Electrophysiological and neurochemical effects of long-term vagus nerve stimulation on the rat monoaminergic systems

Stella Manta¹, Mostafa El Mansari¹, Guy Debonnel² and Pierre Blier¹,²,³
¹ University of Ottawa Institute of Mental Health Research, Ottawa, Ontario, Canada
² Department of Psychiatry, McGill University, Montreal, Quebec, Canada
³ Department of Cellular and Molecular Medicine, Ottawa, Ontario, Canada

Abstract

Vagus nerve stimulation (VNS) is an adjunctive treatment for resistant epilepsy and depression. Electrophysiological recordings in the rat brain have already shown that chronic VNS increases noradrenaline (NE) neuronal firing activity and, subsequently, that of serotonin (5-HT) neurons through an activation of their excitatory α₁-adrenoceptors. Long-term VNS was shown to increase the tonic activation of post-synaptic 5-HT₁A receptors in the hippocampus. This study was aimed at examining the effect of VNS on extracellular 5-HT, NE and dopamine (DA) levels in different brain areas using in vivo microdialysis, on NE transmission in the hippocampus, and DA neuronal firing activity using electrophysiology. Rats were implanted with a VNS device and stimulated for 14 d with standard parameters used in treatment-resistant depression (0.25 mA, 20 Hz, 500 μs, 30 s on–5 min off). The results of the present study revealed that 2-wk VNS significantly increased extracellular NE levels in the prefrontal cortex and the hippocampus and enhanced the tonic activation of post-synaptic α₂-adrenoceptors on pyramidal neurons. The electrophysiological experiments revealed a significant decrease in ventral tegmental area DA neuronal firing rate after long-term VNS; extracellular DA levels were nevertheless increased in the prefrontal cortex and nucleus accumbens. Chronic VNS significantly increased extracellular 5-HT levels in the dorsal raphe but not in the hippocampus and prefrontal cortex. In conclusion, the effect of VNS in increasing the transmission of monoaminergic systems targeted in the treatment of resistant depression should be involved, at least in part, in its antidepressant properties observed in patients not responding to many antidepressant strategies.

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Introduction

Vagus nerve stimulation (VNS) is an adjunctive treatment for resistant epilepsy which has proven efficacy in treating resistant depression. This procedure was approved by the US Food and Drug Administration in 2005 as an adjunctive therapy for the treatment of non-psychotic unipolar and bipolar depressed patients that had failed to respond to at least four antidepressant trials. The latest open-label multicentre study showed a 53% response rate and a 33% remission rate after a year of VNS in 74 treatment-resistant patients (Schlaepfer et al. 2008), effects that were sustained 2 yr after VNS surgery (Bajbouj et al. 2010). VNS efficacy was also supported by depressive relapses that occurred in patients that had their VNS therapy interrupted (Ashton, 2010; Conway et al. 2008; Martinez & Zboyan, 2006).

