British Journal of Pharmacology

DOI:10.1111/bph.12782
www.brjpharmacol.org

RESEARCH PAPER

Multimodal antidepressant vortioxetine increases frontal cortical oscillations unlike escitalopram and duloxetine – a quantitative EEG study in rats

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Received
28 October 2013
Revised
27 April 2014
Accepted
3 May 2014

BACKGROUND AND PURPOSE

EEG studies show that 5-HT is involved in regulation of sleep–wake state and modulates cortical oscillations. Vortioxetine is a 5-HT_3, 5-HT_7, and 5-HT_{1D} receptor antagonist, 5-HT_{1B} partial agonist, 5-HT_{1A} agonist, and 5-HT transporter inhibitor. Preclinical (animal) and clinical studies with vortioxetine show positive impact on cognitive metrics involving cortical function. Here we assess vortioxetine's effect on cortical neuronal oscillations in actively awake rats.

EXPERIMENTAL APPROACH

Telemetric EEG recordings were obtained with the following treatments (mg·kg^{-1}, s.c.): vehicle, vortioxetine (0.1, 1.0, 3.0, 10), 5-HT_{1A} agonist flesinoxan (2.5), 5-HT_3 antagonist ondansetron (0.30), 5-HT_7 antagonist SB-269970-A (10), escitalopram (2.0), duloxetine (10) and vortioxetine plus flesinoxan. Target occupancies were determined by *ex vivo* autoradiography.

KEY RESULTS

Vortioxetine dose-dependently increased wakefulness. Flesinoxan, duloxetine, ondansetron, but not escitalopram or SB-269970-A increased wakefulness. Quantitative spectral analyses showed vortioxetine alone and with flesinoxan increased θ (4–8 Hz), α (8–12 Hz) and γ (30–50 Hz) power. Duloxetine had no effect on θ and γ, but decreased α power, while escitalopram produced no changes. Ondansetron and SB-269970 (≈31–35% occupancy) increased θ power. Flesinoxan (≈41% occupancy) increased θ and γ power.

CONCLUSIONS AND IMPLICATIONS

Vortioxetine increased wakefulness and increased frontal cortical activity, most likely because of its 5-HT_7 and 5-HT_3 antagonism and 5-HT_{1A} agonism. Vortioxetine differs from escitalopram and duloxetine by increasing cortical θ, α and γ oscillations. These preclinical findings suggest a role of vortioxetine in modulating cortical circuits known to be recruited during cognitive behaviours and warrant further investigation as to their clinical impact.

Abbreviations

AHP, after hyper-polarization SB-269970-A; BBB, blood–brain barrier; DSI, Data Sciences International; LMA, locomotor activity; qEEG, quantitative EEG; REM, rapid eye movement sleep; ROI, region of interest; SERT, 5-HT (serotonin) transporter; SNRI, 5-HT (serotonin) / noradrenaline reuptake inhibitor; SSRI, 5-HT (serotonin) reuptake inhibitor

Table of Links

TARGETS	LIGANDS
SERT, 5-HT transporter	ACh
5-HT_{1A} receptors	Dopamine
5-HT_{1B} receptors	Glutamate
5-HT_{1D} receptors	Histamine
5-HT_{3} receptors	Noradrenaline
5-HT_{7} receptors	Vortioxetine
	Escitalopram
	Flesinoxan
	Duloxetine
	Ondansetron
	8-OH-DPAT
	SB-269970-A

This Table lists protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a, Alexander *et al.*, 2013b).

Introduction

Treatment response with current antidepressant medications is limited (Rush *et al.*, 2006) and calls for new drugs with improved response rates (Fatemi *et al.*, 1999; Artigas *et al.*, 2006). Among the significant unmet medical needs in depression is adequate treatment of deficits in cognitive function such as memory, executive function and speed of processing. Furthermore, deficits in cognitive function may persist beyond clinical recovery from the depressive symptoms (Bhardwaj *et al.*, 2010; Hasselbalch *et al.*, 2011). Thus ameliorating such deficits is important in future treatments (Clark *et al.*, 2009; Marazziti *et al.*, 2010).

The 5-HT system, with ascending projections from the raphe nuclei throughout the cortex, is implicated in regulating mood, attention, and learning and memory (Roth, 1994; Roth *et al.*, 2004; Enge *et al.*, 2011; Chandler *et al.*, 2013; Sumiyoshi and Higuchi, 2013). Furthermore, during wakefulness, this transmitter system is in close interaction with other neurotransmitter systems, including ACh, glutamate, dopamine, noradrenaline, histamine and orexin (hypocretin), and is involved in the regulation of circadian and cognitive processes (Sebban *et al.*, 1999; Monti, 2011; Miyamoto *et al.*, 2012; Wright *et al.*, 2012). These neuromodulatory functions of 5-HT are mediated through various receptor subtypes.

The multimodal antidepressant vortioxetine {Lu AA21 004; 1-[2-(2,4-dimethylphenyl-sulfanyl)-phenyl]-piperazine} is an antagonist at the 5-HT_{3} receptor ligand-gated ion channel, a 5-HT_{7} and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and 5-HT transporter (SERT) inhibitor *in vitro* (Bang-Andersen *et al.*, 2011; Westrich *et al.*, 2012). Microdialysis studies in rats have shown that vortioxetine enhances extracellular levels of 5-HT, ACh, noradrenaline, dopamine, and histamine in brain regions involved in the regulation of emotional and cognitive functions, such as the medial prefrontal cortex (mPFC) and ventral hippocampus (Leiser *et al.*, 2009; Bang-Andersen *et al.*, 2011; Mørk *et al.*, 2012; 2013; Pehrson *et al.*, 2013). Furthermore, the cellular localization of the receptors upon which vortioxetine acts suggest that it may also have a modulatory role on GABA and glutamate function, and through these mechanisms may mediate antidepressant and pro-cognitive effects (Pehrson and Sanchez, 2014). Preclinical studies have demonstrated the antidepressant potential of vortioxetine (Bang-Andersen *et al.*, 2011; Li *et al.*, 2012; Mørk *et al.*, 2012; Westrich *et al.*, 2012; Bétry *et al.*, 2013; Pehrson *et al.*, 2013), and its antidepressant efficacy has been demonstrated in clinical studies (Adell, 2010; Alvarez *et al.*, 2012; Baldwin *et al.*, 2012a,b,c; Boulenger *et al.*, 2012; Henigsberg *et al.*, 2012; Katona *et al.*, 2012; McIntyre *et al.*, 2013). In rats, vortioxetine prolongs acquisition and retention of time-dependent contextual fear memory and time-dependent object recognition memory in rats (Mørk *et al.*, 2013). Furthermore, clinical studies of depressed patients with cognitive dysfunction, vortioxetine has shown beneficial effects on several cognitive domains compared with placebo either as a pre-specified secondary outcome measure (Katona *et al.*, 2012), or as the primary outcome measure (McIntyre *et al.*, 2013).

Yet, there is an interest in linking target engagement, receptor localization and neurotransmission to such antidepressive and pro-cognitive end points. EEG might help bridge this gap. Quantitative EEG (qEEG) enables characterization of defined cellular and cerebral circuitries and has been used by others to study EEG changes during cognitive behaviours and depression (Başar *et al.*, 1999; 2000; Başar, 2008; Kucewicz *et al.*, 2011; Millan *et al.*, 2012; Harmony, 2013; Harvey *et al.*, 2013). Moreover, specific EEG patterns have emerged linking the ascending arousal system of the hypothalamus and brainstem to the corticothalamic system thought to play an important role in mood and depression (Robinson *et al.*, 2011). Specifically, although neocortical activation is maintained by multiple, parallel neural systems through indirect pathways

(e.g. amygdala, locus coeruleus, superior colliculus and orbitofrontal cortex), involving noradrenaline, dopamine, histamine and glutamate, it is the direct cholinergic inputs from the basal forebrain and 5-HT inputs from the midbrain raphe pathways that are essential for cortical activation (Vanderwolf, 1988; Dringenberg and Vanderwolf, 1998). Furthermore, substantial evidence shows oscillations act coherently as resonant communication networks through large populations of neurons and, as such, play a role in mood, memory and integrative (cognitive) processes (Başar *et al.*, 2000). The full breadth of the association between neural oscillations and cognition is outside the scope of this paper. However, as reviewed by Ward, specific oscillations, at least in humans, can be identified with particular cognitive processes: theta and gamma rhythms with memory encoding and retrieval, alpha and gamma rhythms with attention or focusing, and gamma synchronization with conscious awareness (Ward, 2003).

