Escitalopram increases synaptic density in the human brain over weeks: A randomized controlled trial

Annette Johansen  
Rigshospitalet and University of Copenhagen  https://orcid.org/0000-0003-0264-2368

Sophia Armand  
Pontus Plavén-Sigray  
Copenhagen University Hospital, Rigshospitalet

Arafat Nasser  
Brice Ozenne  
Ida Petersen  
Sune Keller  
Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, Copenhagen

Jacob Masdsen  
Vincent Beliveau  
Rigshospitalet and Medical University of Innsbruck  https://orcid.org/0000-0001-7805-279X

Kirsten Møller  
Rigshospitalet University of Copenhagen  https://orcid.org/0000-0003-3058-1072

Alexandra Vassilieva  
Christelle Langley  
University of Cambridge  https://orcid.org/0000-0001-5061-2820

Claus Svarer  
Neurobiology Research Unit  https://orcid.org/0000-0001-7811-1825

Dea Stenbæk  
Barbara Sahakian  
University of Cambridge  https://orcid.org/0000-0001-7352-1745

Gitte Knudsen  (gmk@nru.dk)  
Rigshospitalet and University of Copenhagen  https://orcid.org/0000-0003-1508-6866

Keywords:

Posted Date: June 15th, 2023
Abstract

Selective serotonin reuptake inhibitors (SSRIs) are widely used for treating neuropsychiatric disorders. However, the exact mechanism of action and why effects can take several weeks to manifest is not clear. The neuroplasticity hypothesis is supported by preclinical studies, but the evidence in humans is limited. Here, we investigate the effects of the SSRI escitalopram on presynaptic density as a proxy for synaptic plasticity. In a double-blind placebo-controlled study (NCT04239339), 32 healthy participants were randomized to receive daily oral dosing of either 20 mg escitalopram (n = 17) or a placebo (n = 15). After an intervention period of 3-5 weeks, participants underwent a $[^{11}\text{C}]$UCB-J PET scan to quantify synaptic vesicle glycoprotein 2A (SV2A) density in the hippocampus and the neocortex. Group means were compared using t-tests, and effect of intervention duration was assessed with linear models. Whereas there was only a small difference in $[^{11}\text{C}]$UCB-J binding between the escitalopram and placebo groups after an average of 29 (range: 24-38) days of intervention (Cohen’s d of 0.31-0.42, p values > 0.26), we identified time-dependent group effects (neocortex: $p = 0.020$; hippocampus: $p = 0.058$). Linear models showed positive associations between $[^{11}\text{C}]$UCB-J binding and duration of escitalopram intervention: $p_{\text{Neocortex}} = 0.016$; $p_{\text{Hippocampus}} = 0.11$). Our findings suggest that brain synaptic plasticity evolves over 3-5 weeks in healthy humans following daily intake of escitalopram. This is the first in vivo evidence to support the hypothesis of neuroplasticity as a mechanism of action for SSRIs in humans, and it offers a plausible biological explanation for the delayed treatment response commonly observed in patients treated with SSRIs.

Introduction

Drugs targeting the serotonin system, specifically the serotonin transporter, have long been the primary pharmacological treatment for affective and anxiety-related disorders (1). The most widely used group is the selective serotonin reuptake inhibitors (SSRIs), presumed to work by increasing serotonergic neurotransmission (2). Serotonin plays an important modulatory role in the brain, including regulation of mood, sleep, cognition, and behavior, and in the early development of the central nervous system (3,4). Further, years of preclinical studies have established a link between the serotonin system and cellular processes such as cytoskeletal rearrangements, long-term potentiation, and neuronal firing – processes that collectively are regarded as forms of neuroplasticity (2,5). Functionally, neuroplasticity can be thought of as the ability of the brain to change and adapt to physiological or psychological stimuli to uphold homeostasis (6).