Previous experiments conducted in our laboratory were aimed at elucidating possible mechanisms of action for VNS in depression. These experiments were first conducted in the dorsal raphe nucleus (DRN) and the locus coeruleus (LC) to assess the neuronal firing activity of these two major neurotransmitter systems involved in the pathophysiology of depression. The main results were that VNS promptly increases...
the basal firing activity of LC norepinephrine (NE) neurons after only 1 h and secondarily that of DRN serotonin (5-HT) neurons after 14 d (Dorr & Debonnel, 2006). An increase in the firing rate of 5-HT neurons is commonly associated with an increased 5-HT neuronal release (Bosker et al. 1994; Hery et al. 1986) that activates the 5-HTI<sub>A</sub> somatodendritic autoreceptors. These autoreceptors are presumed to be involved in the short negative feedback loop which, in response to 5-HT release from dendrites within the DRN, decreases the firing activity of 5-HT neurons (Blier et al. 1998). Thus, acute administration of antidepressants such as selective serotonin reuptake inhibitors (SSRIs), which initially enhances 5-HT extracellular levels, decreases the firing rate of 5-HT neurons. However, following long-term administration of SSRIs, 5-HTI<sub>A</sub> autoreceptors desensitize and DRN 5-HT neurons recover their normal firing rate (see Pineyro & Blier, 1999). Surprisingly, no change in 5-HTI<sub>A</sub> autoreceptor sensitivity was observed following VNS, thus suggesting an alternative mechanism to increase 5-HT neuronal firing. Further studies then showed that the effect of VNS on 5-HT neuronal firing was indirect and mediated by LC NE neurons through the enhanced activation of excitatory α<sub>2</sub>-adrenoceptors located on DRN cell bodies (Manta et al. 2009a). These data were consistent with microdialysis studies that revealed that VNS increases NE release in projection areas of the LC such as the hippocampus (HPC) and cortex (Roosevelt et al. 2006). This study, however, provided information only about acute VNS delivery with different stimulation intensities than the one already shown to be optimal in increasing 5-HT neuronal firing (Manta et al. 2009b). The HPC plays an important role in the mediation of the therapeutic effect of various classes of antidepressant treatments (Santarelli et al. 2003). As the rat HPC receives a dense noradrenergic innervation arising from the LC (Jones & Moore, 1977) and that NE generally decreases CA3 pyramidal neuronal activity through the activation of post-synaptic α<sub>2</sub>-adrenoceptors (Curet & de Montigny, 1988a, b), a possible change in the degree of activation of these receptors following VNS treatment, as well as that of the α<sub>1</sub>-adrenoceptors, needed to be investigated.

Recently, dopamine (DA) has also been suggested to be implicated in the pathophysiology of depression (Dunlop & Nemeroff, 2007). For instance, lower concentrations of DA and its metabolite homovanillic acid (HVA) had been reported in the cerebrospinal fluid (CSF) of depressed patients (Lambert et al. 2000; Mitani et al. 2006). Interestingly, a comparison between sham vs. active VNS in 21 adults with treatment-resistant major depression revealed a significant VNS-associated increase in CSF HVA (Carpenter et al. 2004). In addition, mood disorders are highly prevalent in diseases directly affecting the dopaminergic system such as Parkinson’s disease (Merschdorf et al. 2003) and the D<sub>2</sub>/D<sub>4</sub> receptor agonist pramipexole has been shown to be effective in the treatment of depression (Corrigan et al. 2000) mainly by increasing DA and 5-HT neurotransmission (Chernoloz et al. 2011). DA neurons in the ventral tegmental area (VTA) project to the cerebral cortex and limbic system, the latter including the anterior cingulate and prefrontal cortices, the HPC, amygdala, and the nucleus accumbens (NAc), which is particularly important for motivation, hedonia, and reward. However, despite the role of DA in the pathophysiology of depression there is no preclinical data on the effects of VNS on the DA system.

The present study therefore first aimed to examine the effect of long-term VNS on the extracellular levels of 5-HT, NE and DA neurotransmitters in brain areas of interest; second, to assess the tonic activation of post-synaptic α<sub>2</sub> and α<sub>1</sub>-adrenoceptors in the HPC, and finally, to characterize VTA DA neuronal firing activity and pattern using standard stimulation parameters for the treatment of resistant depression.

**Method**

**Animals**

The experiments were performed on male Sprague–Dawley rats (Charles River, Canada) weighing between 275 and 300 g at the time of the implantation of the VNS device and housed individually under standard laboratory conditions [12 h light/dark cycle (lights on 07:00 hours) with access to food and water available ad libitum]. Body temperature was kept at 37 °C during surgery, microdialysis and electrophysiological experiments. All experiments were performed in accordance with the Canadian Council on Animal Care and the local animal care committee.

