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Partial inhibition of catecholamine activity and enhanced responsiveness to NMDA after sustained administration of vortioxetine

Mohammad Ebrahimzadeh, Mostafa El Mansari, Pierre Blier*

University of Ottawa Institute of Mental Health Research, 1145 Carling Avenue, Ottawa, ON, K1Z 7K4, Canada

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ABSTRACT

Vortioxetine is a multimodal drug that blocks serotonin (5-HT) reuptake and directly modulates 5-HT receptors. The effects of subacute and long-term administration of vortioxetine on various aspects of catecholamine and glutamate systems were investigated using single-unit extracellular recordings and microiontophoresis in the rat brain. The firing rate of dopamine (DA) neurons was significantly decreased (26%) after 14, but not 4 days of vortioxetine administration (vortioxetine-containing chow, 1.8 g/kg vortioxetine). Same 14- and 4-day regimens of vortioxetine decreased the firing activity of norepinephrine (NE) neurons (by 27% and 41%, respectively). For DA and NE neurons, 14-day vortioxetine exposure also decreased the number of bursts per minute, without changing the number of spikes per burst, percentage of spike firing in burst and the number of spontaneously active neurons per track. However, this vortioxetine-induced suppression of DA and NE neuronal activity is less than that obtained in previous studies with the selective 5-HT reuptake inhibitor (SSRI) escitalopram. In the CA3 region of the hippocampus, 14 days of vortioxetine exposure did not change the sensitivity of postsynaptic α_2-adrenoceptors nor did it increase the tonic activation of α_1-and α_2-adrenoceptors. Vortioxetine administration for 14 days increased the N-methyl-D-aspartate (NMDA)-, but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-evoked responses of CA3 pyramidal neurons. Taken together, the results of the current study suggest that vortioxetine might produce a lesser inhibition of DA and NE neuronal activity when compared to those induced by escitalopram as reported in previous studies.

1. Introduction

Sustained administration of selective serotonin (5-HT) reuptake inhibitors (SSRIs) elevates extracellular 5-HT concentrations, which initially decreases the firing rate of 5-HT neurons of the dorsal raphe nucleus (DRN) by activating 5-HT_{1A} autoreceptors (Blier and El Mansari, 2013). Such increased 5-HT levels also suppresses the firing activity of ventral tegmental area (VTA) dopamine (DA) neurons with no effect on the number of spontaneously active DA neurons (Dremencov et al., 2009). This has been shown to be mediated by activation of 5-HT_{2C} receptors controlling DA neuronal firing (Di Matteo et al., 2001; Dremencov et al., 2009). Hence, while 5-HT neurons regain their baseline firing rate with SSRI treatment prolongation, due to desensitization of 5-HT_{1A} autoreceptors (Blier and de Montigny, 1983), the firing rate of DA neurons remains attenuated (Dremencov et al., 2009). Due to the critical role of DA neuronal activity in motivation, anhedonia and reward (Nestler and Carlezon, 2006), the inhibition of DA neuronal firing might contribute, in some patients with major depressive disorder (MDD), to an incomplete or lack of response to SSRIs.

Sustained SSRI administration also inhibits the firing activity of locus coeruleus (LC) norepinephrine (NE) neurons by activating 5-HT_{2A} receptors, located on GABA neurons controlling NE neuronal activity (Szabo and Blier, 2001a, b). This persistent attenuation of NE neuronal firing activity has been hypothesized to contribute (Blier and El Mansari, 2013; Montoya et al., 2016), at least in part, to incomplete or lack of response to SSRIs in some patients with MDD. The reversal of the attenuated firing activity of these neurons has been documented with aripiprazole, olanzapine, risperidone and

* Corresponding author. University of Ottawa Institute of Mental Health Research, Mood Disorders Research Unit, 1145 Carling Avenue, Ottawa, ON, K1Z 7K4, Canada.
E-mail address: pierre.blier@theroyal.ca (P. Blier).

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paliperidone (Chernoloz et al., 2009; Seager et al., 2005; Dremencov et al., 2007a,b). Furthermore, this action was shown to be exerted through blockade of 5-HT_{2A} receptors, which implies that using 5-HT_{2A} receptor antagonists in SSRI-resistant patients could produce a therapeutic response.

Beyond the role of monoamines, the glutamate system has also been implicated in the therapeutics of MDD. In particular, the N-methyl-D-aspartate (NMDA) antagonist ketamine has been shown to exert a fast onset of therapeutic response, even in cases of treatment-resistant MDD (Sanacora et al., 2017). Based on findings of preclinical studies, the potential contribution of increased throughput of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to NMDA receptors has been hypothesized to underlie the rapid antidepressant effect of ketamine (Du et al., 2006). Consistent with this hypothesis, it has been reported that acute low doses of ketamine increased the responsiveness of AMPA receptors of the pyramidal neurons in the rat hippocampus (El Iskandrani et al., 2015). Interestingly, it has been reported that the multimodal serotonergic agent vortioxetine, but not the SSRI escitalopram, increases the firing activity of glutamate pyramidal neurons in the prefrontal cortex (Riga et al., 2016).

