Sub-chronic vortioxetine (but not escitalopram) normalizes brain rhythm alterations and memory deficits induced by serotonin depletion in rats

Maurizio S Riga^{a,b,c,#}, Connie Sanchez^d, Pau Celada^{a,b,c}, Francesc Artigas^{a,b,c,#}

^a Depart. de Neuroquímica i Neurofarmacologia, CSIC-Institut d'Investigacions Biomèdiques de Barcelona, Barcelona, Spain.

^b Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

^c Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain.

^d H Lundbeck A/S, Ottiliavej 9, Valby-Copenhagen 2500, Denmark at the time the study was conducted, Translational Neuropsychiatry Unit, Clinical Medicine, Aarhus University, Skovagervej 2 8240, Risskov, Denmark.

[#] Corresponding authors:

Maurizio Riga, PhD. Rosselló 161, 6th floor. 08036 Barcelona, Spain. Email: mauririga@gmail.com; Phone: +343638315; Fax: +343638301.

Francesc Artigas, PhD. Rosselló 161, 6th floor. 08036 Barcelona, Spain. Email: francesc.artigas@iibb.csic.es; Phone: +343638315; Fax: +343638301

Abstract (250)

Introduction (724)

Material and Methods (1517)

Results (1241)

Discussion (1806)

Conclusions (171)

References (101)

Abstract

Major depressive disorder (MDD) is a chronic and disabling psychiatric disorder characterized by a wide range of signs/symptoms, including cognitive dysfunction. Vortioxetine (VOR) is a multimodal antidepressant drug with pro-cognitive actions in animal models and MDD patients. The VOR-mediated blockade of 5-HT₃-R in a subpopulation of GABA interneurons enhances pyramidal neuron activity in rat medial prefrontal cortex, an effect possibly underlying its pro-cognitive action. Brain oscillations are involved in regulation of cognitive function. We therefore examined VOR effects on oscillatory activity in four brain areas of freely-moving rats (prelimbic cortex, PrL; nucleus accumbens, NAc; dorsal hippocampus, dHPC; paraventricular thalamic nucleus, PVA), in standard and in serotonin-depleted rats showing recognition memory deficits. 4-chloro-DL-phenylalanine (*p*CPA) markedly reduced low frequency oscillations (LFO, mainly 1 Hz oscillations) and enhanced theta oscillations in PrL and NAc. It also reduced gamma and high frequency oscillations (HFO) in PVA. Subchronic VOR and escitalopram (ESC) treatments had little effect on oscillatory activity in standard rats. However, VOR –but not ESC– prevented recognition memory deficits in 5-HT-depleted rats, and normalized LFO and theta powers in PrL and NAc. In parallel, VOR –but not ESC– prevented the deficit in PrL-dHPC gamma coherence, but not the decrease in gamma and HFO powers in PVA. Overall, this supports a prominent role of serotonergic neurotransmission on brain oscillatory activity, particularly in cortico-striatal pathways linked to short-term recognition memory. Further, VOR prevented *p*CPA-induced cognitive deficits by normalizing oscillatory activity at lower frequencies in the PrL-NAc pathway, also normalizing the PrL-dHPC coherence at gamma frequencies.

Keywords: 5-hydroxytryptamine (serotonin); 5-HT₃ receptors; brain oscillations; escitalopram; short-term memory; vortioxetine

Highlights

- 5-HT controls oscillatory activity in brain pathways linked to recognition memory
- Subchronic vortioxetine restores brain rhythms in 5-HT depleted rats
- Subchronic vortioxetine prevents recognition memory deficits in 5-HT depleted rats
- Acute vortioxetine enhances oscillatory activity only in 5-HT depleted rats
- Pro-cognitive actions of vortioxetine may involve changes in brain oscillations

1. Introduction

Major depressive disorder (MDD) is a chronic and debilitating psychiatric disorder characterized by a wide range of psychic and somatic signs and symptoms, of which a persistent negative mood, lack of interest and pleasure, and feelings of guilt and worthlessness are most common. Likewise, cognitive dysfunction is now widely recognized as an important component of MDD, with alterations in executive functions, difficulties in attention and concentration, and slow processing speed, among other aspects (Cotrena et al., 2016; Snyder, 2013).

Vortioxetine (VOR) is an antidepressant drug (Alvarez et al., 2012; Sanchez et al., 2015) that shows pro-cognitive efficacy in animal models (Pehrson et al., 2018; Sanchez et al., 2015; Wallace et al., 2014; Westrich et al., 2015; Bennabi et al., 2019) and improves aspects of cognitive dysfunction in MDD patients (Al-Sukhni et al., 2015; Bennabi et al., 2019; Katona et al., 2012; McIntyre et al., 2014; 2015; Mahableshwarkar et al., 2015a, 2015b; Rosenblatt et al., 2015; Bennabi et al., 2019)). VOR is a multimodal drug, being a 5-HT₃-R, 5-HT₇-R and 5-HT_{1D}-R antagonist, 5-HT_{1B}-R partial agonist, 5-HT_{1A}-R agonist, and serotonin (5-HT) transporter (SERT) inhibitor (Mørk et al., 2012; Sanchez et al., 2015). VOR produces a dose-dependent occupancy of all these targets at clinical doses, with a preferential occupancy of 5-HT₃-R (Sanchez et al., 2015), for which VOR shows high affinity (3.7 nM; Mørk et al., 2012). 5-HT₃-Rs are ion channels present in a subpopulation of cortical and hippocampal GABAergic interneurons, which in the neocortex, are located in upper (I-III) layers (Lee et al., 2010; Morales and Bloom, 1997; Puig et al., 2004; Santana and Artigas, 2017). The physiological activation of 5-HT₃-R by endogenous 5-HT markedly excites GABA interneurons in rat medial prefrontal cortex (mPFC) (Puig et al., 2004) and hippocampus (Varga et al., 2009).

Notwithstanding the exclusive localization of 5-HT₃-R in layers I-III rat mPFC, their blockade by VOR or the selective 5-HT₃-R antagonist ondansetron led to the disinhibition and subsequent increase in firing activity of a very large percentage (~70%) of midbrain-projecting layer V pyramidal neurons (Riga et al., 2016a). Further, subchronic oral VOR treatment, at doses evoking a SERT occupancy similar to that found in treated MDD patients, markedly enhanced the discharge of putative pyramidal neurons in rat mPFC (Riga et al., 2017). The selective serotonin reuptake inhibitor (SSRI) escitalopram (ESC) did not alter pyramidal neuron activity in any experimental condition (Riga et al., 2016a, 2017). These observations suggest a critical role of 5-HT₃-R in PFC function and in cortical-subcortical communications. Given the

involvement of PFC in higher brain functions, including working memory and executive functions, and its top-down control of neuronal activity in cortical and subcortical brain structures (Fuster, 2008; Miller and Cohen, 2001), the VOR-mediated increase of pyramidal neuron activity in PFC may be the neural substrate for its pro-cognitive activity.