**VNS surgery**

Using sterile surgical techniques, animals were operated under equithesine, 1 ml i.p./300 g rat (4.26% chloral hydrate and 0.96% sodium pentobarbital). Supplemental doses of equithesine were given i.p., 0.1 ml at a time, to maintain constant anaesthesia and to prevent any nociceptive reaction to a tail pinch. A horizontal incision was made in the ventral aspect of the neck. The skin and muscles were meticulously
separated and the left vagus nerve, which lies laterally to the carotid artery, was isolated. Bipolar leads were wrapped around the left carotid artery and the vagus nerve, allowing close contact between the electrodes and the vagus nerve. The leads were sutured in place to the underlying muscle. The leads were then tunneled subcutaneously towards an incision made in the back and were then connected to the stimulator. The stimulator was then placed in a dorsal pocket made under the back skin wiped with iodine and antibiotics, and fluid replacement given to ease recovery. Sham animals underwent the same surgical procedure with leads and a dummy 103-pulse stimulator. After a 2-d recovery period, the stimulator was turned on for 2 wk in treated rats and programmed using standard parameters (0.25 mA, 20 Hz, 500 μs pulse width, 30 s on–5 min off continuously; Sackeim et al. 2001).

**Microdialysis**

Rats were anaesthetized with chloral hydrate 400 mg/kg i.p. and mounted in a stereotaxic apparatus (David Kopf Instruments, USA). The microdialysis probe (CMA/11, Cuprophan membrane, molecular cut-off of 6000 Da; Solna, Sweden) was implanted into the DRN, the HPC, the prefrontal cortex (PFC) or the NAc. According to the rat brain atlas (Paxinos & Watson, 2007), the stereotaxic coordinates relative to the interaural line were: DRN (AP +1.1 mm, L 0 mm, V –7 mm); HPC (AP +4 mm, L +4.8 mm, V –6 mm); PFC (AP +12.7 mm, L +0.6 mm, V –4.6 mm); and NAc (AP +11 mm, L +1.5 mm, V –8 mm). The active length of the dialysis membrane was 1 mm for the DRN, 3 mm for the HPC and PFC, and 2 mm for the NAc. The probe was continuously perfused, at a flow rate of 1 μl/min (CMA/400 microdialysis pump), with the following artificial cerebrospinal fluid: 147 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1.2 mM CaCl₂, adjusted to pH 7.4 with 2 mM sodium phosphate buffer. After a stabilization period of 120 min, dialysate fractions (30 μl) were collected every 30 min for 3 h to measure dialysate neurotransmitter concentrations. Locations of dialysis probes were confirmed histologically on serial coronal brain sections at the end of each experiment. Different sets of rats were used for each structure and each neurotransmitter studied. Six to eight rats were used per group.

**Zero net flux method**

As described by Guiard et al. (2008), five fractions were collected to determine basal hippocampal and cortical 5-HT levels before local perfusion of increasing concentrations of 5-HT (5, 10, 20 nm). The dialysate 5-HT concentrations ([5-HT]_	ext{out}) obtained during perfusion of the various concentrations of 5-HT ([5-HT]_	ext{in}) are used to construct a linear regression curve where [5-HT]_	ext{in} is plotted on the x-axis and the difference between [5-HT]_	ext{in} and [5-HT]_	ext{out} appears on the y-axis. The extracellular 5-HT concentration corresponds to a point at which there is no net diffusion of neurotransmitter between the extracellular space and the microdialysis membrane, i.e. when [5-HT]_	ext{in} – [5-HT]_	ext{out} = 0.

**Biochemical assays**

**NE and DA determination**

Dialysate samples were applied onto a high-performance liquid chromatography (HPLC) system coupled with electrochemical detection. Samples (25 μl) were injected by a Hitachi model L-7200 autosampler into a 2.6 μm C18 reverse phase analytical column (Kinetex 150 × 3.0 mm, Phenomenex, USA). The mobile phase (containing 114 mM NaH₂PO₄, 150 μM EDTA, 3 mM sodium octylsulfonate and 5% methanol, adjusted to pH 3.5 with phosphoric acid for NE and containing 70 mM NaH₂PO₄, 100 μM EDTA, 1 mM sodium octylsulfonate and 15% methanol, adjusted to pH 4.5 with phosphoric acid for DA) was delivered by a Hitachi model L-7100 pump at a flow rate of 0.45 ml/min. Electrochemical detection was performed by an ESA Coulochem III detector (ESA, USA) and analysis of neurotransmitter peak area under the curve using the Empower Pro software (Waters Corporation, USA).

