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In / exclusion criteria

Inclusion criteria:

1. Healthy females and males aged 18-45 years.

Exclusion criteria:

1. Current or former primary psychiatric disorder (DSM-V or WHO ICD-11 diagnostic classification).
2. Current or previous neurological disease, severe somatic disease, or the consumption of drugs likely to influence the test results.
3. Current psychoactive medication.
4. Abnormal ECG.
5. Postural orthostatic tachycardia syndrome.
6. Hypotension (blood pressure < 100/70 mmHg) or hypertension (blood pressure > 140/90 mmHg).
7. Head injury or concussion resulting in loss of consciousness for more than 2 min.
8. Pregnancy.
9. Lactation.
10. Alcohol or drug abuse.
11. Nicotine addiction.
12. Recreational drug use other than tobacco and alcohol within the last 30 days.
13. Cannabis use > 50 x lifetime.
14. Recreational drug use > 10 x lifetime (for each substance).
15. Non-fluent in Danish or pronounced visual or auditory impairments.
16. Current or past learning disability.
17. Severe physical impairments affecting eyesight or motor performance.
18. Participation in experiments with radioactivity (>10 mSv) within the last year or other significant exposure to radioactivity.
19. Contraindications for magnetic resonance imaging (MRI).
20. Allergy to compounds used for PET scan or intervention.

Figure S1: Study design

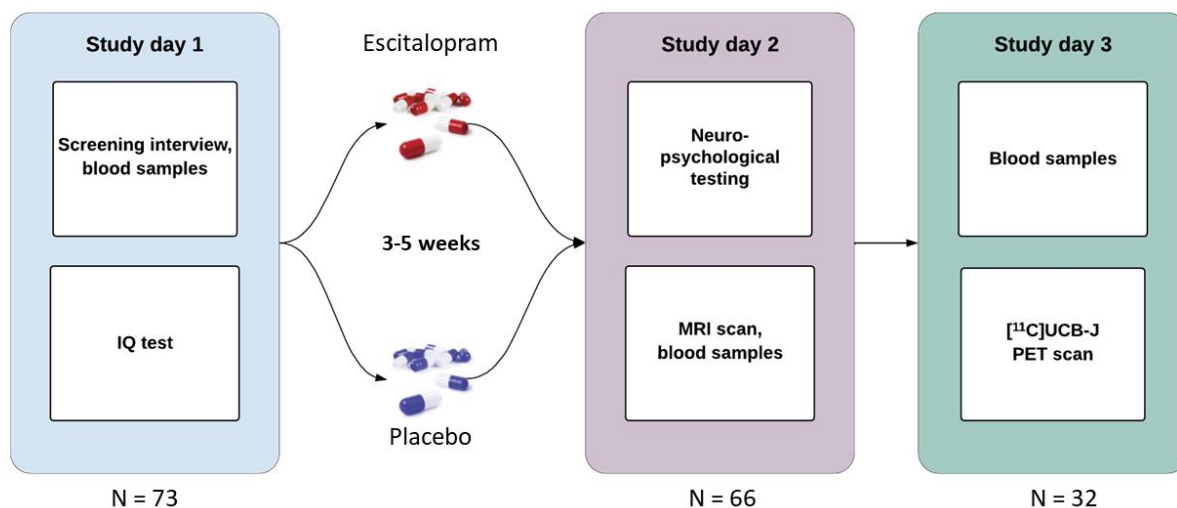


Figure S1: Study design. Participants were screened and randomized on Study day 1. Within 1-5 days, participants started the intervention with either escitalopram or placebo capsules. After 3-5 weeks of full dose intervention, participants came back for MRI scan and neuropsychological testing on Study day 2 and PET scan on Study day 3.

Radiosynthesis of $[^{11}\text{C}]\text{UCB-J}$

Proton irradiation of the target material (nitrogen-14 gas) was performed using the cyclotron: Scanditronix MC-32 with aluminium high-pressure gas target. Irradiations for carbon-11 were performed at 16 MeV. The target gas used was 10% hydrogen in nitrogen. Following irradiation, the target gas was transferred to the radiochemistry system (Scansys Aps) through stainless steel capillaries. $[^{11}\text{C}]\text{methyl iodide}$ was synthesized from $[^{11}\text{C}]\text{methane}$ by a standard circulation procedure.