In order to expand the knowledge of the role of 5-HT₃-R in cognition, and particularly, in the mechanism of action of VOR, we examined the effects of subchronic VOR treatment on the short-term memory deficits induced by the 5-HT synthesis inhibitor para-chlorophenylalanine (*p*CPA) in rats, using the novel object recognition test (NORT). Ethical reasons prevent the use of *p*CPA in humans. However, acute tryptophan depletion –a procedure also reducing CNS serotonergic function– evokes a series of cognitive alterations in healthy volunteers, affecting, among others the consolidation of verbal episodic memories. (Mendelsohn et al., 2009). In rodent, *p*CPA induces a cognitive deficit, which is partially or totally reversed by VOR (du Jardin et al., 2014; Wallace et al., 2014) though the blockade of 5-HT₃-R (Betry et al., 2015). Likewise, to examine the neural substrate of VOR actions in these experiments, we recorded drug effects on oscillatory activity in 4 different brain areas of freely-moving rats, as follows: the prelimbic subdivision (PrL) of the mPFC, the core of the nucleus accumbens (NAc), the dorsal hippocampus (dHPC) and the paraventricular nucleus of the thalamus, anterior part (PVA). The PrL and the dHPC were chosen due to their involvement in memory, together with the presence of local 5-HT₃-R in GABA interneurons in both areas (see above). The PVA was selected due to its high density of 5-HT₇-R (Gustafson et al., 1996), for which VOR shows high affinity (Sanchez et al., 2015). Finally, the NAc was selected due to its integration of cognitive and emotional information (Floresco, 2015), and also to examine distal effects of 5-HT₃-R blockade in mPFC, given 1) the absence of 5-HT₃-R in NAc (Laporte et al., 1992; Morales et al., 1996; Tecott et al., 1993), and 2) the existence of dense inputs from layer V pyramidal neurons of the PrL and infralimbic subdivisions of mPFC, both in the ipsilateral and contralateral hemispheres (Gabbott et al., 2005).

2. Materials and Methods

2.1 Animals

Male albino Wistar rats (Charles River, France) weighing 200-250g at the beginning of the treatment period (**Supplemental Fig. S1**) were used. Animals were kept in a controlled environment (12 h light/dark cycle and 21 ± 1 °C room temperature) with food and water provided *ad libitum*.

The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, Animals (Scientific Procedure) Act 1986 and in accordance with ARRIVE guideline (Kilkenny et al., 2010) during the awake phase. Animal care followed European Union regulations (directive 2010/63 of 22 September 2010) and was approved by the Institutional Animal Care and Use Committees.

2.2 Drugs and treatments

Vortioxetine (VOR) hydrobromide and escitalopram oxalate (ESC) were provided by H. Lundbeck A/S. 4-chloro-DL-phenylalanine-methylester hydrochloride (pCPA) was from Sigma-Aldrich. VOR was administered p.o. in the food at dose of 0.26 g VOR/kg chow as previously described (Riga et al., 2017). This dose evoke SERT occupancies in the rat (40-50%) similar to those achieved in patients treated with the clinical doses of 5 mg/day VOR (Leiser et al., 2015; Wallace et al., 2014). From 5 days before starting drug treatments, the regular rat chow was switched to Purina 5001 Rodent chow (*control food*), with the same nutritional content as in the VOR-enriched chow (Leiser et al., 2015; Wallace et al., 2014). Animals were fed *ad libitum*.

ESC was administered subcutaneously (s.c.) via osmotic minipumps (Alzet, model M2L2) at the dose of 10 mg/kg/day (oxalate salt, corresponding to 7.5 mg/kg free base) as previously reported (Riga et al., 2017). Osmotic minipumps were implanted under anesthesia (100 mg/kg Ketamine + 10 mg/kg Xylazine i.p.). An analgesic (Buprenorphine: 0.5 mg/kg p.o. every 12 h) and a prophylactic antibiotic (Enofloxacin 7.5 mg/kg s.c.) were given during 3 consecutive days after surgery.

The irreversible inhibitor of tryptophan hydroxylase pCPA (86 mg/kg free base, s.c.) was administrated daily during 4 consecutive days (from day 11 to day 14) (**Supplemental Fig.**

S1) to induce cognitive impairment through inhibition of 5-HT synthesis (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014). *p*CPA was injected at a volume of 1 ml/kg and was prepared daily. pH was adjusted to ~6 using NaOH 0.1 M.

At the beginning of treatments (day 1), animals were single-housed and randomly assigned to one of the six following experimental groups as shown in **Supplemental Fig. S1**: 3 groups of standard + 3 groups of *p*CPA-treated rats treated with: 1) control food, 2) VOR-enriched food and 3) ESC minipumps. Treatments lasted two consecutive weeks (from day 1 to day 14).

Moreover, some standard and *p*CPA-treated rats fed with control food were administered at day 15 with a subcutaneous injection of VOR (5 mg/Kg as free base) (**Supplemental Fig. S1**). Solutions of VOR were prepared daily in hydroxyl-propyl- β -cyclodextrin 30%. Injected volume was 10 ml/Kg. The dose used was according to Leiser et al., (2014).

2.3 Electrophysiology: local field potential (LFP) recordings in freely moving rats

A total of 57 rats (standard: 9 control food, 11 VOR, 7 ESC; *p*CPA-treated: 12 control food, 10 VOR, 8 ESC) were implanted with Plastics One electrodes (Virginia, USA) under isoflurane anesthesia (induction: 5%; maintenance: 2-3%) three days before starting treatments (**Supplemental Fig. S1**). The stereotaxic coordinates (in mm) were taken from bregma and brain surface according to the rat brain atlas (Paxinos and Watson, 2005), as follows: prelimbic subdivision (PrL) of the medial prefrontal cortex (mPFC) AP + 3.4, ML-0.7, DV-2.1; nucleus accumbens, core (NAc) AP+1.7, ML-1.6, DV-6.8; paraventricular thalamic nucleus, anterior part (PVA) AP -1.8; ML -1.8; DV -5.3 electrode implanted with an angle of 20°; dorsal hippocampus CA1 (dHPC) AP -3-7; ML -2.0; DV -2.1. A ground screw and three stabilizer screws were also implanted. The implant was fixed with dental cement. Buprenorphine (0.5 mg/kg p.o every 12 h) and a prophylactic antibiotic (Enrofloxacin 7.5 mg/kg s.c.) were given during 3 consecutive days after surgery.

Local field potential (LFP) recordings were performed in a dimly illuminated (40 lx) 40 × 40 cm open field using a digital Lynx system and Cheetah software (Neuralynx, Montana, USA). The signal was obtained at 3.2 kHz sampling rate and filtered between 0.1 and 200 Hz. All recordings were subsequently downsampled 10 times before analysis.