**5-HT determination**

Samples (25 μl) were injected into a 2.6 μm C18 reverse phase analytical column (Kinetex 50 × 3.0 mm; Phenomenex, USA). The mobile phase containing 150 mM NaH₂PO₄, 4.76 mM citric acid, 50 μM EDTA, 1.5 mM sodium dodecyl sulfate, 10% methanol and 15% acetonitrile, adjusted to pH 5.6 with NaOH was delivered at a flow rate of 0.3 ml/min.

**Electrophysiological experiments**

Rats were anaesthetized with chloral hydrate (400 mg/kg i.p) and mounted in a stereotaxic apparatus (David Kopf Instruments, USA). The experiments were performed with the VNS device in place but inactivated for the duration of the experiments to avoid electrical interference.
Recordings of VTA DA neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from λ): AP +3.0 to +3.8, L 0.6–1, V 6.5–9. The presumed DA neurons were identified according to the well-established electrophysiological properties in vivo: a typical triphasic action potential with a marked negative deflection; a characteristic long duration (>2.5 ms) often with an inflection or ‘notch’ on the rising phase; a slow spontaneous firing rate (0.5–9 Hz) with an irregular single spiking pattern with slow bursting activity (characterized by spike amplitude decrement; Grace & Bunney, 1983). Additionally, as previously described, a criterion of duration (>1.1 ms) from the start of the action potential to the negative trough was used (Ungless et al. 2004). The firing patterns of DA neurons were analysed by interspike interval burst analysis following the criteria set by Grace & Bunney (1984). The onset of a burst was defined as the occurrence of two spikes with an interspike interval <0.08 s. The termination of a burst was defined as an interspike interval of ≥0.16 s. Five to six rats were used per group.

Recordings of CA3 pyramidal neurons of dorsal HPC

Extracellular recording of CA3 pyramidal neurons and microiontophoresis were performed with five-barreled glass micropipettes. The central barrel used for the unitary recording was filled with a 2 M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The four side barrels were filled with the following solutions: 5-HT creatinine sulfate (15 mM in 200 mM NaCl; pH 4), (±)-NE bitartrate (10 mM in 200 mM NaCl; pH 4), quisqualic acid (1.5 mM in 200 mM NaCl; pH 8), and the last barrel was filled with a 2 M NaCl solution used for automatic current balancing. Prior to the electrophysiological recordings a catheter was inserted in a lateral tail vein for systemic i.v. injection of pharmacological agents. The microelectrodes were lowered into the dorsal CA3 region of the HPC using the following coordinates: 4 mm anterior to lambda and 4.2 mm lateral. Pyramidal neurons were found at a depth of 4.0±0.5 mm below the surface of the brain. Since the pyramidal neurons do not discharge spontaneously in chloral hydrate-anaesthetized rats, a small ejection current or a leak of quisqualate was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1973). Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel & Spencer, 1961). The duration of microiontophoretic application of 5-HT and NE was 50 s. Neuronal responsiveness to the microiontophoretic application of 5-HT and NE was assessed by determining the number of spikes suppressed per nanoampere (nA) per Hz. The degree of tonic activation of post-synaptic α1 and α2-adrenoceptors was assessed by injecting the selective antagonists idazoxan and prazosin, respectively. Indeed, after lowering and obtaining a steady firing baseline, idazoxan (1000 μg/kg) and prazosin (100 μg/kg) were administered i.v., always in this order, to assess the percentage of changes in the firing activity in sham and VNS-treated rats. Five to six rats were used per group.

Drugs and materials

Components of HPLC mobile phases, artificial CSF, neurotransmitters standards and drugs were obtained from Sigma-Aldrich (USA). The leads, model 103-pulse stimulators and dummy stimulators were provided by Cyberonics Inc. (USA).

Statistical analysis

Statistical analysis of changes in basal extracellular 5-HT, NE, and DA levels, firing rate and burst activity of DA neurons, responsiveness to 5-HT and NE application in the HPC and tonic activation of post-synaptic α1 and α2-adrenoceptors, between sham and VNS-treated groups were performed using a two-tailed t test. Zero net flux data were analysed by linear regression. All values are expressed as mean±S.E.M. and the level of significance set at p<0.05.