Vortioxetine is a 5-HT transporter (5-HTT) inhibitor, a 5-HT_{1A} receptor agonist, a 5-HT_{1B} receptor partial agonist and a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist that can improve cognitive function independently of its actions on mood (Katona et al., 2012; Mahableshwarkar et al., 2015; McIntyre et al., 2014). Because of inhibition of 5-HT reuptake, chronic vortioxetine may dampen firing activity of NE and DA neurons. However, this dampening may not occur because of the vortioxetine's 5-HT_{1A} agonism, known to increase NE and DA neural firing activity (Díaz-Mataix et al., 2005; Piercey et al., 1994). The present study was thus aimed at assessing the effects of vortioxetine on catecholamine neurons, and on glutamatergic pyramidal neurons in the hippocampus, using electrophysiological paradigms in rat.

2. Materials and methods

2.1. Experimental preparations

The experiments were conducted on male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada). Animals were kept (two per cage) in a controlled environment with (12:12 light-dark cycle) and ad libitum access to food and water. Following arrival, rats went through a treatment-free acclimatization period for 5–7 days. Animal weighed between 280 and 320 g at the time of electrophysiological recordings. Rats were assigned, randomly, to the treatment or the control group (4 and 14 days). The experiments were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, Ontario, Canada) and were carried out in accordance with the Canadian Council on Animal Care, for the care and use of laboratory animals.

For electrophysiological recordings, rats were anesthetized with intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg) and supplemental doses (100 mg/kg, i.p.) were given to keep the animal anesthetized. Following anesthesia, rats were mounted on a stereotaxic apparatus and an intravenous catheter was inserted in a lateral tail vein for acute drug delivery. During the electrophysiological recordings, body temperature was maintained at 37 °C using a temperature-controlled heating pad.

2.2. Treatment

Based on the results of previous experiments (Wallace et al., 2014), vortioxetine administration was done via ad libitum access to vortioxetine-containing chow. No significant difference in intake of chow was found in rats that received vortioxetine compared to vehicle (Smagin et al., 2016). This chow diet was chosen in order to reach 5-HTT occupancy (Wallace et al., 2014) comparable to that achieved with therapeutic doses of SSRIs (Meyer, 2007). Moreover the chow diet was used to achieve pharmacologically relevant occupancies of 5-HT_{1A} (Ki = 230 nM) and 5-HT_7 (Ki = 200 nM) receptors because of low affinity of vortioxetine to these receptors in rats (Pehrson and Sanchez, 2014). Purina 5001 rodent chow (control) had the same nutritional content than the one containing vortioxetine.

2.3. Compounds

Purina rodent chow 5001 with 1.8 g/kg vortioxetine or vehicle was manufactured by Research Diet, Inc (New Brunswick, NJ, US) and provided to us by Lundbeck A/S pharmaceutical company, Ltd. (Valby, Denmark). All other compounds were purchased from Sigma Aldrich (Oakville, ON, Canada). Norepinephrine (noradrenaline) bitartrate (4-[(1R)-2-amino-1-hydroxyethyl]-1,2-benzenediol (L-(+))-bitartrate salt), 5-HT creatinine sulfate (3-[2-aminoethyl]-5-hydroxyindole creatinine sulfate complex), quisqualic acid (b-(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)-L-alanine), AMPA hydrobromide ((±)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide) and NMDA (N-methyl-D-aspartate) were dissolved in 0.2 M NaCl. The α_1-adrenoceptor antagonist prazosin (100 μg/kg) and the α_2-adrenoceptor antagonist idazoxan (1 mg/kg) were dissolved in distilled water.

2.4. In vivo electrophysiological recordings

Firing activity of VTA DA and LC NE neurons were recorded extracellularly using a single-barrel glass micropipette, with an impedance of 2–6 MΩ, which was filled with 2 M NaCl. Recordings of hippocampus pyramidal neurons were done with a five-barrel glass micropipette.

2.5. Electrophysiological recording of DA neurons

Recordings of putative DA neurons were obtained by lowering (6–8.5 from the surface of the brain) a single barrel microelectrode in the following coordinates: 3.2–3.6 mm anterior to lambda and 0.6–1.0 mm to the midline suture (Paxinos and Watson, 1998). DA neurons were identified by their established electrophysiological criteria: a regular firing rate (2–10 Hz), an irregular single spiking pattern, a long duration (>2.5 ms) action potential often with a notch on the rising phase and a slow bursting activity with the amplitude of the action potentials progressively decreasing in a given burst and a low pitch sound on the audiometer (Ungless and Grace, 2012). As previously described (Valenti et al., 2011) the number of neurons per track was identified by recording multiple tracks in a 400 μm × 400 μm grid.

2.6. Electrophysiological recording of LC NE neurons

For assessing the firing activity of NE neurons, a single barrel microelectrode was lowered (4.5–6.0 mm from the surface of the brain) in the following coordinates: 0.9–1.2 mm posterior to lambda and 0.9–1.3 mm to the midline suture. NE neurons were identified by their regular firing rate (0.5–5 Hz), long duration (~2 ms) and biphasic action potentials. Another identifying factor was a quiescent period following a volley of spike discharges in response to a nociceptive pinch of the contralateral hind paw (Marwaha and Aghajanian, 1982).