Despite years of research, the question of how inhibition of the serotonin transporter leads to symptom relief in neuropsychiatric conditions remains unresolved. One hypothesis is that strengthened serotonergic neurotransmission induces neuroplasticity and, in turn, improves cognitive and emotional processing (7–9). Neuroplastic effects have foremost been demonstrated for the visual system; in adult rats, chronic treatment with the SSRI fluoxetine has been shown to reactivate a critical period-like plasticity in the visual cortex (10,11). However, whether neuroplasticity is central to the effects of SSRIs in
humans has been difficult to investigate, mainly due to the lack of specific biomarkers. A suggested proxy is change in cortical thickness or brain volume, as measured with MRI, in response to, e.g., learning new skills or tasks, such as juggling (12). However, by using PET, it is possible to non-invasively quantify molecular biomarkers that more specifically reflect plasticity in vivo. Here, we use the PET radioligand [11C]UCB-J that binds to the Synaptic Vesicle glycoprotein 2A (SV2A), which enables visualization and quantification of pre-synaptic density (Finnema et al., 2016), as a proxy for synaptic plasticity.

PET studies on several neuropsychiatric disorders linked to synaptic dysfunction, including depression, have found lower cerebral SV2A density in patients compared to healthy individuals (13–18). So far, the only longitudinal investigation of a pharmacological intervention on SV2A density in humans is a study that examined the acute effect of a single administration of the rapid-acting antidepressant ketamine and they found no changes 24 hours after the intervention (19). Whereas ketamine’s psychoactive effects are hyper-acute, with antidepressant effect reaching a maximum one day after administration (20), the clinical effect of SSRIs emerges much slower. Some studies suggest that SSRIs have acute or subacute effects on cognition, e.g., affective processing bias (21–23), but it generally takes several weeks before symptom relief occurs in patients with depression (24–27). This suggests that clinical effects result from neurobiological changes that emerge gradually, likely over the course of several weeks.

Given the limited knowledge of SSRIs’ neurobiological effects in humans, such as their capacity to induce neuroplasticity, we here aim to investigate if SSRI administration over several weeks can alter synaptic density in the healthy human brain, specifically in the hippocampus and the neocortex. The hippocampus is often the target of research on neuroplasticity as it is a key region in learning and memory, and patients with severe depression have been found to have lower SV2A in the hippocampus and several neocortical regions (14). Although categorized as a mood disorder, symptoms of depression indicate global affection of the brain, with deficits related to, e.g., memory and executive function, that can improve independent of change in depression scores following SSRI treatment (28). Here, we used a double-blind, semi-randomized, placebo-controlled design, to test the hypothesis that healthy participants receiving daily SSRI administration would have higher SV2A binding in the hippocampus and the neocortex than those receiving a placebo. We further hypothesized that SV2A binding would be positively associated with the duration of escitalopram.

1. Arterial blood acquisition and analysis

Methods

i. Study design

The study was conducted in conjunction with a cross-sectional (i.e., single-scan), double-blinded, semi-randomized, placebo-controlled study (see Supplementary Fig S2) on the cognitive effects of escitalopram (29) preregistered at ClinicalTrials.gov (NCT04239339). The study was conducted at the Copenhagen University Hospital, Rigshospitalet, between May 2020 and October 2021. Approval was
granted by the Danish ethics committee for the capital region of Copenhagen (journal ID: H-18038352, with amendments 71579, 73632, and 78565).

All participants were recruited from a database of individuals who had expressed interest in participating in brain imaging studies. Following information about the study, including potential side effects of escitalopram, participants gave their written consent. Next, participants underwent a screening procedure, including medical history, physical and neurological examination, and screening for current or previous psychiatric disorders according to in- and exclusion criteria (see Supplementary file for complete list). Following the screening procedure and neuropsychological testing of IQ and reaction time, participants were semi-randomized to receive either escitalopram (20 mg daily in capsules of 10 mg) or a placebo in identical capsules that were manufactured and distributed by the Capital Region Pharmacy. Randomization balanced with regards to age, sex, and IQ was done by a research administrator not otherwise involved in data collection or analysis. Participants were instructed to take one capsule daily by mouth for three days and then increase to two capsules daily (i.e., full dose). The aim was an intervention period of minimum 3 weeks, and for logistical purposes and to allow room for unforeseen events (e.g., illness or technical issues), participants could continue the intervention for up to 5 weeks. After the intervention period, all participants came in for extensive neuropsychological testing and MRI examination. On intervention day 10 and the day of neuropsychological testing and MRI, a blood sample was collected to measure s-escitalopram steady-state levels as confirmation of drug adherence. Participants were instructed only to take their daily dose of medication after the blood sample had been drawn. S-escitalopram was measured with ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS; Filadelphia Epilepsy Hospital, Dianalund, Denmark).