Although there has yet to be a consensus whether rat and human EEG spectral oscillations are directly or indirectly comparable, evidence does suggest the cellular substrate as well as neuroanatomical projections are relatively conserved. Importantly, there is increasing evidence that EEG methodologies have high translational value from rodent to human (Ruigt, 2002; Drinkenburg and Ahnaou, 2004; Paterson *et al.*, 2007; Javitt *et al.*, 2008; 2011; Day *et al.*, 2011; Leiser *et al.*, 2011; Wilson *et al.*, 2013). For example, citalopram decreased δ and β power in Møll-Wistar rats (Neckelmann *et al.*, 1996) and healthy human subjects (Lader *et al.*, 1986). Fluoxetine decreased delta, but elicited no change in theta, alpha, beta or gamma in Long-Evans rats (Dringenberg *et al.*, 2000) or in healthy subjects (Saletu, 1982; Saletu and Grünberger, 1985). Paroxetine and fluvoxamine decreased power in delta, theta, alpha, beta and gamma in Fisher rats (Dimpfel, 2003) as well as healthy subjects (Knott *et al.*, 2002; Saletu *et al.*, 1996). Lastly, similar effects on rapid eye movement (REM) sleep and wakefulness were also found for rat and healthy subjects for each of these drugs and other antidepressants (Staner *et al.*, 1999; Rijnbeek *et al.*, 2003).

Given the location of the receptors upon which vortioxetine acts, we hypothesized that vortioxetine would function differently than a selective 5-HT (serotonin) reuptake inhibitor (SSRI) or 5-HT/noradrenaline reuptake inhibitor (SNRI) antidepressant by increasing frontal cortical oscillations. In the present study, the effect of vortioxetine on frontal cortical activity was explored via qEEG in awake, freely behaving rats in their home-cages and related to escitalopram (SSRI) and duloxetine (SNRI). Furthermore, vortioxetine's receptor mechanisms were investigated by studying flesinoxan (5-HT_{1A} receptor agonist), ondansetron (5-HT_3 receptor antagonist) and SB 269970-A (5-HT_7 receptor antagonist). *Ex vivo* autoradiography was used to determine levels of target occupancy.

Methods

Animals

All animal care and experimental procedures complied with guidance on the care and use of laboratory animals by Lundbeck and the National Research Council (2011). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 32 animals were used in the experiments described here. Male Sprague-Dawley rats (250–300 g) from Charles River were individually housed under a 12 h light/dark cycle and temperature- (21 ± 2°C) and humidity- (60 ± 10%) control with chow and water *ad libitum*.

Ex vivo *target occupancy*

Determination of target occupancies using *ex vivo* autoradiography was undertaken, as previously described and reported (du Jardin *et al.*, 2013; Pehrson *et al.*, 2013), in a group of satellite animals. Additionally, 5-HT_{1A} and 5-HT_7 occupancies were determined as described below. In the previously published data animals were dosed with the appropriate vehicle or vortioxetine at 0.001, 0.01, 0.1, 1, 3 or 10 mg·kg^{-1}; escitalopram at 0.16, 0.49 or 1.6 mg·kg^{-1}; duloxetine at 1 or 10 mg·kg^{-1}; flesinoxan at 2.5 mg·kg^{-1}; ondansetron at 0.3 mg·kg^{-1}; or SB269970 at 10 mg·kg^{-1} (du Jardin *et al.*, 2013; Pehrson *et al.*, 2013). In all studies, three animals were used per dose. One hour after drug administration, rats were anaesthetized using CO_2, decapitated and their brains quickly harvested, flash-frozen, then sectioned coronally 20 µm thick and mounted on slides. A minimum of three replicate slices were used for each brain per experiment. Tissue was sectioned beginning at approximately +1.2 mm anterior from Bregma for SERT and 5-HT_{1B} receptors, –2.1 mm posterior from Bregma for 5-HT_7 receptors, and –4.8 mm posterior from Bregma for 5-HT_{1A} and 5-HT_3 receptors. The region of interest (ROI) for each assay was selected *a priori* by receptor mapping studies (Table 1), showing that the ROI had a reliable specific binding signal for the relevant radioligand. Although subcortical regions were used to estimate target occupancy, these estimates are thought to represent receptors throughout the brain, including in cortical regions. This assumption is based on two principles: (i) according to the law of mass action, fractional receptor occupancy depends only on the concentration of the drug in the biophase and its affinity for the target receptor, and (ii) drugs that penetrate the blood–brain barrier (BBB) reach an equilibrium concentration in the biophase that is similar everywhere within the BBB.

Autoradiography

Slides were defrosted, briefly preincubated in appropriate buffer (see below), allowed to dry then incubated in an assay buffer that included the appropriate tritiated radioligand at a concentration determined *a priori* by saturation binding experiments. Non-specific binding was determined by incubating slices from a vehicle-treated animal in assay buffer that contained the appropriate radioligand and a high concentration of a non-radioactive competitor for the target. After incubation, slides were washed twice for 5 min in cold (4°C) assay buffer. Finally, slides were transferred to a vacuum desiccator for at least 1 h before being exposed in a Beta-imager (Biospace Labs) for 15–24 h. Specific details for each assay are noted in Table 1. Surface radioactivity (counts per minute per mm^2) was measured and averaged from three replicate brain slices from each rat using Beta-vision plus software (Biospace Labs). Specific binding was ascertained by subtracting non-

Table 1

Receptor and SERT occupancy assay conditions for each target

	5-HT$_3$	5-HT$_7$	5-HT$_{1B}$	5-HT$_{1A}$	SERT
Preincubation buffer	50 mM Tris HCl 150 mM NaCl	50 mM Tris HCl 4 mM $CaCl_2$ 0.5 μM L-ascorbic acid	170 mM Tris HCl 4 mM $CaCl_2$ 5.67 mM L-ascorbic acid	None	None
Preincubation time	1 × 5 min	1 × 3 min	1 × 3 min	None	None
Assay buffer	50 mM Tris HCl 150 mM NaCl 4 mM $CaCl_2$	170 mM Tris HCl 4 mM $CaCl_2$ 0.5 uM L-ascorbic acid 100 μM pargyline	170 mM Tris HCl 4 mM $CaCl_2$ 5.67 mM L-ascorbic acid 10 μM pargyline	170 mM Tris HCl 4 mM $CaCl_2$ 5.67 mM L-ascorbic acid 10 μM pargyline	50 mM Tris HCl 150 mM NaCl 5 mM KCl
Radioligand	[^{3}H]LY278584 2 nM	[^{3}H]SB269970[a] 5.9 nM	[^{3}H]GR125743 1 nM	[^{3}H]8-OH-DPAT[b] 3 nM	[^{3}H]escitalopram 4.5 nM
Non-specific binding agent	ondansetron 10 μM	SB269970 1 μM	SB216641 1 μM	WAY-100635 1 uM	Paroxetine 1 uM
Incubation time	1 h	1 h	1 h	1 h	1 h
Region of Interest	amygdala, piriform cortex	Anterior paraventricular thalamic nucleus interanteromedial thalamic nucleus, other medial thalamic regions	Caudate, putamen, nucleus accumbens	Hippocampus	Lateral septum, medial septum, olfactory tubercle

Specific details of each assay for each target are provided. See also du Jardin *et al.*, 2013 and Pehrson *et al.*, 2013.
[a][^{3}H]SB269970 was supplied by Amersham (Piscataway, NJ, USA), and has a specific activity of 33 Ci/mmol and radioactivity concentration of 0.2 mCi/mL. Given a counting efficiency of 44%, the cpm/mL equivalent of 5.9 nM [^{3}H]SB269970 is approximately 190 000.
[b][^{3}H]8-OH-DPAT was supplied by Perkin Elmer (Waltham, MA, USA) and has a specific activity of 154.2 Ci/mmol and radioactivity concentration of 1.0 mCi/mL. Given a counting efficiency of 44%, the cpm/mL equivalent of 3 nM [^{3}H]8-OH-DPAT is approximately 452 000.

specific binding from total binding. For each brain, specific binding was normalized to the average specific binding from brains in the vehicle-treated group. These values were expressed as a percentage of vehicle-specific binding levels, and were finally subtracted from 100 to obtain the percentage of occupancy and determine ED_{50} values. Where appropriate, doses were log-transformed and a non-linear regression analysis was applied using a sigmoidal dose–response curve. Values were constrained to 0–100, while the Hill coefficient was not constrained.

5-HT$_{1A}$ receptor occupancy

Slides were incubated for 1 h in assay buffer consisting of 170 mM Tris HCl (pH = 7.4), 4 mM $CaCl_2$, 5.67 mM L-ascorbic acid and 10 μM of pargyline that contained 3 nM of the 5-HT$_{1A}$ receptor agonist [^{3}H]8-OH-DPAT. Although [^{3}H]8-OH-DPAT has some affinity at 5-HT$_7$ receptors, empirical *in vitro* competition experiments performed in this laboratory suggest that 5-HT$_7$ receptor-specific bound radioactivity is a negligible proportion of the total binding observed under the assay conditions defined earlier.

5-HT$_7$ receptor occupancy

Defrosted slides were preincubated for 3 min at 4°C in a buffer containing 50 mM Tris HCl (pH = 7.4), 4 mM $CaCl_2$, and 0.5 μM L-ascorbic acid. After drying, slides were incubated for 1 h in assay buffer consisting of 50 mM Tris HCl (pH = 7.4), 4 mM $CaCl_2$, 0.5 μM L-ascorbic acid and 100 μM of pargyline that contained 5.9 nM of the 5-HT$_7$ receptor-selective radioligand [^{3}H]SB269970.

