1521-0103/358/3/472–482$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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http://dx.doi.org/10.1124/jpet.116.233924
J Pharmacol Exp Ther 358:472–482, September 2016

Task- and Treatment Length–Dependent Effects of Vortioxetine on Scopolamine-Induced Cognitive Dysfunction and Hippocampal Extracellular Acetylcholine in Rats[S]

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Received March 25, 2016; accepted July 8, 2016

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ABSTRACT

Major depressive disorder (MDD) is a common psychiatric disorder that often features impairments in cognitive function, and these cognitive symptoms can be important determinants of functional ability. Vortioxetine is a multimodal antidepressant that may improve some aspects of cognitive function in patients with MDD, including attention, processing speed, executive function, and memory. However, the cause of these effects is unclear, and there are several competing theories on the underlying mechanism, notably including regionally-selective downstream enhancement of glutamate neurotransmission and increased acetylcholine (ACh) neurotransmission. The current work sought to evaluate the ACh hypothesis by examining vortioxetine's ability to reverse scopolamine-induced impairments in rodent tests of memory and attention. Additionally, vortioxetine's effects on hippocampal extracellular ACh levels were examined alongside studies of vortioxetine's pharmacokinetic profile. We found that acute vortioxetine reversed scopolamine-induced impairments in social and object recognition memory, but did not alter scopolamine-induced impairments in attention. Acute vortioxetine also induced a modest and short-lived increase in hippocampal ACh levels. However, this short-term effect is at variance with vortioxetine's moderately long brain half life (5.1 hours). Interestingly, subchronic vortioxetine treatment failed to reverse scopolamine-induced social recognition memory deficits and had no effects on basal hippocampal ACh levels. These data suggest that vortioxetine has some effects on memory that could be mediated through cholinergic neurotransmission, however these effects are modest and only seen under acute dosing conditions. These limitations may argue against cholinergic mechanisms being the primary mediator of vortioxetine′s cognitive effects, which are observed under chronic dosing conditions in patients with MDD.

Introduction

Major depressive disorder (MDD) patients commonly experience impairments in cognitive function, including deficits in cognitive domains such as attention, executive function, speed of processing, and memory (McIntyre et al., 2013). These impairments are clinically important from the perspective that they may predict poor response to treatment with selective serotonin (5-HT) reuptake inhibitors (Dunkin et al., 2000; Withall et al., 2009), tend to remain prominent after recovery of mood symptoms (Kuny and Stassen, 1995; Herrera-Guzman et al., 2009, 2010), and are associated with poor functional recovery (Jaeger et al., 2006). Thus, it is important to identify effective treatment strategies for MDD-associated cognitive dysfunction in order to achieve a functional recovery in MDD patients.

Vortioxetine is a multimodal antidepressant that is approved for the treatment of MDD. Evidence from clinical trials suggests that vortioxetine ameliorates some aspects of MDD-associated cognitive impairment, for example, speed of processing, executive function, and memory. This is supported by evidence from a number of preclinical experiments (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014; Li et al.,

Alan Pehrson, Arne Mørk, Gennady Smagin, Dekun Song, David Budac, Manuel Cajina, and Connie Sanchez were employees of Lundbeck Research USA while these studies were conducted. Lundbeck employees played a role in study design, execution, and analysis, as well as in the writing of and decision to submit this manuscript. Nasser Haddjeri received funding from Lundbeck Research USA to conduct the object recognition experiments presented in this work. Joseph Porter received funding from Lundbeck Research USA to conduct the VSDT experiments presented in this work. Other authors have no conflicts to report.
dx.doi.org/10.1124/jpet.116.233924.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CE, collision energy; D-ACh, deuterated ACh; 5-HT, serotonin; IT, information trial; MDD, major depressive disorder; RT, retention trial; SERT, serotonin transporter; VSDT, visual signal detection task.

2015). Much of the recent research efforts from our laboratory have been aimed at understanding the biologic mechanism by which vortioxetine exerts these beneficial effects on cognitive function. Vortioxetine is a 5-HT transporter protein (SERT) inhibitor, a 5-HT_{1A} receptor agonist, 5-HT_{1B} receptor partial agonist, and 5-HT_{1D}, 5-HT_3, and 5-HT_7 receptor antagonist (Sanchez et al., 2015). Interestingly, vortioxetine's 5-HT receptor activity may confer the ability to indirectly modulate signaling through several other neurotransmitter systems, including norepinephrine, dopamine (Pehrson et al., 2013), acetylcholine (ACh) and histamine (Mørk et al., 2013), γ-aminobutyric acid (Pehrson and Sanchez, 2015; Dale et al., 2016), and glutamate (Dale et al., 2014; Pehrson and Sanchez, 2014; Riga et al., 2016). Several of these neurotransmitter systems have known relationships to cognitive function, and we have hypothesized that vortioxetine's cognitive effects may be specifically related to indirect modulation of γ-aminobutyric acid and glutamate neurotransmission (Pehrson and Sanchez, 2014), ACh, or histamine neurotransmission (Mørk et al., 2013). Although these ideas are not necessarily mutually exclusive, it is important to empirically investigate the relationship between vortioxetine's effects on these neurotransmitter systems and cognition.

ACh neurotransmission has a well-documented relationship to cognitive function. Speaking generally, improvements of cognitive function can be consistently observed after cholinergic receptor activation, whereas impairments tend to be observed after cholinergic receptor antagonism. For example, antagonism of muscarinic cholinergic receptors using scopolamine consistently impairs cognitive functions in domains such as attention, learning, and memory in rodents and humans (Collerton, 1986; Molchan et al., 1992), and genetic models featuring deletions of muscarinic M_2 receptors show impairments in behavioral flexibility and working memory, as well as reductions in hippocampal long-term potentiation, an electrophysiological measure conceptually associated with memory function (Seeger et al., 2004). Antagonism of nicotinic $\alpha 7$ or $\alpha 4\beta 2$ cholinergic receptors also impairs memory function in rodents (Levin, 1992, 2002; Dobryakova et al., 2015). Moreover, improvements in cognitive performance or related electrophysiological measures have been observed after pharmacological activation of muscarinic or nicotinic receptor targets (Levin, 1992; Levin and Rezvani, 2002; Dennis et al., 2016). Acetylcholinesterase inhibitors such as donepezil, which increase synaptic availability of ACh by reducing the rate of catabolism, improve memory function not only in rodent models (e.g., Zarrindast et al., 2002), but also induce small but significant improvements in memory function in humans, whether in schizophrenic patients [see meta-analysis (Ribeiz et al., 2010)], or in patients with Alzheimer's disease [see meta-analysis (Birks, 2006)]. Thus, it appears in general that activation of cholinergic neurotransmission, whether by direct pharmacological action on receptors or by increased ACh availability, may be a mechanism that can be leveraged to improve cognition across several different clinical populations.

These data may suggest that vortioxetine's indirect effects on cholinergic neurotransmission could represent a relevant mechanism for the observed improvements in cognitive function among MDD patients. Therefore, the purpose of the present study is to empirically evaluate the relationship between vortioxetine's effects on ACh neurotransmission and cognition in preclinical animal models.

Materials and Methods

Animals

A total of 321 adult male rats was used, that is, 144 Wistar rats (Charles River Laboratories, France) for the social recognition memory tests, 69 Sprague Dawley OFA rats (Charles River Laboratories, Saint Germain sur l'Arbresle, France) for the object recognition experiments, 16 Sprague Dawley rats (Harlan Laboratories, Frederick, MD) for the visual signal detection task (VSDT), and 92 Sprague Dawley rats (Charles River Laboratories, Indianapolis, IN) for the in vivo microdialysis, ex vivo receptor occupancy, and pharmacokinetic interaction and exposure time course studies. Additionally, social recognition memory experiments used juvenile Wistar rats as stimulus animals that were not otherwise subjected to any experimental manipulations. All rats were group-housed in plastic cages under temperature- and humidity-controlled conditions, a 12-hour light/dark cycle, and with ad libitum access to food and water, except where noted below. All procedures were performed in a manner consistent with local guidelines on ethical research in animals and were approved by the relevant authority or animal care and use committee prior to the start of experiments.

