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ARTICLE

Effect of clinically relevant doses of vortioxetine and citalopram on serotonergic PET markers in the nonhuman primate brain

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Vortioxetine is a multimodal antidepressant approved for treatment of major depressive disorder. Preclinical studies have demonstrated that the mechanism of action of vortioxetine might be different from selective serotonin reuptake inhibitors (SSRIs), including larger serotonin (5-HT) release and direct modulation of several 5-HT receptors. In the current positron emission tomography (PET) study, we evaluated the mechanism of action of vortioxetine by comparing its effect to the SSRI citalopram on the binding of [^{11}C]AZ10419369 to the 5-HT$_{1B}$ receptor in the nonhuman primate brain. Initially, the 5-HT transporter (5-HTT) binding of vortioxetine was determined by [^{11}C]MADAM PET measurements before and after administration of vortioxetine (0.1–3.0 mg/kg) and data were used to confirm clinically relevant dosing in subsequent PET measurements with [^{11}C]AZ10419369. The 5-HT$_{1B}$ receptor binding was significantly decreased after 0.3 mg/kg of citalopram in the dorsal raphe nucleus (5%), as well as after 0.3 mg/kg of vortioxetine in six brain regions (~25%) or 1.0 mg/kg of vortioxetine in all 12 examined regions (~48%). Moreover, there was no effect of 1.0 mg/kg of vortioxetine on the binding of [^{11}C]Cimbi-36 to the 5-HT$_{2A}$ receptor, which has comparable sensitivity to 5-HT release as [^{11}C]AZ10419369 binding. In conclusion, at clinically relevant doses, vortioxetine induced larger reductions in [^{11}C]AZ10419369 binding than citalopram. These observations suggest that vortioxetine binds to the 5-HT$_{1B}$ receptor at clinically relevant doses. Future studies are warranted to evaluate the role of the 5-HT$_{1B}$ receptor in the therapeutic effects of vortioxetine and as a potential target for the development of novel antidepressant drugs.

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INTRODUCTION

Major depressive disorder (MDD) is a leading cause of disease burden worldwide [1]. Serotonin (5-HT) is one of the main neurotransmitters in brain and of central interest in the pathophysiology and treatment of MDD. One reason is that selective serotonin reuptake inhibitors (SSRIs) or serotonin–norepinephrine reuptake inhibitors (SNRIs) have been shown to increase synaptic 5-HT concentrations via inhibition of the 5-HT transporter (5-HTT) in the animal brain [2] and are the current first choice among therapeutics for MDD due to the combined efficacy and tolerability profile of these drugs. However, the therapeutic outcome of current MDD treatment strategies with SSRIs or SNRIs is suboptimal and there is a large need for more effective treatments [3, 4].

Vortioxetine is a recent developed antidepressant, which was approved in 2013 by EU and US authorities for the treatment of MDD in adults [5, 6]. In addition to 5-HTT inhibition, vortioxetine has in preclinical studies been characterized as a 5-HT$_3$, 5-HT$_7$, and 5-HT$_{1D}$ receptor antagonist, a partial agonist at the 5-HT$_{1B}$ receptor and a full agonist at the 5-HT$_{1A}$ receptor [6–9]. Moreover, it has been shown that the mechanism of action of vortioxetine in animals differ from SSRIs. In rat microdialysis studies, vortioxetine has been shown to induce an around twofold higher increase in extracellular 5-HT concentration when compared to SSRIs, and it has been suggested that the mechanism for antidepressant effect of vortioxetine may include binding to one or several of the 5-HT receptor subtypes [6, 10, 11]. Importantly, species differences in the binding of vortioxetine to some of the 5-HT receptor subtypes has to be taken into account, e.g., higher affinity for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and lower affinity for 5-HT$_{1A}$ and 5-HT$_7$ receptors for rat than for recombinant human receptors [6–9]. It is therefore of interest to evaluate the mechanism of action of vortioxetine directly in the primate brain in vivo.

Positron emission tomography (PET) imaging studies have previously demonstrated that clinically relevant doses of SSRIs occupy ~60–90% of the 5-HTT in healthy subjects [12] and in treated MDD patients [13, 14]. Clinically relevant doses of vortioxetine have shown slightly lower occupancies, typically around 60%, of the 5-HTT in the human brain [15, 16]. This potential difference in 5-HTT occupancy between clinically relevant doses of SSRIs and vortioxetine might relate to a different mechanism of action as described above. Although the level of target occupancy is important for dose selection in initial clinical studies [17], the 5-HTT occupancy of

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Table 1. Binding affinities of vortioxetine and citalopram to recombinant human and rat 5-HTT and 5-HT receptors reported in vitro

	Vortioxetine (nmol/L)		Citalopram (nmol/L)	
	Human	Rat	Human	Rat
5-HTT	1.6[a]	8.6[b]	4.7[d]	1.5[d]
5-HT_{1B}	33[a]	16[c]	>10,000[d]	>10,000[e]
5-HT_{2A}	180[a]	NA	>10,000[d]	>1000[f]

NA not available, *5-HTT* serotonin transporter, *5-HT* serotonin
[a]Bang-Andersen et al. (2011)[7]
[b]Westrich et al. (2012)[8]
[c]Mork et al. (2012)[9]
[d]Millan et al. (2001)[22]
[e]Wong et al. (1991)[23]
[f]Sánchez et al. (1999)[24]

vortioxetine in the nonhuman primate (NHP) brain has not been reported so far.

We have previously demonstrated that the binding of [^{11}C]AZ10419369, a 5-HT_{1B} receptor partial agonist, is significantly reduced in the primate brain following a high but not clinically relevant dose of escitalopram [18]. A likely interpretation is that [^{11}C]AZ10419369 binding is sensitive to drug-induced increases in 5-HT concentration. Previous rodent microdialysis studies have demonstrated that the increases in 5-HT concentration (two- to fivefold from baseline) induced by a clinically relevant dose of vortioxetine [10] are comparable to increases induced by high doses of SSRIs [19, 20]. It can thus be anticipated that [^{11}C]AZ10419369 binding may also be sensitive to increases in 5-HT concentration induced by a clinically relevant dose of vortioxetine.

It is worth noting that vortioxetine has nanomolar range affinity to recombinant human 5-HT_{1B} receptors [7]. Consequently, in addition to increases in 5-HT concentration, vortioxetine might also alter [^{11}C]AZ10419369 binding by direct occupancy of the 5-HT_{1B} receptors. More recently, [^{11}C]Cimbi-36, a 5-HT_{2A} receptor agonist radioligand, has been reported to have similar sensitivity as [^{11}C]AZ10419369 to increases in 5-HT concentration [21]. Vortioxetine has fivefold higher affinity for the recombinant human 5-HT_{1B} receptor ($K_i = 33$ nmol/L) than the 5-HT_{2A} receptor ($K_i = 180$ nmol/L) (Table 1) [7]. Therefore, evaluation of the effect of vortioxetine on [^{11}C]Cimbi-36 binding may help differentiate between a direct effect of vortioxetine on [^{11}C]AZ10419369 binding and an indirect effect from increased 5-HT concentration. Specifically, if there is no effect on [^{11}C]Cimbi-36 binding, it would support direct vortioxetine binding to the 5-HT_{1B} receptors.