EEG

Stainless-steel screw electrodes for EEG and wire electrodes for EMG of dorsal neck muscles were implanted in each animal under anaesthesia as described previously (Vogel *et al.*, 2002; Bastlund *et al.*, 2004). Bipolar (differential) EEG screw electrodes were placed supradural approximately 2.0 mm anterior and 2.0 mm lateral to Bregma bilaterally for frontal cortical EEG and intrahemispheric at 4.0 mm posterior and 2.0 mm lateral to Bregma for fronto-parietal EEG. Electrodes were connected to a sterile multi-channel telemetric device (TL10M3-F50-EEE; Data Sciences International, DSI) that was implanted s.c. on the flank. These transmitters also digitally monitor locomotor activity (LMA). Data from 16 rats were recorded simultaneously. To accomplish the multiple drug arm comparison presented herein, two sets of animals were subjected to a cross-over study wherein each rat would receive each compound in a random order, with a minimum of 3 days wash-out between dosing. The results presented combine these data since all animals were housed, handled, dosed, and recorded from identically. In support of combining the data there was no statistical significant difference between vehicle groups across the two studies (ANOVA). Recordings were started 90 min before injection (~10:00 h; 4 h into light cycle) and for up to 4 h post-injection using Dataquest A.R.T software (DSI) at a sampling rate of 500 Hz.

Offline, using NeuroScore (DSI), artefacts were removed from the data and sleep stages assigned manually for every 10 s epoch using EEG, EMG and LMA by conventional methods as previously described (Ivarsson *et al.*, 2005; Parmentier-Batteur *et al.*, 2012) using the fronto-parietal EEG, LMA and EMG: active wake (less regular, low-amplitude EEG with high EMG and LMA activity); quiet wake (less regular, low-amplitude EEG, with low EMG and no LMA activity); slow wave sleep (or non-REM, NREM, sleep), consisting of high-amplitude waves with predominant delta (1–4 Hz), low EMG and no LMA; paradoxical or REM sleep exhibited stable, low-amplitude waves dominated by theta (4–8 Hz) with near absent EMG and no LMA. All data were scored into these stages however only Active Wake is shown in this paper in order to maximize the likelihood of translatability (Maire *et al.*, 2013). The amount of time in each stage was calculated as a percentage change for each animal and used to compare versus vehicle (treatment × stage ANOVA).

Spectral frequencies (1–50 Hz) for the active wake state of frontal cortical EEG only were calculated in NeuroScore (DSI) with 1 Hz resolution. Frequencies were binned into delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta-I (12–18 Hz), beta-II (18–30 Hz), and gamma (30–50 Hz) power bands. Pharmacologically induced changes were evaluated by averaging the 10-s bins constituting 15–75 min before ('Baseline') and 45–90 min post-treatment. This post-dose time was chosen to match the time receptor occupancy was measured (Figure 1). Using each rat as its own control, the percentage of change from baseline was calculated for each spectral bin from the pre- and post-dose averages using the formula: [(MeanPost – MeanPre)/MeanPre × 100%]. This percentage of change allowed for standardization of power changes for comparisons across all rats without the need for normalizing to total or computing relative power. A one-way ANOVA with Fisher's least significant difference (LSD) *post hoc* test (Statistica, StatSoft, Inc., Plymouth Meeting, PA, USA) was used to determine differences of each power band for each treatment group versus vehicle. This *post hoc* test was chosen because it is well suited for testing multiple hypotheses and performing specific multiple comparisons in a large dataset. The ANOVA for timeseries data was computed as Treatment × Epoch (15 min) for each power band; *post hoc* LSD test was used to compare each treatment versus vehicle at each time epoch for each power band.

Materials

Vortioxetine, escitalopram, duloxetine and flesinoxan were synthesized by Lundbeck. Ondansetron and SB-269970-A were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in 20% aqueous β-cyclodextrin and administered s.c. in a volume of 2.0 mL·kg^{-1}. Doses were chosen based on target occupancies measured by *ex vivo* autoradiography. Doses expressed as mg·kg^{-1} of the base, and number of rats in parentheses were: vehicle (n = 12), vortioxetine 0.1 (n = 9), 1.0 (n = 8), 3.0 (n = 8), 5.0 (n = 8), and 10.0 (n = 8) mg·kg^{-1}; SB-269970-A 10.0 mg·kg^{-1} (n = 8); ondansetron 0.3 mg·kg^{-1} (n = 8); escitalopram 2.0 mg·kg^{-1} (n = 8); duloxetine 10.0 mg·kg^{-1} (n = 8); and flesinoxan 2.5 mg·kg^{-1} (n = 9). As vortioxetine has lower affinity at the rat than the human 5-HT$_{1A}$ receptor (K_i 15 vs. 230 nM), a combination of vortioxetine and the 5-HT$_{1A}$ receptor agonist flesinoxan (2.5 mg·kg^{-1}, s.c.) was chosen to mimic the estimated occupancy level in humans at this receptor in the rat: vortioxetine 1.0 (n = 8), 3.0 (n = 8), 5.0 (n = 9) and 10.0 (n = 9) mg·kg^{-1} in combination with flesinoxan.

Results

Ex vivo *target occupancy*

Acute vortioxetine administration engendered dose-dependent increases in occupancies at each of its receptor targets that were in line with the rank order of *in vitro*

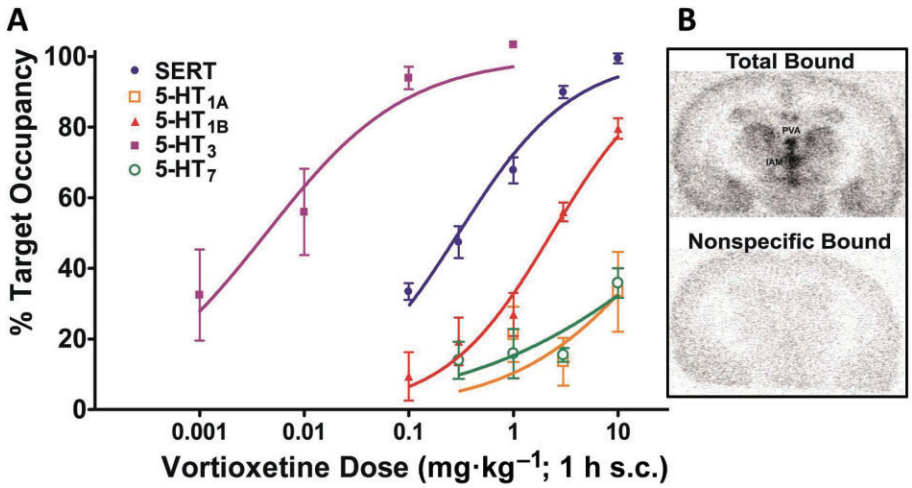

Figure 1

Ex vivo occupancies of vortioxetine. Occupancies of the multimodal drug vortioxetine for each of its targets (A) and the regions of interest used in the 5-HT$_7$ receptor assay (B) are shown. The percentage of target occupancies for the 5-HT$_3$, 5-HT$_7$, 5-HT$_{1B}$, 5-HT$_{1A}$ receptors and SERT elicited by vortioxetine are graphed as a function of dose. PVA, paraventricular thalamus; IAM, interanteromedial nucleus.

Table 2

Summary of *ex vivo* occupancies in the rat and *in vitro* binding affinities of vortioxetine

Target	*Ex vivo* occupancy in the rat ED_{50} ($mg \cdot kg^{-1}$) [95% CI]	*In vitro* binding affinity, K_i (nM)	
		Rat	Human
5-HT_3	0.004 [0.0016–0.011]	1.1	3.7
5-HT_7	NC	190	19
5-HT_{1D}	ND	3.7	54
5-HT_{1B}	2.3 [1.7–3.2]	16	33
5-HT_{1A}	NC	230	15
SERT	0.3 [0.24–0.37]	8.6	1.6

The *ex vivo* occupancy ED_{50} values in the rat were derived based on occupancy–dose curves of vortioxetine for the 5-HT_3, 5-HT_7, 5-HT_{1B}, 5-HT_{1A} receptors and the SERT as shown in Figure 1. The previously published *in vitro* binding affinities (Bang-Andersen *et al.*, 2011; Mørk *et al.*, 2012) are also shown in order to highlight the species differences for some of the targets. NC, not calculated due to limited range; ND, not determined.

affinities (Figure 1). ED_{50} values with 95% confidence intervals are shown in Table 2. Time course data for vortioxetine (not shown) suggests that receptor occupancies are at a relative maximum from 1–4 h and thus the values reported earlier are a good estimate of target engagement over the course of the EEG data described later. At 1 hour post s.c. dose, escitalopram administration at 0.16, 0.49 and 1.6 $mg \cdot kg^{-1}$ engendered mean SERT occupancies of 83 ± 3, 87 ± 4 and 95 ± 1%, respectively, while 1 and 10 $mg \cdot kg^{-1}$ duloxetine corresponded to 86 ± 3% and 97 ± 1%, and 2.5 $mg \cdot kg^{-1}$ flesinoxan lead to 41 ± 9% 5-HT_{1A} receptor occupancy, while 10 $mg \cdot kg^{-1}$ SB-269970 caused 31 ± 3% 5-HT_7 receptor occupancy, and 0.3 $mg \cdot kg^{-1}$ ondansetron engendered 12 ± 5% 5-HT_3 receptor occupancy.