Drugs and Chemicals

Vortioxetine HBr was synthesized by H. Lundbeck A/S scopolamine HBr trihydrate, and paroxetine maleate was purchased from Sigma-Aldrich. Donepezil HCl monohydrate was purchased from either Sigma-Aldrich (St. Louis, MO) or Sequoia Research Products (Pangbourne, United Kingdom). SB216641 HCl was purchased from Tocris Biosciences (Minneapolis, MN). Vortioxetine was dissolved in a 20% (w/v) aqueous solution of 2-hydroxypropyl-β-cyclodextrin (Roquette America, Keokuk, IA) and injected s.c. at a volume of 2 mL/kg. Scopolamine and donepezil were dissolved in saline and injected s.c. at a volume of 1 mL/kg. Paroxetine and SB216641 were used in ex vivo autoradiography experiments and were dissolved in dimethylsulfoxide (Sigma-Aldrich). Specific information on the magnitude and timing of doses is reported below for each experiment type. All doses are expressed as the mass of the active base. Vortioxetine doses (10 mg/kg, s.c. acute, and 1.8 g per kg/food subchronically) were chosen to target the top of the clinically-relevant vortioxetine dose range based on ex vivo rat SERT occupancy experiments (Leiser et al., 2014). Doses of scopolamine and donepezil were chosen independently for each behavioral task based on the results of pilot experiments. In general, the doses used were the minimum required to induce a reliable effect in each behavioral task.

[^{3}H]Escitalopram (70 Ci/mmol; 1 mCi/mL) was synthesized at Amersham (Piscataway, NJ), whereas the 5-HT_{1B} receptor antagonist [^{3}H]GR125743 (76 Ci/mmol; 0.1 mCi/mL) was purchased from PerkinElmer (Boston, MA).

Behavioral Experiments

Social Recognition Memory Task. The social recognition test was conducted as described by Lemaire et al. (1994) with minor modifications. The test consists of three steps, as follows: an information trial (IT), a delay, and a retention trial (RT). During the IT, an unfamiliar juvenile rat was introduced into the home cage of an adult rat for 5 minutes, then removed for the duration of the delay, and reintroduced to the adult rat for the RT. During the IT and RT, the time the adult rat spent investigating the juvenile was recorded. Investigations were defined as sniffing, grooming, or closely following the juvenile rat.

Control experiments were conducted to determine whether the pharmacological manipulations alter investigation behavior per se. For that purpose, adult rats were injected with vehicle or vortioxetine (10 mg/kg, 1 hour s.c.) and scopolamine (0.25 mg/kg, 30 minutes s.c.) before an initial contact with an unfamiliar juvenile rat. The time spent investigating the juvenile rat was recorded. The rats used in

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these preliminary experiments were evenly distributed throughout the experimental groups and used in the main social recognition memory experiments after a 5-day washout.

In the study of acute vortioxetine, adult rats were randomly injected with vehicle or vortioxetine (0.1, 1, 3, or 10 mg/kg, 1 hour s.c.) and vehicle or scopolamine (0.25 mg/kg, 30 minutes s.c.) prior to the IT. The delay lasted 30 minutes.

In the study of subchronic vortioxetine, adult rats were fed ad libitum for 14–15 days with either a vehicle food (Purina 5001, Nestlé, St. Louis, MO) or food infused with 1.8 g vortioxetine per kg food. On the test day, rats received vehicle or scopolamine (0.25 mg/kg, s.c.), and testing was performed, as described above.

Object Recognition Memory Task. The object recognition task was performed in a Plexiglas Y-maze apparatus (45 × 15 × 33 cm) placed in a room illuminated only by a halogen lamp orientated toward the ceiling, which produced a uniform dim light in the maze of approximately 60 lux (Bartolini et al., 1996; Bertaina-Anglade et al., 2011). The objects were different in shape, color, and texture. In addition, the objects were made of glass (white) and metal (gray), about 14 cm high, and were too heavy to be moved by the rats. Each pair of objects was validated in previous experiments to ensure the absence of spontaneous preference for one of the pair. In each experiment, the role (familiar versus novel object) as well as the relative position of the two objects were counterbalanced and randomly permuted. Rats were placed in the experimental room for at least 30 minutes prior to testing.

The experiment consisted of four sessions. On days 1 and 2, rats received habituation sessions to the area and test room environment, in which animals were allowed to freely explore the apparatus for 10 minutes per day. On the third day, rats were submitted to two exploration trials with a 1-hour intertrial interval. During the IT (day 3, trial 1), animals were placed in the Y-maze containing two identical objects and were given 5 minutes to explore the objects before being returned to the home cage for the intertrial interval. Exploration was defined as the animal having its head close to the object while looking at, sniffing, or touching the object. Any rat not exploring the objects for at least 15 seconds during the IT was excluded from the experiments. In the RT (day 3, trial 2), animals were exposed to an identical copy of one of the objects previously seen during the first trial and a novel object. The RT lasted 5 minutes. The floor and walls of the Y-maze, as well as the objects, were washed with 10% ethanol and dried thoroughly after each trial. On day 3, rats were injected with vehicle, vortioxetine (3.9 or 7.9 mg/kg, s.c.), or donepezil (0.4 or 1.3 mg/kg, s.c.) 1 hour and with vehicle or scopolamine (3.47 mg/kg, s.c.) 55 minutes before the start of the IT.

Visual Signal Detection Task. The VSDT was conducted in four identical operant chambers enclosed within sound-attenuating cubicles (Med-Associates, Fairfax, VT). Each chamber was equipped with a signal light mounted above a food cup (centered on the front panel), a house light, two retractable levers (on either side of the food cup), and a fan to provide ventilation and white noise. The visual signal consisted of an increased illumination intensity lasting for 500 ms.

The rats were trained and tested according to procedures adapted from previously published experiments (Hillhouse and Prus, 2013; Freitas et al., 2015; Hillhouse et al., 2015). Briefly, each trial started with the house light and signal light on (background illumination of 0.9 lux). During training, a trial started with a consistent presignal interval of 4 seconds, which is the attention portion of the trial. After the presignal interval, rats experienced either a blank or signal trial for 500 ms. Under blank trial conditions, there was no change in the signal light illumination, whereas under signal trial conditions there was a 1.5-lux increase in illumination intensity of the signal light to 2.4 lux for 500 ms. Next, a postsignal interval of 1 second preceded the left and right levers extending into the chamber. If a rat pressed the signal lever (randomly assigned as either the right or left lever) during a signal trial, it was recorded as a hit and the rat received a food pellet. If a rat pressed the blank lever during a blank trial, it was recorded as a correct rejection and the rat received a food pellet. Levers were retracted after a response or 5 seconds (whichever occurred first). If rats failed to make a response in 5 seconds, then it was counted as an omission. Incorrect responses (i.e., blank lever during a signal trial or signal lever during a blank trial) and trial omissions resulted in no food pellet delivery and a 2-second timeout (all lights in test chamber turned off). Rats were trained until a criterion of ≥70% choice accuracy was obtained for three consecutive sessions.

Test sessions were identical to training sessions except that three signal intensities were used (i.e., 0.4-, 0.6-, and 1.5-lux increase above blank conditions, order randomized) and presignal interval delays of 3, 6, and 12 seconds (order randomized) were used. Test sessions consisted of 90 blank trials and 90 signal trials (i.e., 30 low, 30 moderate, and 30 high signal intensity trials). Once the training criterion was met, test sessions occurred every third day. Animals received at least one training session immediately preceding a test session. Test sessions were conducted no more than twice per week (typically Tuesdays and Fridays) and were separated by at least 72 hours. On test days, animals were injected with vehicle or vortioxetine (10 mg/kg, s.c.) 1 hour, followed by scopolamine (0.1 or 0.25 mg/kg, s.c.) 30 minutes prior to test session start. The order of drug combinations was determined by a randomized Latin-square design. The initial scopolamine 0.25 mg/kg dose was chosen because pilot experiments had demonstrated reliable impairment in VSDT performance at this dose. The 0.1 mg/kg dose was included to assess the extent to which vortioxetine's effects on scopolamine-induced VSDT performance depended on scopolamine dose.