To assess the effects of subchronic drug treatments, LFP recordings were made at day 14 in rats performing the *habituation trial* (T0, 15 min) of the novel object recognition test

(NORT). To assess effects of acute VOR in standard and *p*CPA-treated rats, oscillatory activity was recorded at day 15 (afternoon) after *learning* (T1) and *testing* (T2) trials of the NORT (**Supplemental Figure S1**). In the latter case, after recording 10 min of basal activity, an acute injection of VOR (5 mg/Kg s.c.) was administrated (time 0) and recording was kept for 30 min.

2.4 Novel object recognition test

To assess drug effects on short-term memory, we used the novel object recognition test (NORT) while simultaneously recording brain oscillatory activity. A protocol previously described (du Jardin et al., 2014) was adapted for LFP recordings. Briefly, animals were handled for 5 days before starting NORT sessions. On the afternoon of the day 14 (i.e. after the final day of *p*CPA injections) and 30 min after acclimation in the test room, each rat was placed in the open field for 15 min of free exploration (*habituation trial*, T0) while recording oscillatory activity (**Supplemental Figure S1**). On the morning of day 15 (i.e. 24 h after last *p*CPA injection), two identical metallic objects were positioned in the open field. Subsequently, each rat was allowed to freely explore the cage for 15 min (*learning trial*, T1). During the second 3-min trial (*test trial*, T2), one of the objects was replaced by a novel one. In each experimental group, the preference (familiar *versus* novel object) as well as the relative position of the two objects were counterbalanced and randomly permuted. The time rats spent exploring the objects was measured in T1 and T2, and a recognition index (RI) was calculated as follows: $RI = [(TN - TF) / (TN + TF)] * 100$, where TN is the time exploring the new object and TF is the time exploring the familiar object on T2. The inter trial interval (ITI) was 45 min. Rats were considered to explore if they directed their nose towards an object or if they touched it. Animals were excluded from the study if they did not explore for more than 5 s in T1 or if they failed to explore objects in T2 (**Supplemental Table S1**).

2.5 Histology

At the end of recordings, rats were euthanatized by an anesthetic overdose. The histological localization of electrodes was performed by passage of current (intensity: 0.8 mA; duration: 10 s). Brain sections were stained according to standard procedures, to verify recordings sites (**Supplemental Figure S2**). A piece of the PFC (~30-100 mg) was dissected out, weighed and frozen at -80°C for subsequent analysis of the tissue 5-HT concentration, performed by high

performance liquid chromatography (HPLC) of tissue homogenates, as previously described (Adell et al. 1989).

2.6 Data and statistical analysis

LFPs recordings were analyzed *off-line* with the Spike2 software (Cambridge Electronic Design, Cambridge, UK) using built-in and home-made scripts. The frequency bands analyzed were: low frequency oscillation (LFO, 0.15–4 Hz) and its dominant wave (1 Hz, 0.15–1.22 Hz), theta (4–12 Hz), beta (12–30 Hz), gamma (30–90 Hz) and high frequency oscillation (HFO, 90–200 Hz). The 50 Hz noise was filtered and removed from the recordings.

Power spectra were calculated using Fast Fourier Transformations (FFT) of 10-s signal intervals with a resolution of 0.3 Hz (FFT size of 8192). LFPs with the same resolution and of 60-s periods for the corresponding band in both areas were used for phase coherence analysis. For coherence analysis, phase synchronization between brain regions at 1 Hz was not calculated given the short frequency interval used (0.15–1.22 Hz) and the low reliability of the values obtained. Spectral analysis was carried out in LFP recordings free of artefacts. Incomplete or noisy recordings were also excluded from the calculations. **Supplemental Table S1** shows the number of animal used for each experimental group following histological and electrophysiological exclusion criteria.

Mean powers for each frequency band were quantified by averaging 6 consecutive 10-s periods (for each minute) and then generating the mean of 15 min corresponding to the T0 of the NORT. Similarly, mean coherences at different frequency bands were calculated by averaging 15 consecutive 1-min periods. Comparisons between experimental groups were made by using relative powers (%) and coherences (from 0 to 1) calculated for each frequency band. On the other hand, comparisons between the effects of acute VOR in standard and *p*CPA-treated rats on power spectra were made by using % of basal power values. In this case, data were averaged for 5-min periods. Tissue 5-HT concentrations in control and *p*CPA-depleted rats are given as fmol/mg tissue.

Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using one- or two-way ANOVAs for independent samples (*pre-treatment* and *treatment* as main factors) or two-way repeated measures ANOVA (*pre-treatment* and *time* as main factors) followed by post-hoc analysis using LSD's test, as appropriate. Statistical significance has been set at the 95% confidence level (two tailed).

3 Results

3.1 Effects of treatments on rat weight gain

We first assessed whether the pharmacological treatments used (*p*CPA, VOR, ESC) and/or the implantation of subcutaneous minipumps altered food intake in rats by comparing the weight gain (weight at day 14 – weight at day 1, in g) in all experimental groups. As previously observed (Riga et al., 2017), *p*CPA pre-treatment significantly reduced weight gain ($p < 0.01$), without significant *post-hoc* differences between treatments (**Supplemental Tables S2 and S3**).

3.2 Effects of 5-HT depletion on brain rhythms

The magnitude of 5-HT depletion induced by *p*CPA in the mPFC was 94% (49 ± 4 vs. 789 ± 69 pmol/g in *p*CPA-treated ($n=30$) and control ($n=27$) rats; $p < 0.0001$) similar to that found previously using this treatment regime (du Jardin et al., 2014; Jensen et al., 2014; Riga et al., 2017).

*p*CPA impairs short-term memory and reversal learning (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014). Given the involvement of oscillatory activity in mnemonic processes (Harmony, 2013; Hermann et al., 2010; Lachaux et al., 2012; Lundqvist et al., 2016; Nyhus and Curran, 2010), we examined the effects of 5-HT depletion on oscillatory activity in four brain regions of awake, freely moving rats (**Table 1**). 5-HT depletion led to a very marked and significant reduction (to ~55% that in standard conditions) of the 1 Hz oscillation in the prelimbic subdivision (PrL) of the mPFC [$F(1,16)=9.57$; $p < 0.01$]. This reduction was accompanied by a moderate increase of the theta power [$F(1,16)=4.72$; $p < 0.05$], with no significant changes in other oscillatory bands. Similar changes, yet of a smaller effect size for the 1 Hz oscillation, were found in the core of NAc [1 Hz: $F(1,19)=8.19$; $p < 0.01$; theta: $F(1,19)=6.10$; $p < 0.05$], also without affecting higher frequency bands. No significant alterations were found in the dHPC, whereas in the thalamic PVA nucleus, the 5-HT depletion was associated with significant reductions of gamma [$F(1,17)=4.60$; $p < 0.05$] and high frequency oscillation [$F(1,17)=11.38$; $p < 0.01$], perhaps due to the lack of activation by endogenous 5-HT of excitatory 5-HT₇-R, densely expressed in several thalamic nuclei, including the PVA (Gustafson et al., 1996).