Wakefulness

Sleep staging was performed in order to obtain active wake periods for spectral analyses. Results of the sleep staging analyses are not included as it is outside the scope of this paper. Analysis revealed a significant treatment by stage effect [$F(48, 544) = 51.8$, $P < 0.001$, ANOVA]. Focusing on active wake only, *post hoc* comparisons versus vehicle showed a significant dose-dependent increase in the time spent in active wake with vortioxetine (3, 5 and 10 $mg \cdot kg^{-1}$, $P < 0.05$, Figure 2). As

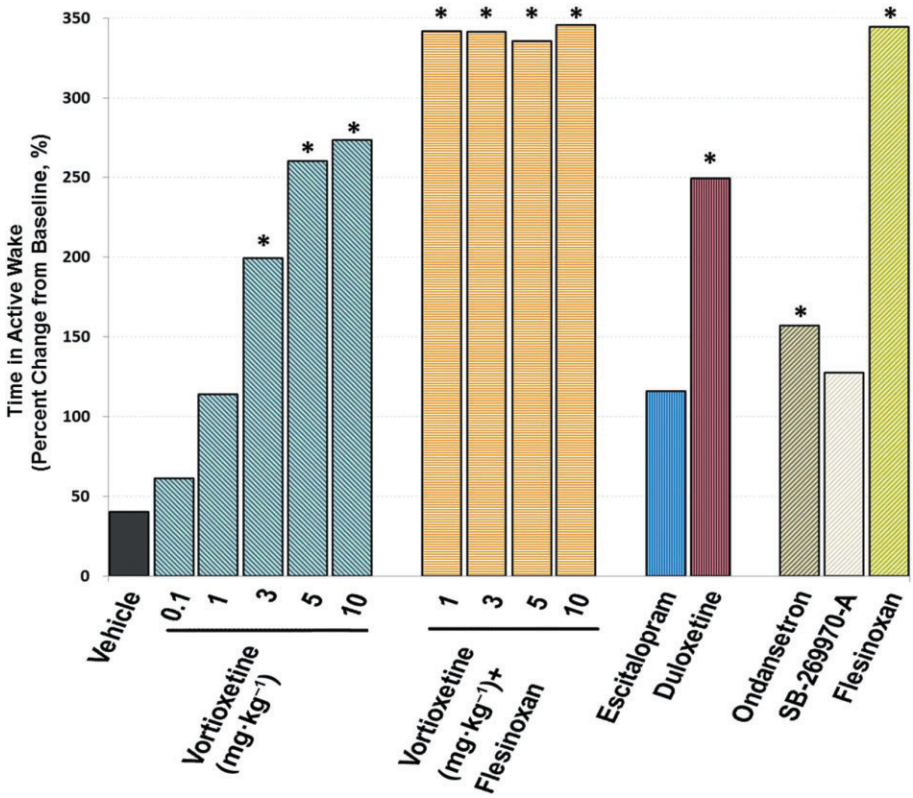

Figure 2

Comparison of vortioxetine and other treatments on impacting wakefulness. The effects on active wake in the rat by different treatment regimens measured by EEG are shown: vehicle, vortioxetine 0.1–10 $mg \cdot kg^{-1}$, vortioxetine 1–10 $mg \cdot kg^{-1}$ plus flesinoxan 2.5 $mg \cdot kg^{-1}$, SB-269970-A 10 $mg \cdot kg^{-1}$, ondansetron 0.3 $mg \cdot kg^{-1}$, escitalopram 2 $mg \cdot kg^{-1}$, duloxetine 10 $mg \cdot kg^{-1}$ and flesinoxan 2.5 $mg \cdot kg^{-1}$. Data are expressed as percentage of change from baseline. *$P < 0.05$, post-dose versus vehicle, one-way ANOVA with LSD *post hoc* comparison.

Table 3

Summary of changes in EEG power spectra induced by drug treatment

Treatment (Dose mg·kg^{-1})	Changes in the amplitude of EEG spectral components					
	Delta (1–4 Hz)	Theta (4–8 Hz)	Alpha (8–12 Hz)	Beta-I (12–18 Hz)	Beta-II (18–30 Hz)	Gamma (30–50 Hz)
Vehicle	NC	NC	NC	NC	NC	NC
Dose comparisons						
Vortioxetine (0.1)	NC	↑	↑↑	↑	NC	NC
Vortioxetine (1)	↑↑*	↑	NC	NC	↓	NC
Vortioxetine (3)	↑	↑	NC	NC	NC	NC
Vortioxetine (5)	↑↑*	↑↑*	↑	NC	NC	↑↑**
Vortioxetine (10)	↑	↑↑*	↑	NC	NC	↑↑*
Clinical comparisons						
Vortioxetine (5)	↑↑*	↑↑*	↑	NC	NC	↑↑**
Escitalopram (2)	↓	NC	NC	NC	NC	NC
Duloxetine (10)	↓	↓↓	↓↓*	↓↓	↓↓	NC
Mechanism of action comparisons						
5HT-3 antagonist Ondansetron (0.3)	↑↑	↑↑*	↑	↑↑	↑	↑
5HT-7 antagonist SB-269970 (10)	NC	↑↑	↑↑	↑↑	↑	↑
5HT-1A agonist Flesinoxan (2.5)	NC	↑↑	↑↑	NC	↑↑*	↑↑**
Combination 5HT-1A receptor agonism comparisons						
Vor (1) + Fles	NC	NC	↑↑*	NC	↑↑*	↑↑**
Vor (3) + Fles	↑↑**	↑↑*	↑↑	↑↑	↑↑**	↑↑**
Vor (5) + Fles	↑↑	↑↑*	↑↑	NC	↑↑*	↑↑**
Vor (10) + Fles	↑↑	↑↑↑**	↑↑↑**	↑↑*	↑↑↑**	↑↑↑**

Changes in power in conventional frequency bands (delta, theta, alpha, beta, and gamma) in response to drug treatments are categorically summarized as follows: NC, no change (<10%); ↑, increase <25%; ↑↑, increase >25%; ↑↑↑, increase >75%; ↓, decrease <25%; ↓↓, decrease >25%. Statistical significance: *P < 0.05, **P < 0.01, one-way ANOVA with LSD *post hoc* analysis.

vortioxetine has a considerably lower affinity for rat, compared with human, 5-HT$_{1A}$ receptors (Table 2), combined treatments with flesinoxan (2.5 mg·kg^{-1}) were included to mimic 5-HT$_{1A}$ occupancy in clinical settings. With flesinoxan, all doses of vortioxetine (1, 3, 5 and 10 mg·kg^{-1}) resulted in a significant increase in active wake (P < 0.05). The increase in active wake reached a physiological maximum, as flesinoxan alone produced the same magnitude increase (P < 0.05). Additionally, the 5-HT$_3$ receptor antagonist ondansetron (0.3 mg·kg^{-1}) and the SNRI duloxetine (10 mg·kg^{-1}), but not the 5-HT$_7$ receptor antagonist SB-269970-A (10 mg·kg^{-1}) or the SSRI escitalopram (2 mg·kg^{-1}), increased active wake time (P < 0.05), although these magnitudes were smaller.

Active wake state-specific quantitative EEG

Quantitative analyses of the spectral frequencies on frontal cortical EEG were performed when the animal was in the active wake state. *Post hoc* comparisons were made to vehicle. Table 3 summarizes the effects across all power bands. The effects on theta, alpha, and gamma power are shown in Figures 3, 5 and 7. Bar graphs show mean changes during 45–90 min post-treatment with respect to pretreatment baseline; this time matches when receptor occupancy was measured. Time series data for up to 240 min post-dose is also presented for these spectra for comparisons of vortioxetine with and without flesinoxan to vehicle, escitalopram, and duloxetine (Figures 4, 6, and 8).

There was a significant treatment effect for frontal cortical theta power [F(14, 113) = 3.0, P < 0.001, one-way ANOVA] (Figure 3). Vortioxetine (5 and 10 mg·kg^{-1}) significantly increased theta power alone and with flesinoxan (P < 0.05), whereas SB-269970-A and flesinoxan produced an increase but did not reach statistical significance (P = 0.096 and P = 0.079, respectively). Ondansetron significantly increased theta power (P = 0.026). In contrast, whereas escitalopram had little effect, duloxetine caused a modest but not significant decrease. These effects can be seen throughout the time-course as well (Figure 4).