Drug Exposure Studies

To assess a possible pharmacokinetic interaction between vortioxetine and scopolamine, a separate set of rats was treated with vehicle or vortioxetine (10 mg/kg, s.c.) 1 hour and scopolamine (0.1 mg/kg, s.c.) or vehicle 30 minutes before sampling of plasma and brains. In addition, a separate set of experiments was made to determine the relationship between pretreatment time and plasma and brain concentration of 10 mg/kg vortioxetine, s.c. Toward this end, blood and brains were collected at 1, 2, 4, 8, 12, and 16 hours after dosing. Rats were anesthetized using CO_2 and decapitated using a sharpened guillotine. Blood was collected into vacutainers containing EDTA and gently mixed for 30 seconds before placing on ice. Later, blood was centrifuged at 3000 rpm for 15 minutes at 4°C. The plasma layer was collected and frozen at −20°C until use. Brains were quickly dissected, flash frozen on powdered dry ice, and stored at 20°C until analysis.

Frozen brains were weighed and homogenized in 3× (weight/volume) brain homogenization buffer (50% water, 30% 2-propanol, and 20% dimethylsulfoxide). A 150 μL internal standard (Lundbeck compound AA34745) solution was added to 50 μL homogenized brain sample, which was then vortexed and centrifuged. The supernatant was collected for analysis of vortioxetine or scopolamine exposure. For plasma samples, the internal standard solution was added to the sample, as described above, before thorough mixing. In addition, standard curves for vortioxetine and scopolamine (0–4000 ng/ml in brain or 0–1000 ng/mL for plasma) were generated. The samples were injected directly into an Aria TLX2 coupled with a TSQ Quantum Ultra (both systems Thermo Electron, San Jose, CA). A Gemini column (Kinetex, 2.6 μm C18, 50 × 2.1 mm; Phenomenex, Torrance, CA) was used for analytical separation. A typical 3-minute gradient with the following mobile phases was used: 0.1% formic acid in water (solvent A) and 0.01% formic acid in acetonitrile (solvent B). The mass spectrometer was equipped with a heated electrospray ionization probe, and the source conditions were as follows: vaporizer temperature 450°C, spray voltage 3000, sheath gas at 40°C, ion sweep gas and aux gas at 20°C, and capillary temperature at 300°C. Spectra were acquired in positive selected reaction monitoring mode with the parent masses of the following: vortioxetine 299.16 (m/z) and daughter ion (1) 109 (m/z) at 37 collision energy (CE), daughter ion (2) 150 (m/z) at 27 CE both at tube lens of 120; scopolamine 304.36 (m/z) and daughter ion (1) 138 (m/z) at 20 CE and daughter ion (2) 103 (m/z) at

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25 CE, both at tube lens of 115; internal standard 552.32 (m/z) and daughter ion 203 (m/z) at 50 CE at tube lens of 120.

In Vivo Microdialysis Experiments

Anesthetized (isoflurane, 2%, 800 mL/min O_2) rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, California) and implanted with CMA12 guide cannulas (CMA Microdialysis, Kista, Sweden) in the ventral hippocampus (coordinates: anteroposterior axis = −5.3 mm relative to bregma; mediolateral axis = −4.8 mm relative to midline; dorsoventral axis = −8.0 mm relative to dura) (Paxinos, 1998). Skull screws were implanted, and a head cap was constructed using dental cement. Lidocaine was used for local anesthesia, and carprophen (5 mg/kg s.c.) was used as a pre- and postoperative analgesic. After surgery, animals were singly housed and allowed to recover for at least 7 days prior to the microdialysis experiments.

At 5 PM the day prior to the microdialysis experiments, a CMA12 microdialysis probe (polyarylethersulphone, 4-mm membrane length, 100-kDa pore size) was inserted into the guide cannula, and the rat was placed in a plastic shoebox cage with bedding, connected to a two-channel swivel (Instech Solomon, Plymouth Meeting, PA), and allowed to move freely. The probes were perfused at a constant flow rate of 1 μL/min with a sterile artificial CSF solution (aCSF; CNS Perfusion Fluid; CMA Microdialysis, Kista, Sweden; Harvard Apparatus, Holliston, MA). The aCSF consisted of the following (in mM): 148 NaCl; 4 KCl; 0.8 $MgCl_2$, 1.4 $CaCl_2$, and 1.2 Na_2HPO_4 (pH 7.2). At 8 AM the following morning, samples were collected into a refrigerated fraction collector (Honeycomb fraction collector; Bioanalytical Systems) in 30-minute intervals for 3 hours to define the baseline. The first two samples were discarded from analysis. For the acute study, all animals were treated with saline upon collection of the final baseline sample, and subsequently with either vehicle or vortioxetine (10 mg/kg, s.c.) 1 h after completion of baseline sample collection. Samples were then collected for 4 hours. Experiments assessing the effects of subchronic vortioxetine on extracellular ACh were conducted as described above, with a few modifications. After surgery, rats were singly housed for 14 days, during which they were given 30 g/d Purina 5001 rodent diet (vehicle), or the same amount of food infused with 1.8 g vortioxetine per kg food. On day 15 probes were inserted and rats were perfused with aCSF at 1.5 μL/min. After a 4-hour stabilization period, samples were collected in 20-minute intervals for 4 hours to determine basal ACh levels. Given that the goal in this experiment was only to compare basal ACh levels in vehicle- and subchronic vortioxetine-treated groups, the small differences in perfusion rate and fraction collection time between the subchronic and acute dosing experiments are not theoretically important. After the completion of experiments, rats were anesthetized by CO_2 and decapitated. Brains were quickly dissected from the skull, flash frozen on powdered dry ice, and stored at −20°C until used in ex vivo receptor occupancy experiments.

ACh standards were prepared in aCSF and were diluted prior to analysis by combining 10 μL standard solution with 10 μL 1.0 ng/mL deuterated ACh (D-ACh) solution prepared in distilled water. The range of standards was 10–100,000 pg/mL. Microdialysis samples were prepared in the same manner (10 μL sample and 10 μL D-ACh stock solution) to provide 20 μL sample for analysis. Samples and standards were prepared in plastic tubes (Bioanalytical Systems) that were loaded into a 96-deep-well plate and then heat sealed (Thermo Scientific Easy Peel, Waltham, MA) prior to placing them onto the Acquity sample organizer for analysis.

A Waters Acquity high-pressure liquid chromatography system (Milford, MA) equipped with a Sunshell RP-Aqua 2.1 × 100-mm, 2.6-μm particle column was used to isolate ACh prior to detection using a Waters Quattro Premier XE triple-quadrupole mass spectrometer operating in the mass spectrometry/mass spectrometry mode. A full loop injection using a 5 μL loop with a 4× overfill, requiring a total sample volume of 20 μL, was used to deliver samples onto the liquid chromatography/mass spectrometry/mass spectrometry system. Column and tubing prior to column were maintained at 30°C. The mobile phase consisted of an aqueous component (A: 100 mM ammonium acetate in milliQ water) and an organic component (B: 100% acetonitrile). Isocratic elution of ACh (retention time 1.77 minutes) using 100% A followed by a wash of the column using a fast ramp to 100% B and then a re-equilibration of the column back to 100% A permitted separation of the two analytes with a total run time of 5 minutes. Detection of the analytes was performed by monitoring unique fragments formed from parent ions of ACh [parent (146.05 Da) to fragment (86.3 Da)]. To correct for sample and instrument variability, a D-ACh was incorporated into the samples to act as an internal standard [parent (149.95 Da) to fragment (90.15 Da)]. Utilizing this technique, a lower limit of detection of 5 pg/mL was achieved.