The main study included 66 healthy participants, for which we have reported the neuropsychological outcomes (29). A subset of 32 participants underwent \(^{11}C\)UCB-J PET scanning after the main study program was completed and while still double-blinded to the intervention. Participants were asked at the time of inclusion whether they, in addition to the described study program, agreed to undergo a PET scan. The sample size for the PET cohort (16 participants in each group) was calibrated to detect a 10% change (Cohen's d \(\cong\) 1) in \(^{11}C\)UCB-J \(V_T\) in the hippocampus, at 80% power and a significance level of 0.05, based on data from Finnema et al. (30). The data presented here are based on these 32 participants.

**ii. MRI acquisition and preprocessing**

All participants underwent MRI scans in a Siemens Magnetom Prisma 3T scanner (Siemens AG, Erlangen, Germany) using a Siemens 32-channel head coil. Structural T1- and T2-weighted images were acquired (T1 protocol: Isotropic 0.9x0.9x0.9 mm\(^3\) resolution, repetition time = 2000 ms, echo time = 2.58 ms, inversion time = 972 ms, and flip angle = 8°; T2 protocol: Isotropic 0.9x0.9x0.9 mm\(^3\) resolution, repetition time = 3200 ms, echo time = 408 ms). Grey matter masks for PET processing were extracted from T1- and T2-weighted images using the multispectral segmentation routine in SPM12 (Functional Imaging
Laboratory, the Wellcome Trust Centre for NeuroImaging, London, UK). Cortical thickness and hippocampal volume were derived from the T1-weighted images using the standard anatomical processing stream (recon-all) from FreeSurfer (v. 7.2, https://surfer.nmr.mgh.harvard.edu/) (31), with manual refinement of the pial surface using the T2-weighted images.

iii. PET acquisition

Radiosynthesis of $[^{11}\text{C}]$UCB-J was modified on the basis of Nabulsi et al. (32), as described in detail in the Supplementary file. All participants were scanned with a high-resolution research tomography (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA). Following a six-min transmission scan, a 120 min PET scan was started at the time of intravenous $[^{11}\text{C}]$UCB-J bolus injection (over ~20 sec). PET data were acquired in 3D list mode and reconstructed into 40 frames (8 x 15 s, 8 x 30 s, 4 x 60 s, 5 x 120 s, 10 x 300 s, 5 x 300 s) using a 3D OP-OSEM algorithm with modeling of the point-spread-function (33,34), and attenuation corrected using the HRRT maximum a posteriori transmission reconstruction method (MAP-TR) (35). Each image frame consisted of 207 planes of 256 x 256 voxels of 1.22x1.22x1.22 mm$^3$.

iv. Arterial blood acquisition and analysis

For determination of the arterial input function, arterial blood samples were collected from a 20G catheter which had been placed in the radial artery under local anesthesia. For the first 15 min of each scan, whole blood radioactivity was continuously measured (2-sec intervals, flow = 8 mL/min) using an Allogg ABSS autosampler (Allogg Technology, Mariefred, Sweden). In addition, manual blood samples were drawn at 2.5, 5, 10, 25, 40, 60, 90, and 120 min for measuring radioactivity in blood and plasma using a gamma counter (Cobra II auto-gamma, Packard, Packard Instrument Company, Meriden, CT, USA) that was cross-calibrated to the PET scanner biweekly. Plasma was extracted after centrifugation of arterial blood at 2246xg for 7 min at 4 °C. To measure intact tracer and radiolabeled metabolites, plasma samples up until 90 min were analyzed using radio-HPLC (see the Supplementary file for full detail).

The plasma free fraction ($f_P$) of $[^{11}\text{C}]$UCB-J was determined by the equilibrium dialysis method as described in the Supplementary file.

v. PET image processing

All PET images were motion corrected using the AIR software with the reconcile command (Automated Image Registration, v. 5.2.5, LONI, UCLA, http://bishopw.loni.ucla.edu/air5/). Tissue time-activity curves were extracted from automatically defined ROIs using the PVElab software (https://nru.dk/index.php/testmenu/category/37-pvelab). The PVElab pipeline used an unfiltered summation PET image that was automatically co-registered to the participant's T1-weighted MR image using SPM12. Segmented T1- and T2-weighted MR images were then used to extract grey matter values
from each ROI defined with a brain atlas, as previously described (36). Co-registration and ROI placement were visually inspected for each subject; no manual correction was needed. No correction for partial volume effects was applied. The ROI for the centrum semiovale (white matter) was obtained from the PVElab region with the Müller-Gartner partial volume correction method, and was further eroded twice with a 3D erosion operator to minimize partial volume effects. Final volume had a mean (SD) of 7.45 (2.63) mL.