The aim of the present study was to examine the mechanism of action of vortioxetine in the NHP brain. PET measurements with [^{11}C]MADAM were performed to estimate the 5-HTT affinity of vortioxetine in vivo in the NHP brain. The effect of vortioxetine and citalopram, a reference SSRI with high affinity for the 5-HTT (Table 1) [22–24], on the binding of [^{11}C]AZ10419369 to the 5-HT_{1B} receptor were then compared at clinically relevant and comparable doses. Finally, the effect of vortioxetine on [^{11}C]Cimbi-36 binding to the 5-HT_{2A} receptor was evaluated.

MATERIALS AND METHODS

Subjects

The study was approved by the Animal Research Ethical Committee of the Northern Stockholm region (Dnr N185/14). Five female rhesus monkeys (*Macaca mulatta*) with body weight: 7.7 ± 2.7 kg (mean ± standard deviation) were included. The caring and experimental procedures were performed according to the "Guidelines for planning, conducting and documenting experimental research" (Dnr 4820/06-600) of Karolinska Institutet and the "Guide for the Care and Use of Laboratory Animals: Eighth Edition" [25].

Preparation of radioligands

[^{11}C]MADAM [26], [^{11}C]AZ10419369 [27], and [^{11}C]Cimbi-36 [28] were prepared according to procedures reported previously.

Study design

A total of 32 PET measurements (10 with [^{11}C]MADAM, 18 with [^{11}C]AZ10419369, and 4 with [^{11}C]Cimbi-36) were performed on 16 experimental days. On each experimental day, following a baseline PET measurement, a consecutive PET measurement was conducted after pretreatment with vortioxetine or citalopram. The two PET measurements were performed ~3 h apart.

There were four stages in the experiments. First, to support that the 5-HTT affinity of citalopram in rhesus monkeys is comparable to that previously reported for cynomolgus monkeys [29], one rhesus monkey (NHP1) underwent PET measurements with [^{11}C]MADAM before and after administration of 2.0 mg/kg of citalopram. Second, to determine the 5-HTT affinity of vortioxetine in rhesus monkeys, four monkeys (NHP2–NHP5) underwent PET measurements with [^{11}C]MADAM before and after administration of one dose of vortioxetine (0.1, 0.3, 1.0, or 3.0 mg/kg, respectively). Third, to compare the effects of citalopram and vortioxetine on 5-HT_{1B} receptor binding, three monkeys (NHP1, NHP3, and NHP4), each underwent PET measurements with [^{11}C]AZ10419369 before and after administration of 0.3 mg/kg of citalopram, 0.3 mg/kg of vortioxetine or 1.0 mg/kg of vortioxetine. These doses were selected based on literature data and the comparison in the first part of the present study to assure clinically relevant 5-HTT occupancy levels. Finally, to evaluate the effect of vortioxetine on 5-HT_{2A} receptor binding, two monkeys (NHP2 and NHP3) underwent PET measurements with [^{11}C]Cimbi-36 before and after administration of 1.0 mg/kg of vortioxetine.

Pretreatment administration

Citalopram was formulated in phosphate-buffered saline (PBS). Vortioxetine was formulated in a mixture of 10% hydroxypropyl beta cyclodextrin dissolved in PBS. All drug solutions were infused (1 mL/kg) over 30 min, starting 45 min before injection of radioligand.

PET experimental procedures

Anesthesia was initiated by intramuscular injection of ketamine hydrochloride (~10 mg/kg) and maintained by a mixture of sevoflurane (2–8%), oxygen and medical air. PET measurements were conducted in the High Resolution Research Tomograph (HRRT); a six min transmission measurement (using a single ^{137}Cs source) was followed by the acquisition of list-mode data for 123 min after a bolus injection of radioligand. Eleven venous blood samples (at −55, −14.5, −5, 15, 30, 45, 60, 75, 90, 105, and 120 min after injection of radioligand) were collected for determination of plasma drug concentrations of vortioxetine and citalopram.

Determination of plasma drug concentrations

Plasma drug concentrations for vortioxetine and citalopram were determined using ultra performance liquid chromatography (UPLC) followed by tandem mass spectrometry (MS/MS) detection according to procedures reported previously [29] and as described in Supplementary Materials and Methods. The mean of the plasma drug concentration at eight time points after injection of radioligand and during time of PET data acquisition was used to represent plasma drug concentration in each PET experiment.

Magnetic resonance imaging (MRI)
Each monkey was examined by MRI to define brain volumes of Interest (VOIs). T1-weighted MRI images were acquired on a GE 1.5 Tesla Signa MRI scanner (Milwaukee, WI) using a 3D spoiled gradient recalled (SPGR) protocol: repetition time 21 ms, flip angle 35°, FOV 12.8, matrix 256 × 256 × 128, 128 × 1.0 mm^2 slices.

Image data analysis
Preprocessing of MRI images included manual reorientation to the anterior-posterior commissure (AC-PC) plane, manual removal of non-brain tissue and N4 bias field correction. PET images were preprocessed according to previously reported methods [30] with reconstructed image frames binned as: 9 × 10, 2 × 15, 3 × 20, 4 × 30s, 4 × 60, 4 × 180, and 17 × 360 s. Following imaging data analyses were performed using PMOD (version 3.604; PMOD Technologies, Zurich, Switzerland).

Each monkey's baseline summed PET image (average of time frames corresponding to 15–69 min for [^{11}C]MADAM, 5–18 min for [^{11}C]AZ10419369 and 12–63 min for [^{11}C]Cimbi-36) was co-registered to its individual MRI brain image by the Rigid matching algorithm in the PMOD Fuse It Tool (PFUSEIT). The resulting transformation matrices were applied to the corresponding two PET measurements performed on the same day.

Twelve VOIs were defined based on the NeuroMaps atlas in the INIA19 rhesus template [31], including four regions for the basal ganglia: putamen, caudate nucleus (CN), ventral striatum (VS), and globus pallidum (GP); two neocortical regions: frontal cortex (FC) and occipital cortex (OC); three limbic regions: anterior cingulated cortex (ACC), amygdala and hippocampus; and VOIs for thalamus, midbrain, and cerebellum. Each monkey's brain MRI image was normalized to the INIA19 rhesus template with the Deformable matching algorithm in the PFUSEIT and the resulting normalization matrix was used to inversely transform the template VOIs into individual MRI space. In addition, the dorsal raphe nuclei (DRN) was manually delineated on each monkey's coregistrated summed [^{11}C]MADAM PET image in a sagittal plane, including 5–6 slices from the level of the superior colliculus to the level of the inferior colliculus [32].