There was also a significant treatment effect for frontal cortical alpha power [F(14, 113) = 3.0, P < 0.001, one-way ANOVA] (Figure 5). All doses of vortioxetine non-significantly increased alpha power (>3 times vehicle) with vortioxetine (1 and 10 mg·kg^{-1}) plus flesinoxan significantly increasing alpha power (P < 0.05). Interestingly, flesinoxan alone also non-significantly increased alpha, suggesting an additive effect with vortioxetine's other 5-HT targets. Ondansetron and SB-269970-A modestly increased alpha power. Escitalopram had little effect, whereas duloxetine elicited a robust and

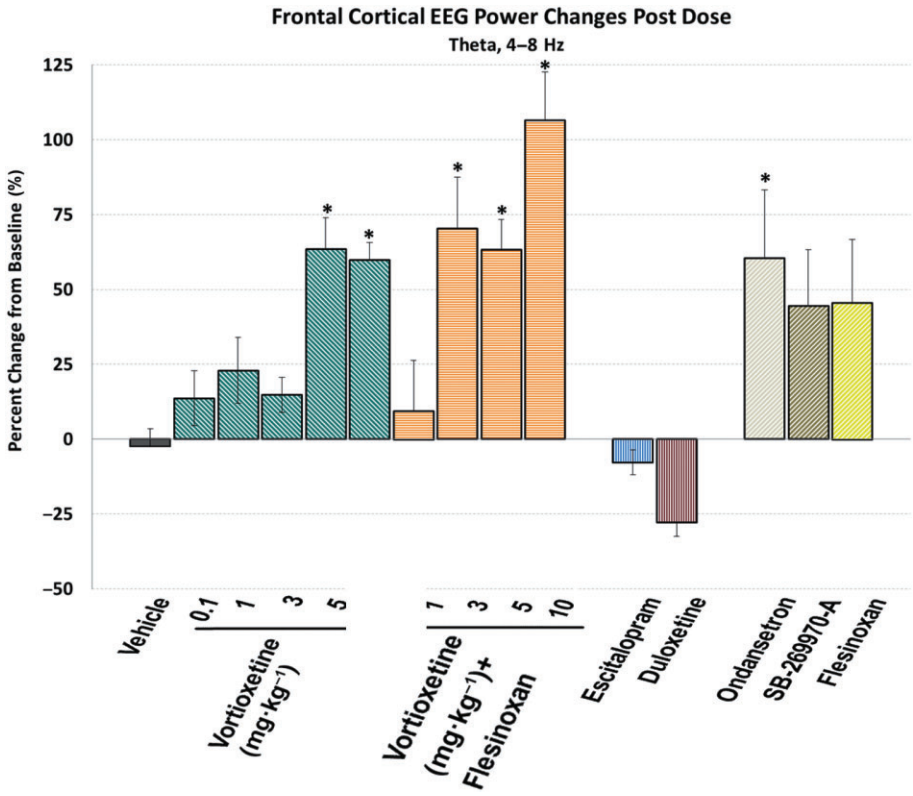

Figure 3

Vortioxetine increases frontal cortical theta power. Frontal cortical theta power during active wake, expressed as the percentage of change from baseline, is shown for each different treatment. A negative value indicates a decrease in EEG power from baseline. *$P < 0.05$, post-dose versus vehicle, one-way ANOVA with LSD *post hoc* comparison.

significant decrease ($P < 0.05$). These changes can be seen throughout the time course (Figure 6).

Finally, there was a significant treatment effect for frontal cortical gamma power [$F(14, 113) = 5.2$, $P < 0.001$, one-way ANOVA] (Figure 7). Vortioxetine (5 and 10 mg·kg^{-1}) significantly increased gamma power alone and at all doses in combination with flesinoxan ($P < 0.05$). Flesinoxan alone significantly increased gamma power ($P < 0.05$), while SB-269970-A and ondansetron non-significantly elevated gamma ($P = 0.11$ and 0.06 respectively). Escitalopram and duloxetine elicited no change. These changes are observable throughout the time course with a clear increase in gamma oscillations by vortioxetine plus flesinoxan (Figure 8).

Discussion

There is considerable evidence linking selective attention, memory and cognitive efficiency to arousal (Hebb, 1955; Dickman, 2002; Mair *et al.*, 2011; Mather and Sutherland, 2011). Thus, the first step in investigating vortioxetine's role in modulating cortical oscillations was to assess its effect on arousal, here measured as increased wakefulness. Cognitive and affective processes vary over the 24 h day, with peak performance occurring when the ascending brainstem, basal forebrain and hypothalamic arousal systems are activated, producing enhanced wakefulness. The sleep–wake cycle is regulated by complex neurobiological circuits; waking is regulated via ACh, dopamine, noradrenaline, 5-HT, histamine and orexin (hypocretin) systems (Datta and Maclean, 2007), while sleep is regulated via GABAergic and cholinergic systems within select brain regions, including the cortex (Murillo-Rodriguez *et al.*, 2009; Brown *et al.*, 2012). As vortioxetine has been shown to enhance extracellular levels of ACh, dopamine,noradrenaline, 5-HT and histamine in the prefrontal cortex (Mørk *et al.*, 2013; Pehrson *et al.*, 2013), it appeared reasonable to hypothesize that vortioxetine can increase frontal cortical oscillations in a manner that may act to prime the cortex to respond better to incoming afferent information.

We found vortioxetine significantly and dose-dependently increased wakefulness, as measured as increased time spent in active wake (Figure 2). From the results shown in Figures 1 and 2, it is apparent that active doses of vortioxetine in our study in rats were in clinically equivalent range corresponding to 50–90% SERT occupancy as seen in clinical PET studies (Areberg *et al.*, 2012). Taking into consideration the lower affinity of vortioxetine for rat, compared with human, 5-HT$_{1A}$ receptors (Table 2), results of combined dosing of vortioxetine and flesinoxan indicate that 5-HT$_{1A}$ agonism plays a critical role in mediating the effects of vortioxetine on wakefulness. Flesinoxan administered alone or with vortioxetine, produced a maximal increase in wakefulness. The flesinoxan dose corresponded to ≈40% occupancy,

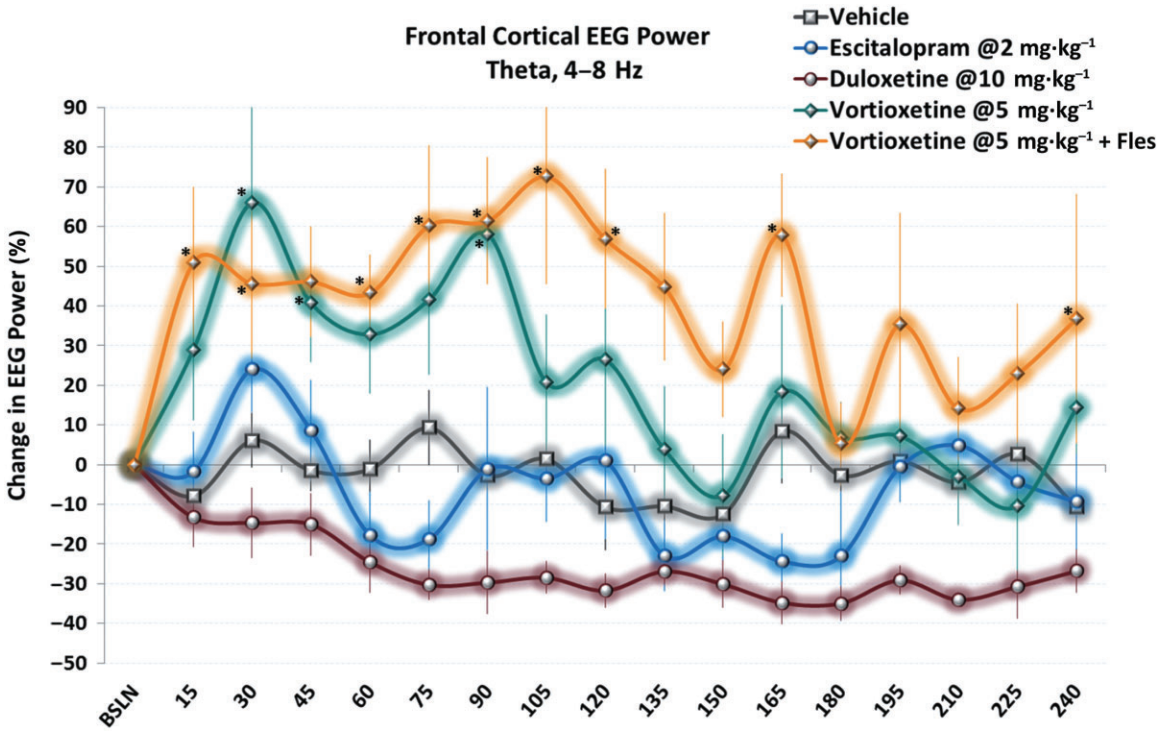

Figure 4

Vortioxetine increases frontal cortical theta power unlike escitalopram and duloxetine. Time-series data show elevated theta power immediately following acute doses of vortioxetine alone or with flesinoxan. Only data during active wake is shown. *$P < 0.05$ versus vehicle, LSD *post hoc* comparison.