vi. Kinetic modeling

Kinetic modeling of $[^{11}\text{C}]$UCB-J PET data was performed in R (v. 4.2.2, R Foundation, Vienna, Austria) using the kinfitr package (v. 0.6 (37). Time-activity curves from all ROIs were fitted to the one-tissue compartment model (1TCM) using the subject’s metabolite-corrected arterial input function to estimate the total volume of distribution ($V_T$), an index of SV2A binding. The fraction of blood volume ($v_B$) was excluded from the model as it did not improve the model fits or change $V_T$ estimates, which is in agreement with previous kinetic evaluations (30). In addition, time-activity curves from the hippocampus and neocortex were fitted to the simplified reference tissue model 2 (SRTM2) using the white matter region centrum semiovale as the reference region, and the median $k_2$ from 1TC modeling of centrum semiovale as a global $k_2'$ (0.035 min$^{-1}$).

vii. Statistical analyses

The distributions of demographic variables and PET scan parameters were visually compared between the groups and formally tested with a Welch two-sample t-test for continuous variables and Chi squared tests for group sex ratios. Our primary hypothesis of higher $[^{11}\text{C}]$UCB-J $V_T$ in the hippocampus and the neocortex in the escitalopram group compared to the placebo group was tested using Welch two-sample t-tests. $B_P$NDs from the SRTM2 model were likewise compared with two-sample t-tests.

As a secondary analysis, we investigated if there was an effect on $[^{11}\text{C}]$UCB-J $V_T$ dependent on escitalopram intervention duration: using a likelihood-ratio test, we compared a linear regression model including a group-by-intervention duration interaction term to a nested model where the group term was excluded. We further investigated the effect of s-escitalopram concentration on $[^{11}\text{C}]$UCB-J $V_T$ using linear regression. Here, the values were divided by 60 (~1 SD) to make estimates easier to interpret. Effects of age and sex have not been established for $[^{11}\text{C}]$UCB-J binding estimates, and because of our relatively narrow age range and balanced randomization, we did not include age and sex in the analyses of $[^{11}\text{C}]$UCB-J binding.

Group means for $[^{11}\text{C}]$UCB-J $V_T$ estimates for other regions are listed in the Supplementary file. These include neocortical ROIs: Orbital frontal, anterior cingulate, insula, superior temporal gyrus, parietal, medial inferior temporal gyrus, superior frontal, occipital, sensory-motor, dorsolateral prefrontal gyrus,
ventrolateral prefrontal gyrus. Subcortical ROIs: Centrum semiovale, thalamus, caudate, putamen, entorhinal cortex, amygdala, raphae nuclei.

As exploratory analyses, we investigated effects of escitalopram versus placebo, and s-escitalopram on hippocampus volume adjusted for age, sex, and intracranial volume (ICV). Lastly, for the neocortical subregions frontal, parietal, temporal, occipital, and insular cortex, we examined if there was a group effect on cortical thickness using linear regressions, as described for $^{[11]}$CUCB-J $V_T$s. As cortical thickness varies with age and sex (38,39), these parameters were included as covariates in the models.

All tests were performed as two-sided tests. Secondary and exploratory analyses were corrected for comparisons across multiple brain regions when applicable, using the Bonferroni-Holm method. Statistical analyses were performed in R (v. 4.2.2).

**Results**

i. **Demographics and scan-related parameters**

The escitalopram and placebo groups were similar in age and sex distribution and PET-related variables, including $^{[11]}$CUCB-J plasma free fraction (Table 1). This was also the case when leaving out three participants without full arterial input functions, all from the placebo group. Serum-escitalopram (s-escitalopram) measurements confirmed correct group assignment and that all participants in the escitalopram group had been compliant.
Table 1. Subject demographics and $^{[11]}$CUCB-J PET scan-related parameters