Quantification
For each VOI, a decay-corrected time–activity curve (TAC) was generated from the co-registered dynamic PET data. Binding potential (BP_{ND}) values were calculated using the simplified reference tissue model (SRTM) [33], with cerebellum as the reference region, as has been validated in previous studies [34–37].

Relative change in BP_{ND} values (ΔBP_{ND}) (%) was calculated using the following equation:

$$\Delta BP_{ND}(\%) = \frac{BP_{ND}\text{Pretreatment} - BP_{ND}\text{Baseline}}{BP_{ND}\text{Baseline}} \times 100, \quad (1)$$

For illustration purposes, parametric BP_{ND} images were generated using SRTM2 [38] and normalized to the INIA19 rhesus template [31] by using the methods described in Supplementary Materials and Methods.

Relationship between pretreatment drugs and 5-HTT occupancy
According to the law of mass action, the relationship between radioligand binding and the concentration of drug at equilibrium can be described by a one-site binding hyperbola, as expressed by the following equation:

$$\text{Decrease in } BP_{ND}(\%) = I_{max} \times \frac{\text{drug dose (or } C_P)}{\text{drug dose (or } C_P) + \text{ID50 (or } K_i)}, \quad (2)$$

where I_{max} is the maximal inhibition (%), C_P is the plasma drug concentration and ID_{50} or K_i corresponds to the drug dose or the plasma drug concentration at which 5-HTT occupancy is 50%, respectively [29, 39].

Based on Eq. (2), the drug-induced decrease in [^{11}C]MADAM BP_{ND} (%) (equal to the ΔBP_{ND} (%) with opposite sign) in putamen and CN were plotted against the values of corresponding drug dose or plasma drug concentration [29]. The mean plasma drug concentration of 8 blood samples taken during the PET measurement was used to represent the plasma drug concentration during the PET measurement period. An unconstrained I_{max} value was applied to the model [29], and the ID_{50} or K_i value was calculated using GraphPad Prism (version 6.05; GraphPad Software Inc., La Jolla, CA, USA).

Statistical analysis
A paired *t*-test was used to assess changes in parameters between the two PET measurements performed on the same day. All statistical analyses were performed in GraphPad Prism. The threshold of significance was set as $P < 0.05$ (two-tailed).

RESULTS

Radiochemistry
The 32 PET measurements in rhesus monkeys were conducted according to plans and the three radioligands were synthesized to high specific radioactivity. There were no statistically significant differences in injected radioactivity or injected mass at time of injection between baseline and pretreatment conditions (Supplementary Table 1).

Plasma concentrations of pretreatment drugs
In the measurements conducted to estimate 5-HTT occupancy using [^{11}C]MADAM, following administration of 2.0 mg/kg citalopram, the plasma drug concentration was 952 nmol/L. Following administration of vortioxetine at 0.1, 0.3, 1.0mg/kg and 3.0 mg/kg, the plasma drug concentration was 16, 43, 192, and 562 nmol/L, respectively.

In the measurements to evaluate the effects of pretreatment drugs on [^{11}C]AZ10419369 binding, the time course of plasma concentrations of citalopram or vortioxetine are presented in Supplementary Fig. 1. The group mean of plasma drug concentration was 119, 38, and 114 nmol/L for 0.3 mg/kg of citalopram, 0.3 mg/kg of vortioxetine, and 1.0 mg/kg of vortioxetine, respectively. In the measurements with [^{11}C]Cimbi-36, the group mean of plasma drug concentration was 157 nmol/L following administration of 1.0 mg/kg of vortioxetine.

5-HTT binding after administration of pretreatment drugs
After administration of 2.0 mg/kg of citalopram in a single rhesus monkey, there was a marked decrease in [^{11}C]MADAM binding. The 5-HTT occupancy was 56% in putamen and 63% in CN. This level is comparable to previous data in cynomolgus monkeys (60%, with $ID_{50} = 0.059$ mg/kg and $I_{max} = 62\%$) [29].

There was a decrease of [^{11}C]MADAM binding in all four monkeys examined after administration of vortioxetine (Fig. 1). The estimated ID_{50} and K_i values for vortioxetine were 0.25 ± 0.09 mg/kg (95% CI = 0.03–0.47; $R^2 = 0.89$; $I_{max} = 69\%$) and 38.9 ± 12.1 nmol/L (95% CI = 10.2–67.7; $R^2 = 0.91$; $I_{max} = 68\%$), respectively.

5-HT_{1B} receptor binding after administration of pretreatment drugs
After administration of 0.3 mg/kg of citalopram, there were numerical increases in BP_{ND} values for [^{11}C]AZ10419369 binding to the 5-HT_{1B} receptor across several brain regions, with a trend level of significance for the FC (Table 2, Figs. 2, 3, and Supplementary Fig. 2). Interestingly, there was a small but significant reduction in BP_{ND} for the DRN.

Following administration of 0.3 or 1.0 mg/kg of vortioxetine, the binding of [^{11}C]AZ10419369 to the 5-HT_{1B} receptor was reduced in

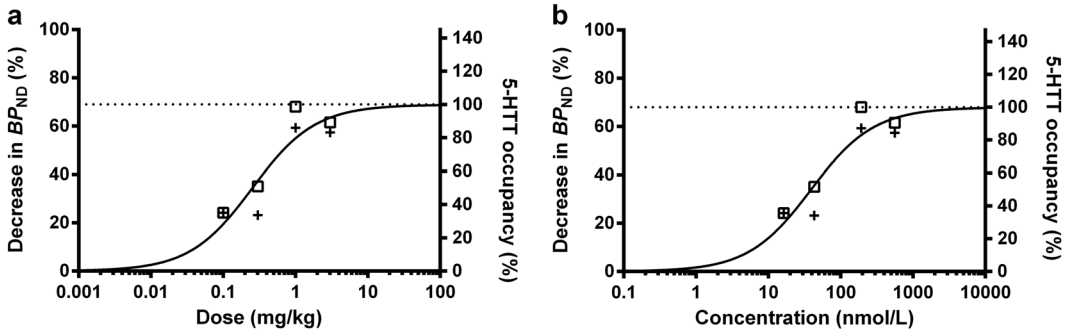

Fig. 1 Decreases in [^{11}C]MADAM binding potential (BP_{ND}) in caudate nucleus (CN, open square symbol, □) and putamen (Put, plus sign, +) after intravenous administration of vortioxetine in four nonhuman primates. The solid lines represent fitting of an unconstrained one-site binding hyperbola for both regions. The dotted lines represent the estimated maximal inhibition (I_{max}). The corresponding serotonin transporter (5-HTT) occupancy is shown along the right *y*-axis. **a** Doses of vortioxetine versus decreases in BP_{ND}. **b** Plasma concentrations of vortioxetine versus decreases in BP_{ND}