Preparation of the precursor: The precursor (1.5 mg) was dissolved in MeOH (70 μL) and 1 M HCl (15 μL) was added. The resulting mixture reacted overnight at room temperature. On the day of synthesis, the liquid was removed by a stream of nitrogen to complete dryness.

Preparation of the labelling mixture: 4-5 mg $\text{P}(\text{o-tolyl})_3$ was dissolved in DMF (1.8 mL) and H_2O (0.2 mL) in a capped vial and degassed with nitrogen. Pd_2dba_3 (4-5 mg) was weighed out in a capped vial and flushed with nitrogen. Immediately before labelling, the $\text{P}(\text{o-tolyl})_3$ solution was added to the Pd_2dba_3 . From here, 350 μL was withdrawn and added to a 0.9 mL vial containing 0.5 M K_2CO_3 (20 μL). The formed $[^{11}\text{C}]\text{methyl iodide}$ was trapped in the 0.9 mL glass vial containing the $\text{K}_2\text{CO}_3/\text{P}(\text{o-tolyl})_3/\text{Pd}_2\text{dba}_3$ mixture. After trapping the $[^{11}\text{C}]\text{methyl iodide}$, the hydrolyzed DM-BF₃-UCB-J precursor was re-dissolved in DMF (150 μL) and reacted with the mixture by heating at 100 $^\circ\text{C}$ for 300 seconds to give $[^{11}\text{C}]\text{UCB-J}$. The reaction mixture was diluted

with 4 mL 0.1% H₃PO₄ and automatically injected onto a preparative HPLC-column (Onyx™ Monolithic C-18, 100 × 10 mm equipped with a SecurityGuard Cartridge Lux Cellulose-4, 4 × 3.0 mm; flow: 6 mL/min; eluent: 13/87 [ethanol (96%)/0.1 M phosphoric acid]). The radioactive fraction corresponding to the radiolabeled product (retention time (rt) app. 300 s) was collected by diverting the flow from the column outlet through a 0.22 µm sterile filter and directly into a sterile stoppered and a capped vial containing phosphate buffer (9 mL, pH 7). The identity and molar activity of the product was determined by using a C-18 column (Kinetex 2.6 µm, C18, 100Å, 50 × 4.6 mm, Phenomenex) eluted with 67% 25 mM citrate buffer (pH 5.4)/33% acetonitrile ; injection volume 50 µL; flow rate 1.5 mL/min; on-line UV (261 nm) and radioactivity detection. Rt UCB-J 2.1 min.

Radiometabolite analysis

For measurement of intact tracer and radiolabeled metabolites, plasma samples up until 90 min were filtered through a 0.45 µm syringe filter (Whatman GD/X 13 mm, Cytiva, Buckinghamshire, UK) and subsequently diluted 1:1 with 20 mM disodium hydrogen phosphate and 5 mM sodium-1-decanesulfonate pH 7.2 with 2% isopropanol. Samples were analyzed in a fully automated column-switching HPLC system (UltiMate 3000, Thermo Fisher Scientific, Hvidovre, Denmark) connected to a radio-HPLC detector (PosiRam Model 2, LabLogic Systems, Sheffield, UK)(Gillings, 2009). The HPLC system was equipped with a small extraction column (Shimpack MAYI-ODS 30×4.6 mm, Shimadzu, Ballerup, Denmark) combined with an analytical column (Onyx Monolithic C18 50×4.6 mm, Phenomenex, Brønshøj, Denmark). For extraction and elution, the mobile phase consisted of 100% 20 mM disodium hydrogen phosphate and 5 mM sodium-1-decanesulfonate pH 7.2 with 2% isopropanol and 59% 100 mM sodium dihydrogen phosphate and 2 mM sodium-1-decanesulfonate pH 2.6 and 41% methanol, respectively. Samples were injected in a volume of 4 mL, and the analysis was run at a flow of 5 mL/min at 25 °C. The total runtime for each sample was 8.55 min with a 4 min extraction step, 4 min elution step and 0.55 min of equilibration. Four eluate fractions were collected in 2 min intervals, and radioactivity was subsequently measured using a gamma counter (Wizard 2480, Perkin Elmer, Wallac Oy, Turku, Finland). The parent tracer fraction was calculated as follows: % parent fraction = (radioactivity of parent eluate/total amount of collected radioactivity) × 100%.