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=15$^1$)</th>
<th>Escitalopram (N=17)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (53%)</td>
<td>12 (71%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Male</td>
<td>7 (47%)</td>
<td>5 (29%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>22.8 (2.9)</td>
<td>25.2 (5.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>21.7 [19.9, 31.6]</td>
<td>22.7 [19.6, 41.9]</td>
<td></td>
</tr>
<tr>
<td>Intervention duration (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>30.4 (4.7)</td>
<td>28.2 (3.3)</td>
<td>0.14</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>32.0 [22.0, 38.0]</td>
<td>27.0 [24.0, 35.0]</td>
<td></td>
</tr>
<tr>
<td>S-escitalopram, day 10 (nmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0 (0)</td>
<td>86 (75)</td>
<td>-</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>0 [0, 0]</td>
<td>68 [28, 338]</td>
<td></td>
</tr>
<tr>
<td>S-escitalopram, follow-up (nmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0 (0)</td>
<td>84 (56)</td>
<td>-</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>0 [0, 0]</td>
<td>69 [28, 263]</td>
<td></td>
</tr>
<tr>
<td>Injected dose (MBq)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>401 (101)</td>
<td>410 (63)</td>
<td>0.77</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>415 [124, 550]</td>
<td>414 [251, 526]</td>
<td></td>
</tr>
<tr>
<td>Injected mass (ng/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>12.2 (19.5)</td>
<td>8.9 (7.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>8.8 [1.2, 80.9]</td>
<td>6.7 [1.4, 29.3]</td>
<td></td>
</tr>
<tr>
<td>$f_P$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.36 (0.05)</td>
<td>0.36 (0.05)</td>
<td>0.87</td>
</tr>
<tr>
<td>Median [Min, Max]</td>
<td>0.37 [0.29, 0.46]</td>
<td>0.38 [0.26, 0.42]</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Including 1 subject with a suicide attempt.
Table 1. P-values refer to two-sample t-tests for continuous variables and chi-square tests for categorical variables. Includes three participants in the placebo group who did not have a complete arterial input function. Group characteristics did not change markedly when leaving out these participants.

ii. Primary analyses

There was no statistically significant difference in $[^{11}C]$UCB-J binding between the escitalopram and placebo group in our primary ROIs, the hippocampus and the neocortex, after an average intervention period of 29 days (Fig. 1). The mean (SD) $V_T$ in the hippocampus was 15.1 (2.2) mL/cm$^3$ for escitalopram ($n=17$) vs. 14.3 (1.9) mL/cm$^3$ for placebo ($n=12$), corresponding to Cohen's $d = 0.43$ (95% CI [-0.36, 1.20], $p = 0.26$). In the neocortex, the mean (SD) $V_T$ was 18.3 (2.5) mL/cm$^3$ for escitalopram ($n=17$) vs. 17.6 (2.0) mL/cm$^3$ for placebo ($n=12$) corresponding to Cohen's $d = 0.31$, (95% CI [-0.47, 1.08], $p = 0.41$).

For completeness, we also evaluated the non-displaceable binding potential (BP$_{ND}$) based on reference tissue modelling using the white matter as reference region. Mean (SD) BP$_{ND}$ in the hippocampus was 2.65 (0.36) for escitalopram ($n=17$) vs. 2.70 (0.38) for placebo ($n=15$) corresponding to Cohen's $d = -0.15$ (95% CI [-0.57, 0.88], $p = 0.67$), while BP$_{ND}$ in the neocortex was 3.42 (0.38) for escitalopram ($n=17$) vs. 3.57 (0.42) for placebo ($n=17$) corresponding to Cohen's $d = -0.19$ (95% CI [-0.53, 0.92], $p = 0.31$).

Neocortical subregions and subcortical regions were not included in our a priori hypothesis; none of these regions showed significant differences in $[^{11}C]$UCB-J $V_T$ estimates between escitalopram and placebo groups (Supplementary Table S1).

iii. Secondary analyses

a. Effect of intervention duration on $[^{11}C]$UCB-J binding

As the length of the intervention period ranged from 24 to 35 days for the escitalopram group, we investigated if longer exposure to escitalopram was associated with higher $[^{11}C]$UCB-J $V_T$. A likelihood-ratio test between a linear regression model including a group-by-intervention duration interaction term and a nested model where the group term was excluded, indicated a time-dependent group effect of escitalopram: the test resulted in a p-value of 0.020 ($p_{adj.} = 0.039$) for the neocortex and 0.058 ($p_{adj.} = 0.058$) for the hippocampus. We also modelled the drug-specific effect of the length of exposure on $[^{11}C]$UCB-J $V_T$: in the neocortex (Fig. 2A) we found a positive effect of time for the escitalopram group, estimated to be $+0.41$ mL/cm$^3$ per day ($p = 0.016$), whereas it was $-0.12$ mL/cm$^3$ per day ($p = 0.38$) for the placebo group. Similarly, for the hippocampus (Fig. 2B), the effect of time on $[^{11}C]$UCB-J $V_T$ was $+0.25$
mL/cm$^3$ per day ($p = 0.11$) for the escitalopram group, whereas for the placebo it was -0.14 mL/cm$^3$ per day ($p = 0.26$).