Ex Vivo Target Occupancy Experiments

Ex vivo occupancy at the SERT and the 5-HT_{1B} receptor was determined after acute (10 mg/kg, s.c., 1 hour) and subchronic (1.8 g/kg food for 15 days) vortioxetine. Rats were anesthetized with CO_2 and decapitated, and brains were dissected, frozen, and stored, as described above. Ex vivo occupancy experiments were performed, as described previously (du Jardin et al., 2014; Wallace et al., 2014). Briefly, frozen brain tissue was sectioned coronally at 20-μm thickness using a cryostat (MicroM, Waldorf, Germany) beginning at ~1.5–1.2 mm anterior to Bregma (Paxinos, 1998). Slices were thaw mounted on glass microscope slides and stored at −20°C after being thoroughly dried. On the day of autoradiography experiments, plastic boxes containing slides were defrosted at room temperature for at least 30 minutes under a constant airflow. Following a brief preincubation at 4°C (5-HT_{1B} receptor occupancy only), slides were incubated for 1 h in an assay buffer containing 50 mM Tris HCl, 150 mM NaCl, and 5 mM KCl (pH 7.4) and 4.5 nM [^{3}H]escitalopram (SERT occupancy) or 170 mM Tris HCl, 4mM $CaCl_2$, and 0.1% (w/v) L-ascorbic acid and 1 nM [^{3}H]GR125743 (5-HT_{1B} receptor occupancy). Nonspecific binding was determined on a separate slide by including with the radioligand a high concentration of a nonradioactive competitor selective for the target in question, that is, 1 μM paroxetine or 1 μM SB216641 for SERT and 5-HT_{1B} receptor occupancy, respectively. After incubation, slides were washed twice in cold assay buffer and briefly dipped in cold distilled water. Slides were air dried under a fan for 30 minutes before being transferred to a vacuum desiccator for at least 1 hour. Finally, an image of the slides was taken using a β-imager (Biospace Laboratory, Nesles-la-Vallée, France) for 16 hours.

Statistics

All data are presented as mean ± S.E.M. The criterion for statistical significance was set at $\alpha = 0.05$, unless noted otherwise. Statistical analyses were conducted using either GraphPad Prism 6.0 for windows (GraphPad Software, La Jolla, CA), or MATLAB (Mathworks, Natick, MA).

Social Recognition Memory Task. For assessment of drug effects on social exploration, the dependent measure was the time(s) spent investigating a juvenile stimulus animal during an initial exposure, and data were analyzed using an independent samples *t* test. Within the memory experiments, the dependent measure was a recognition index, defined as IT/(IT + RT) × 100, where IT is defined as the investigation time during the information trial, and RT is the investigation time during the retention trial. Outliers were identified using Pierce's criterion (Ross, 2003) and removed. The data were subsequently analyzed by a one-way analysis of variance (ANOVA), followed where appropriate by Fisher's protected *t* post hoc tests.

Object Recognition Task. Data were collected as time(s) spent actively exploring the familiar (F) or novel (N) object during RT. Recognition memory data are presented as a recognition index, defined as follows: [(N − F)/(N + F)] × 100. Animals with low level

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of object exploration (novel + familiar <5 seconds) were excluded from the data analysis. Outliers were identified using Pierce's criterion (Ross, 2003) and removed. The data were subsequently analyzed by a one-way ANOVA, followed where appropriate by Fisher's protected *t* post hoc tests.

Visual Signal Detection Task. The dependent variables were as follows: 1) percent hits = (number of correct responses on signal trials/the number of signal trials completed) × 100; 2) percent correct rejections = (number of correct responses on blank trials/the number of blank trials completed) × 100; 3) response latency = total time elapsed from when the levers were extended to when a lever press occurred/the number of trials completed (these data were collapsed across signal and blank trials); and 4) response omissions = total number of trials where no response occurred (these data were collapsed across signal and blank trials). A two-factor repeated measures ANOVA was applied with signal intensity and treatment condition as factors for percentage of hits. The *F*-values reported in text for this dependent measure relate to the treatment × stimulus intensity interaction effects. A one-way repeated measures ANOVA was used to assess the effect of treatment condition on percent correct rejections, response latency, or trial omissions. A significant ANOVA model was followed by a Fisher's protected *t* post hoc test.

Drug Exposure Experiments. Scopolamine or vortioxetine plasma concentrations are expressed as nM and μM, respectively, whereas brain concentrations are expressed as either nmol/kg or μmol/kg. The brain to plasma ratio was calculated by dividing the brain concentration by plasma concentration. Data were analyzed using independent samples *t* tests. In vortioxetine time course experiments, the peak vortioxetine level was determined empirically, and the elimination of vortioxetine from the plasma and blood was modeled using a one-phase exponential decay regression model.

Statistical analysis of microdialysis experiments was performed, as reported previously (Pehrson et al., 2013). For the acute dosing study, the raw ACh concentrations were normalized to the average basal ACh concentrations observed in the vehicle group. Subsequently, the area under the curve for each individual animal's normalized extracellular ACh concentration was calculated using the trapezoid method for fractions 4–9 (0–150 minutes on Fig. 4), which constituted the entirety of vortioxetine's apparent time course on extracellular ACh concentrations. Area under the curve data from the vehicle and vortioxetine groups were compared using unpaired two-tailed independent samples *t* tests. This analytical method was preferred for its simplicity; however, similar significance values were observed using a more traditional two-factor ANOVA of normalized extracellular ACh concentrations, including data from all dialysate fractions. For the subchronic dosing study, the basal extracellular ACh concentration was defined for each animal by taking the average of all dialysate fractions collected. These basal values were normalized to the average basal concentration for the vehicle animals and compared using a two-tailed independent samples *t* test.

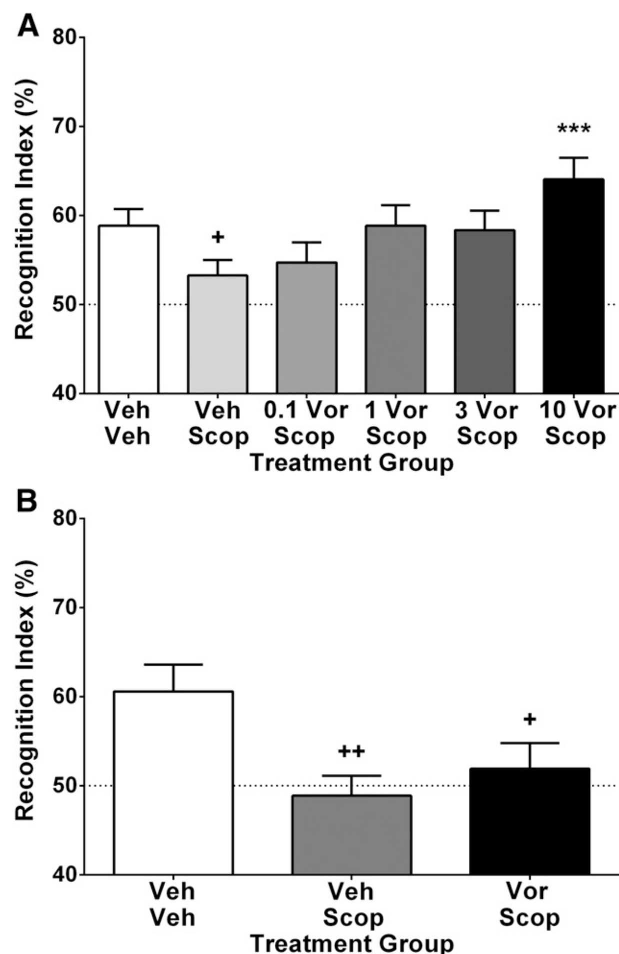

Fig. 1. Vortioxetine effects on scopolamine-induced social recognition memory impairments. (A) Acute treatment with 0.25 mg/kg scopolamine significantly impaired social recognition memory performance. Acute vortioxetine treatment at 0.1–3 mg/kg did not significantly reverse scopolamine-induced impairments in this task, although 10 mg/kg vortioxetine did. (B) Acute scopolamine impaired social recognition memory performance, but subchronic treatment with vortioxetine (1.8 g vortioxetine/kg food, p.o. ad libitum) failed to reverse scopolamine-induced deficits. N = 12–24 rats per group. Plus signs represent significant differences in post hoc tests from the vehicle/vehicle condition ($^{+}P < 0.05$; $^{++}P < 0.01$), whereas asterisks represent significant differences versus the vehicle/scopolamine treatment group ($^{***}P < 0.001$).

Results

Social Recognition Memory Test. Vortioxetine treatment did not alter the basic drive for social interaction observed for scopolamine. Adult rats that were administered vehicle followed by 0.25 mg/kg scopolamine exhibited an investigation time of 55 ± 7 seconds, whereas rats administered 10 mg/kg vortioxetine followed by 0.25 mg/kg scopolamine investigated the juvenile stimulus animal for 51 ± 7 seconds ($t(18) = 0.46$, N.S.).

