore, systemic IL-1β may bind to this cells and activate the vagal afferents which terminate in the NTS. Indeed, peripheral administration of IL-1β and LPS produce c-fos activation in the NTS (115). The NTS has projections to the paraventricular nucleus (PVN) which contains a population of CRF-immunoreactive neurons which activate the HPA axis in response to peripheral cytokines. *The activation of the HPA axis may reduce the cytokine production in the peripheral organs.*

On the other hand, it has been demonstrated that a stimulation of the vagus nerve afferents induces and increases the expression of IL-1β mRNA in the hypothalamus and the hippocampus as well as increases the expression of mRNA in the hypothalamus and ACTH plasma levels. Since corticoids are known to suppress IL-1β transcription and mRNA stability, the afferent VNS may play an important anti-inflammatory effect (116).

In conclusion, it can be hypothesized that VNS through both afferent and efferent fibers can modulate the immune system response which is associated with HPA axis. As cytokines activate the HPA axis, the suppression of the cytokine production will decrease HPA axis which is found overexpressed in depression.

However, some findings are controversial with the monoaminergic hypothesis of depression and the modification of these neurotransmitters by VNS. It has been described that administra-

tion of cytokines induce the release of NE in the hypothalamus and increases brain concentrations of tryptophan, which is a metabolite of 5-HT(39). These findings are supported by the fact that a subdiaphragmatic vagotomy prevented the increases in the concentration of the monoamines in the mouse (117).

### 3.6. TRANS AURICULAR VAGUS NERVE STIMULATION

All the information above determines that VNS is an effective therapy to treat MDD. However, the application of this treatment has been limited to patients with TRD and no other treatment option because of the involvement of surgery.

Lately, a non-invasive trans-cutaneous VNS technique has been developed also termed trans-auricular VNS (taVNS) (118). It is based on the fact that the ear is the only place on the human body where afferent vagus nerve fibers are distributed. Therefore, it was postulated that direct stimulation of these afferent fibers may produce a similar effect to classic VNS (119,120).

Some studies showed that robust taVNS can induce a decreased fMRI signal in limbic areas including amygdala, hippocampus, parahippocampal gyrus and middle and superior temporal gyrus, as well as an increase of fMRI signal in the insula, precentral gyrus and thalamus (121,122). Moreover, other fMRI studies in humans showed a significant increase in the NTS when the subjects were treated with taVNS (123). These findings are consistent with the hypothesis that taVNS could be the non-invasive alternative to classical VNS to treat TRD.

In addition, it was demonstrated that taVNS can significantly modulate the resting state connectivity in the default mode network in the brain which is associated with emotions and affect regulation (119).

Lately, several clinical studies have tried to explore the effectiveness of this therapy. It has been demonstrated that there was a greater symptoms improvement in those MDD patients treated with taVNS compared with sham taVNS (120,124). Moreover, Rong P et al assessed that taVNS is effective in depressed patients with both mild and severe depressive symptoms (125).

Consistent with that, a recent study has determined the fMRI patterns of taVNS in depressed patients. The results showed that taVNS produces an increase in the activation of the anterior insula during the first stimulation session. Therefore, the effect of the taVNS could be primarily achieved through the modulation of the left anterior insula (126)

In conclusion, the taVNS may be an effective and noninvasive treatment for MDD which could be used as co-adjuvant of classical treatments in order to improve the symptomatology for TRD patients.

## 4. CONCLUSIONS

In this review the basic pathophysiological hypothesis of MDD have been reviewed in relation with the mechanism of action of a novel strategy to treat MDD symptoms, the VNS.

It can be concluded that in the pathophysiology of MDD many factors are involved. First of all, the monoamine hypothesis determines that MDD is caused by an alteration of monoamine levels in the brain. Secondly, the neurotrophic hypothesis postulates that neural atrophy in certain brain regions is found in depressive patients due to a reduction in the levels of neurotrophic factors. Third, the neuroendocrine hypothesis of depression describes a dysregulation in the HPA axis in MDD patients. Finally, it has been postulated a relationship between

MDD and an acute-phase inflammatory response. Although these hypothesis are widely accepted independently, the MDD can be understood as an interactive matrix of all of them.

Moreover, it is known that the vagus nerve is a crucial bidirectional link between the central nervous system and the peripheral organs. Such interconnection makes it a good target to treat several diseases that affect either the central nervous system, such as epilepsy or depression, or peripheral systems such as chronic inflammatory diseases.

The VNS produces in the central nervous system of depressed patients an increase of the monoamine levels, regulates the HPA axis and enhances the levels of neurotrophic factors and neurogenesis in the atrophied brain regions.

Furthermore, the VNS stimulates the efferent fibers of the vagus nerve acting in the periphery, reducing immune system activity. Therefore, this therapy is appropriate to treat diseases in which there is an imbalance of the immune system such as rheumatoid arthritis and Crohn's disease. Moreover, this therapy is also useful for those diseases in which inflammation is an important pathogenic factor such as acute kidney injury.

The main limitation of the VNS is the fact that the device has to be implanted using surgery. However, a non-invasive alternative to stimulate the vagus nerve, the taVNS, has been developed in order to make this technique more useful and safe.

For all the reasons above, the VNS is a promising therapy to treat depressive symptoms, especially in TDR patients. This conclusion is now supported by recent studies that show that patients with TDR are most likely to respond to the treatment and increase their remission rate if they are also treated with VNS as an adjunctive treatment of antidepressants and psychological treatment (127,128).

In conclusion, this review shows the relevance of the vagus nerve as an important bidirectional link between the central nervous system and the peripheral organs which activity can be modulated for therapeutic purposes for a number of pathological conditions. Further studies should define the optimal VNS conditions in terms of intensity, frequency and length of treatment to benefit from such powerful therapeutic tool.

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