b. Effect s-escitalopram concentration on $[^{11}\text{C}]$UCB-J binding

We also investigated the effect of participants’ s-escitalopram level on $^{11}\text{C}]$UCB-J $V_T$. The estimate of the effect was +0.28 mL/cm$^3$ per SD of s-escitalopram ($p = 0.60, p_{adj.} = 1.0$) in the hippocampus, and 0.1 mL/cm$^3$ per SD of s-escitalopram ($p = 0.85, p_{adj.} = 1.0$) in the neocortex. For this analysis, one outlier was excluded due to a mean s-escitalopram concentration ~3.5 standard deviations above the mean.

i. Exploratory analyses

a. Effects of escitalopram on hippocampus volume

The mean (SD) hippocampus volume was 4572 (389) mm$^3$ in the escitalopram group versus 4767 (329) mm$^3$ in the placebo group. When compared using a linear regression model controlling for age, sex, and intracranial volume the estimated difference was reduced to -97 mm$^3$ ($p = 0.33$). A model including the group-by-intervention duration interaction term did not improve the model fit, as compared using a likelihood-ratio test ($p = 0.62$). The effect of s-escitalopram concentration on hippocampus volume was estimated to be -36 mm$^3$ per SD of s-escitalopram ($p = 0.62$). All model estimates are listed in Table S2.

b. Effects of escitalopram on cortical thickness

Linear regression models with age and sex as covariates showed no difference in cortical thickness between escitalopram compared to placebo for the neocortical subregions (minimum $p = 0.22, p_{adj.} = 1.0$). Estimates for individual regions are listed in Table S3. Likelihood-ratio tests between linear regression models including a group-by-intervention duration interaction term and nested models where the group term was excluded, did not support a time-dependent effect of escitalopram on cortical thickness in any of the subregions after correcting for multiple comparisons (minimum $p = 0.033, p_{adj.} = 0.16$). Individual estimates are listed in Table S4. Lastly, s-escitalopram concentration also was not associated with cortical thickness (minimum $p = 0.36, p_{adj.} = 1.0$) (Table S5).

Discussion

In this study, we examine the effects of the SSRI escitalopram on brain synaptic density in SSRI-naïve healthy volunteers, as indexed by SV2A density measured with $[^{11}\text{C}]$UCB-J PET. Administering the drug to healthy participants allowed us to study potential effects on synaptic plasticity in the absence of clinical symptoms or brain pathology. The mean $[^{11}\text{C}]$UCB-J $V_T$s were higher in the escitalopram group with estimated Cohen's $d$ values of 0.43 and 0.31 for the hippocampus and the neocortex. However, these effects sizes were associated with considerable uncertainty as reflected in the 95% confidence intervals,
and thus the crude group analysis did not support our primary hypothesis that $[^{11}\text{C}]\text{UCB-J}$ binding would be higher in the escitalopram group than the placebo group following 3-5 weeks of drug intervention. When adjusting for differences in the length of the intervention period within the escitalopram group, we found a time-dependent effect of escitalopram intervention on $[^{11}\text{C}]\text{UCB-J} V_T$, an effect that was more pronounced for the neocortex than the hippocampus. The time-dependent effect of escitalopram was reflected in the linear regression models estimating higher $[^{11}\text{C}]\text{UCB-J} V_T$ with increasing number of days of escitalopram intervention. This positive association with escitalopram intervention duration suggests that a reason why we do not find a group difference in the primary analysis could be that an average of 28 days of escitalopram intervention is too short for synaptic effects to fully emerge. Delayed effects of the escitalopram intervention align with the clinical observations that when SSRIs are used for treating, e.g., depression, at least 2-4 weeks of treatment is required before effects on symptoms can be expected (25–27). As our participants were healthy and relatively young and without cognitive impairments or a history of neuropsychiatric illness, it is also plausible that synaptic wiring, hippocampus volume, and cortical thickness, on which we saw no effect of escitalopram, are less affected by SSRIs.