2.7. Extracellular recording and microiontophoresis in dorsal hippocampus CA3 pyramidal neurons

Recordings of hippocampal pyramidal neurons were carried out with a five-barrel glass micropipette. The NaCl-filled (2 M) central barrel of the pipette, with an impedance of 2–5 M Ω, was used for extracellular unitary recording and a side barrel, also filled with 2 M NaCl solution was used for automatic current balancing. The other side barrels were filled with the following solutions depending on each experiment: quisqualic acid (1.5 mM in 0.2 M NaCl, pH 8), norepinephrine bitartrate (10 mM in 0.2 M NaCl, pH 4), 5-HT creatinine sulfate (15 mM in 0.2 M NaCl, pH 4), AMPA hydrobromide (5 mM in 0.2 M NaCl, pH 8) and NMDA (10 mM in 0.2 mM NaCl, pH 8).

The micropipette was lowered (4.0 ± 0.5 mm from the surface of the brain) into the hippocampus CA3 region at the following coordinates: 4.0–4.2 mm anterior to lambda and 4.2 mm to the midline suture. Since these pyramidal neurons do not discharge spontaneously under chloral hydrate anesthesia, a small current of quisqualic acid was used to activate them within their physiological range (10–15 Hz; Ranck, 1975). The used identification criteria for pyramidal neurons included: long duration, large amplitude and simple action potentials alternating with complex spike discharges (Kandel and Spencer, 1961).

2.8. In vivo determination of 5-HT and NE reuptake

Microiontophoretic ejection of 5-HT creatinine sulfate and NE bitartrate was used to reduce the firing activity of CA3 pyramidal neurons to approach near complete suppression. Following the termination of microiontophoretic ejection, pyramidal neurons gradually regained their firing activity due to the active reuptake process of 5-HT and NE, by the 5-HTT and NE transporters (NET) respectively. The time bracket from the end of the microiontophoretic ejection to where the activity of the neuron has recovered to half of its initial firing activity is defined as the RT50 value and is used as a reliable *in vivo* index for activity of the 5-HTT and NET (de Montigny et al., 1980; Piñeyro et al., 1994; El Mansari et al., 2015a). The RT50 values were determined to ensure that ad libitum regimen of vortioxetine-containing chow has resulted in relevant concentrations of vortioxetine and a significant blockade of 5-HTT.

2.9. Determination of sensitivity of α_2- and tonic activation of α_2- and α_1-adrenoceptors in the hippocampus

Sensitivity of the α_2-adrenoceptors was assessed by determining the neuronal responsiveness to the iontophoretic application of NE (Curet and de Montigny, 1988). After establishing a proper baseline firing activity, NE was iontophoretically ejected for 50 s to inhibit the firing activity of pyramidal neurons. The responsiveness of the recorded neuron was measured by calculating the number of spikes within the 50 s period prior to NE ejection minus the number of spikes during the ejection divided by the applied current. This gives the number of spikes suppressed by the amount of current ejected.

The selective adrenoceptor antagonists, idazoxan and prazosin, were used for investigating a possible tonic activation of postsynaptic α_2- and α_1-adrenoceptors, respectively. After obtaining a lowered and steady state firing rate, idazoxan (1 mg/kg) and prazosin (100 μg/kg) were sequentially administered intravenously to investigate a possible disinhibition of pyramidal neuronal activity. The used doses of idazoxan and prazosin have been reported as physiologically effective in an electrophysiological setting (Chernoloz et al., 2012).

2.10. Determination of AMPA- and NMDA-evoked activity of CA3 pyramidal neurons

Drug effect was assessed by measuring the degree of excitation of pyramidal neurons (measured as number of spikes generated for 60 s ejection) induced by iontophoretic application of NMDA and AMPA. Two of the side barrels of the electrode were filled with AMPA hydrobromide and NMDA and another one was filled with quisqualate. Both the duration (60 s) and ejection currents of AMPA and NMDA were kept constant in control and vortioxetine-administered rats. To activate and locate the neurons, a small current of quisqualate was used (−2 to −5 nA). Once a pyramidal neuron was found, the quisqualate pump was stopped and AMPA and NMDA were applied. When AMPA and NMDA were not ejected, a retention current of +15 nA was applied to prevent leakage from the barrels.

Effect of acute intravenous (i.v.) injection of vortioxetine (6 mg/kg; El Mansari et al., 2015b) on the responsiveness of neurons was measured by recording the firing activity of neurons in response to iontophoretically ejected AMPA (−1 nA) and NMDA (−8 nA) before and after the injection. Prior to the vortioxetine injection, rats received an i.v. injection of saline followed by measuring the AMPA- and NMDA-evoked firing activity of pyramidal neurons. Two minutes after vortioxetine injection, recording the responsiveness of neurons to AMPA and NMDA was started and continued for thirty minutes.

The effects of long-term vortioxetine exposure (vortioxetine-containing chow, 1.8 g/kg) was assessed by comparing the responsiveness of pyramidal neurons to 60 s of AMPA (0,-1,-2 nA) and NMDA (−7,-8,-9 nA) ejection in control and vortioxetine-exposed rats. The acquired data were used to construct a dose response curve representing the iontophoretic ejection current and number of generated spikes per 10 seconds.