Results

Effects of long-term VNS on extracellular NE levels

A 2-wk VNS delivery significantly increased extracellular NE levels in the PFC by 58% (2.63±0.10 pg/sample in sham vs. 4.15±0.22 pg/sample in VNS-treated rats, p<0.001; Fig. 1a) and in the HPC by 14% (2.55±0.10 pg/sample in sham vs. 2.91±0.11 pg/sample in VNS-treated rats, p=0.028; Fig. 1b). No change in extracellular NE levels was observed in the DRN between sham and VNS-treated rats (2.36±0.13 pg/sample in sham vs. 2.64±0.13 pg/sample in VNS-treated rats, p=0.148; Fig. 1c).

Effects of long-term VNS on the responsiveness of CA3 pyramidal neurons to exogenous NE and 5-HT

The microiontophoretic application of NE and 5-HT suppressed the firing activity of CA3 pyramidal
neurons in the HPC in a current-dependent manner in sham rats, expressed as number of spikes suppressed per nA/Hz (Fig. 2a). A 2-wk VNS delivery did not result in a modification of the suppressant effect of local application of NE and 5-HT on hippocampal firing rate revealing an unaltered sensitivity of post-synaptic $\alpha_2$-adrenoceptors and 5-HT$_{1A}$ receptors in the HPC (Fig. 2b,c).

**Effects of long-term VNS on the tonic activation of post-synaptic $\alpha_2$- and $\alpha_1$-adrenoceptors in the HPC**

The i.v. administration of the selective $\alpha_2$- and $\alpha_1$-adrenoceptor antagonists idazoxan and prazosin, respectively, did not modify the firing activity of dorsal HPC CA3 pyramidal neurons in sham rats, as previously reported by Ghanbari et al. (2011). After a 2-wk VNS delivery, the administration of idazoxan significantly increased the firing activity of CA3 pyramidal neurons (from $20 \pm 16\%$ in sham rats to $107 \pm 46\%$ in VNS-treated rats, $p=0.03$; Fig. 3), whereas the administration of prazosin had no effect ($p=0.96$), therefore indicating a greater tonic activation of only the post-synaptic $\alpha_2$-adrenoceptors.

**Effects of long-term VNS on VTA DA firing activity**

A 2-wk VNS delivery significantly decreased VTA DA neuronal firing rate by $23\%$ (from $3.24 \pm 0.24$ Hz in sham rats to $2.50 \pm 0.35$ Hz in VNS-treated rats, $p=0.025$; Fig. 4a). No change was observed between sham and VNS-treated rats regarding the firing pattern of these neurons such as the number of bursts/min, number of spikes/burst, % of spikes occurring in burst, and mean burst length (Fig. 4b).

**Effects of long-term VNS on extracellular DA levels**

A 2-wk VNS delivery significantly increased extracellular DA levels in the PFC by $26\%$ (1.15 $\pm$ 0.11 pg/sample in sham vs. 1.55 $\pm$ 0.07 pg/sample in VNS-treated rats, $p=0.005$; Fig. 5a) and in the NAc by $27\%$ (2.91 $\pm$ 0.18 pg/sample in sham vs. 3.7 $\pm$ 0.13 pg/sample in VNS-treated rats, $p<0.001$; Fig. 5b).

**Effects of long-term VNS on extracellular 5-HT levels**

A 2-wk VNS delivery significantly increased extracellular 5-HT in the DRN by $30\%$ compared to sham rats (1.57 $\pm$ 0.14 pg/sample in sham vs. 2.04 $\pm$ 0.16 pg/sample in VNS-treated rats, $p=0.03$; Fig. 6a). Conventional microdialysis and zero net flux method were employed to assess extracellular 5-HT concentrations in the HPC and PFC. Both methods showed no change in basal 5-HT extracellular concentrations between sham and long-term VNS-treated rats in either the HPC or the PFC, respectively (1.0 $\pm$ 0.10 pg/sample in sham vs. 1.04 $\pm$ 0.10 pg/sample in VNS-treated rats, $p=0.77$; 1.15 $\pm$ 0.13 pg/sample in sham vs. 1.35 $\pm$ 0.19 pg/sample in VNS-treated rats, $p=0.31$; Fig. 6b,c).
Discussion