In acute administration experiments, the vehicle plus scopolamine–treated group had significantly reduced recognition index scores compared with vehicle controls [$F(5,96) = 3.63$, $P < 0.01$; Fig. 1A], suggesting an impairment in social recognition memory. Post hoc tests revealed that recognition index scores for animals pretreated with 0.1–3 mg/kg vortioxetine before scopolamine administration were not significantly different from vehicle plus scopolamine–treated subjects. However, rats administered 10 mg/kg vortioxetine plus scopolamine had significantly higher recognition index scores as compared with vehicle plus scopolamine–treated rats ($P < 0.001$).

In subchronic administration experiments, animals fed vehicle food for 2 weeks followed by 0.25 mg/kg scopolamine 30 minutes s.c. on the day of experiments had significantly reduced recognition index scores versus animals fed vehicle food ad libitum followed by vehicle injection [$F(2,32) = 3.7$, $P < 0.05$; Fig. 1B]. Post hoc tests demonstrated that recognition index scores in animals fed 1.8 g/kg vortioxetine food ad libitum followed by 0.25 mg/kg scopolamine 30 minutes s.c. were not significantly different from those observed in vehicle plus scopolamine animals.

Object Recognition Memory. Acute administration of scopolamine significantly reduced object recognition scores

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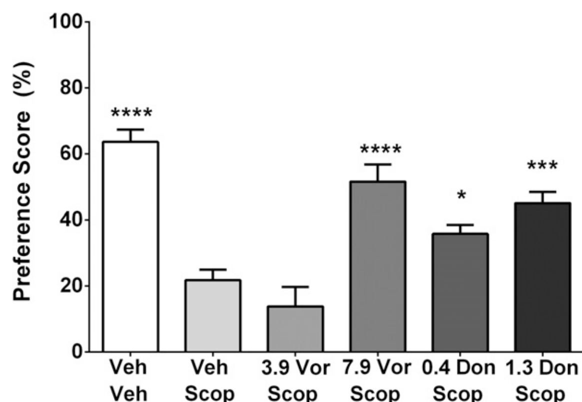

Fig. 2. Vortioxetine effects on scopolamine-induced object recognition memory impairments. Acute treatment with 3.47 mg/kg scopolamine significantly impaired object recognition memory performance. The 3.9 mg/kg vortioxetine did not reverse scopolamine-induced impairments, but 7.9 mg/kg induced a significant improvement in performance compared with scopolamine alone. Donepezil (Don) treatment at either 0.4 or 1.3 mg/kg significantly improved object recognition performance compared with scopolamine alone. N = 9–18 rats per group. Plus signs represent significant differences from the vehicle/vehicle treatment group, whereas asterisks represent significant differences versus the vehicle/scopolamine group (*$P < 0.05$; ***$P < 0.001$; ****$P < 0.0001$).

versus vehicle control subjects [$F(5,63) = 23.02$, $P < 0.001$; Fig. 2]. Recognition index scores in animals treated with 3.9 mg/kg vortioxetine were not significantly different from those in animals in the vehicle plus scopolamine group. However, recognition index scores for animals in the 7.9 mg/kg vortioxetine plus scopolamine group were significantly higher than those of animals in the vehicle plus scopolamine group. Additionally, animals in the 0.4 mg/kg donepezil plus scopolamine and 1.3 mg/kg donepezil plus scopolamine groups had significantly higher recognition index scores as compared with the vehicle plus scopolamine group.

Visual Signal Detection Task. Scopolamine 0.25 mg/kg produced a significant reduction in percent hits [$F(6,84) = 32.08$, $P < 0.001$; Fig. 3A] and correct rejections [$F(3,42) = 29.49$, $P < 0.001$; Fig. 3B] versus the vehicle plus vehicle condition. Vortioxetine 10 mg/kg did not influence scopolamine-induced deficits on percent hits or correct rejections. Vortioxetine by itself did not produce any significant changes in percent hits or correct rejections (Fig. 3, A and B). Similar effects were observed in other VSDT-dependent measures. Scopolamine 0.25 mg/kg significantly increased response latencies [$F(3,42) = 120.20$, $P < 0.001$; Supplemental Table 1] and response omissions [$F(3,42) = 59.27$, $P < 0.001$; Supplemental Table 1] versus vehicle controls, and vortioxetine significantly enhanced these effects. When administered alone, vortioxetine did not affect response latency or omissions.

Scopolamine 0.1 mg/kg also produced a significant reduction in percent hits [$F(6,90) = 30.75$, $P < 0.001$; Fig. 3C] and correct rejection [$F(3,45) = 22.92$, $P < 0.001$; Fig. 3D] versus vehicle controls. Pretreatment with 10 mg/kg vortioxetine did not alter these scopolamine-induced deficits. Consistent with the previous experiment, vortioxetine alone did not have any effect (Fig. 3, C and D). As observed with the higher scopolamine dose, 0.1 mg/kg scopolamine significantly increased the response latencies [$F(3,45) = 128.30$, $P < 0.001$; Supplemental Table 1] and omissions [$F(3,45) = 58.13$, $P < 0.001$; Supplemental Table 1] versus the vehicle plus vehicle control group and vortioxetine enhanced these effects. Once again, vortioxetine by itself did not produce a significant effect.

Finally, the effects of the acetylcholinesterase inhibitor donepezil on the deficits induced by 0.1 mg/kg scopolamine were studied. As observed previously, 0.1 mg/kg scopolamine significantly impaired signal detection performance by decreasing the accuracy of percent correct hits [$F(6,84) = 8.77$, $P < 0.001$; Fig. 3E] and rejections [$F(3,42) = 13.97$, $P < 0.001$; Fig. 3F] versus the vehicle control group. Pretreatment with 3 mg/kg donepezil significantly improved scopolamine-induced deficits on percent correct hits (selectively at 2.4-lux stimulus intensity) and rejection accuracy (Fig. 3, E and F). Donepezil had no significant effects on percent correct hits or rejections when administered alone. As observed in previous trials, scopolamine increased response latencies [$F(3,42) = 16.94$, $P < 0.001$; Supplemental Table 1] and omissions [$F(3,42) = 3.3$, $P < 0.05$; Supplemental Table 1] versus vehicle controls. Donepezil had no effect by itself on these measures, but donepezil pretreatment reversed scopolamine-induced increases in response omissions (although scopolamine effects on omissions were small).

Drug Exposure Studies. Scopolamine exposure levels after 0.1 mg/kg scopolamine were unaffected by pretreatment with 10 mg/kg vortioxetine [plasma ($t[14] = 1.56$, N.S.), brain ($t[14] = 0.23$, N.S.); Table 1]. The brain to plasma ratio of scopolamine was also unchanged [$t(14) = 0.85$, N.S.]. Rats treated with vortioxetine showed significantly higher vortioxetine plasma concentrations than animals treated with vortioxetine plus 0.1 mg/kg scopolamine [$t(14) = 2.74$, $P < 0.05$]. However, this did not translate into a difference in vortioxetine brain concentrations between the two treatment groups [$t(14) = 0.13$, N.S.], or in the brain to plasma ratio [$t(14) = 1.19$, N.S.].

Simple visual inspection of the time course for plasma and brain levels after 10 mg/kg vortioxetine shows peak plasma (2.1 ± 0.13 μM) and brain (660 ± 19 μmol/kg) exposure approximately 2 hours after dosing (Fig. 4). Further analysis of these data using nonlinear regression revealed an apparent plasma half life of 3.9 hours (95% confidence interval: 2.8–6.5 hours), and the half life in brain was calculated to be 5.1 hours (95% confidence interval: 3.4–10 hours).

In Vivo Microdialysis Measurement of Extracellular ACh in the Ventral Hippocampus. In the acute dosing experiment, the mean basal extracellular ACh concentrations were 66 ± 15 and 69 ± 11 pg/mL, respectively, for the vehicle- and vortioxetine-treated groups (Fig. 5). Acute vortioxetine injections produced a relatively small, but statistically significant increase in extracellular ACh concentration versus the vehicle group [$t(12) = 2.486$, $P < 0.05$]. The increase was short-lived and peaked at approximately 150% of basal concentrations from 60–90 minutes postvortioxetine dose before quickly returning to basal levels by 3 hours postdose.