Table 2. Effect of citalopram and vortioxetine on mean regional binding potential (BP_{ND}) values of [^{11}C]AZ10419369 and [^{11}C]Cimbi-36 in rhesus monkeys

	[^{11}C]AZ10419369 BP_{ND} ($n = 3$)									[^{11}C]Cimbi-36 BP_{ND} ($n = 2$)			
	Bas	CIT 0.3	ΔBP_{ND} (%)[a]	Bas	VOR 0.3	ΔBP_{ND} (%)[a]	Bas	VOR 1.0	ΔBP_{ND} (%)[a]	Bas	VOR 1.0	ΔBP_{ND} (%)[a] NHP2	ΔBP_{ND} (%)[a] NHP3
Put	0.73	0.80	10.0	0.82	0.69	−15.1[#]	0.84	0.56	−34.2**	0.45	0.45	−5.7	7.9
CN	0.68	0.74	9.1	0.72	0.56	−22.3*	0.71	0.42	−40.8***	0.55	0.50	1.7	−18.1
VS	1.44	1.49	5.1	1.47	1.11	−24.5**	1.41	0.84	−41.6**	0.62	0.54	−0.4	−23.9
GP	1.53	1.52	−0.4	1.66	1.32	−20.3*	1.60	1.00	−38.1**	0.38	0.48	17.3	34.3
FC	0.73	0.83	14.7[#]	0.75	0.68	−9.0	0.79	0.58	−27.2**	1.56	1.53	1.0	−4.6
OC	1.28	1.52	18.1	1.29	1.01	−22.4*	1.28	0.66	−48.3*	1.09	1.27	10.9	22.8
ACC	0.87	0.96	12.1	0.94	0.83	−11.5	1.00	0.72	−29.2**	2.06	1.93	8.8	−21.2
Amyg	1.05	1.11	6.4	1.09	0.94	−13.6	1.14	0.75	−34.9***	0.91	0.67	−37.1	−10.6
HC	0.76	0.79	4.5	0.75	0.66	−12.0[#]	0.74	0.59	−20.4*	0.94	0.96	−4.3	7.1
Thal	0.95	0.96	1.4	1.01	0.76	−24.3*	0.95	0.60	−37.2***	0.49	0.49	−2.9	2.2
MB	0.98	1.02	4.8	1.10	0.86	−22.7***	1.00	0.57	−43.2*	0.43	0.53	38.2	13.5
DRN	1.15	1.09	−4.8**	1.15	0.93	−19.2[#]	1.07	0.60	−44.0*	0.60	0.46	−24.5	−22.0

Bas baseline, *CIT 0.3* citalopram 0.3 mg/kg, *VOR 0.3* vortioxetine 0.3 mg/kg, *VOR 1.0* vortioxetine 1.0 mg/kg, *Put* putamen, *CN* caudate nucleus, *VS* ventral striatum, *GP* globus pallidum, *FC* frontal cortex, *OC* occipital cortex, *ACC* anterior cingulated cortex, *Amyg* amygdala, *HC* hippocampus, *Thal* thalamus, *MB* midbrain, *DRN* dorsal raphe nucleus

[#]$0.05 \le P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (two-tailed) by paired *t*-test

[a]$\Delta BP_{ND}(\%) = \frac{BP_{ND}\,Pretreatment - BP_{ND}\,Baseline}{BP_{ND}\,Baseline} \times 100$

a dose-dependent fashion when compared to baseline (Table 2, Figs. 2 and 3, and Supplementary Fig. 2). After 0.3 mg/kg of vortioxetine, the reduction in BP_{ND} values was statistically significant in six brain regions (CN, VS, GP, OC, thalamus, and midbrain). After 1.0 mg/kg of vortioxetine, the reduction in BP_{ND} values was statistically significant in all 12 examined brain regions (Table 2).

5-HT_{2A} receptor binding after administration of vortioxetine

Following administration of 1.0 mg/kg of vortioxetine, there was no conspicuous change in [^{11}C]Cimbi-36 binding across different brain regions in the two monkeys (Table 2, Figs. 2 and 3, and Supplementary Fig. 3).

DISCUSSION

In the current PET study, we evaluated the mechanism of action of vortioxetine by comparing the effect of clinically relevant doses of vortioxetine and citalopram on serotonergic PET markers. A main observation was that vortioxetine significantly reduced [^{11}C]AZ10419369 binding to the 5-HT_{1B} receptor in several cortical and subcortical regions while citalopram reduced [^{11}C]AZ10419369 binding only in DRN. Moreover, there was no significant effect of vortioxetine on [^{11}C]Cimbi-36 binding to the 5-HT_{2A} receptor though [^{11}C]Cimbi-36 binding has been demonstrated to have similar sensitivity for the 5-HT concentration as [^{11}C]AZ10419369 binding. In conclusion, the large and dose-dependent decreases in [^{11}C]AZ10419369 binding induced by vortioxetine suggest direct drug binding to the 5-HT_{1B} receptor. The observations also suggest that vortioxetine significantly occupies the 5-HT_{1B} receptor when administered at clinically relevant doses. This finding appears to differentiate vortioxetine from the reference SSRI citalopram. However, a difference in their ability to elevate 5-HT concentration cannot be excluded.

The K_i value determined for vortioxetine binding to the 5-HTT (39 ± 12 nmol/L) was modestly higher than previously reported for humans (16–20 nmol/L) [15, 16]. Similar level of discrepancy between monkey and human 5-HTT K_i values have in previous PET