Free fraction measurement

[¹¹C]UCB-J free fraction was measured in the following way: plasma spiked with 1 µL/mL of tracer was added into one dialysis chamber (500 µL chambers, Harvard Apparatus, Holliston, MA, USA) and dialyzed against an equal volume of phosphate-buffered saline through a cellulose membrane (MWCO 10,000 Dalton, Harvard

Apparatus, Holliston, USA). The system was incubated at 37 °C for 30, 60, 120, 150 and 180 min. After completion of dialysis, samples were extracted and analyzed for radioactivity using the Cobra II gamma counter (Packard Instrument Company, Meriden, USA). The ratio of buffer:plasma radioactivity was plotted over time and fitted according to eq. 1 with a fixed average value of the diffusion coefficient (k_D), using GraphPad Prism (v. 9.0, GraphPad Software, San Diego, CA, USA) to determine $f_{P_{eq}}$.

$$f_P(t) = \frac{f_{P_{eq}} \times t}{k_D + t} \quad (\text{eq. 1})$$

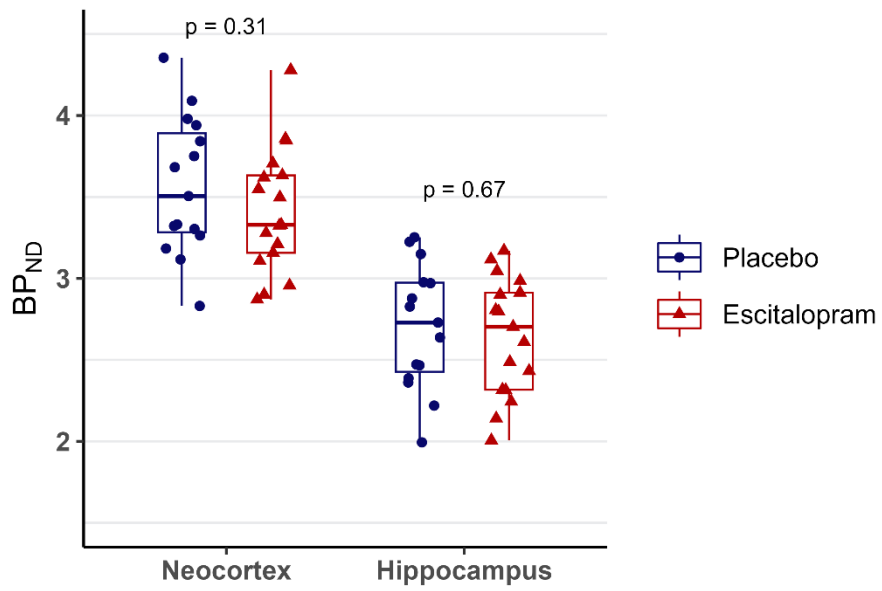
[^{11}C]UCB-J plasma free fraction did not differ between intervention groups or sexes, but correlated with age ($\beta_{\text{esc}} = 0.01$, $p = 0.52$; $\beta_{\text{male}} = -0.003$, $p = 0.85$; $\beta_{\text{age}} = -0.005$ /year, $p = 0.005$). The effect of age was slightly reduced and not statistically significant when omitting one age-outlier of 41.9 years ($\beta_{\text{age}} = -0.004$, $p = 0.1$). As there was no group difference nor association between [^{11}C]UCB-J V_T estimates and f_P in the neocortex ($\beta f_P = 7.0 \text{ mL/cm}^3$, $p = 0.50$), it was not used in the analyses.