Moreover, *p*CPA enhanced synchronization at low frequencies, increasing the PrL-NAc coherence at LFO [$F(1,16)=6.48$; $p<0.05$] and theta bands [$F(1,16)=6.87$; $p<0.05$], the PrL-dHPC coherence at the theta band [$F(1,16)=4.54$; $p<0.05$], and NAc-dHPC coherence at theta [$F(1,19)=9.68$; $p<0.01$] and beta bands [$F(1,19)=4.52$; $p<0.05$] (**Table 1**). In contrast, 5-HT depletion decreased coherences at higher frequencies, with a lower PrL-dHPC coherence at gamma [$F(1,16)=11.57$; $p<0.01$] and HFO bands [$F(1,16)=11.81$; $p<0.01$], and NAc-dHPC coherence at HFO [$F(1,19)=11.47$; $p<0.01$] (**Table 1**).

3.3 Effects of acute vortioxetine administration on brain rhythms in standard and 5-HT depleted animals.

We previously reported that low and high subchronic doses of VOR (0.26 and 1.8 g VOR/Kg chow, respectively), enhanced pyramidal neuron activity in both standard and 5-HT-depleted rats (Riga et al., 2017). The effect of VOR in 5-HT-depleted rats suggested a non-canonical interaction of VOR with 5-HT₃-R, independent of the presence of 5-HT, a view also supported by behavioral studies showing that VOR prevents cognitive deficits induced by 5-HT depletion in rats, likely through a 5-HT₃-R-dependent mechanism (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014) (see Section 4 for extended discussion on this point).

Therefore, we examined the effect of single VOR administration (5 mg/kg s.c.) to rats on control food in the standard and *p*CPA-treated groups ($n=8$ per treatment group). VOR induced a sustained increase of LFO, theta and beta powers in PrL (**Fig. 1A-C**), NAc (**Fig. 1F-H**), PVA (**Fig. 1K-M**) and dHPC (**Fig. 1P-R**) in 5-HT-depleted but not in standard rats. In the latter group, some transient elevations were noted, similar to those previously reported (Leiser et al., 2014). Conversely, VOR administration induced a sustained decrease of gamma and HFO powers in the NAc of 5-HT-depleted rats (**Fig. 1I-J**). *Post-hoc* differences between standard and *p*CPA groups were found at different time points after VOR administration (**Fig. 1A-T**). Statistical analysis is shown in **Supplemental Table S3**. Representative spectrograms showing the effects of acute VOR administration on low frequencies (up to 30 Hz) mentioned above are shown in **Fig 2**. The administration of VOR facilitated the emergence of simultaneous bursts of oscillatory activity at low frequencies, particularly in PrL, which were more marked in 5-HT-depleted rats. Unlike during the performance of NORT, the behavior of rats was not videotaped during these recordings, which prevents establishing an association of the increased oscillatory activity with rats' movement or exploratory behavior.

Taken together, these data indicate a preferential effect of acute VOR administration on low frequency bands (up to 30 Hz), more marked in 5-HT-depleted rats. These effects on brain oscillations add to previous observations indicating that VOR can modulate neuronal activity in absence of 5-HT at doses preferentially occupying 5-HT₃-R and SERT (Riga et al., 2017).

3.4 Effects of subchronic vortioxetine and escitalopram on brain rhythms in standard conditions

We recorded the brain oscillatory activity in rats treated subchronically with VOR (0.26 g/kg chow p.o.) and ESC (ESC, 10 mg/kg per day s.c.) in standard conditions, and compared power and coherence values with those found in rats fed with control food (control group) during the T0 of the NORT (15 min) (**Table 2**).

Subchronic VOR (but not ESC) increased the power of gamma oscillations in the PrL [$F(2,19)=5.41$; $p<0.05$]. In parallel, VOR (but not ESC) decreased the power of LFO [$F(2,20)=7.62$; $p<0.01$] and its dominant 1 Hz oscillation [$F(2,20)=7.39$; $p<0.01$] and marginally increased the power of theta [$F(2,20)=7.14$; $p<0.01$] and beta [$F(2,20)=7.22$; $p<0.01$] oscillations in PVA thalamic nucleus. On the other hand, sub-chronic ESC moderately increased the power of LFO in PVA while it marginally decreased theta and beta rhythms. Neither VOR nor ESC significantly affected the power of any frequency band in NAc and dHPC.

Subchronic VOR or ESC did not affect the values of coherence between any of the recorded areas (PrL-NAc, PrL-dHPC and NAc-dHPC). Mean and deviation values, statistical significances and the number of animal used for each area, band and treatment are shown in **Table 2**.

3.5 Effects of subchronic vortioxetine and escitalopram on brain rhythms and recognition memory impairment induced by 5-HT depletion

We next examined the ability of subchronic VOR and ESC treatments to prevent the deficits in recognition memory induced by *p*CPA and the associated changes in oscillatory activity in the four brain areas examined. Neither treatment altered the learning time at T1 (**Fig. 3A**). At T2, subchronic VOR –but not subchronic ESC- treatment restored short-term recognition memory in *p*CPA-treated rats, as assessed by the significant increase in the recognition index (%) (**Fig. 3B**). Subchronic VOR treatment (but not ESC) prevented the *p*CPA-induced alterations on 1 Hz and theta waves in the PrL (**Fig. 3C-D**) and NAc (**Fig. 3E-F**), with similar effects in both brain areas. This indicates that the increase in recognition memory induced by VOR is associated to

an enhancement of the 1 Hz wave, together with a decrease of theta power in the PrL-NAc pathway. Subchronic VOR treatment did not prevent the *p*CPA-induced changes in gamma and HFO waves in PVA, suggesting that the changes in PVA were not associated to the memory deficits nor to its reversal. In parallel, VOR (but not ESC) selectively restored the *p*CPA-induced fall in the PrL-dHPC coherence at gamma frequencies without affecting the gamma power in these areas (**Fig. 4**). Representative examples of the effects of subchronic VOR in restoring brain oscillatory activity in 5-HT depleted rats are shown in **Fig. 5**. Detailed statistical analysis is shown in **Supplemental Table S3**.

4 Discussion

The interest of the present study lies in two main points, i.e., the role of serotonergic neurotransmission in the modulation of oscillatory activity, and the prevention by VOR of the parallel effects of *pCPA* on recognition memory and brain oscillations.

We show that 5-HT depletion induced simultaneous changes in short-term recognition memory and in slow rhythms in the PrL-NAc pathway (reduced 1 Hz and increased theta wave powers), together with a reduced PrL-dHPC synchronization at high frequencies. Subchronic VOR treatment prevented both effects, suggesting a strong link between behavioral and electrophysiological variables. This association is strengthened by the inability of ESC to prevent the effects of *pCPA* on oscillatory activity and -as previously observed (Jensen et al., 2014)- on recognition memory deficits. Other changes in oscillatory activity induced by *pCPA* such as the reduced power of gamma and HFO power in PVA, are likely unrelated to recognition memory deficits since they were not countered by subchronic VOR treatment.