In the subchronic dosing experiment, extracellular ACh concentrations measured over a period of 3 hours were 47 ± 7 and 50 ± 14 pg/mL, respectively, in vehicle- and vortioxetine (1.8 g/kg food)-treated animals (basal concentrations normalized to the average vehicle baseline were 100 ± 16% and 106 ± 30%, respectively; Fig. 6). These values did not differ statistically significantly [$t(12) = 0.17$, N.S.].

Ex Vivo Autoradiography Experiments. Acute vortioxetine (10 mg/kg, s.c., 1 hour) produced ~99% SERT and 80% 5-HT_{1B} receptor occupancy (Table 2). Similarly, animals

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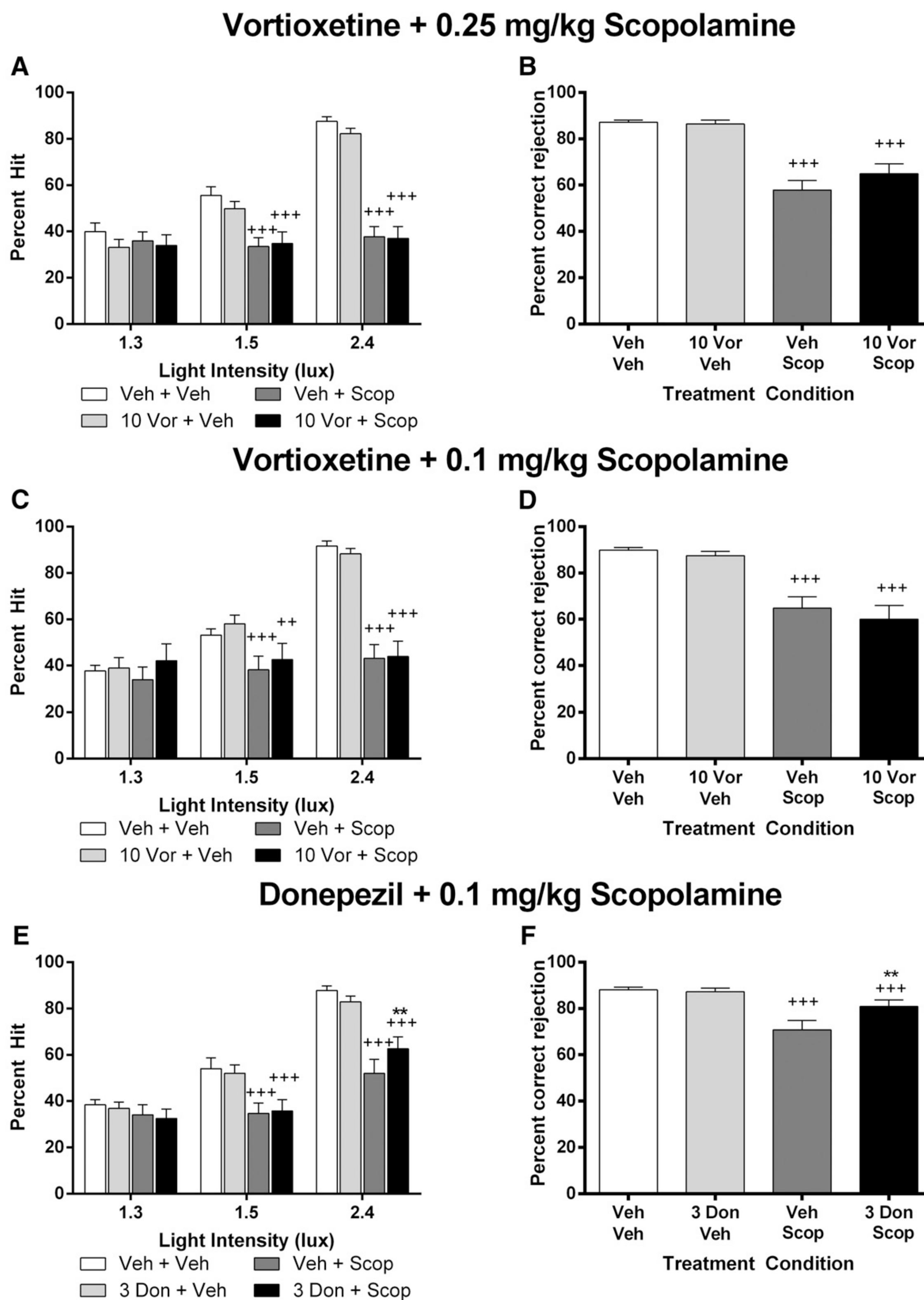

Fig. 3. Effects of vortioxetine and donepezil on scopolamine-induced impairments of VSDT performance. Left panels show effects of a given treatment on scopolamine-induced deficits in percent correct hits. Right panels show effects of a given treatment on scopolamine-induced deficits in percent correct rejections. (A and B) Acute treatment with 0.25 mg/kg scopolamine (Scop) significantly impaired VSDT performance, and these impairments were not reversed by 10 mg/kg vortioxetine. (C and D) Acute treatment with 0.1 mg/kg scopolamine also significantly impaired VSDT performance, and again 10 mg/kg vortioxetine did not reverse these effects. (E and F) The 0.1 mg/kg scopolamine once again significantly impaired VSDT performance. Acute treatment with 3 mg/kg donepezil (Don) induced a small, but significant improvement compared with scopolamine alone, which was limited to the 2.4-lux stimulus intensity. $N = 15–16$ rats per group. Asterisks represent significant differences from vehicle/vehicle ($^{++}P < 0.01$; $^{+++}P < 0.001$). Plus signs represent significant differences from vehicle/scopolamine ($^{**}P < 0.01$).

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TABLE 1
No pharmacokinetic interactions exist between acute vortioxetine and scopolamine
Bioanalysis of plasma and brain exposure to vortioxetine and scopolamine using administration routes and times relevant for the behavioral experiments presented in this study reveals no pharmacokinetic interactions between vortioxetine and scopolamine. Data are presented as mean ± S.E. N = 8 rats per group.

Treatment Group	Vortioxetine			Scopolamine		
	Plasma (μM)	Brain (μmol/kg)	Brain:Plasma	Plasma (nM)	Brain (nmol/kg)	Brain:Plasma
Scopolamine (0.1 mg/kg, s.c., 30 minutes)	0	0	0	60 ± 6.2	330 ± 60	5.7 ± 1.1
Vortioxetine (10 mg/kg, s.c., 1 hour)	2.3 ± 0.1[a]	34 ± 2.6	15 ± 1.1	0	0	0
Vortioxetine (10 mg/kg, s.c., 1 hour) + scopolamine (0.1 mg/kg, s.c., 30 minutes)	1.9 ± 0.08	34 ± 2.9	17 ± 1.4	74 ± 7.1	340 ± 60	4.6 ± 0.6

[a]Significant difference from vortioxetine + scopolamine group.

treated subchronically with vortioxetine-infused food had 99% of SERT and 81% of 5-HT_{1B} receptors occupied.

Discussion

This is the first in-depth study to empirically evaluate the proposed relationship between vortioxetine's effects on cognitive function and ACh neurotransmission. We found that acute vortioxetine reversed scopolamine-induced deficits in social and object recognition memory-related tasks, but not in an attention-related VSDT. However, vortioxetine had no effect on social recognition memory after subchronic administration at an equivalent dose (Table 2). In line with previous observations in the prefrontal cortex (Mørk et al., 2013), acute vortioxetine caused a small and transient, but significant increase of hippocampal ACh. The transient nature of this effect on hippocampal ACh levels cannot be explained by vortioxetine's kinetics, because brain exposure did not peak until 2 hours postadministration, and the brain elimination half life is 5.1 hours. Furthermore, subchronic vortioxetine administration at an equivalent dose failed to alter basal hippocampal ACh levels. Overall, these data suggest that vortioxetine's effects on cholinergic neurotransmission are relatively small and short-lived, reducing the likelihood that altered cholinergic neurotransmission is the primary mechanism mediating vortioxetine's sustained positive effects on cognitive function.