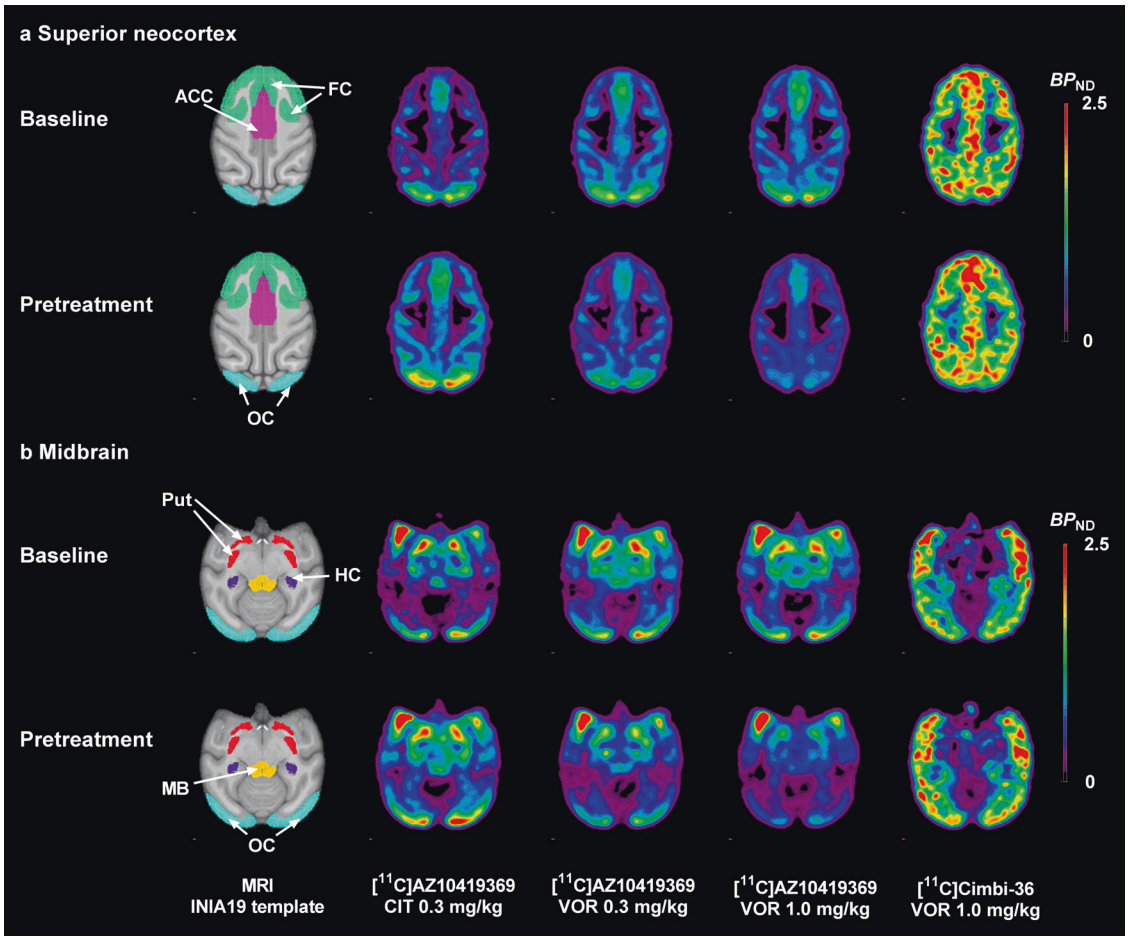

Fig. 2 Mean parametric binding potential (BP_{ND}) images of [^{11}C]AZ10419369 or [^{11}C]Cimbi-36 derived by SRTM2 at baseline and after pretreatment with citalopram or vortioxetine ($n = 3$ for each pretreatment condition measured by [^{11}C]AZ10419369 and $n = 2$ for vortioxetine 1.0 mg/kg measured by [^{11}C]Cimbi-36). The images were normalized to the INIA19 rhesus template. **a** Axial view of images at the level of superior neocortex. **b** Axial view of images at the level of midbrain. ACC anterior cingulated cortex, FC frontal cortex, HC hippocampus, MB midbrain, OC occipital cortex, Put putamen

studies been reported for escitalopram [12, 29] and venlafaxine [13, 40]. Species differences in pharmacokinetics or the different route of drug administration may have contributed to these modest discrepancies [29, 40, 41]. The estimated K_i value for 5-HTT binding in the monkey brain was therefore considered to be in line with previous human results.

It is crucial to select adequate doses when comparing the effects of vortioxetine and citalopram. We first confirmed that the 5-HTT affinity of citalopram is comparable between rhesus and cynomolgus monkeys [29]. Since the 5-HTT affinity of citalopram and vortioxetine was estimated using the same methodology, it is feasible to use the in vivo affinity data to select doses, which achieve comparable 5-HTT occupancy [18, 29, 42]. In the measurements of binding to the 5-HT_{1B} receptor, the doses of 0.3 mg/kg of citalopram and 1.0 mg/kg of vortioxetine were chosen as they should correspond to about 80% 5-HTT occupancy, which is considered a representative effective occupancy level at clinical treatment with SSRIs [13, 14]. A lower dose of vortioxetine (0.3 mg/kg) was selected to achieve about 55% 5-HTT occupancy, and to represent the lower end of reported 5-HTT occupancy values at the clinical dose range for vortioxetine (5–10 mg/day) [5, 15, 16].

The clinical relevance of the selected doses was further supported by the plasma concentration of current pretreatment drugs (119 nmol/L for 0.3 mg/kg of citalopram; 38 and 114 nmol/L for 0.3, and 1.0 mg/kg of vortioxetine, respectively). The concentration values are comparable to clinical data for 20–60 mg/day of citalopram (130–400 nmol/L) [43] and 5–20 mg/day of vortioxetine (30–110 nmol/L) [5]. It can thus be assumed that the current results can be extrapolated to human studies at which a therapeutic antidepressant effect has been documented.

In the current study, in contrast to citalopram, clinically relevant doses of vortioxetine significantly decreased [^{11}C]AZ10419369 binding in several brain regions. With regard to the mechanism of action of these two drugs, the divergent effects might originate from a difference in the ability to change the 5-HT concentration or, alternatively, a difference in affinity for the 5-HT_{1B} receptor. The decreases in [^{11}C]AZ10419369 binding induced by 1.0 mg/kg of vortioxetine were two- to fourfold higher than those previously reported for a high dose of an SSRI [18] and comparable to the decreases induced by 5.0 mg/kg of fenfluramine, one of the strongest 5-HT releaser [21]. Moreover, [^{11}C]Cimbi-36 binding has been reported to have similar sensitivity as [^{11}C]AZ10419369 to the 5-HT release induced by fenfluramine [21]. Accordingly, if the vortioxetine induced reductions in [^{11}C]AZ10419369 binding mainly originate from increases in 5-HT concentration, it would be expected that 1.0 mg/kg of vortioxetine could significantly reduce [^{11}C]Cimbi-36 binding. The lack of effect of vortioxetine on [^{11}C]Cimbi-36 binding in the NHP brain suggests that the increases in 5-HT concentration induced by clinically relevant doses of vortioxetine might not be detectable by [^{11}C]AZ10419369 binding. Thus, the observed reductions in [^{11}C]AZ10419369 binding are