Brain oscillatory activity arises from the coordinated activity of neuronal ensembles in a given brain structure or network, and contributes to the communication between different brain areas. Inhibitory neurotransmission (e.g., GABA) plays a critical role in brain oscillations by allowing excitatory neurons to alternate between periods of low and high excitability (Mathalon and Sohal, 2015). Given their selective expression in GABA interneurons (see above) 5-HT₃-R may thus play a critical role in the modulation of brain oscillatory activity.

When small neuronal populations locally integrate their activity, microcircuits are assembled that, in turn, built dynamic and synchronous long-range networks linking distal brain areas (Buzsaki and Draguhn, 2004; Buzsaki et al., 2012; Engel et al., 2001; Fries, 2005; Varela et al., 2001). Accordingly, brain rhythms play an important role in many brain functions, including cognition (Buzsaki and Draguhn, 2004; Jensen and Tesche, 2002; Jensen et al., 2002; Roux et al., 2012), and disruptions of oscillatory activity lead to aberrant information encoding, often associated with neurological and psychiatric disorders (Coburn et al., 2006; Huges and John, 1999; Palop and Murcke, 2016; Voytek and Knight, 2015). Hence, changes in brain oscillations have been associated to depressive states and as predictors of treatment response (Baskaran et al., 2012; Iosifescu, 2011; Nystrom et al., 1986).

Surprisingly, despite the involvement of the hippocampal formation in episodic and long-term memory (Banneman et al., 2014; Battaglia et al., 2011; Buzsaki and Moser, 2013; Eichenbaum, 2014; Preston and Eichenbaum, 2013; Zeidman and Maguire 2016), *pCPA* did not affect oscillatory activity in the dHPC. However, it markedly reduced the coherence between PrL and dHPC at gamma and HFO frequencies and increased theta coherence, likely affecting the interplay between both brain areas during the encoding and retrieval of sensory and spatial information (Preston and Eichenbaum, 2013).

4.1 Role of 5-HT in brain oscillations

5-HT modulates PFC function through the stimulation of different receptor subtypes located in a large proportion of pyramidal neurons and GABA interneurons in different subfields and cortical layers (Santana and Artigas, 2017). Their activation by endogenous 5-HT or by selective agonists modulates pyramidal neuron activity in a complex manner, involving direct and GABA-mediated responses (Celada et al., 2013; Lladó-Pelfort et al., 2012; Puig et al., 2003; Puig et al., 2005; Riga et al., 2016b). However, the role of 5-HT on oscillatory activity is still poorly known, although existing evidence supports a role in the regulation of LFO (particularly 1 Hz oscillations) in PFC. LFO reflect the spontaneous changes between periods of neuronal activity (UP states) and silence (DOWN states) in large neuronal ensembles, resulting from alternation of periods of membrane depolarization and hyperpolarization, respectively (Steriade et al., 1993; Contreras and Steriade, 1995; Mukovski et al., 2007). LFO serve as a temporal template to coordinate neuronal discharge in a given brain area. The power of LFO is high during slow-wave sleep and under anesthesia. In the latter experimental conditions, 5-HT_{2A}-R stimulation by serotonergic hallucinogens dramatically reduced 1 Hz oscillations in rat mPFC (Celada et al., 2008; 2013; Riga et al., 2016b). On the other hand, the selective 5-HT_{1A}-R agonists 8-OH-DPAT and BAY x 3702 reversed the fall in 1 Hz oscillations induced by the non-competitive NMDA-R antagonist phencyclidine in the mPFC of anesthetized rats (Lladó-Pelfort et al., 2016). Along the same lines, 5-HT_{1A}-R activation by the mixed 5-HT_{1A}-R/5-HT_{2A}-R agonist 5-MeO-DMT in 5-HT_{2A}-R knockout mice enhances the power of all oscillatory bands in the mPFC and visual cortex (except gamma waves) of awake, freely moving mice (Riga et al., 2018). However, the 5-HT_{1A}-R antagonist WAY-100635 did not reduce the power of 1 Hz oscillation in anesthetized rats (Puig and Gullledge, 2011). The present results add to this body of evidence by showing an important role of 5-HT in the maintenance of LFO (in particular the 1 Hz oscillation).

A causal relationship between the memory deficits and the fall in 1 Hz oscillations in the PrL-NAc pathway cannot be established from the present study and deserves further investigation. However, in close similarity with the present observations, alterations in the mPFC-NAc synchrony appear to underlie object recognition deficits in the subchronic phencyclidine model (Asif-Malik et al., 2017). Theta and gamma oscillations play a key role in cognition and memory (Başar et al., 2001; Buzsaki, 2006; Buzsaki and Wang, 2012; Herrmann et al., 2010; Herweg et al., 2020; Jensen et al., 2007; Nyhus and Curran, 2010; Sauseng et al., 2010). Less attention had been paid to the role of LFO (Başar et al., 2001; Harmony, 2013) although human studies show that boosting LFO (0.75 Hz) during slow-wave sleep enhances the retention of declarative memories (Marshall et al., 2006). Likewise, enhancing LFO in rat PFC modulates cortical network activity and enhances recognition memory in a radial maze task (Binder et al., 2014). Moreover, a recent study by the same group showed that the optogenetic silencing of the hippocampal-PFC pathway during slow-wave sleep (i.e., during high LFO power) prevented spatial memory consolidation in mice (Binder et al., 2019). In line with these studies, our results support a role of LFO in short-term memory.

4.2 Parallel prevention by vortioxetine of memory deficits and alterations in oscillatory activity

The neural mechanism(s) by which subchronic VOR treatment prevents the *p*CPA-induced changes in oscillatory activity and recognition memory are still not fully understood. A normalization of brain 5-HT levels by VOR can be fully excluded, given its pharmacological profile. In a previous study, the same subchronic VOR treatment induced a ~2-fold elevation of pyramidal discharge in standard and *p*CPA-treated anesthetized rats (Riga et al., 2017). Here we show that this increased discharge occurs within a pattern of normalized oscillatory activity, given the VOR-induced normalization of slow waves (1 Hz and theta) in PrL and NAc, as well as by the normalized PrL-dHPC synchrony at gamma frequency.