2.11. Data analysis

The acquired data are presented as mean values ± S.E.M. In VTA and LC, comparisons between controls and treated groups were carried out using Kruskal-Wallis One-Way analysis of variance (ANOVA) on ranks followed by Dunn's method. Interspike interval (ISI) burst analysis was used for analyzing the firing patterns of DA and NE neurons. The initiation of a burst was defined as the occurrence of two spikes with ISI <0.08 s and the termination of the bursts were defined as ISI >0.16 s (Grace and Bunney, 1984). The burstiDAtor software was used in this project for burst analysis (https://github.com/nno/burstiDAtor).

Paired *t*-test and Mann-Whitney U tests were used for measuring the effect of acute i.v. injection of vortioxetine on AMPA- and NMDA-evoked firing activity of pyramidal neurons. For analyzing the data related to tonic activation of adrenoceptors and comparing AMPA- and NMDA-evoked firing of pyramidal neurons in control and 14-day vortioxetine administered rats, Two-Way analyses of variance with repeated measures were used, with treatment as the main factor. Throughout the analyses, statistical significance was taken as $p < 0.05$. These statistical comparisons were analyzed using the software Graphpad (Prism software Inc, La Jolla, CA).

3. Results

3.1. Effect of sustained vortioxetine exposure on the activity of the 5-HTT and NET

The RT50 values were determined as an *in vivo* measure for activity of 5-HTT and NET. RT50 values were significantly increased in rats exposed to vortioxetine for 4 (240%) and 14 days (650%),

compared to control rats indicating a potent blockade of the 5-HTT (Fig. 1A). This increase of the recovery time of firing activity of pyramidal neurons after 5-HT ejection (increased RT50 value) indicated the presence of physiologically relevant doses of vortioxetine, enough to inhibit the activity of 5-HTT.

Microiontophoretic applications of NE were carried out in the vehicle group and in rats exposed with vortioxetine for 14 days. As illustrated in Fig. 1B, the RT50 values did not significantly change in the controls compared to rats exposed to vortioxetine for 14 days, indicating that NET was not blocked.

3.2. Effects of 4- and 14-day vortioxetine administration on the firing activity of VTA DA neurons

The mean firing activity of DA neurons in the 4- and 14-day control groups were not significantly different (4-day control group: 4.6 ± 0.2 Hz, n = 93 versus 14-day control group: 4.2 ± 0.2 Hz, n = 80) and consequently these two groups were combined.

While vortioxetine administration for 4 days did not significantly change the firing activity of DA neurons (Fig. 2), its administration for 14 days, however, significantly decreased their activity by 26% (Fig. 2).

Vortioxetine administration for 14 days, but not 4 days, decreased the burst frequency of DA neurons without changing the bursting pattern (spikes/burst and percentage of spikes in burst) and the number of spontaneously active DA neurons per track (Table 1).

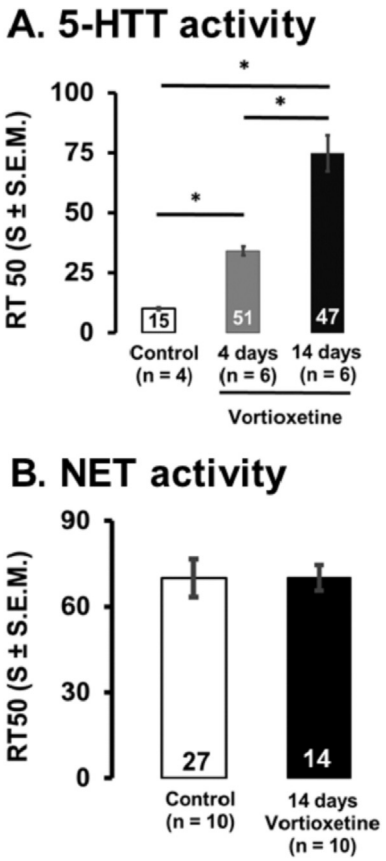

Fig. 1. Effect of 4- and 14-day vortioxetine exposure on the recovery time, expressed as RT50 values, from microiontophoretic applications of 5-HT (A) and NE (B) with 20 nA. (A) There was a significant increase in 5-HTT-related RT50 values in 4- and 14-day vortioxetine administered rats, when compared to the control group. (B) Vortioxetine administration for 14 days did not have any statistically significant effect on NET-related RT50 values when it is compared to the control group. Number of neurons are indicated at the bottom of each graph. Number of rats are indicated in the brackets. *$p < 0.05$.

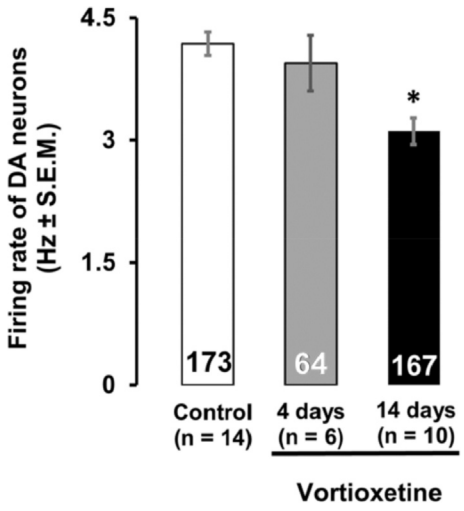

Fig. 2. Firing rates of VTA DA neurons (mean ± S.E.M.) in control, 4- and 14-day vortioxetine-administered rats (1.8 g/kg, vortioxetine-infused chow). Number of neurons are indicated at the bottom of each graph. Number of rats are indicated in the brackets. *$p < 0.05$ compared to the control group.