Effects sizes and temporal dynamics might be different in patients, as data from a recent $[^{11}\text{C}]\text{UCB-J}$ PET study by Holmes et al. (14) suggest that patients with depression have synaptic deficits that correlate with symptom severity. If replicated, it would be interesting to examine whether SSRI treatment normalizes SV2A levels and if such normalization is associated with clinical improvement.

The reason for the delay in symptom relief following initiation of SSRI treatment is unclear, although both biological and neuropsychological hypotheses have been proposed, e.g., affective bias and reward sensitivity (8,9,23,40). Even though inhibition of the serotonin transporter occurs immediately after SSRI dosing (41), the net effect on synaptic serotonin levels is more dynamic. A meta-analysis investigating the temporal effect of SSRIs on brain serotonin levels in rats found an initial dip in the frontal cortex followed by a linear increase over three weeks, in contrast to the hippocampus, where a marked increase was found on day three followed by a modest increase from day 6-21 (42). Our data similarly estimate a larger average effect size for escitalopram in the hippocampus compared to the neocortex, but weaker association with intervention duration. The downstream effects of SSRIs on synaptic structures might be even further delayed and depend on the regional level of serotonergic innervation. One example of this was found in the rat hippocampus in response to the SSRI fluoxetine; in the subregion CA1, synaptic density was equally elevated following 5 and 14 days of intervention, whereas in the subregion CA3, the increase in synaptic density was significantly higher after 14 days than after five days of intervention (43).

Aside from the intervention duration, the drug dose is also an important aspect to consider. Despite substantial variation in drug concentration, we saw no association between $[^{11}\text{C}]\text{UCB-J} V_T$ estimates and s-escitalopram concentration. This could be because we used a high daily dose of 20 mg escitalopram, which we expected to lead to a near-maximum occupancy of 70-80% of the serotonin transporter (41). However, concentrations beyond the point of saturation of the serotonin transporter may be important for
the engagement of low-affinity targets. Escitalopram is considered the most selective of the SSRIs (44), but could have important off-target effects according to a recent study: An allosteric binding site at the tropomysin receptor kinase B (Trk-B) was identified as a low-affinity target of drugs representing several classes of antidepressants, including the SSRIs (45). The Trk-B receptor activates neurotrophic signaling cascades when activated by brain-derived neurotrophic factor (BDNF). BDNF is known to have antidepressant effects and is increased in response to SSRIs, which forms a strong link between SSRIs and neuroplasticity (11). It remains to be determined whether all SSRIs, including escitalopram, exert positive allosteric modulation of the Trk-B receptor at clinically relevant doses. This will be important for mapping out the mechanisms of SSRIs and could be a potential target for dual-action drugs promoting neuroplasticity. In this context, evaluating synaptic markers such as SV2A may prove to be a valuable tool.

Few other studies have investigated the effect of drug interventions on SV2A quantified with radioligand techniques. Using $^{[3]}$H]UCB-J in vitro autoradiography, we recently showed that a single administration of the 5-HT$_{2A}$ receptor agonist psilocybin was associated with higher hippocampal SV2A levels in awake pigs (46). In contrast, another study found no effect of ketamine on SV2A in humans measured with $^{[1]}$C]UCB-J PET 24 hours after the drug intervention (19).

So far, most other SV2A PET imaging studies have been cross-sectional case-control studies of neurodegenerative and psychiatric disorders for which causal relationships cannot be determined. Yet, indications of how modifiable SV2A is in the human brain may potentially be derived indirectly: A study on SV2A binding in cocaine-use disorder by Angarita et al. found a negative correlation between $^{[1]}$C]UCB-J binding and duration of cocaine abstinence, whereas years of lifetime use was unrelated to SV2A binding (47). In contrast, another study found no association with the frequency of cannabis use in participants with cannabis use disorder (48). Although exploratory, such analyses can indicate to which extent SV2A is a modifiable state marker or a stable trait marker of synapses.