which based on extrapolations from *in vitro* human and rat affinities and *ex vivo* occupancy measurements in rats, in principle should be attainable by vortioxetine in clinical settings. The effects of flesinoxan are in line with published studies where the full 5-HT_{1A} receptor agonist, 8-OH-DPAT, was found to increase wakefulness (Wilson *et al.*, 2005). The 5-HT_7 receptor antagonist SB-269970, at dose corresponding ≈31% occupancy, increased active wake moderately to a level corresponding to vortioxetine at 1 mg·kg^{-1}. The time course of EEG results suggests that the 1 h time point used for 5-HT_3 receptor occupancy determinations was after the time of maximal brain exposure for ondansetron because 1 h after administration 5-HT3 receptor occupancy was only about 12%, yet 0.3 mg·kg^{-1} ondansetron also increased active wake to a level similar to that seen with vortioxetine. Thus, it is plausible that not only 5-HT_{1A} receptor agonism, but also 5-HT_3 and 5-HT_7 receptor antagonism may contribute to vortioxetine's increase of wakefulness and thereby increase attention and vigilance. In addition, vortioxetine has lower *in vitro* affinity for the rat versus human 5-HT_7 receptor (Table 2) making it likely that the effects of vortioxetine mediated by 5-HT_7 receptor antagonism may be greater in humans.

In line with previous studies (Katoh *et al.*, 1995; Sanchez *et al.*, 2007), our data show that duloxetine, 10 mg·kg^{-1} corresponding to >80% SERT occupancy, increased wakefulness significantly. The effect size was comparable with that of vortioxetine at 5 mg·kg^{-1}. We further showed that escitalopram, 2 mg·kg^{-1} corresponding to >80% SERT occupancy, only moderately increased wakefulness similarly to vortioxetine at 1 mg·kg^{-1}. These results are consistent with previous findings and the difference between duloxetine and escitalopram is ascribed to the increased noradrenergic neurotransmission elicited by duloxetine (Sanchez *et al.*, 2007). Clinical literature has substantially provided evidence that SERT occupancy of ~80% is necessary to achieve therapeutic effects of SSRI and SNRI (Meyer *et al.*, 2004; Takano *et al.*, 2006; Shang *et al.*, 2007; Voineskos *et al.*, 2007; Kasper *et al.*, 2009). Thus, the doses used in our studies are clinically equivalent doses.

The next step was to explore vortioxetine's effects on spectral frequency domains suggested to play important roles in cognitive function. Although complex and still evolving, some fundamental understandings about each brain rhythm have emerged (Başar *et al.*, 1999; 2000; 2001; Klimesch, 1999; 2012; Hajós *et al.*, 2003a; Başar, 2008; Millan *et al.*, 2012). Vortioxetine, unlike escitalopram and duloxetine, elicited an increased power in key spectra, demonstrating the multimodal mechanism of action of vortioxetine involving modulation of 5-HT receptors and SERT inhibition results in a distinct profile compared with antidepressants acting through inhibition of monoamine transporters (Nutt *et al.*, 2010). Although in this study, EEG from rats was not recorded during a cognitive task, but rather innate exploratory behaviour, we believe our findings set a framework for cortical activation that warrants further study both in animals and in humans.

Delta waves are believed to be generated by the summation of long-lasting after hyper-polarizations (AHPs) in pyramidal neurons (layers II–III or V) and increases reflect

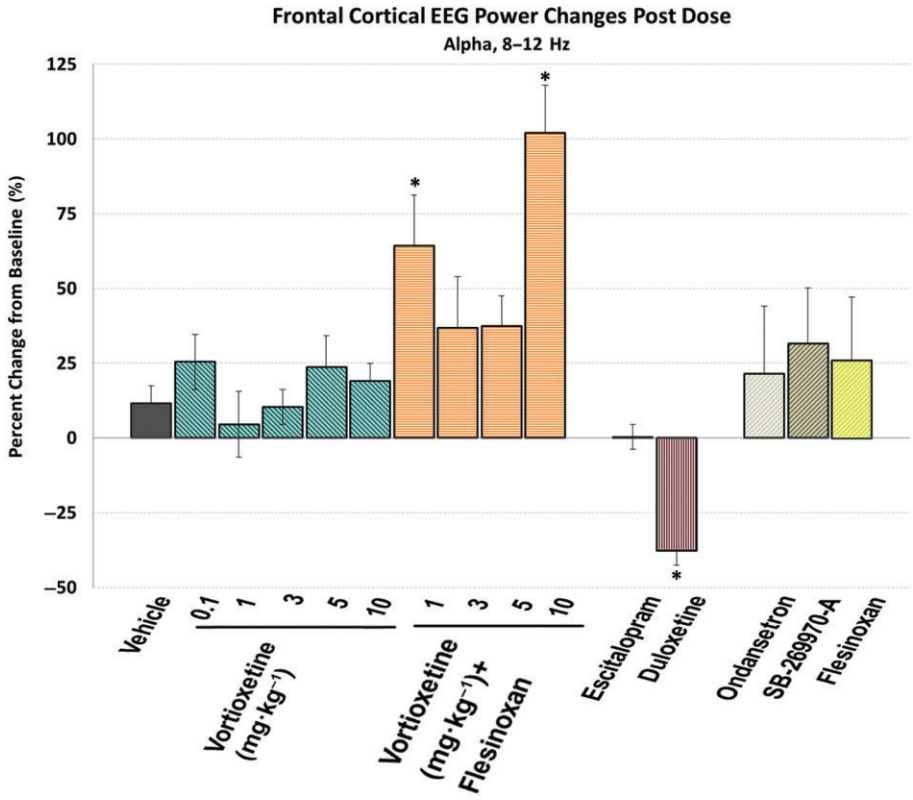

Figure 5

Vortioxetine increased frontal cortical alpha power. Frontal cortical alpha power during active wake, expressed as the percentage of change from baseline, is shown for each different treatment. A negative value indicates a decrease in EEG power from baseline. *$P < 0.05$, post-dose versus vehicle, one-way ANOVA with LSD *post hoc* comparison.

more cells exhibiting AHPs (inhibition of pyramidal neurons by local-circuit cells) (Steriade, 2005). Further, cortical delta activity has been shown to increase during concentration demand (Harmony, 2013). Our data show vortioxetine produced an increase in delta power, alone and in combination with flesinoxan, whereas duloxetine and escitalopram produced a non-significant decrease. The 5-HT_3 receptor antagonism of vortioxetine appears to contribute to this effect as ondansetron did increase delta activity significantly ($P < 0.05$, Fisher's LSD), as seen previously (Bo *et al.*, 1993), while neither SB-269970-A nor flesinoxan elicited any change.

Theta oscillations have been associated with cognitive functions. For example, preclinical and clinical data suggest that theta oscillations tend to increase during memory tasks, especially during encoding (Klimesch, 1996; 1999; Başar *et al.*, 2000). Theta oscillations are predominantly driven via hippocampal–entorhinal–cortical projections. Extensive coupling of theta oscillations throughout the midline cortices and hippocampus has been demonstrated, and this coherence is believed to reflect binding of cortical and hippocampal pathways into functional units by behavioural demands (Young and McNaughton, 2009). Moreover, hippocampal theta power is linked to memory, particularly spatial memory in rats, while in humans, an increase in theta correlated with improved performance on working memory tasks (Caplan *et al.*, 2001; Raghavachari *et al.*, 2001; 2006). Cortical theta power is proposed to reflect coordinating neural networks involved in monitoring behaviour and the environment as well as facilitating task-specific adaptive changes in performance. Modulation by 5-HT is known to play an important role in the generation and regulation of theta power (Stäubli and Xu, 1995; Vertes and Kocsis, 1997; Bland *et al.*, 1999; Dragoi *et al.*, 1999; Buzsaki, 2002; Hajós *et al.*, 2003b; McNaughton *et al.*, 2007; Sörman *et al.*, 2011). Our data show vortioxetine (5 and 10 mg·kg^{-1}) significantly increased frontal cortical theta power. While flesinoxan (≈35% 5-HT_{1A} receptor occupancy) alone only modestly increased theta power, vortioxetine (3, 5, and 10 mg·kg^{-1}) plus flesinoxan significantly increased theta power, indicating an additive effect. Similarly, 8-OH-DPAT, a 5-HT_{1A} receptor agonist, has increased theta power in hippocampal EEG in cats (Bjorvatn *et al.*, 1997). Ondansetron significantly increased theta power in the present study, consistent with previously published data on the augmentation of theta power in hippocampal EEG recordings from freely-moving rats (Stäubli and Xu, 1995). Similarly, ondansetron dose-dependently increased hippocampal theta rhythm as well as the magnitude and duration of CA1 long-term potentiation in rat (Stäubli and Xu, 1995). The 5-HT_7 receptor antagonist SB-269970-A increased theta power significantly even at a low level of occupancy. In contrast, escitalopram and duloxetine produce non-