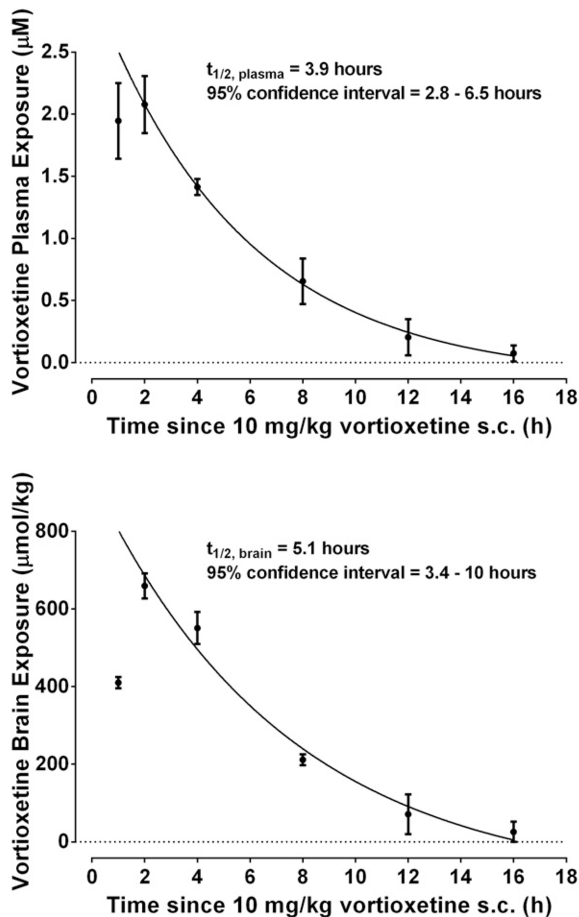

Fig. 4. Time course of vortioxetine exposure in plasma and brain after acute 10 mg/kg s.c. treatment. Plasma vortioxetine exposure peaked at 2 hours postdose and had an apparent half life of 3.9 hours (95% confidence interval 2.8–6.5 hours). Brain vortioxetine exposure peaked at 2 hours postdose and had an apparent half life of 5.1 hours (95% confidence interval 3.4–10 hours). N = 3 per group.

In line with published evidence, we observed that dysregulating cholinergic neurotransmission via administration of the nonselective muscarinic receptor antagonist scopolamine induced significant deficits in social and object recognition memory performance in rats (Winslow and Camacho, 1995; Lieben et al., 2005; de Bruin and Pouzet, 2006; Millan et al., 2007; Riedel et al., 2009; de Bruin et al., 2010). Acute vortioxetine administration significantly reversed scopolamine-induced memory deficits in these tasks, but only at the high end of the clinically relevant dose range (Leiser et al., 2014), that is, a modest but significant effect at 10 mg/kg in the social recognition memory task and at 7.9 mg/kg in the object recognition task. Given the well-characterized relationship between dose and target engagement for this drug, these data may provide some hints at the receptor mechanisms that are relevant for vortioxetine's ability to reverse scopolamine-induced deficits. Vortioxetine selectively and fully occupies 5-HT_3 receptors at low doses (ED_{80} = 0.1 mg/kg), and as the dose increases vortioxetine engages SERT (ED_{80} = 1.2 mg/kg), 5-HT_{1B} receptors (ED_{80} = 9 mg/kg), and 5-HT_{1A} and 5-HT_7 receptors (du Jardin et al., 2014; Leiser et al., 2014). Therefore, 5-HT_3 receptor antagonism and SERT inhibition appear to be insufficient alone, and either 5-HT_{1B} receptor partial agonism, 5-HT_{1A} receptor agonism, 5-HT_7 receptor antagonism, or some combination thereof is necessary for vortioxetine's effects in these tasks. However, because 5-HT receptor-mediated modulation of scopolamine-induced recognition memory deficits is poorly studied, it is difficult to further evaluate our findings. Although acute vortioxetine reversed scopolamine-induced social recognition memory deficits, a similar effect was not observed in animals treated subchronically with an equivalent vortioxetine dose. The cause of this loss of efficacy is not yet understood, but may involve desensitization of the relevant receptor effect.

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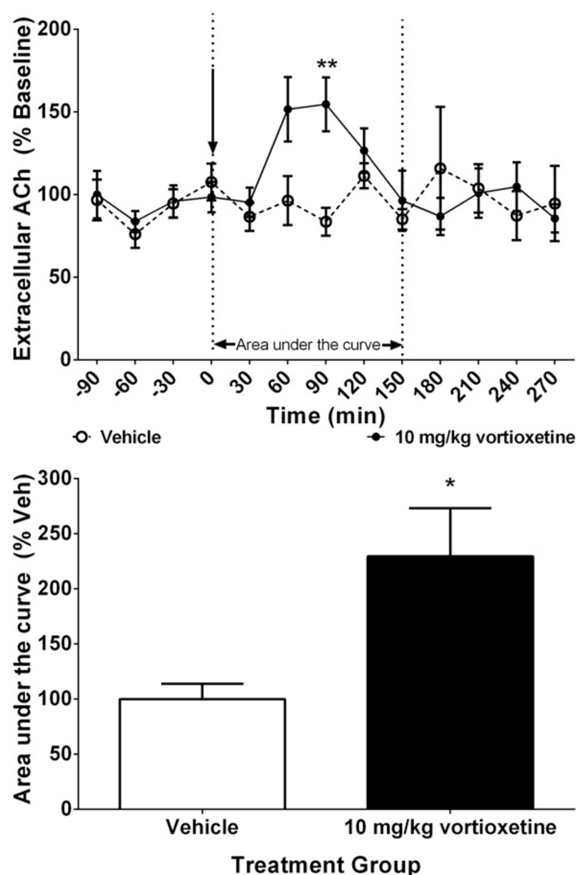

Fig. 5. Acute vortioxetine effects on extracellular acetylcholine concentrations in the ventral hippocampus. Acute 10 mg/kg vortioxetine treatment induced a transient and relatively small, but significant increase in hippocampal extracellular ACh compared with vehicle control animals. Comparison of the area under the curve for vehicle- and vortioxetine-treated animals. N = 6–8 rats per group. Asterisks represent significant differences from the vehicle control group (*$P < 0.05$; **$P < 0.01$).

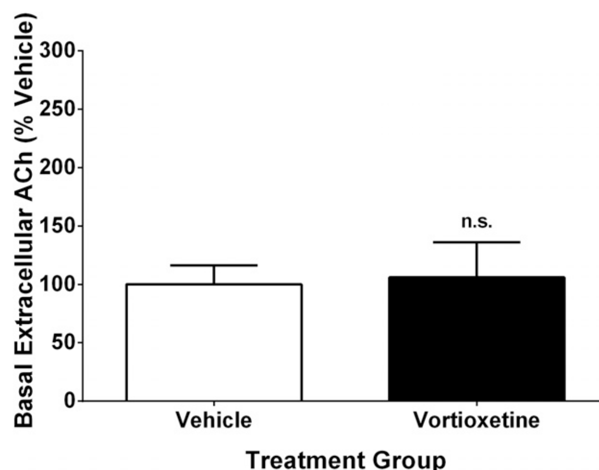

Fig. 6. Subchronic vortioxetine effects on basal extracellular acetylcholine concentrations in the ventral hippocampus. Fourteen-day treatment with vortioxetine-infused food (1.8 g vortioxetine/kg food, p.o., ad libitum) failed to alter basal extracellular ACh concentrations compared with vehicle controls. N = 5–7 rats per group (n.s., not significant).

To examine whether elevation of extracellular ACh is mechanistically relevant for reversing scopolamine-induced object recognition memory deficits, we investigated the effects of acute treatment with the cholinesterase inhibitor donepezil. We found that acute donepezil (0.4 and 1.3 mg/kg) significantly attenuated scopolamine's effects. This agrees with published data showing that the cholinesterase inhibitors galantamine and metrifonate reversed scopolamine's effects in this model (Lieben et al., 2005; de Bruin and Pouzet, 2006). We did not test donepezil in the social recognition memory task, but the literature has consistently shown that cholinesterase inhibition reverses scopolamine-induced deficits in this model (Winslow and Camacho, 1995; Millan et al., 2007; Riedel et al., 2009; de Bruin et al., 2010). Thus, it appears that elevating extracellular ACh concentrations can consistently reverse scopolamine's negative effects on recognition memory, although the precise receptor mechanism is unclear.