3.3. Effects of 4- and 14-day vortioxetine administration on the firing activity of LC NE neurons

The firing activity of NE neurons in the 4- and 14-day control groups were not statistically different (4-day control group: 2.0 ± 0.1, n = 65 versus 14-day control group: 2.4 ± 0.2 Hz, n = 78). Hence, the data from these two groups were combined.

Vortioxetine administration for 4 days significantly decreased the firing rate of NE neurons by 41% (Fig. 3). Following administration of vortioxetine for 14 days, the firing rate of NE neurons remained dampened when compared to the control group, but to a significant lesser extent in comparison to the 4-day group (27%; Fig. 3).

As illustrated in Table 2, vortioxetine administration for 14 days, decreased the burst frequency of NE neurons without changing the bursting pattern (spikes/burst and percentage of spikes in burst) and the number of spontaneously active NE neurons per track.

3.4. Effect of prolonged administration of vortioxetine on the sensitivity of α_2- and tonic activation of α_2- and α_1-adrenoceptors of the pyramidal neurons of the hippocampus

The sensitivity of the α_2-adrenoceptors, measured by the number of spikes inhibited by iontophoretic ejection of NE per nanoampere, was not altered by vortioxetine exposure for 14 days.

In 14-day vortioxetine-administered rats, systemic administration of the α_2- and α_1-adrenoceptor antagonists, idazoxan (1 mg/kg) and prazosin (100 μg/kg) respectively (Fig. 4), did not result in any change in firing activity of pyramidal neurons of the hippocampus compared to the control group (Fig. 5). These results indicate that there was no increase in NE neurotransmission in the hippocampus after 14 days of vortioxetine administration.

3.5. Effects of acute injection and sustained exposure to vortioxetine on AMPA- and NMDA-induced firing activity of pyramidal neurons of the CA3 region of the hippocampus

Intravenous vortioxetine administration (6 mg/kg; El Mansari

Table 1
Bursting activity (mean ± S.E.M.) of VTA DA neurons. *$p < 0.05$, compared to the control group; n indicates the number of neurons.

	Burst/min	Spikes/burst	% spikes in burst	Neurons/track
Control (n = 151)	22 ± 2	3.2 ± 0.2	29 ± 2	1.5 ± 0.1 (14 rats)
4-day vortioxetine (n = 54)	19 ± 3	2.8 ± 0.2	26 ± 4	1.6 ± 0.3 (6 rats)
14-day vortioxetine (n = 91)	14 ± 2*	2.8 ± 0.1	21 ± 3	1.5 ± 0.2 (10 rats)

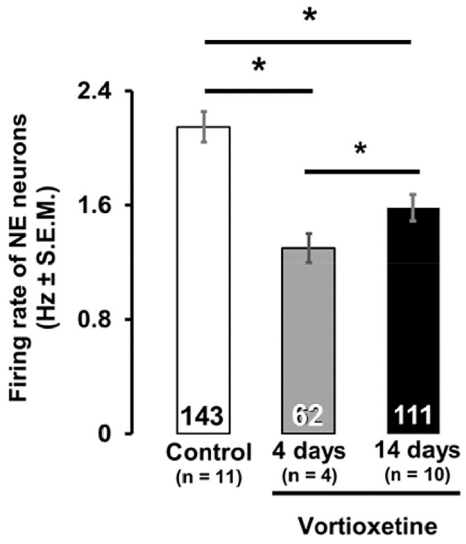

Fig. 3. Mean (±S.E.M.) firing rate of LC NE neurons in control and vortioxetine-administered rats for 4 and 14 days. Number of neurons are indicated at the bottom of each graph. Number of rats are indicated in the brackets. *$p < 0.05$.

et al., 2015b) significantly decreased AMPA-evoked firing activity in 7 out of 11 pyramidal neurons (mean firing activity prior to vortioxetine injection was 13 ± 1.4 versus 7 ± 1.9 Hz after vortioxetine injection; $p = 0.02$). In the remaining four neurons, vortioxetine did not cause a significant change in the AMPA-evoked firing activity.

However, in 14-day vortioxetine administered rats, there was no alteration in responsiveness of pyramidal neurons to iontophoresed AMPA, using incremental currents of ejection (Fig. 6A).

Intravenous administration of vortioxetine (6 mg/kg) significantly decreased NMDA-induced firing activity of pyramidal neurons in 8 out of 10 tested pyramidal neurons (mean firing rate prior to vortioxetine injection was 12 ± 1.2 versus 6 ± 1.1 Hz after vortioxetine injection; $p = 0.01$). In the two other neurons, injection of vortioxetine had no effect on the NMDA-evoked firing activity (mean firing rate prior to vortioxetine injection was 12 ± 1.3 versus 13 ± 2.3 Hz after vortioxetine injection).