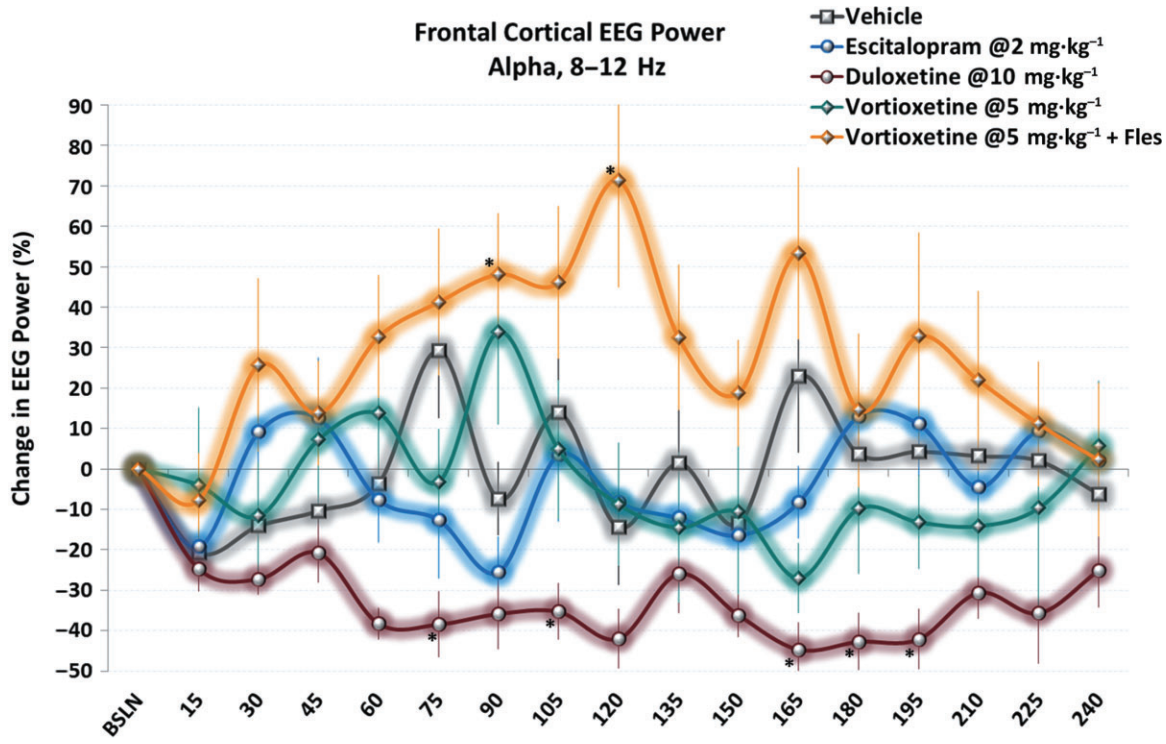

Figure 6

Time-series data show elevated alpha power following acute doses of vortioxetine with flesinoxan. Effects are more transient than other spectra; however, clear differences can still be seen between vortioxetine with flesinoxan and escitalopram and duloxetine. Only data during active wake is shown. *$P < 0.05$ versus vehicle, LSD *post hoc* comparison.

statistically significant decreases of theta power. In a previous study, escitalopram was found to decrease theta (5–9 Hz) after acute treatment during active wake in rats (Vas *et al.*, 2013). This further differentiates vortioxetine from escitalopram and duloxetine. Thus, our results suggest that 5-HT_3 and 5-HT_7 receptor antagonism and 5-HT_{1A} receptor agonism may collectively contribute to the enhancement of both the coordination and synchronization of neuronal networks, and this is likely a functional mechanistic component in mediating the actions of vortioxetine in humans.

Here we show vortioxetine in combination with flesinoxan significantly increased alpha power. The numerical yet not significant increases in alpha power following treatment of vortioxetine at 5 and 10 mg·kg^{-1}, SB-269970-A, ondansetron, and flesinoxan, suggest that converging action at multiple 5-HT receptors is necessary and sufficient to elicit changes in alpha wave generation as caused by vortioxetine plus flesinoxan, at a dose that should mimic clinical levels of 5-HT_{1A} occupancy. Of further interest is that duloxetine caused a significant decrease in alpha power, while escitalopram had no effect. Although, interpretation and identification of alpha power and its generators are still a matter of debate (Klimesch *et al.*, 1999; Shaw, 2003), there is some consensus that alpha waves reflect synchronization of neurons for sensorimotor integration (coordinated cortical output to afferent input) and is generated by thalamocortical feedback loops (excitatory and inhibitory) and cortico-cortical networks involving layer V pyramidal neurons (Steriade *et al.*, 1990; Lopes da Silva, 1991) and cognitive tasks elicit increases in alpha power (Klimesch *et al.*, 1999; Bastiaansen *et al.*, 2002; Schack and Klimesch, 2002; Meltzer *et al.*, 2007). In conclusion, the implication of vortioxetine's effects on alpha power and in general the role of alpha power in cognition remains to be studied further.

Gamma synchronization occurs across neuronal networks that represent related features of an object ('feature binding') to generate a coherent percept via networks of inhibitory interneurons acting at pyramidal neurons. Gamma power is a near-ubiquitous feature of ongoing cortical activity and is involved in multiple aspects of cognitive computation, sensory representation and short-term memory (Engel *et al.*, 2001; Kaiser and Lutzenberger, 2005; Kaiser *et al.*, 2008; Ainsworth *et al.*, 2011). Moreover, gamma power is associated with transient coupling of brain areas during memory refreshment, memory formation and experience-dependent plasticity (Ward, 2003; Jutras and Buffalo, 2010; Fell and Axmacher, 2011; Headley and Weinberger, 2011). There is also a putative link between gamma power and the speed of processing in a neural network, where reduced gamma power was linked to age-related cognitive and behavioural slowing (Insel *et al.*, 2012). Given that the neural basis of gamma oscillations have been associated with the GABAergic interneuronal system (Shin *et al.*, 2011) and 5-HT_{1A}, 5-HT_3 and 5-HT_7 receptors are present on GABAergic interneurons, we hypothesized gamma power would be modulated by vortioxetine.

We showed a robust and significant increase in gamma power following vortioxetine at 5 and 10 mg·kg^{-1}, as well as at

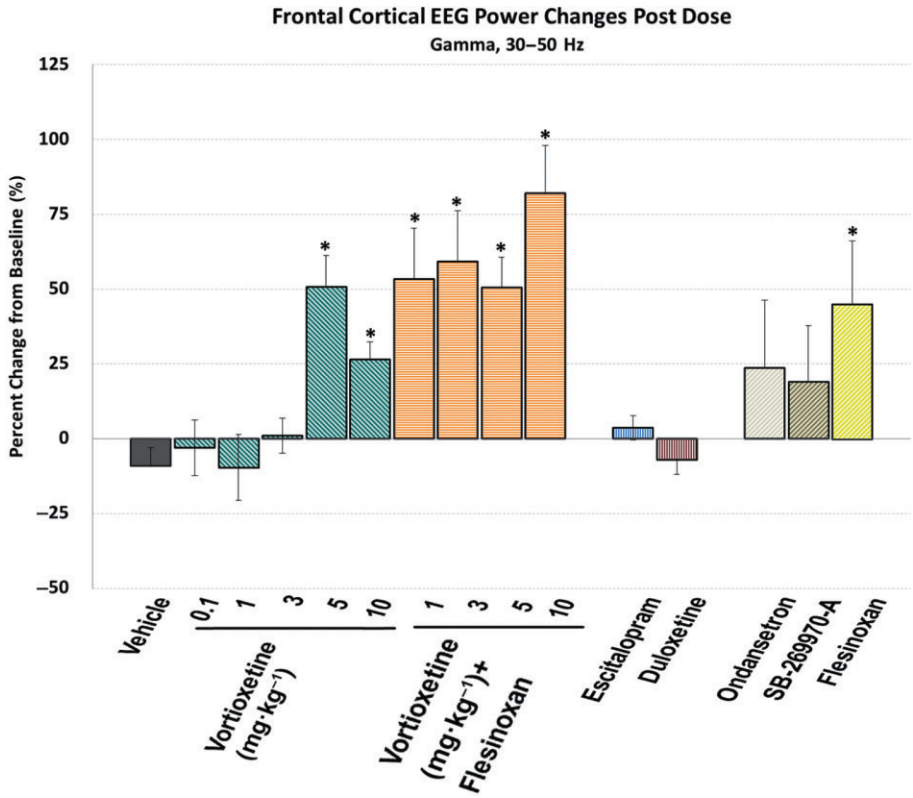

Figure 7

Vortioxetine increases frontal cortical gamma power. Frontal cortical gamma power during active wake, expressed as the percentage of change from baseline, is shown for each different treatment. A negative value indicates a decrease in EEG power from baseline. *$P < 0.05$, post-dose versus vehicle, one-way ANOVA with LSD *post hoc* comparison.