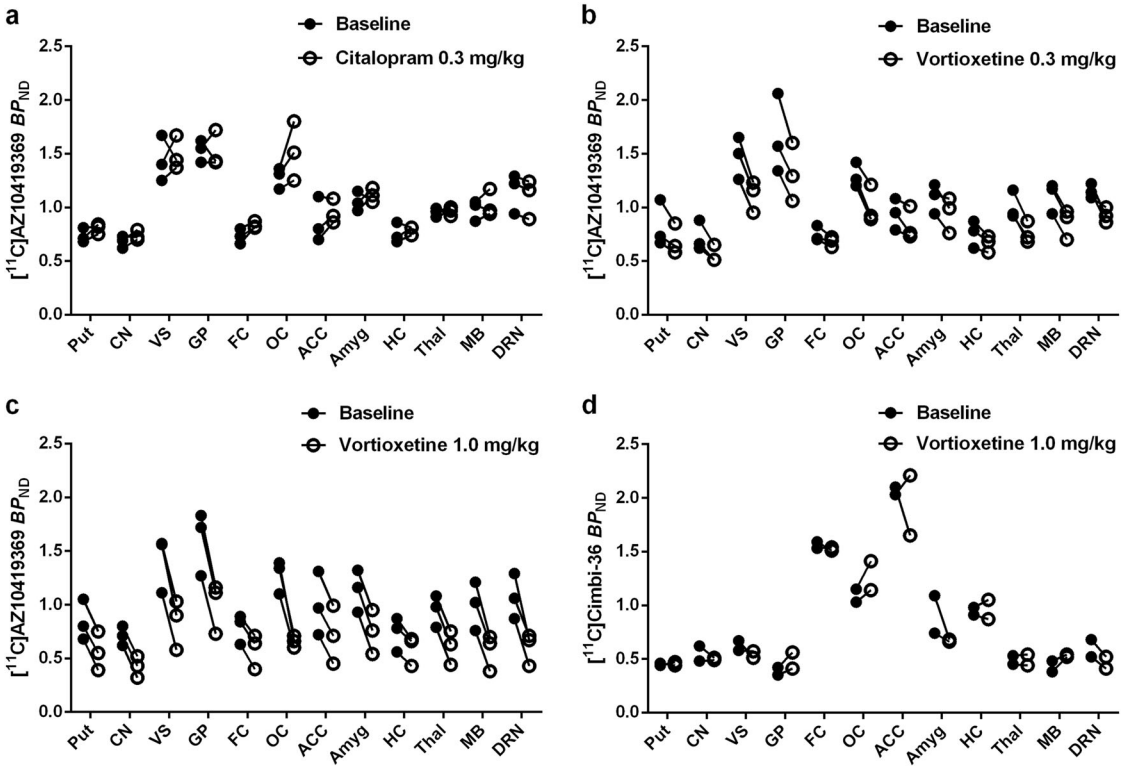

Fig. 3 Individual [^{11}C]AZ10419369 or [^{11}C]Cimbi-36 BP_{ND} values before and after pretreatment with citalopram or vortioxetine in 12 predefined volumes of interest, including putamen (Put), caudate nucleus (CN), ventral striatum (VS), globus pallidum (GP), frontal cortex (FC), occipital cortex (OC), anterior cingulated cortex (ACC), amygdala (Amyg), hippocampus (HC), thalamus (Thal), midbrain (MB), and dorsal raphe nuclei (DRN). **a** Citalopram 0.3 mg/kg and [^{11}C]AZ10419369 ($n = 3$). **b** Vortioxetine 0.3 mg/kg and [^{11}C]AZ10419369 ($n = 3$). **c** Vortioxetine 1.0 mg/kg and [^{11}C]AZ10419369 ($n = 3$). **d** Vortioxetine 1.0 mg/kg and [^{11}C]Cimbi-36 ($n = 2$)

more likely explained by direct binding of vortioxetine to the 5-HT_{1B} receptors. This interpretation is also in line with the markedly different binding affinities to the 5-HT_{1B} receptors between these two drugs reported in vitro (Table 1) [7–9, 22–24]. In summary, the current results support that there might be direct drug occupancy of 5-HT_{1B} receptors at the administered doses of vortioxetine.

It is important to note that the lack of effect of vortioxetine on [^{11}C]Cimbi-36 binding did not exclude the possibility that vortioxetine induces larger increases in 5-HT concentration than citalopram in the NHP brain. Vortioxetine has been reported to increase extracellular 5-HT concentration about twice as large as for an SSRI in the rat hippocampus [10]. Considering the low statistical power in the current study due to a small sample size, the current results might reflect that the 5-HT release induced by administration of vortioxetine was below the threshold that could be reliably measured by the study protocol using [^{11}C]Cimbi-36. Future studies with a larger sample size to evaluate the effect of 1.0 mg/kg of vortioxetine on [^{11}C]Cimbi-36 binding might further corroborate our findings. In addition, it cannot be excluded that the effect of vortioxetine on [^{11}C]Cimbi-36 binding might differ between NHPs and humans.

The current results suggest that vortioxetine binds to the 5-HT_{1B} receptor at clinically relevant doses. The 5-HT_{1B} receptors are widely distributed throughout the brain. The receptors are predominantly located on axon terminals where they serve as autoreceptors on serotonergic neurons and heteroreceptors on non-serotonergic neurons. Importantly, the functional role of the autoreceptors is to regulate the release of 5-HT [44–46]. On the basis of a series of experimental studies, the 5-HT_{1B} receptor has been proposed to be involved in the pathophysiology of depression and to be a potential target for drug treatments [44–48]. The occupancy of vortioxetine at the 5-HT_{1B} receptor might also contribute to cognitive benefits, which in clinical studies has been reported to be independent of the antidepressant effect [49, 50]. Although desensitization of hippocampal 5-HT_{1B} autoreceptors following vortioxetine administration has been shown to be related to antidepressant effects in rodent models [51], the contribution of 5-HT_{1B} receptor occupancy by vortioxetine in relation to its antidepressant effect in patients remains to be clarified. Future studies are warranted, including application of the current PET paradigm in human subjects [52, 53].

Considering the affinity of vortioxetine for multiple neuroreceptors [6, 10, 11], it is challenging to dissect the clinical effect of 5-HT_{1B} receptor binding from the summed effect of the engaged targets. While several selective 5-HT_{1B} receptor compounds have been reported to demonstrate antidepressant-like effects in different animal models of depression [54–56], none of them have been examined in clinical studies [46]. Extended work with compounds that are selective for the 5-HT_{1B} receptor may not only aid identification of mechanisms of action of vortioxetine but also, in a wider sense, assessment of the 5-HT_{1B} receptor as a potential target for development of novel antidepressant drugs.

A potential limitation of the current PET study is the use of anesthesia. Anesthetic doses of ketamine might influence the binding of radioligand to 5-HTT and result in overestimation of ID_{50} or K_i values [29, 57]. Ketamine has also been reported to increase [^{11}C]AZ10419369 binding in the NHP brain, but had no effect on decreases in [^{11}C]AZ10419369 binding induced by fenfluramine, a 5-HT releaser [57]. So far, there have been no studies evaluating the effect of sevoflurane on [^{11}C]AZ10419369 binding or the effect of anesthesia on [^{11}C]Cimbi-36 binding. Importantly, as the same anesthesia regimen was applied across the experiments, potential anesthesia effects were similar between citalopram and vortioxetine in 5-HTT occupancy studies

and between [^{11}C]AZ10419369 and [^{11}C]Cimbi-36 pretreatment studies using vortioxetine. Accordingly, potential anesthesia effects were not likely to affect the main observations of the current study.