The present results demonstrated major impacts of long-term VNS on monoaminergic systems. Chronic VNS markedly enhanced extracellular NE levels in the PFC and to a lesser extent in the HPC and increased the degree of activation of post-synaptic α2-adrenoceptors located on CA3 pyramidal neurons. However, no change in extracellular NE levels was observed in the DRN. The electrophysiological experiments revealed a decrease in VTA DA neuronal firing rate after 2 wk VNS without any change in their firing pattern; however, a significant increase of extracellular DA levels was detected in the PFC and the NAc. Chronic VNS significantly increased extracellular 5-HT levels in the DRN but not in two post-synaptic structures, namely the HPC and PFC.

Fig. 2. Integrated firing rate histogram of a CA3 pyramidal neuron in the hippocampus of a control rat during the microiontophoretic application of (a) 5-HT and norepinephrine (NE), and responses of CA3 pyramidal neurons to different currents of (b) NE or (c) 5-HT in sham and VNS-treated rats. Horizontal bars indicate the duration of iontophoretic ejection and current values in nA. Data are expressed as mean ± S.E.M. of the number of spikes suppressed per nA/Hz. Numbers at the bottom of the columns represent the number of neurons tested.

Fig. 3. (a) Integrated firing rate histogram of a CA3 pyramidal neuron in the hippocampus of a control rat (upper panel) and a 14-d vagus nerve stimulation (VNS)-treated rat (lower panel) during the i.v. administration of idazoxan and prazosin; and graph bars representing the overall changes of firing activity of CA3 pyramidal neurons after systemic injections of (b) idazoxan and (c) prazosin in sham and VNS-treated rats. Numbers at the bottom of the columns represent the number of rats used. * p < 0.05 between sham and VNS-treated rats.
The NE neurotransmitter has been proposed to be implicated in the pathophysiology of depression for many years (El Mansari et al. 2010; Schildkraut, 1974). In the present study, a marked increase of extracellular NE levels was observed in the PFC and in the HPC following 14 d VNS. These results are in keeping with those of previous studies also showing an enhancement of NE levels in these brain areas after acute VNS (Raedt et al. 2011; Roosevelt et al. 2006). They are also consistent with the net increased firing rate and burst activity of the LC NE neurons reported in our previous experiments (Manta et al. 2009a). Moreover, following the 14-d delivery of VNS, the degree of activation of \( \alpha_2 \)-adrenoceptors was enhanced while that of \( \alpha_1 \)-adrenoceptors remained unaltered. A similar increase in the tonic activation of \( \alpha_2 \)-adrenoceptors has already been found following a long-term administration with the antidepressant bupropion (Ghanbari et al. 2011).

Interestingly, the hippocampal \( \alpha_2 \)-adrenoceptors have recently been shown to be implicated in the seizure-suppressing effect of acute VNS treatment (Raedt et al. 2011). Thus, this robust action of VNS on extracellular NE levels in post-synaptic structures as well as the increased NE neurotransmission that results may be involved, at least in part, in the antidepressant effect of VNS. Indeed, this action of VNS on the NE system is different from that of SSRIs, for example, which exert a negative influence on NE neuronal firing activity and NE release (Dremencov et al. 2007; Kawahara et al. 2007; Szabo et al. 2000). Furthermore, this negative impact of SSRIS on the NE system could be in part responsible for residual symptoms such as fatigue, concentration difficulties, and cognitive impairment in patients receiving such treatment (Blier & Briley, 2011; Kasper et al. 2011).