Fourteen days of vortioxetine exposure, however, significantly enhanced NMDA-induced firing of pyramidal neurons (Fig. 6B, $F[1, 103] = 5.2$; $p = 0.03$). Moreover there was a significant effect of current ($F[1, 103] = 36.9$; $p = 0.001$) as well as a significant interaction (treatment x current, $F[1, 103] = 3.7$; $p = 0.03$).

4. Discussion

The present *in vivo* electrophysiological experiments showed that prolonged vortioxetine administration dampened the firing activity of catecholamine neurons, although to a lesser degree than that observed with the SSRI escitalopram. Following this same regimen, the current results also showed that the tonic activation of the α_1- and α_2-adrenoceptors on hippocampus pyramidal neurons was not changed. On the other hand, while 14 days of vortioxetine exposure did not change the AMPA-evoked firing activity of pyramidal neurons, the NMDA-evoked activity of these neurons was increased after 14 days of vortioxetine regimen.

The current study showed an increase of the RT50 values for 5-HT in hippocampus indicating blockade of 5-HTT by vortioxetine. The RT50 values also significantly increased from 4- to 14-day vortioxetine administration, suggesting that the steady-state level of vortioxetine was not achieved after 4 days of treatment.

Acute administration of vortioxetine has been shown to cause no change in hippocampal and cortical DA levels as well as VTA DA neuronal firing activity (Pehrson et al., 2013). In the present study, although 4-day vortioxetine administration did not change the firing activity of DA neurons, its sustained administration significantly dampened firing activity of these neurons by 26%. Since vortioxetine has no significant affinity for any DA receptors or the DA transporter (Bang-Andersen et al., 2011), the latter effect may be due to an increase of 5-HT levels around DA neuron cell bodies. Indeed, evidence has shown that 5-HT neurons exert an inhibitory effect on DA neurons through 5-HT_{2C} receptors (Di Matteo et al., 2001; Gobert et al., 2000; Prisco et al., 1994). In addition, this suppression of DA neurons firing was blocked by the selective 5-HT_{2C} receptor antagonist SB 242084 (Dremencov et al., 2009). Although in the current study vortioxetine exposure has suppressed the activity of DA neurons, this decrease was lower compared to one induced by the SSRI escitalopram, as found in previous studies (40–50%; Chernoloz et al., 2009; Dremencov et al., 2009). Likewise, a lower decrease of firing activity of DA neurons compared to escitalopram was found with vilazodone (28%), which is an inhibitor of 5-HTT (Ki = 0.5 nM) and an agonist with high affinity for 5-HT_{1A} receptors in rats (Ki = 0.2 nM; Dawson and Watson, 2009; El Mansari et al., 2015a). It might be argued that this relative weak suppression of DA neuronal activity by vortioxetine and vilazodone may be due to an activation of 5-HT_{1A} receptors by these compounds. Indeed activation of 5-HT_{1A} receptors by 5-HT_{1A} receptor agonists was shown to markedly increase firing and bursting activities of DA neurons in the VTA, much more so than for NE neurons (Gronier, 2008; Lejeune and Millan, 1998). This enhancing action of a 5-HT_{1A} receptor agonist is abolished by 5-HT_{1A} receptor antagonist WAY100635 (Ichikawa et al., 2001). However, due to weak occupation of 5-HT_{1A} receptors by vortioxetine in rats (Ki = 230 nM) compared to humans (Ki = 15 nM), the involvement of the latter receptor in this effect remains uncertain.

In the LC, the current study showed that firing activity of NE

Table 2
Bursting activity of LC NE neurons (mean ± S.E.M.). *$p < 0.05$, compared to the control group; n indicates the number of neurons.

	Burst/min	Spikes/burst	% spikes in burst	Neurons/track
Control (n = 80)	5.8 ± 1.2	2.2 ± 0.0	6 ± 1	2.9 ± 0.3 (11 rats)
4-day vortioxetine (n = 19)	4.5 ± 2.8	2.1 ± 0.1	6 ± 4	3.1 ± 0.5 (4 rats)
14-day vortioxetine (n = 37)	1.9 ± 0.4*	2.5 ± 0.3	4 ± 1	3.4 ± 0.3 (10 rats)