Another potential limitation of the current study is the inclusion of female monkeys only. In addition to higher prevalence of MDD in females than in males, gender differences in the 5-HT system have been reported [58, 59]. Therefore, it cannot be excluded that gender differences influenced the current results, although we are not aware of PET studies indicating gender differences in drug-induced 5-HTT occupancy or 5-HT release.

In conclusion, our results reveal that the effect of clinically relevant doses of vortioxetine and citalopram on [^{11}C]AZ10419369 binding to the 5-HT$_{1B}$ receptor was different in the NHP brain. The results might mainly originate from a difference in 5-HT$_{1B}$ receptor affinity between these two drugs although it cannot be excluded that a difference in their ability to elevate 5-HT concentration may also contribute. These observations suggest that vortioxetine binds to the 5-HT$_{1B}$ receptor at clinically relevant doses. Future studies are warranted to evaluate the role of the 5-HT$_{1B}$ receptor in the therapeutic effects of vortioxetine and as a potential target for the development of novel antidepressant drugs.

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ADDITIONAL INFORMATION

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REFERENCES

1. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet. 2013;382:1575–86.
2. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455:894–902.
3. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. Lancet. 2018;391:1357–66.
4. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term out- comes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163:1905–17.
5. Garnock-Jones KP. Vortioxetine: A review of its use in major depressive disorder. CNS drugs. 2014;28:855–74.
6. Sanchez C, Asin KE, Artigas F. Vortioxetine, a novel antidepressant with multimodal activity: Review of preclinical and clinical data. Pharm Ther. 2015;145:43–7.
7. Bang-Andersen B, Ruhland T, Jørgensen M, Smith G, Frederiksen K, Jensen KG, et al. Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressive disorder. J Med Chem. 2011;54:3206–21.
8. Westrich L, Pehrson A, Zhong H, Nielsen SM, Frederiksen K, Stensbol TB, et al. In vitro and in vivo effects of the multimodal antidepressant vortioxetine (Lu AA21004) at human and rat targets. Int J Psychiatry Clin Pract. 2012;16:47 (Informa healthcare telephone house, 69–77 paul street, London EC2A 4LQ, England, 2012).
9. Mørk A, Pehrson A, Tottrup Brennum L, Moller Nielsen S, Zhong H, Lassen AB, et al. Pharmacological effects of Lu AA21004: A novel multimodal compound for the treatment of major depressive disorder. J Pharm Exp Ther. 2012;340:666–75.
10. Pehrson AL, Cremers T, Bétry C, van der Hart MGC, Jørgensen L, Madsen M, et al. Lu AA21004, a novel multimodal antidepressant, produces regionally selective increases of multiple neurotransmitters-A rat microdialysis and electrophysiology study. Eur Neuropsychopharmacol. 2013;23:133–45.
11. du Jardin KG, Jensen JB, Sanchez C, Pehrson AL. Vortioxetine dose-dependently reverses 5-HT depletion-induced deficits in spatial working and object recognition memory: A potential role for 5-HT1A receptor agonism and 5-HT3 receptor antagonism. Eur Neuropsychopharmacol. 2014;24:160–71.
12. Lundberg J, Christophersen JS, Petersen KB, Loft H, Halldin C, Farde L. PET measurement of serotonin transporter occupancy: a comparison of escitalopram and citalopram. Int J Neuropsychopharmacol. 2007;10:777–85.
13. Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ, et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. Am J Psychiatry. 2004;161:826–35.
14. Lundberg J, Tiger M, Landén M, Halldin C, Farde L. Serotonin transporter occupancy with TCAs and SSRIs: a PET study in patients with major depressive disorder. Int J Neuropsychopharmacol. 2012;15:1167–72.
15. Areberg J, Luntang-Jensen M, Søgaard B, Nilausen D. Occupancy of the serotonin transporter after administration of Lu AA21004 and its relation to plasma concentration in healthy subjects. Basic Clin Pharm Toxicol. 2012;110:401–4.
16. Stenkrona P, Halldin C, Lundberg J. 5-HTT and 5-HT1A receptor occupancy of the novel substance vortioxetine (Lu AA21004). A PET study in control subjects. Eur Neuropsychopharmacol. 2013;23:1190–8.
17. Lee C-M, Farde L. Using positron emission tomography to facilitate CNS drug development. Trends Pharm Sci. 2006;27:310–6.
18. Nord M, Finnema SJ, Halldin C, Farde L. Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. Int J Neuropsychopharmacol. 2013;16:1577–86.
19. Invernizzi R, Belli S, Samanin R. Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. Brain Res. 1992;584:322–4.
20. Gartside SE, Umbers V, Hajos M, Sharp T. Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. Br J Pharm. 1995;115:1064–70.
21. Yang K-C, Stepanov V, Martinsson S, Ettrup A, Takano A, Knudsen GM, et al. Fenfluramine reduces [11C]Cimbi-36 binding to the 5-HT2A receptor in the nonhuman primate brain. Int J Neuropsychopharmacol. 2017;20:683–91.
22. Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet J-M, Auclair A, et al. S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. J Pharm Exp Ther. 2001;298:565–80.
23. Wong D, Threlkeld P, Robertson D. Affinities of fluoxetine, its enantiomers, and other inhibitors of serotonin uptake for subtypes of serotonin receptors. Neuropsychopharmacology. 1991;5:43–7.
24. Sánchez C, Hyttel J. Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. Cell Mol Neurobiol. 1999;19:467–89.
25. Council NR. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: The National Academies Press; 2011.
26. Halldin C, Lundberg J, Sóvágó J, Gulyás B, Guilloteau D, Vercouillie J, et al. [11C] MADAM, a new serotonin transporter radioligand characterized in the monkey brain by PET. Synap (New Y, NY) 2005;58:173–83.
27. Andersson JD, Pierson ME, Finnema SJ, Gulyás B, Heys R, Elmore CS, et al. Development of a PET radioligand for the central 5-HT1B receptor: Radiosynthesis and characterization in cynomolgus monkeys of eight radiolabeled compounds. Nucl Med Biol. 2011;38:261–72.