Some methodological aspects of the current study should be considered. First, the use of SV2A as a proxy for pre-synaptic density. Although SV2A is ubiquitously expressed throughout the brain, it cannot be excluded that changes (or lack thereof) in SV2A binding estimates could have several different causes, such as number of vesicles per synapse or differential effects on excitatory and inhibitory synapses. Preclinical studies comparing in vivo SV2A PET imaging with in vitro methods will help advance our understanding and interpretations of SV2A imaging studies. Second, as our study did not include baseline $^{[1]}$C]UCB-J PET scans, we make an assumption of no group differences in cerebral SV2A binding before the intervention was initiated; this assumption is justified on the basis of balanced group randomization that took age and sex into account. The present study design also eliminates issues of long-term test-retest bias which has been reported to occur with $^{[1]}$C]UCB-J PET in some instances (49). Finally, the sample size was targeted to detect larger effect sizes, which limits us in detecting subtler differences and subgroup differences (e.g., sex). As such, the study should be replicated in an
independent sample, ideally with an even longer intervention period to confirm the results and map the temporal dynamics more closely.

In summary, this is the first study to investigate the effect of an SSRI intervention, using clinically relevant doses and duration (i.e., 3-5 weeks), on pre-synaptic density in the human brain. Our data suggest that escitalopram has a time-dependent effect on cerebral SV2A, i.e., that over 3-5 weeks, escitalopram induces synaptic neuroplasticity in the human brain. This offers a biological explanation for the delayed response commonly observed in patients treated with SSRIs. These results have important implications for future studies investigating the effects of SSRIs, especially concerning the duration of intervention studies. Our study adds a novel perspective to the growing literature on synaptic alterations in neuropsychiatric conditions.

Declarations

Acknowledgments

Assistance from Lone Freyr, Anna Søndergaard, Elisabeth Pedersen, Dorthe Givard, Peter Steen Jensen, Lucas Andreasen, Oliver Overgaard-Hansen, Caroline Lund, Ida Likaj, Anton Lund, Ida Møller Larsen, Christina Schulze, and Vibeke Jensen, is greatly acknowledged. The John and Birthe Meyer Foundation is gratefully acknowledged for donating the Cyclotron and PET scanner. The Kirsten and Freddy Johansen Foundation is gratefully acknowledged for donating the MRI scanner. The Toyota Foundation is gratefully acknowledged for the donation of the HPLC equipment.

Conflicts of interest

GMK has received honoraria as a speaker for Sage Biogen and H. Lundbeck, and is a consultant for Onsero, Pangea, and Gilgamesh, Abbvie, PureTechHealth. BJS consults for Cambridge Cognition and receives technology transfer fees from PopReach via Cambridge Enterprise. All other authors declare no conflicts of interest. Funding agencies did not impact the study and played no role in manuscript preparation and submission.

Author contributions:

GMK and BJS conceptualized the study and designed the main study together with CL and DSS. BJS and GMK acquired funding for the study. AJ designed the PET experiments and data processing pipeline with input from CS, PPS, GMK, and SHK. SA was responsible for recruitment and MRI acquisition. AJ acquired the PET data with assistance from AN, KM, and AV. INP and JM were responsible for radiochemistry. VB was responsible for processing of volumetric MR data. AJ analyzed the data with assistance from PPS,
GMK, BO, CS, SHK, and VB. AJ drafted the manuscript in consultation with GMK. All authors critically reviewed the manuscript.

Data availability

Upon completion of the study, all data will be uploaded to the existing CIMBI Database (50). Researchers may apply for access to the data. Code generated and used in the production of this manuscript is available on reasonable request.

References


2. Branchi I. The double edged sword of neural plasticity: Increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover. Psychoneuroendocrino. 2011;36(3):339–51.


Figures
Figure 1

Comparison of $[^{11}\text{C}]$UCB-J binding in healthy individuals following 3-5 weeks of intervention with escitalopram or placebo. **A:** $[^{11}\text{C}]$UCB-J total volume of distribution ($V_T$) quantified using the 1TCM ($n = 29$). **B:** $[^{11}\text{C}]$UCB-J binding quantified using the SRTM2 with centrum semiovale as the reference region ($n = 32$). Within each box, the horizontal line denotes the median value, and boxes extend from the 25th to 75th percentile.

Figure 2
Relationship between the duration of intervention and $[^{11}\text{C}]$UCB-J binding ($V_T$). (A) neocortex ($n = 29$) and (B) the hippocampus ($n = 29$). The shaded grey area represents the 95% CI.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- 20230417HCSSRIUCBJSupplementary.docx