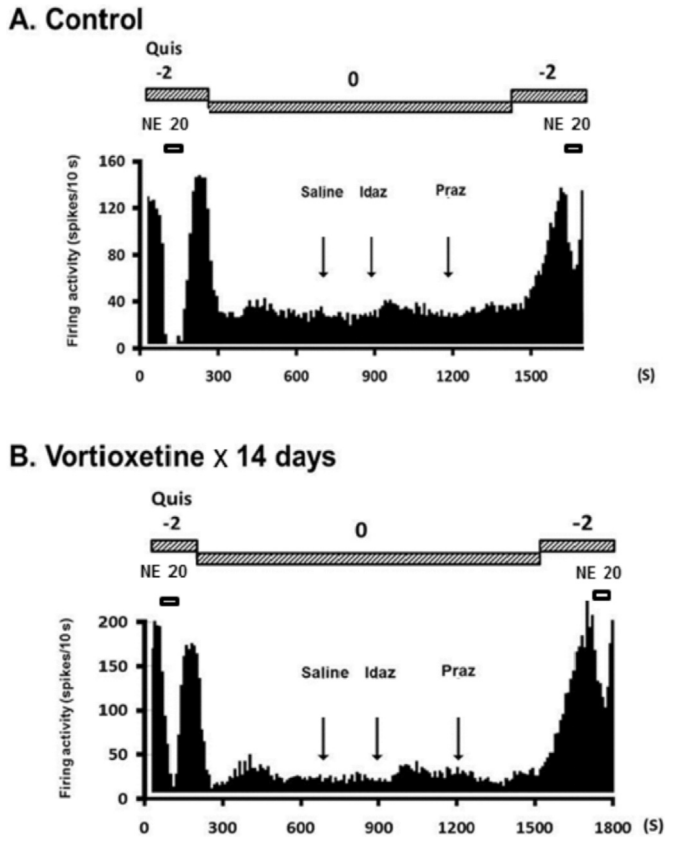

Fig. 4. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating their responsiveness to microiontophoretic ejection of NE (20 nA) and systemic administration of idazoxan (1 mg/kg; Idaz) and prazosin (100 μg/kg; Praz) in a control rat (A) and a 14-day vortioxetine-treated rat (B). Note the decreased inhibition of firing produced by NE following the injection of the noradrenergic antagonists.

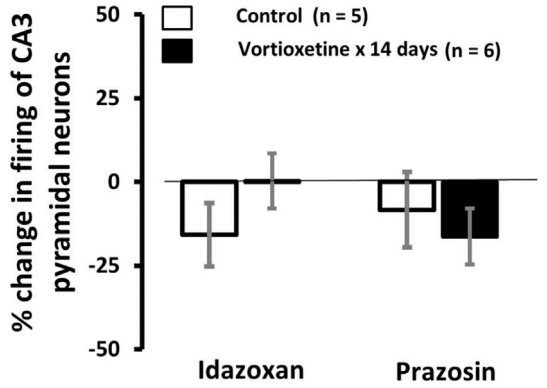

Fig. 5. Percentage of change in firing frequency of pyramidal neurons in response to systemic administration of idazoxan (1 mg/kg) and prazosin (100 μg/kg) in control and 14-day vortioxetine-treated rats. Number of rats are indicated at the top of the graph.

neurons was significantly dampened after 4 days of exposure to vortioxetine as it was reported in another study after acute injection (Pehrson et al., 2013). In the current study, after 14 days of exposure, the firing activity of NE neurons remained decreased but to a lesser degree. The inhibitory action of vortioxetine on NE neuronal firing may be due to an enhancement of 5-HT levels resulting from a potent blockade of the 5-HTT (Fig. 1A), as shown in the present study and previous microdialysis experiments (Pehrson

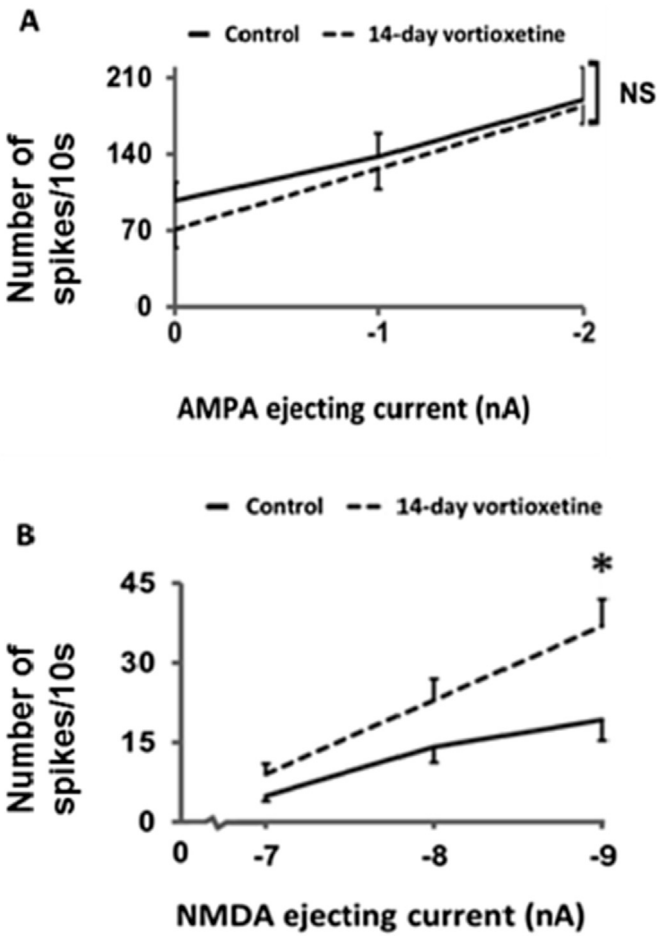

Fig. 6. The effects of 14-day vortioxetine administration on the responsiveness of AMPA and NMDA receptors. Responsiveness is measured by calculating the number of spikes (per 10 s) generated by microiontophoretic application of AMPA (0, −1 and −2 nA) and NMDA (−7,-8 and −9 nA). NS: non-significant. $^{*}p < 0.05$.