28. Ettrup A, Hansen M, Santini MA, Paine J, Gillings N, Palner M, et al. Radiosynthesis and in vivo evaluation of a series of substituted 11C-phenethylamines as 5-HT2A agonist PET tracers. Eur J Nucl Med Mol Imaging. 2011;38:681–93.
29. Finnema SJ, Halldin C, Bang-Andersen B, Bundgaard C, Farde L. Serotonin transporter occupancy by escitalopram and citalopram in the non-human primate brain: a [(11)C]MADAM PET study. Psychopharmacol (Berl) 2015;232:4159–67.
30. Varrone A, Sjoholm N, Eriksson L, Gulyás B, Halldin C, Farde L. Advancement in PET quantification using 3D-OP-OSEM point spread function reconstruction with the HRRT. Eur J Nucl Med Mol Imaging. 2009;36:1639–50.
31. Rohlfing T, Kroenke CD, Sullivan EV, Dubach MF, Bowden DM, Grant Ka, et al. The INIA19 template and NeuroMaps atlas for primate brain image parcellation and spatial normalization. Front Neuroinform. 2012;6:27.
32. Kranz GS, Hahn A, Savli M, Lanzenberger R. Challenges in the differentiation of midbrain raphe nuclei in neuroimaging research. Proc Natl Acad Sci USA. 2012;109:E2000.
33. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. NeuroImage. 1996;4:153–8.
34. Lundberg J, Odano I, Olsson H, Halldin C, Farde L. Quantification of 11C-MADAM binding to the serotonin transporter in the human brain. J Nucl Med. 2005;46:1505–15.
35. Varnäs K, Nyberg S, Halldin C, Varrone A, Takano A, Karlsson P, et al. Quantitative analysis of [11C]AZ10419369 binding to 5-HT1B receptors in human brain. J Cereb Blood Flow Metab. 2011;31:113–23.
36. Finnema SJ, Stepanov V, Ettrup A, Nakao R, Amini N, Svedberg M, et al. Characterization of [11C]Cimbi-36 as an agonist PET radioligand for the 5-HT2A and 5-HT2C receptors in the nonhuman primate brain. NeuroImage. 2014;84:342–53.
37. Ettrup A, da Cunha-Bang S, McMahon B, Lehel S, Dyssegaard A, Skibsted AW, et al. Serotonin 2A receptor agonist binding in the human brain with [11C]Cimbi-36. J Cereb Blood Flow Metab. 2014;34:1188–96.
38. Wu Y, Carson RE. Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. J Cereb Blood Flow Metab. 2002;22:1440–52.
39. Farde L, Wiesel F-A, Halldin C, Sedvall G. Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry. 1988;45:71–6.
40. Takano A, Halldin C, Farde L. SERT and NET occupancy by venlafaxine and milnacipran in nonhuman primates: A PET study. Psychopharmacol (Berl) 2013;226:147–53.
41. Areberg J, Søgaard B, Hojer AM. The clinical pharmacokinetics of Lu AA21004 and its major metabolite in healthy young volunteers. Basic Clin Pharm Toxicol. 2012;111:198–205.
42. Selvaraj S, Turkheimer F, Rosso L, Faulkner P, Mouchlianitis E, Roiser JP, et al. Measuring endogenous changes in serotonergic neurotransmission in humans: a [11C]CUMI-101 PET challenge study. Mol Psychiatry. 2012;17:1254–60.
43. Bezchlibnyk-Butler K, Aleksic I, Kennedy SH. Citalopram–a review of pharmacological and clinical effects. J Psychiatry Neurosci. 2000;25:241–54.
44. Sari Y. Serotonin1B receptors: from protein to physiological function and behavior. Neurosci Biobehav Rev. 2004;28:565–82.
45. Ruf BM, Bhagwagar Z. The 5-HT1B receptor: a novel target for the pathophysiology of depression. Curr Drug Targets. 2009;10:1118–38.
46. Tiger M, Varnäs K, Okubo Y, Lundberg J. The 5-HT1B receptor-a potential target for antidepressant treatment. Psychopharmacol (Berl) 2018;235:1317–34.
47. Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, Yacoubi ME, et al. Alterations in 5-HT1B receptor function by p11 in depression-like states. Sci (New Y, NY). 2006;311:77–80.
48. Nautiyal KM, Tritschler L, Ahmari SE, David DJ, Gardier AM, Hen R. A lack of serotonin 1B autoreceptors results in decreased anxiety and depression-related behaviors. Neuropsychopharmacology. 2016;41:2941–50.
49. Mahableshwarkar AR, Zajecka J, Jacobson W, Chen Y, Keefe RSE. A randomized, placebo-controlled, active-reference, double-blind, flexible-dose study of the efficacy of vortioxetine on cognitive function in major depressive disorder. Neuropsychopharmacology. 2015;40:2025.
50. McIntyre R, Harrison J, Loft H, Jacobson W, Olsen C. The effects of vortioxetine on cognitive function in patients with major depressive disorder: a meta-analysis of three randomized controlled trials. Int J Neuropsychopharmacol. 2016;19:pii: pyw055.
51. El Mansari M, Lecours M, Blier P. Effects of acute and sustained administration of vortioxetine on the serotonin system in the hippocampus: electrophysiological studies in the rat brain. Psychopharmacol (Berl) 2015;232:2343–52.
52. Finnema SJ, Scheinin M, Shahid M, Lehto J, Borroni E, Bang-Andersen B, et al. Application of cross-species PET imaging to assess neurotransmitter release in brain. Psychopharmacology. 2015;232:4129–57.
53. Yang K-C, Takano A, Halldin C, Farde L, Finnema SJ. Serotonin concentration enhancers at clinically relevant doses reduce [11C]AZ10419369 binding to the 5-HT1B receptors in the nonhuman primate brain. Transl Psychiatry. 2018;8:132.
54. Dawson LA, Hughes ZA, Starr KR, Storey JD, Bettelini L, Bacchi F, et al. Characterisation of the selective 5-HT1B receptor antagonist SB-616234-A (1-[6-(cis-3,5-dimethylpiperazin-1-yl)-2,3-dihydro-5-methoxyindol-1-yl]-1-[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methanone hydrochloride): In vivo neurochemical and behavioural evidence of anxiolytic/antidepressant activity. Neuropharmacology. 2006;50:975–83.
55. Hudzik T, Smolka J, Litwin L, Porrey T, Pierson E. In vivo pharmacology of AZD1134, a novel 5-HT1B antagonist. Eur Neuropsychopharmacol. 2003;13: S181–2.
56. Hudzik TJ, Yanek M, Porrey T, Evenden J, Paronis C, Mastrangelo M, et al. Behavioral pharmacology of AR-A000002, a novel, selective 5-hydroxytryptamine1B antagonist. J Pharm Exp Ther. 2003;304:1072–84.
57. Yamanaka H, Yokoyama C, Mizuma H, Kurai S, Finnema SJ, Halldin C, et al. A possible mechanism of the nucleus accumbens and ventral pallidum 5-HT1B receptors underlying the antidepressant action of ketamine: a PET study with macaques. Transl Psychiatry. 2014;4:e342.
58. Jans LAW, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry. 2006;12:522.
59. Veldman ER, Svedberg MM, Svenningsson P, Lundberg J. Distribution and levels of 5-HT 1B receptors in anterior cingulate cortex of patients with bipolar disorder, major depressive disorder and schizophrenia–An autoradiography study. Eur Neuropsychopharmacol. 2017;27:504–14.