Because of the implication of DA in depression and the critical role of DA pathways in the regulation of motivation and reward, it was deemed essential to investigate the effect of long-term VNS in that monoaminergic system. In the present study, a significant increase of extracellular DA levels in both PFC and NAc following VNS delivery was unexpected in the presence of an attenuated firing rate of the VTA DA
neurons. The present results revealed that the firing rate and release are not always correlated. Other mechanisms may thus be involved in an increase of extracellular DA levels such as changes in terminal area. This is supported by a previous study showing that an inhibition of VTA DA neuronal firing induced by the infusion of tetrodotoxin in that area did not prevent the enhanced DA release in the medial PFC induced by systemic administration of the atypical antipsychotic asenapine (Franberg et al. 2009). In contrast, in the same study, the inhibition of VTA neuronal firing completely blocked the increase of NAc DA induced by the injection of asenapine, suggesting that this increase is dependent on VTA DA neuronal activity. As VTA DA neuronal firing is decreased following chronic VNS, the increase of extracellular DA levels observed after such treatment in the NAc should involve other mechanisms. An explanation for the enhanced extracellular DA levels despite a diminished VTA DA neuronal firing rate would be a change in terminal DA $D_2$ autoreceptor sensitivity. Indeed, a recent in vitro electrophysiological study demonstrated the ability of terminal $D_2$ receptors, which normally inhibit DA release, to show

Fig. 6. Effects of chronic vagus nerve stimulation (VNS) on basal extracellular serotonin (5-HT) levels in (a) the dorsal raphe nucleus, (b) the hippocampus using the zero net flux (ZNF) method (left) or conventional microdialysis method (right), and (c) the prefrontal cortex using the ZNF method (left) or conventional microdialysis method (right). Graphs represent a linear regression of ZNF data, the x-intercepts corresponds to the extracellular 5-HT levels. Histograms represent mean $\pm$ S.E.M. of pg/sample collected over 3 h after stabilization. Numbers at the bottom of the column represent the numbers of rats used. $^* p < 0.05$ between sham and VNS-treated rats.
a decrease in their effectiveness (Fawaz et al. 2009). It would thus be interesting to assess terminal D2 receptor function following chronic VNS. Finally, despite a diminished firing rate of VTA DA neurons, the increase in extracellular DA levels in post-synaptic areas could be at play in the efficacy of VNS in depression. Among SSRIs, only fluoxetine was shown to increase extracellular DA in the PFC acutely but failed to do so chronically (Bymaster et al. 2002; Tanda et al. 1996b). Moreover, acutely or chronically administered fluoxetine had no effect on extracellular DA in the NAc (Clark et al. 1996; Koch et al. 2002). Other antidepressants such as desipramine, nortriptyline, and mianserin induce an enhancement of extracellular DA levels in the PFC but not in the NAc suggesting a regionally selective effect (Carlson et al. 1996; Tanda et al. 1996a). Thus, the significant increase of DA observed both in the PFC and the NAc after chronic VNS could help improve depressive symptoms when VNS therapy is added to the treatment as usual of resistant-depressed patients.

Antidepressant therapies have been consistently reported to affect 5-HT neuronal activity and principally contributing to an increase in 5-HT neurotransmission after chronic treatment (Blier & de Montigny, 1994), thus revealing an important role of 5-HT in the antidepressant response. The present study showed an increase of extracellular 5-HT levels in the DRN after 14 d VNS delivery. This is congruent with previous electrophysiological studies in the rat brain showing an increase of the firing rate of DRN 5-HT neurons by about 30% after 14 d and by 100% after 90 d of VNS (Dorr & Debonnel, 2006). While such an increase, following antidepressant treatment, is usually attributable to a desensitization of 5-HT1A autoreceptors (see Pineyro & Blier, 1999), a 14-d VNS delivery did not yield such desensitization. Nevertheless, it is now well documented that reciprocal interactions exist between monoaminergic neurons and that an increase in DRN 5-HT neuron firing rate can be obtained through the activation of α1-adrenergic receptors (Baraban & Aghajanian, 1980) and/or D2-like DA receptors (Aman et al. 2007; Chernoloz et al. 2009; Haj-Dahmane, 2001), both located on the soma of 5-HT neurons in the DRN. Although, the present study was not able to detect changes in extracellular NE levels in the DRN, the increase in DRN 5-HT neuronal firing rate after VNS was shown to be mediated by the activation of α1-adrenergic receptors (Manta et al. 2009a). Moreover, α1-adrenoceptors were shown to be implicated in the modulation of 5-HT release, when the infusion of the α1-adrenoceptor antagonist prazosin decreased the citalopram-induced increase in 5-HT levels in the DRN (Rea et al. 2010). Thus, the increased extracellular 5-HT levels in the DRN may be due to the activation of the somatic α1-adrenergic receptors on 5-HT neurons.