1, 3, 5 and 10 mg·kg^{-1} when in combination with flesinoxan as well as flesinoxan alone. Conversely, both escitalopram and duloxetine elicited no change in gamma power. Further, SB-269970-A and ondansetron trended to increase gamma power, but both elicited submaximal effects. Thus, a combined effect of 5-HT$_3$ and 5-HT$_7$ receptor antagonism and 5-HT$_{1A}$ receptor agonism is likely to contribute to vortioxetine's increase of gamma power. That gamma oscillations increased in frontal cortex is substantiated by previous work showing that vortioxetine, but not escitalopram, disinhibits pyramidal cell function by blocking the 5-HT- and mCPBG (5-HT$_3$ receptor agonist)-induced increases in GABA transmission and enhanced theta-burst LTP in the rat hippocampus (Dale *et al.*, 2013) and in a separate study dose-dependently increased the discharge rate of rat mPFC pyramidal neurons projecting to midbrain (Riga *et al.*, 2013).

As mentioned, a blockade of 5-HT$_3$ receptors may contribute to the modulation of cortical activation via the cholinergic system. Previous data suggested beneficial effects of 5-HT$_3$ receptor antagonists on memory function in preclinical studies (Brambilla *et al.*, 1993; Fontana *et al.*, 1995; Pitsikas and Borsini, 1996; Arnsten *et al.*, 1997; Roychoudhury and Kulkarni, 1997). In clinical settings, ondansetron has shown promise in treating cognitive impairment in schizophrenia (Zhang *et al.*, 2006; Akhondzadeh *et al.*, 2009; Khodaie-Ardakani *et al.*, 2013). Previous preclinical studies also demonstrate the memory-enhancing effects of 5-HT$_7$ receptor antagonists, probably through modulation of the glutamatergic or GABAergic systems (Meneses, 2004; McLean *et al.*, 2009; Horiguchi *et al.*, 2011; Horisawa *et al.*, 2011; Nolan and Roman, 2012; Dale *et al.*, 2013). Activation at 5-HT$_{1A}$ receptors, which serves as both a somatodendritic autoreceptor and postsynaptic heteroceptor in cognition-associated brain regions such as the dorsal raphe, entorhinal cortex, hippocampus and central amygdala (Chalmers and Watson, 1991; Polter and Li, 2010), has been linked to memory-enhancing effects (Meneses and Hong, 1999; Meeter *et al.*, 2006; Newman-Tancredi *et al.*, 2009; Depoortere *et al.*, 2010).

Study limitations

We did not measure oscillatory power during a cognitive task and thus we cannot directly tie our observations to cognitive function. Rather, given that vortioxetine does improve cognition in animal models and in clinical trials, we suggest via our EEG data that it may do so via frontal cortical activation and through its action on specific 5-HT receptor subtypes. Also that the acute dose was administered approximately 2 h into the light cycle when rats are typically quiescent may have an impact on performance as different tasks and different task parameters may depend upon circadian phase (Goel *et al.*, 2010). We further propose that the underlying cellular mechanisms generating the oscillations are similar to those

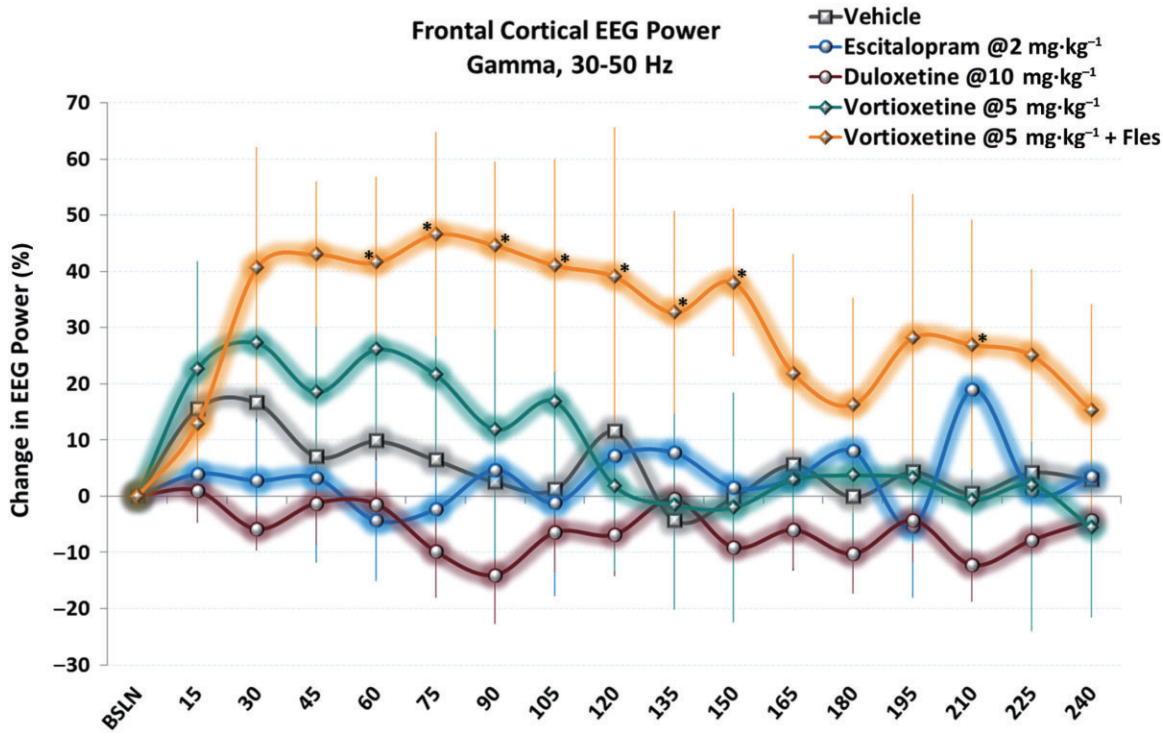

Figure 8

Vortioxetine with flesinoxan elicits a sustained effect on frontal cortical gamma power unlike escitalopram and duloxetine. Timeseries data show elevated gamma power immediately following acute doses of vortioxetine alone (non-significant trend) or with flesinoxan (statistically significant). Only data during active wake is shown. *$P < 0.05$ versus vehicle, LSD *post hoc* comparison.

recruited during cognitive tasks and that these would be relevant in humans, yet we understand and note that data is lacking to conclude on how reliably EEG can directly translate between rat and human. In example, the generators for θ and α remain enigmatic and thus it is unclear whether the increases in rat cortical θ and α power observed can be directly correlated to human cortical θ and α power, yet it is clear that these increases were elicited by vortioxetine and have not been observed for SSRIs or SNRIs to our knowledge, and thus warrants further investigation.

Conclusions

Our data of vortioxetine shows a distinct profile compared with escitalopram and duloxetine. Unlike vortioxetine, escitalopram had no effect on frontal cortical delta, theta, alpha, beta or gamma power. Consistently, a meta-analysis reported that low doses of SSRIs had virtually no effect on delta and theta and in 60% of studies caused small decreases in alpha and increases in beta, while high doses increased low frequencies with no change in alpha and beta power (Dumont *et al.*, 2005). The authors further report 'disappearance of cognitive stimulation' for increasing SSRI doses (Dumont *et al.*, 2005). Meanwhile, increased noradrenergic activity decreases spectral power (Sebban *et al.*, 1999). This is consistent with our findings with duloxetine.

Collectively, these data argue that 5-HT_{1A} receptor agonism, and 5-HT_3 and 5-HT_7 receptor antagonism, in addition to SERT inhibition and possibly other actions not investigated here (e.g. 5-HT_{1D} receptor antagonism and 5-HT_{1B} receptor partial agonism) are likely all important elements in the actions of vortioxetine on modulating cortical oscillations. Given the impact of vortioxetine on cortical oscillations and the putative connection between these oscillations and cognitive function, these observations warrant further investigation of vortioxetine's effects on cognitive functioning in preclinical and clinical settings. Specifically, implementing integrated EEG recordings and behavioural assessments using methods that translate between rodent and humans would be helpful to understand the value of our preclinical data in predicting EEG changes in human and also the relationship of cortical oscillations to cognitive function.

Acknowledgements

The authors would like to thank Drs. David Simpson (Lundbeck) and Huailing Zhong (U-Pharm Laboratories LLC, Parsippany, NJ, USA) for helpful insights and comments, and Huailing Zhong for assistance in preparing the paper.

Author contributions

S. C. L. and C. S. wrote the paper; A. L. P. performed the *ex vivo* autoradiography; P. J. R. performed the EEG recordings and sleep staging; S. C. L. performed the qEEG analyses.

Conflict of interest

The work by all authors was performed as full-time employees or consultants of Lundbeck at the time of the study. This study was funded by H. Lundbeck A/S.

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