et al., 2013). Interestingly, it was shown that the inhibitory effect obtained with the SSRI escitalopram is mediated via activation of 5-HT_{2A} receptors since it is reversed by injection of the selective 5-HT_{2A} receptor antagonist M100907 (Dremencov et al., 2007a; Haddjeri et al., 1997). These results are similar to those obtained with several SSRIs that share blockade of 5-HTTs with vortioxetine (Chernoloz et al., 2009; Dremencov et al., 2007a; Ghanbari et al., 2010; Szabo et al., 2000). The vortioxetine-induced inhibition of NE neuronal firing activity (27%) in the present study was, however, less than the reported decrease following administration of escitalopram, found in earlier studies (40–50%; Chernoloz et al., 2009; Ghanbari et al., 2010). Although vortioxetine and escitalopram were not directly compared, the magnitude of their effect on DA and NE firing activity appears to be different. On the one hand, such a comparison does not take into consideration that vortioxetine has an agonistic activity at 5-HT_{1A} receptors and may have exerted an excitatory influence on NE neuronal activity. Indeed, previous studies have shown that 5-HT_{1A} receptor agonists increase neuronal firing rate and burst activity of NE neurons (Piercey et al., 1994; Szabo and Blier, 2001a, b). On the other hand, although vortioxetine has high affinity for the human 5-HT_{1A} receptors, it has a weaker affinity to these receptors in rats (Pehrson and Sanchez, 2014). By increasing the dose of vortioxetine in the present study, occupancy of 5-HTT was aimed to reach a level (Wallace et al., 2014), that is reached in patients with MDD treated with SSRIs

(Meyer, 2007). It is possible that a lack of recovery of NE firing activity to normal after 14 days of vortioxetine administration may have stemmed from desensitization of the 5-HT_{1A} receptors. This argument is strengthened by a previous report demonstrating that the increasing effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on NE neuronal firing was abolished in rats that received the SSRI citalopram, due to desensitization of 5-HT_{1A} receptor (Szabo et al., 2000). Furthermore, it was shown that long-term administration of vilazodone, a 5-HTT inhibitor which possesses high affinity to 5-HT_{1A} receptors in rats, also resulted in a significant decrease (33%) of neuronal activity of NE neurons, when 5-HT_{1A} receptors were desensitized (El Mansari et al., 2015a). Nonetheless, contribution of these 5-HT_{1A} receptors to this effect remains uncertain due to weak occupation of 5-HT_{1A} receptors by vortioxetine in rats.

Despite an increase in NE levels in the hippocampus after 3-day administration of vortioxetine (Pehrson et al., 2013), the present electrophysiological study did not show an increase in the tonic activation of α_1- and α_2-adrenoceptors following 14-day administration of this compound. This lack of increase cannot be due to a desensitization of α_2-adrenoceptors as these receptors are normosensitive under prolonged vortioxetine regimen as shown in the present work. Although vortioxetine, which possesses no affinity for either adrenoceptors or NET (Bang-Andersen et al., 2011), induced no change in tonic activation of α_2- and α_1-adrenoceptors, an increase in tonic activation of these receptors was present with drugs that have an affinity for adrenoceptors or NET, such as bupropion and quetiapine (Chernoloz et al., 2012; Ghanbari et al., 2010).

It is noteworthy that the measure of tonic activation in this study reflects the net effect of NE transmission because it is determined in postsynaptic neurons, while NE levels measured by microdialysis reflect the changes that take place in the extracellular compartment. In addition, the mentioned microdialysis study (Pehrson et al., 2013) was done with vortioxetine administered subacutely, while tonic activation in the current study was measured after long-term vortioxetine administration, which is more relevant to clinical conditions.

Previous studies have shown that acute and sub-chronic administration of the SSRI fluoxetine increases phosphorylation of the AMPA receptor subunits (Svenningsson et al., 2002). In the present study, acute administration of vortioxetine, which is also a 5-HTT inhibitor, had mixed effects whereby it decreased AMPA-evoked firing of CA3 pyramidal neurons in some neurons and did not alter it in others. However, following long-term administration of vortioxetine, the NMDA- but not AMPA-induced firing of these glutamatergic neurons increased. It is thus possible that this direct effect of vortioxetine contributes to the pro-cognitive effects of vortioxetine, which was reported to be ameliorated following vortioxetine administration in rats (du Jardin et al., 2014; Jensen et al., 2014; Mørk et al., 2013) and in patients with MDD (Katona et al., 2012; Mahableshwarkar et al., 2015; McIntyre et al., 2014). Interestingly, a recent electrophysiological study using the same regimen of vortioxetine as herein (Riga et al., 2017) has shown that prolonged administration of vortioxetine increased the firing activity of glutamatergic pyramidal neurons in the rat medial prefrontal cortex, while the SSRI escitalopram had no effect (Riga et al., 2016).

In summary, results of the present study in comparison with those obtained in previous studies using escitalopram suggest that vortioxetine might have a different effect on the basis of its weaker inhibitory activity on DA and NE neurons. However, only a head-to-head clinical trial would be able to determine whether these drugs have a differential effectiveness on various symptom domains.

Conflicts of interest

M. El Mansari and M. Ebrahimzadeh declare no conflict of interest. P. Blier received grant funding and/or honoraria for lectures and/or participation in advisory boards for Allergan, Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Euthymics, Janssen, Lundbeck, Merck, Otsuka, Pfizer, Pierre Fabre, Servier, Shire, Takeda, and Valeant.

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