In line with previous reports, we observed that acute scopolamine administration significantly impairs VSDT performance (McQuail and Burk, 2006; Rezvani et al., 2009; Freitas et al., 2015). Acute vortioxetine failed to improve scopolamine-induced impairments of VSDT performance measures related to attention (i.e., hits and correct rejections) and accentuated scopolamine-induced deficits in response latency and omissions, measures that may be consistent with changes in arousal or motivation. This is most likely a pharmacodynamic effect because coadministration of scopolamine and vortioxetine had no impact on brain concentrations of either drug. However, the mechanisms driving these effects on response latency and omissions are unknown. Vortioxetine alone consistently failed to alter performance in any of the VSDT-dependent measures, whereas donepezil (3 mg/kg) attenuated scopolamine-induced deficits. However, the effect was only partial and only observed at the highest signal intensity level. The 3 mg/kg donepezil dose is notably higher than the dose needed to reverse scopolamine-induced deficits in the object recognition test (0.4 mg/kg). Furthermore, a s.c. administration of this donepezil dose is expected to produce a marked increase in extracellular ACh. Thus, it appears that increasing extracellular ACh levels is insufficient to fully reverse scopolamine-induced VSDT deficits.

Vortioxetine at 10 mg/kg s.c. caused a relatively small, transient, but statistically significant increase in extracellular ACh in the ventral hippocampus. This observation is similar to our previously reported small and transient extracellular ACh increase in the prefrontal cortex (Mørk et al., 2013). Thus, these data serve to further substantiate the presence of an indirect effect of vortioxetine on extracellular ACh, and to extend this effect to a new region of the brain. The precise receptor mechanism or downstream system-level mechanism that drives vortioxetine's effects on extracellular ACh cannot be empirically discerned based on these data and is outside the intended scope of the experiments presented in this work. However, given the vortioxetine dose-effect curve observed for cortical extracellular ACh (Mørk et al., 2013), the responsible receptor mechanism must be one that becomes occupied to a biologically meaningful level between 2.5 and 10 mg/kg vortioxetine, and is either present in cholinergic terminal regions such as the frontal cortex and hippocampus, or in the basal forebrain, where cholinergic fibers originate (McKinney et al., 1983). Based on these restrictions, the receptor that is most likely to be involved is the 5-HT_{1B} receptor, which is on the ascending portion of its dose-occupancy curve between 2.5 and 5 mg/kg (Leiser et al., 2014), is present at high levels in both the medial frontal cortex and hippocampus (Dale et al.,

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TABLE 2
Estimated fractional target occupancy values at the SERT and 5-HT_{1B} receptor after acute or subchronic vortioxetine administration

Vortioxetine occupied the same fraction of SERT and 5-HT_{1B} receptor targets using acute administration at 10 mg/kg 1 hour s.c. or 14 day p.o. administration via ad libitum feeding with rodent chow infused with 1.8 g vortioxetine/kg food weight. Thus, these dosing regimens are considered equivalent. Data are presented as mean ± S.E.M. N = 6 rats per group.

Treatment Condition	Estimated Target Occupancy (%)	
	SERT	5-HT_{1B} Receptor
Vortioxetine (10 mg/kg, s.c., 1 hour)	99 ± 1.4	80 ± 2.9
Vortioxetine (1.8 g/kg food weight p.o. libitum, 14 days)	99 ± 0.7	81 ± 2.6

2016; Pehrson et al., 2016), and has a demonstrated role in regulating ACh release (Consolo et al., 1996). It is also possible that the mechanism responsible requires a combination of vortioxetine's target pharmacological mechanisms, for example, SERT inhibition and 5-HT_{1B} receptor partial agonism, or 5-HT_3 receptor antagonism and 5-HT_{1B} receptor partial agonism. Alternatively, it is possible that vortioxetine-induced increases of extracellular monoamine concentrations (Pehrson et al., 2013) are driving this effect indirectly at receptors for which vortioxetine has no pharmacological affinity. More empirical studies are required to investigate this mechanism further.

The presence of an acute vortioxetine-induced increase in extracellular ACh, in combination with our demonstration that the acetylcholinesterase inhibitor donepezil reverses some scopolamine-induced cognitive deficits, opens for the possibility that a portion of vortioxetine's effects in these models is due to an indirect increase in cholinergic receptor activation, for example, at nicotinic receptors. Data from other laboratories suggest that activation of $\alpha7$ (Roncarati R et al., 2009) or $\alpha4\beta2$ (Lange-Asschenfeldt et al., 2016) nicotinic receptors is capable of attenuating scopolamine-induced deficits in object recognition memory in rodents. However, whether vortioxetine's effects on scopolamine-induced deficits are mediated via nicotinic receptor mechanisms or some other downstream mechanism cannot be evaluated based on the data presented in this work, and again these concepts are outside the intended scope of the present study.

Indeed, it is noteworthy that vortioxetine's effect on extracellular ACh is consistently transient. Mørk et al. (2013) showed significant increases in cortical extracellular ACh concentrations at 20 and 60 minutes postdose only. In the present study, we observed significantly increased hippocampal ACh concentrations at 60 and 90 minutes postdose. However, as observed in the exposure time course data, vortioxetine reaches its peak exposure at 2 hours postdose and has a brain half life of 5.1 hours. Therefore, vortioxetine's short-lasting effect on ACh cannot be explained by a rapid elimination of drug, but is more likely ascribed to a fast desensitization of vortioxetine-induced activation at the relevant receptors, or the activation of a low-affinity vortioxetine receptor mechanism (for example, agonism at the 5-HT_{1A} receptor or antagonism at the 5-HT_7 receptor), which limits the effects of the receptor mechanism responsible for vortioxetine-induced increases in ACh. The data presented in this work cannot differentiate between these possibilities. In line with the transient nature of the ACh response, it is also important to note that subchronic vortioxetine resulted in no changes in basal extracellular ACh concentrations in the hippocampus. This is consistent with vortioxetine's inability to reverse scopolamine-induced impairments in social recognition memory task after subchronic administration.

There are important caveats to consider when evaluating these data. Importantly, vortioxetine's affinity for rat 5-HT_{1A} and 5-HT_7 receptors is approximately an order of magnitude lower than for the human counterparts (Sanchez et al., 2015). Therefore, if vortioxetine's cholinergic mechanisms are mediated via effects at either of these targets, then our study may have underestimated the impact of the cholinergic system in clinical populations. Additionally, the behavioral data are limited to assessments of social recognition memory, object recognition memory, and attention; thus, caution should be exercised when extrapolating to other cognitive domains that are improved by vortioxetine in the clinic, for example, processing speed and executive function (du Jardin et al., 2014; Jensen et al., 2014; Wallace et al., 2014; Li et al., 2015). Moreover, although the neurochemical data presented in this work may suggest that vortioxetine has limited effects on cholinergic neurotransmission, this should be viewed with caution until empirical evaluations of other cognitive domains have been evaluated. Furthermore, whether vortioxetine can modulate cognitive impairments induced by nicotinic, rather than muscarinic, receptor antagonism has not been evaluated in this study. Finally, given that these studies were conducted only in male rats, these data may not necessarily translate into females. Therefore, further studies are needed to evaluate vortioxetine's effects on nicotine receptor antagonist-mediated cognitive deficits, and to address whether there are sex differences in vortioxetine's effects on ACh neurotransmission.

In conclusion, our data suggest that vortioxetine has some effects on memory that could be mediated through cholinergic neurotransmission. However, the effects are only seen under acute dosing conditions, which may argue against cholinergic mechanisms being the primary mediator of vortioxetine's cognitive effects observed under chronic dosing conditions in patients with MDD.

Acknowledgments

The authors thank Theepica Jeyarajah and Christina Merritt for excellent technical assistance.

Authorship Contributions

Participated in research design: Pehrson, Hillhouse, Haddjeri, Rovera, Porter, Smagin, Budac, Cajina, Sanchez.

Conducted experiments: Pehrson, Hillhouse, Rovera, Song, Budac, Cajina.

Performed data analysis: Pehrson, Hillhouse, Haddjeri, Rovera, Budac, Smagin, Song, Cajina.

Wrote or contributed to the writing of the manuscript: Pehrson, Hillhouse, Haddjeri, Rovera, Budac, Porter, Mørk, Smagin, Song, Cajina, Sanchez.

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