1TCM V_T estimates for additional ROIs

Table S1

Region	Placebo (N=12)	Escitalopram (N=17)	p	Cohen's <i>d</i>
Neocortex	17.55 (1.96)	18.25 (2.51)	0.41	0.31
Hippocampus	14.25 (1.85)	15.12 (2.21)	0.26	0.43
Caudate	20.23 (2.31)	21.83 (3.63)	0.16	0.53
Centrum semiovale	3.85 (0.53)	4.14 (0.61)	0.18	0.51
Insula	18.21 (2.05)	19.38 (2.64)	0.19	0.50
Putamen	20.52 (2.25)	21.77 (3.02)	0.21	0.47
Orbitofrontal ctx	17.78 (2.16)	18.85 (2.94)	0.27	0.41
Entorhinal ctx	14.05 (2.70)	15.19 (2.82)	0.28	0.41
Med inf temp gyrus	17.97 (1.93)	18.90 (2.70)	0.29	0.39
Amygdala	16.66 (2.18)	17.60 (2.75)	0.31	0.38
ACC	18.67 (2.28)	19.65 (2.94)	0.32	0.37
Sup frontal gyrus	16.96 (2.08)	17.81 (2.61)	0.34	0.36
vlPFC	17.95 (2.07)	18.83 (2.75)	0.34	0.36
dlPFC	17.69 (2.14)	18.49 (2.59)	0.37	0.34
Sensory-motor ctx	16.21 (2.02)	16.93 (2.30)	0.38	0.33
Temporal ctx	18.36 (1.97)	19.12 (2.60)	0.38	0.33
Parietal ctx	17.77 (2.09)	18.42 (2.53)	0.45	0.28
Cerebellum (excl. vermis)	13.93 (1.57)	14.41 (1.93)	0.47	0.27
Thalamus	17.35 (2.15)	17.91 (2.41)	0.51	0.25
Sup temp gyrus	18.84 (2.04)	19.42 (2.50)	0.50	0.25
Raphe nuclei	10.98 (1.52)	11.13 (1.25)	0.78	0.11
Occipital ctx	17.50 (1.94)	17.67 (2.39)	0.84	0.08
PCC	18.26 (1.81)	18.40 (2.33)	0.86	0.07

BP_{ND} estimates from the SRTM2 in primary ROIs



Effects on [¹¹C]UCB-J V_T

Table S2: Effect of intervention group

General linear model with age, sex and IQ as covariates to test group effect on [¹¹C]UCB-J V_T

<i>Predictors</i>	Neocortex			Hippocampus			Centrum semiovale		
	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>
Intercept*	17.01	0.92	<0.001	13.75	0.82	<0.001	3.83	0.25	<0.001
Age (per decade)	0.23	0.84	0.79	0.14	0.76	0.86	0.01	0.23	0.98
Sex (Male)	-1.48	0.85	0.09	-1.29	0.76	0.10	-0.22	0.23	0.35
IQ	0.14	0.06	0.027	0.13	0.05	0.026	0.02	0.02	0.37
Intervention (escitalopram)	-0.11	0.88	0.90	0.16	0.79	0.84	0.19	0.24	0.43

* Intercept centered at age = 20 years and IQ = 100.

Table S3: Effect of intervention duration

General linear model with age, sex and IQ as covariates to test effect of intervention duration on [¹¹C]UCB-J V_T

<i>Predictors</i>	Neocortex				Hippocampus				Centrum semiovale			
	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>r_p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>r_p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>r_p</i>
Intercept*	17.18	0.78	<0.001		13.85	0.78	<0.001		3.86	0.24	<0.001	
Age (per decade)	-0.32	0.74	0.67	-0.09	-0.23	0.74	0.76	-0.07	-0.12	0.22	0.61	-0.11
Sex (Male)	-1.65	0.82	0.056		-1.22	0.82	0.15		-0.21	0.25	0.41	
IQ	0.15	0.05	0.008	0.53	0.13	0.05	0.017	0.48	0.02	0.02	0.29	0.22
Intervention (escitalopram)	0.32	0.76	0.68		0.42	0.76	0.59		0.28	0.23	0.23	
Duration (placebo)	-0.01	0.13	0.95	-0.01	-0.06	0.13	0.62	-0.1	-0.02	0.04	0.60	-0.11
Duration (escitalopram)	0.47	0.14	0.003	0.58	0.30	0.14	0.048	0.41	0.10	0.04	0.029	0.45

*Intercept centered at age = 20 years, mean intervention duration (29 days), and IQ = 100. R_p = partial correlation coefficient.