In the PFC, in contrast to chronic administration of SSRIs, which have been shown many times to increase cortical extracellular 5-HT levels (Bel & Artigas, 1993; Ceglia et al. 2004; Tanda et al. 1996b), long-term VNS had no effect on 5-HT levels in that area. In the HPC, as with a variety of antidepressants (Blier & de Montigny, 1994; Haddjeri et al. 1998), our previous results have shown that 14-d VNS delivery increases the degree of activation of post-synaptic 5-HT1A receptors (Manta et al. 2009a), without an alteration of their sensitivity, which reflects an enhancement of 5-HT transmission. However, the present study showed no change in extracellular 5-HT levels in the HPC following VNS delivery even by using the more sensitive zero net flux method. A similar phenomenon was observed when an increase in the α1-adrenergic receptors’ tonic activation already reported was not associated with an increase in extracellular NE levels in the DRN (Manta et al. 2009a). For instance, with SSRIs, many discrepancies exist between studies regarding a possible 5-HT extracellular increase in the HPC after long-term treatment. Several groups reported, for example, that chronic treatments with citalopram, paroxetine or fluoxetine produce an increase in extracellular 5-HT levels in the HPC (Gundlah et al. 1997; Hajos-Korcsok et al. 2000; Kreiss & Lucki, 1995), while other groups did not observe any change after repeated paroxetine or citalopram in the same structure (Gardier et al. 2003; Invernizzi et al. 1995). However, using electrophysiology, it was possible to show an increase in the tonic activation of post-synaptic 5-HT1A receptors after long-term administration with these antidepressants (El Mansari et al. 2005; Haddjeri et al. 1998; Mnie-Filali et al. 2011). It is interesting to note that the electrophysiological approach assesses neurotransmitter activity in the biophase of the receptors, while microdialysis estimates the concentration of neurotransmitters in the extracellular compartment. Thus, despite a lack of change in extracellular 5-HT levels in the HPC, VNS was shown to increase the tonic activation of 5-HT1A post-synaptic receptors suggesting an enhancement of 5-HT neurotransmission in the forebrain.

In conclusion, the present results support the enhancing effect of VNS on NE transmission in forebrain structures when using stimulation parameters currently used in the clinic to treat resistant depression. Moreover, despite a diminished firing activity of VTA...
DA neurons, VNS increased extracellular DA levels in post-synaptic structures, especially in the NAc which plays an important role in reward and hedonia. The enhancement of 5-HT transmission observed using an electrophysiological approach in the HPC was not detected using microdialysis, but the enhanced firing of 5-HT neurons was reflected at their cell bodies in the DRN by an increased extracellular level of 5-HT. Finally, the effect of VNS in increasing the transmission of key monoaminergic systems targeted in the treatment of depression might explain, at least in part, the efficacy of VNS, when added to treatment as usual, in patients failing to respond to previous antidepressant strategies. Moreover, a better understanding of how VNS affects each monoaminergic systems and their projection areas will be very helpful in eventually finding the best combination of VNS with other antidepressants to further improve response and remission rates of treatment-resistant depressed patients.

There are some limitations of this study. First, all experiments were conducted on healthy animals; however, although it would have been relevant to perform them in rats with depressive-like symptoms, there is no consensus yet as to the best animal model of depression. Second, it would have been preferable to perform our experiments after 90-d VNS, which is the average period to observe clinical responses in patients with resistant depression receiving VNS. However, regarding the number of stimulators available for all the experiments (n = 7) it would have been technically difficult to collect our results in a reasonable amount of time. Furthermore, it is difficult to predict how much can be inferred from the effects of 14 d compared to 90 d; however, it is important to note that the effects of VNS on 5-HT and NE neuronal firing activity are already significant after 14 d stimulation but in a less pronounced manner (Dorr & Debonnel, 2006). Thus, the 14-d stimulation period can still give reliable clues regarding the long-term impact of VNS on all three monoaminergic systems.

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Statement of Interest

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