The increase in pyramidal neuron discharge in PrL, together with the normalization of oscillatory activity seen in the present study likely contribute to the VOR-mediated recovery of short-term recognition memory deficit induced by *p*CPA. Studies in primates have shown that the neurobiological substrate of short-term -or working- memory is the emergence and maintenance of patterns of persistent neuronal activity in the dorsolateral PFC (of which the anterior cingulate and prelimbic subdivisions are considered the rodent equivalent). In spatial working memory experiments, the animals must remember the position of a visual stimulus on a screen during a delay period in absence of the stimulus. The emergence of spontaneous –i.e., not motor- or sensory-driven- persistent neuronal activity during the delay period is

considered to be the neurobiological substrate of working memory (Curtis and Esposito, 2003; Fuster and Alexander, 1971; Miller and Coehn, 2001; Wang et al., 2015). Interestingly, a recent study shows that enhancement of neuronal activity in primate dorsolateral PFC induced by blockade of GABA_A-R improves working memory selectivity (Rodermund et al., 2020). Likewise, the partial blockade of AMPA-R led to an overall reduction of persistent activity whereas NMDA-R blockade reduced the firing activity associated with the memory of the neuron's preferred spatial location (van Vugt et al., 2020). Overall, these observations support that an increase in the excitation/inhibition ration in dorsolateral PFC improves working memory in primates.

Accordingly, the enhanced neuronal discharge in the PrL of VOR-treated rats (Riga et al., 2017), together with the normal alternation of UP and DOWN periods (i.e., recovery by VOR of the power of the 1 Hz oscillation; this study) may facilitate working memory processes by pre-setting PFC neurons at a moderately higher discharge rate, thus requiring less energy (excitatory inputs) to attain the required activity level in order to sustain the stored information. These views are in line with previous studies showing that 5-HT₃-R agonists impair short-term and long-term memory in rats (Meneses, 2007), while the 5-HT₃-R antagonists ondansetron and tropisetron improve memory consolidation (Meneses 2013).

Remarkably, subchronic VOR evoked a comparable increase in pyramidal neuron discharge in standard and in 5-HT-depleted rats (Riga et al., 2017), suggesting that VOR may interact with 5-HT₃-R in a non-canonical way, independent on the presence of 5-HT. This possibility is further supported by the present data, showing that acute VOR treatment (5 mg/kg s.c., a dose preferentially targeting 5-HT₃-R and SERT) (Leiser et al., 2014) was more effective to modulate oscillatory activity in 5-HT-depleted rats than in standard rats. Likewise, subchronic VOR treatment, was more effective to modulate oscillatory activity in 5-HT-depleted than in standard rats (present study) and restored recognition memory deficits in 5-HT-depleted rats (du Jardin et al., 2014; Wallace et al., 2014; present study). The much lower affinity and occupancy (as compared to 5-HT₃-R) of VOR for the only receptor for which VOR shows agonist activity (5-HT_{1A}-R; Leiser et al., 2014; Sanchez et al, 2015) allows to discard that the above effects are due to an agonist action of VOR on 5-HT_{1A}-R in 5-HT-depleted rats

Interestingly, the selective 5-HT₃-R antagonist ondansetron stopped gut motility in monoamine-depleted -reserpinized- guinea pigs (Sia et al., 2013). Although using different species and experimental models, both studies indicate that VOR and ondansetron may modulate 5-HT₃-R function by a mechanism independent of endogenous 5-HT. The observations in guinea pigs have been interpreted as resulting from an action of 5-HT₃-R

antagonists on constitutively-active 5-HT₃-R in the gut (Spencer, 2013). Likewise, the complex structure of 5-HT₃-R, including the presence of agonist and antagonist binding sites (Lummis, 2012) as well as the possibility of allosteric modulation (Davies, 2011), opens the possibility that VOR and other 5-HT₃-R antagonists modulate the opening of the channel independently of 5-HT.

4.3 Limitations

The main limitation of the present study is the poor construct validity of the *p*CPA model used, which on the other hand, shows an excellent predictive validity since, as observed in the clinic, VOR –but not ESC- improves cognitive function in MDD patients (see Introduction). Although the translational value of measures of brain oscillatory activity in rodents remains to be fully established, existing evidences suggest common neurobiological bases in humans and rodents. Hence, as in humans, delta, theta and gamma rhythms have been associated to cognitive function in rodents (Fujisawa and Buzsaki, 2011; Gu et al., 2018; Siapas et al., 2005; Spellman et al., 2015) and abnormalities in theta and gamma activities are linked to cognitive dysfunction in animal models of psychiatric disorders (Adhikari et al., 2010; Dzirasa et al., 2011; Fitzgerald and Watson, 2019; Zheng and Zhang, 2015). On the other hand, changes in brain oscillations have been associated to MDD and as predictors of treatment response (Baskaran et al., 2012; Iosifescu, 2011; Nystrom et al., 1986).

5. Conclusions

In summary, the present study supports a prominent role of serotonergic neurotransmission on brain oscillatory activity, particularly in cortico-striatal pathways linked to short-term recognition memory. Further investigation is required to characterize the exact mechanisms and 5-HT receptors involved in the maintenance of the 1 Hz oscillation. On the other hand, VOR prevented the recognition memory deficits induced by 5-HT depletion, and in parallel, it normalized the *p*CPA-induced alterations in the PrL-NAc pathway, also restoring the PrL-dHPC coherence at gamma frequencies. The normalization of oscillatory activity is simultaneous to the enhancement of neuronal activity in the mPFC (both PrL and infralimbic subdivisions) produced by the same VOR treatment in 5-HT-depleted rats (Riga et al., 2017), suggesting that changes in both neuronal discharge and oscillatory activity contribute to the beneficial effect

of VOR on short-term recognition memory. The lack of effect of the SSRI ESC on behavioral and electrophysiological variables indicates that pharmacological activities of VOR other than SERT blockade (most likely 5-HT₃-R blockade) mediate the effects on oscillatory activity and recognition memory.

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Figures and Tables

Figure 1. Effects of acute vortioxetine administration (VOR, 5 mg/Kg s.c.) on the power of low frequency oscillations (LFO), theta, beta, gamma and high frequency oscillations (HFO) in the prelimbic subdivision (PrL) of the medial prefrontal cortex, nucleus accumbens core (NAc), paraventricular thalamic nucleus, anterior part (PVA) and dorsal hippocampus (dHPC, CA1 subfield) in standard and *pCPA*-treated rats during 30 min post administration. Vertical arrow shows the time of VOR administration. Data are given as percentages of basal values in 5 min periods. Note that acute VOR increases LFO, theta and beta powers in all examined areas in *pCPA*-treated but not in standard rats.

Statistical analysis: Two-way repeated measures ANOVA (*pre-treatment* and *time* as main factors) (see Supplemental **Table S3**). Statistical significance: **p* < 0.05 versus basal values; α *p* < 0.05 versus standard rat values. In PrL: *n* = 6 and 7 for standard and *pCPA* rats, respectively; in NAc and dHPC: *n* = 8 for both standard and *pCPA* rats, respectively; in PVA: *n* = 8 and 7 for standard and *pCPA* rats, respectively.