Table S4: Effects of s-escitalopram

General linear model with age and sex as covariates to test effect of s-escitalopram level on [^{11}C]UCB-J V_T

<i>Predictors</i>	Neocortex			Hippocampus			Centrum semiovale		
	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>
Intercept*	17.03	0.92	<0.001	13.80	0.83	<0.001	3.85	0.26	<0.001
Age (per decade)	0.23	0.83	0.78	0.17	0.75	0.83	0.03	0.23	0.91
Sex (Male)	-1.50	0.86	0.01	-1.31	0.78	0.11	-0.22	0.24	0.36
IQ	0.14	0.06	0.028	0.13	0.06	0.026	0.02	0.02	0.37
S-escitalopram (log[ng/L])	-0.04	0.20	0.85	0.01	0.18	0.96	0.03	0.06	0.57

* Intercept centered at age = 20 years.

Effects on hippocampus volume

Table S5: Effects of intervention group and s-escitalopram

General linear model with age, sex and intra-cranial volume (ICV) as covariates to test effect of intervention group and s-escitalopram level on hippocampus volume

<i>Predictors</i>	Group			S-escitalopram		
	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>
Intercept*	4637	99	<0.001	4736	91	<0.001
Age (per decade)	68	104	0.52	61	103	0.56
Sex (Male)	-124	117	0.23	-128	117	0.28
ICV (L)	2048	419	<0.001	2038	423	<0.001
Intervention (Escitalopram)	97	99	0.33			
S-escitalopram (log[ng/L])				-22	23	0.34

Hippocampus volume is in unit of mm³. * Intercept centered at age = 20 years, and mean ICV.

Effects on cortical thickness

Table S6: Effects of intervention group

General linear model with age and sex as covariates to test effect of intervention group cortical thickness

<i>Predictors</i>	Frontal			Parietal			Temporal			Occipital			Insular		
	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>
Intercept*	2.77	0.02	<0.01	2.52	0.03	<0.01	2.90	0.03	<0.01	2.02	0.03	<0.01	3.16	0.04	<0.01
Age (per decade)	-0.07	0.03	0.02	-0.05	0.03	0.13	-0.04	0.04	0.32	-0.01	0.03	0.67	-0.05	0.05	0.29
Sex (Male)	0.06	0.03	0.04	0.06	0.03	0.07	0.08	0.04	0.06	0.06	0.03	0.07	0.06	0.04	0.17
Intervention (Escitalopram)	0.03	0.03	0.22	0.03	0.03	0.26	0.01	0.04	0.77	0.01	0.03	0.83	-0.00	0.04	0.92

Cortical thickness is in the unit of mm. * Intercept centered at age = 20 years. All escitalopram corrected p-values = 1 (Bonferroni-Holm, 5 tests).

Table S7: Effects of intervention duration

Likelihood-ratio tests of model with *group-by-intervention duration* vs. a nested model without group term. Age and sex included in both models

Frontal			Parietal			Temporal			Occipital			Insular		
χ^2	p	$p_{adj.}$	χ^2	p	$p_{adj.}$	χ^2	p	$p_{adj.}$	χ^2	p	$p_{adj.}$	χ^2	p	$p_{adj.}$
6.72	0.03	0.16	3.1	0.21	0.62	1.60	0.45	0.90	6.84	0.03	0.16	1.00	0.61	0.90

($p_{adj.}$ = Bonferroni-Holm, 5 tests).

Table S8: Effects of s-escitalopram

General linear model with age and sex as covariates to test effect of s-escitalopram on cortical thickness

	Frontal			Parietal			Temporal			Occipital			Insular		
<i>Predictors</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>p</i>
Intercept*	2.77	0.02	<0.01	2.52	0.03	<0.01	2.90	0.04	<0.01	2.02	0.03	<0.01	3.16	0.04	<0.01
Age (per decade)	-0.07	0.03	0.02	-0.05	0.03	0.15	-0.04	0.04	0.34	-0.01	0.03	0.67	-0.05	0.04	0.28
Sex (Male)	0.06	0.03	0.03	0.06	0.03	0.07	0.08	0.04	0.06	0.06	0.03	0.07	0.06	0.04	0.18
S-escitalopram (log[ng/L])	0.01	0.01	0.19	0.01	0.01	0.28	0.00	0.01	0.88	0.00	0.01	0.79	-0.00	0.01	0.97

Cortical thickness is in the unit of mm. * Intercept centered at age = 20 years. All escitalopram corrected p-values = 1 (Bonferroni-Holm, 5 tests).