Figure 2. Representative spectrograms showing the effects of acute vortioxetine (VOR, 5 mg/Kg s.c.) on the power of low frequency (LFO), theta and beta oscillations in the prelimbic subdivision (PrL) of the medial prefrontal cortex, nucleus accumbens core (NAc), paraventricular thalamic nucleus, anterior part (PVA) and dorsal hippocampus (dHPC, CA1 subfield) in standard and *pCPA*-treated rats during 30 min post administration. Note that effects are more marked in *pCPA* treated rats, particularly in PrL. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity). Note also the presence of simultaneous bursts of oscillatory activity in the four brain areas examined after VOR administration, which were more marked in 5-HT-depleted than in standard rats.

Figure 3. Effects of subchronic vortioxetine (VOR, 0.26 mg/Kg chow, p.o.) and ESC (ESC, 10 mg/Kg s.c.) on *pCPA*-induced alterations of recognition-memory (**A–B**) assessed by novel object recognition test (NORT) and power spectra (**C–H**) in the prelimbic subdivision (PrL) of the medial prefrontal cortex, nucleus accumbens (NAc) and paraventricular thalamic nucleus, anterior part (PVA). Note that VOR (but not ESC) prevented *pCPA* effects on i) recognition memory (**B**) without altering exploration time during the learning trial (T1) of the NORT (**A**) and on low frequency oscillatory activity (1 Hz and theta) in PrL (**C–D**) and NAc (**E–F**). *pCPA*-induced

alterations in PVA oscillatory activity at gamma (**G**) and high frequency (HFO) (**H**) bands are not avoided by VOR.

Statistical analysis: Two-way ANOVA (*pre-treatment* and *treatment* as factors) (see Supplemental **Table S3**). Statistical significance: * $p < 0.05$ versus control food values; [#] $p < 0.05$ versus *p*CPA values; [#] $p < 0.05$ versus VOR values; ^a $p = 0.059$ and ^b $p = 0.057$ (marginally significant) versus control food values; ^c $p = 0.050$ versus VOR values. For NORT: $n = 7, 8, 6$ and $9, 8, 7$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively; for power spectra: (PrL) $n = 7, 9, 7$ and $11, 9, 6$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively; (NAC) $n = 9, 8, 7$ and $12, 10, 6$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively; (PVA) $n = 9, 7, 7$ and $10, 9, 5$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively.

Figure 4. Effects of subchronic vortioxetine (VOR, 0.26 mg/Kg chow, p.o.) and ESC (ESC, 10 mg/Kg s.c.) on *pCPA*-induced dysfunction of brain synchronizations (coherences) between the prelimbic subdivision (PrL) of the medial prefrontal cortex, nucleus accumbens (NAc) and dorsal hippocampus (dHPC). Note that VOR (but not ESC) prevented *pCPA* effects only on PrL-dHPC synchronization at gamma frequencies (**D**).

Statistical analysis: Two-way ANOVA (*pre-treatment* and *treatment* as factors) (see Supplemental **Table S3**). Statistical significance: * $p < 0.05$ versus control food values; # $p < 0.05$ versus *pCPA* values; ^ $p < 0.05$ versus VOR values; ^ $p = 0.052$ (marginally significant) versus control food values; ^ $p = 0.050$ versus *pCPA* values. For PrL-NAc coherence: $n = 7, 9, 7$ and 11 ,

9, 6 for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively; for PrL-dHPC coherence: $n = 7, 9, 6$ and $11, 9, 6$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively; for NAc-dHPC coherence: $n = 9, 7, 6$ and $12, 10, 6$ for standard rats treated with control food, VOR, ESC and *p*CPA rats treated with control food, VOR, ESC, respectively.

Figure 5. A, B) Representative spectrograms showing the effects of subchronic vortioxetine (VOR, 0.26 mg/Kg-day chow during 14-days, p.o.) in restoring the *pCPA*- induced alteration on slow brain rhythms (<30 Hz) in **(A)** the prelimbic subdivision (PrL) of the medial prefrontal cortex, and **(B)** nucleus accumbens (NAc). Note that, unlike escitalopram (ESC, 10 mg/Kg/day during 14 days, s.c.), VOR selectively reversed the fall of the 1 Hz oscillation and the increase of theta (4-12 Hz) rhythms induced by 5-HT depletion in both areas. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity). **C)** Unlike ESC, subchronic VOR treatment also prevented the *pCPA*-induced decrease of the coherence between the PrL and the dorsal hippocampus (dHPC) at gamma frequencies (30-90 Hz). Three individual 1-min coheregrams for each experimental group are shown, corresponding to the last 3-min recordings (one coheregram per min). Coherence values from 0 (minimum) to 1 (maximum).

Table 1. Effect of the irreversible inhibitor of tryptophan hydroxylase 4-chloro-DL-phenylalanine (*p*CPA) on power spectra **(A)** and coherences **(B)** in the areas and band frequencies analyzed. *p*CPA was injected subcutaneously during 4 days at the dose of 86 mg/kg free base for day. Note that i) *p*CPA mainly affected low frequency oscillations in PrL and NAc, and high frequency oscillations in PVA, and ii) *p*CPA alters phase synchronization, with increases at low frequencies (LFO, theta and beta) and decreases at high frequencies (gamma and HFO). Data are represented as mean $\pm$ SEM of relative power (%) **(A)** or coherences (0–1) **(B)**. *Abbreviations used:* PrL, prelimbic subdivision of the medial prefrontal cortex; NAc, nucleus accumbens core; PVA paraventricular thalamic nucleus, anterior part; dHPC, dorsal hippocampus (CA1 subfield). As indicated in methods, phase synchronizations at 1 Hz were not calculated due to the short frequency interval used (0.15–1.22 Hz) and the subsequent low reliability of data obtained.

Statistical analysis: One-way ANOVAs; Statistical significance: ^a $p < 0.05$ versus control group. Number of animals for each experimental group is shown in brackets.

A) POWER SPECTRA (relative %)						
PrL						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (7)	51.5 ± 7.4	77.0 ± 3.8	13.9 ± 2.6	4.5 ± 0.9	3.7 ± 0.4	1.0 ± 0.1
pCPA (11)	28.6 ± 3.7 ^α	70.5 ± 2.1	19.5 ± 1.2 ^α	5.2 ± 0.5	4.0 ± 0.6	0.9 ± 0.1
NAC						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	49.8 ± 5.1	74.4 ± 3.1	13.0 ± 1.5	4.7 ± 0.5	5.0 ± 0.8	3.3 ± 1.1
pCPA (12)	33.1 ± 3.3 ^α	72.4 ± 1.7	17.8 ± 1.2 ^α	4.9 ± 0.6	3.6 ± 0.3	3.3 ± 0.3
PVA						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	39.6 ± 3.1	62.3 ± 2.8	25.1 ± 2.2	8.0 ± 0.7	3.7 ± 0.2	1.0 ± 0.1
pCPA (10)	30.7 ± 3.8	61.4 ± 3.2	27.7 ± 2.1	7.6 ± 0.8	2.8 ± 0.3 ^α	0.5 ± 0.1 ^α
dHPC						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	31.8 ± 7.8	52.4 ± 5.8	32.9 ± 4.3	10.2 ± 1.3	4.0 ± 0.4	0.7 ± 0.1
pCPA (12)	21.7 ± 1.6	51.0 ± 2.7	35.2 ± 2.1	9.7 ± 0.7	3.6 ± 0.4	0.5 ± 0.1
B) COHERENCE (0 min -1 max)						
PrL-NAC						
	LFO	Theta	Beta	Gamma	HFO	
Control (7)	0.40 ± 0.060	0.32 ± 0.043	0.26 ± 0.037	0.25 ± 0.032	0.28 ± 0.055	
pCPA (11)	0.56 ± 0.031 ^α	0.43 ± 0.022 ^α	0.28 ± 0.017	0.20 ± 0.017	0.20 ± 0.025	
PrL-dHPC						
	LFO	Theta	Beta	Gamma	HFO	
Control (7)	0.16 ± 0.022	0.072 ± 0.008	0.036 ± 0.002	0.057 ± 0.007	0.13 ± 0.032	
pCPA (11)	0.21 ± 0.024	0.114 ± 0.014 ^α	0.054 ± 0.008	0.036 ± 0.002 ^α	0.04 ± 0.002 ^α	
NAC-dHPC						
	LFO	Theta	Beta	Gamma	HFO	
Control (9)	0.19 ± 0.028	0.12 ± 0.009	0.058 ± 0.005	0.067 ± 0.008	0.15 ± 0.034	
pCPA (12)	0.24 ± 0.025	0.19 ± 0.020 ^α	0.092 ± 0.013 ^α	0.054 ± 0.006	0.05 ± 0.004 ^α	

Table 2. Effects of subchronic vortioxetine (VOR, 0.26 mg/Kg chow, p.o.) and escitalopram (ESC, 10 mg/Kg, s.c.) on oscillatory activity in the prelimbic subdivision (PrL) or the medial prefrontal cortex, nucleus accumbens core (NAc), paraventricular thalamic nucleus, anterior part (PVA) and dorsal hippocampus (dHPC, CA1) of standard rats. **A)** Power spectra and **B)** coherence between two areas at different frequency bands. Data are represented as Mean $\pm$ SEM of relative power (%) **(A)** or coherences (0–1) **(B)**. *Abbreviations used:* PrL, prelimbic subdivision of the medial prefrontal cortex; NAc, Nucleus accumbens core; PVA paraventricular thalamic nucleus, anterior part; dHPC, dorsal hippocampus (CA1 subfield).

Statistical analysis: One-way ANOVAs; Statistical significance: * $p < 0.05$ versus control group; # $p < 0.01$ versus VOR values; ^a $p=0.058$, ^b $p=0.057$, ^c $p=0.056$, ^d $p=0.051$ and ^e $p=0.059$ (marginally significant) versus control group. Number of animals for each experimental group is shown in brackets.

A) POWER SPECTRA (relative %)						
PrL						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (7)	51.5 ± 7.4	77.0 ± 3.8	13.9 ± 2.6	4.5 ± 0.9	3.7 ± 0.4	1.0 ± 0.1
VOR (8)	35.0 ± 2.4	68.8 ± 2.4	19.0 ± 1.5	5.8 ± 0.6	5.2 ± 0.4 *	1.3 ± 0.2
ESC (7)	50.9 ± 9.9	78.0 ± 3.7	13.3 ± 2.9	4.4 ± 0.6	3.5 ± 0.4 #	0.9 ± 0.2
NAc						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	49.8 ± 5.1	74.4 ± 3.1	13.0 ± 1.5	4.7 ± 0.5	5.0 ± 0.8	3.3 ± 1.1
VOR (8)	41.2 ± 2.7	72.5 ± 1.3	15.8 ± 0.8	5.5 ± 0.3	4.7 ± 0.6	1.7 ± 0.2
ESC (7)	54.3 ± 7.0	79.2 ± 2.5	10.9 ± 1.5	4.1 ± 0.5	4.3 ± 0.5	1.9 ± 0.4
PVA						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	39.6 ± 3.1	62.3 ± 2.8	25.1 ± 2.2	8.0 ± 0.7	3.7 ± 0.2	1.0 ± 0.1
VOR (7)	27.1 ± 3.1 *	53.1 ± 2.5 ^a	31.7 ± 2.0 ^b	10.1 ± 0.6 ^c	4.5 ± 0.3	0.8 ± 0.1
ESC (7)	50.7 ± 6.1 #	72.1 ± 4.4 * #	18.1 ± 3.0 ^d #	5.9 ± 0.8 ^e #	3.2 ± 0.7	0.8 ± 0.1
dHPC						
	1 Hz	LFO	Theta	Beta	Gamma	HFO
Control (9)	31.8 ± 7.8	52.4 ± 5.8	32.9 ± 4.3	10.2 ± 1.3	4.0 ± 0.4	0.7 ± 0.1
VOR (9)	30.2 ± 4.4	50.9 ± 4.3	33.0 ± 2.9	10.8 ± 1.0	4.6 ± 0.7	0.7 ± 0.1
ESC (6)	25.7 ± 4.0	47.7 ± 4.2	35.2 ± 4.0	12.5 ± 0.8	4.0 ± 0.4	0.6 ± 0.1
B) COHERENCE (0 min -1 max)						
PrL-NAc						
	LFO	Theta	Beta	Gamma	HFO	
Control (7)	0.40 ± 0.060	0.32 ± 0.043	0.26 ± 0.037	0.25 ± 0.032	0.28 ± 0.055	
VOR (8)	0.47 ± 0.057	0.37 ± 0.027	0.30 ± 0.022	0.26 ± 0.023	0.25 ± 0.031	
ESC (7)	0.29 ± 0.077	0.31 ± 0.076	0.25 ± 0.059	0.25 ± 0.048	0.27 ± 0.053	
PrL-dHPC						
	LFO	Theta	Beta	Gamma	HFO	
Control (7)	0.16 ± 0.022	0.072 ± 0.008	0.036 ± 0.002	0.057 ± 0.007	0.13 ± 0.032	
VOR (7)	0.13 ± 0.026	0.057 ± 0.004	0.039 ± 0.004	0.054 ± 0.008	0.068 ± 0.013	
ESC (6)	0.10 ± 0.015	0.081 ± 0.017	0.051 ± 0.009	0.048 ± 0.004	0.075 ± 0.009	
NAc-dHPC						
	LFO	Theta	Beta	Gamma	HFO	
Control (9)	0.19 ± 0.028	0.12 ± 0.009	0.058 ± 0.005	0.067 ± 0.008	0.15 ± 0.034	
VOR (7)	0.20 ± 0.020	0.15 ± 0.026	0.071 ± 0.018	0.072 ± 0.010	0.085 ± 0.015	
ESC (6)	0.16 ± 0.016	0.18 ± 0.049	0.120 ± 0.029	0.078 ± 0.007	0.095 ± 0.021	