## Psychotropic medications and the developing brain

Solleveld, M.M.

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# Chapter 5

## Non-invasive MR assessment of serotonin function: dose-dependent effects of the SSRI citalopram

Anouk Schrantee

Michelle M. Solleveld

Hilde Schwantje

Willem B. Bruin

Henk-Jan J.M. Mutsaerts

Sofie M. Adriaanse

Paul J. Lucassen

Jan Booij

Liesbeth Reneman

*Submitted*

**Abstract**

Purpose: To assess whether pharmacological magnetic resonance imaging (phMRI) can detect dose-dependent hemodynamic responses to selective serotonin reuptake inhibitors (SSRIs) and to investigate the association between the phMRI response and serotonin transporter (SERT) occupancy, as assessed by single photon emission computed tomography (SPECT) measurements.

Methods: The study was approved by the local Institutional Review Board and 45 healthy female participants provided written informed consent. After baseline thalamic SERT [123I]FP-CIT SPECT measurements, participants were randomized to pre-treatment with placebo (N=15), a low (4 mg, N=15) or a clinically standard (16 mg, N=15) oral citalopram dose. After 3 hours, [123I]FP-CIT displacement (representing SERT occupancy) by citalopram was assessed. Subsequently, a phMRI scan was obtained, during which 7.5 mg citalopram was administered intravenously to assess the thalamic cerebral blood flow (CBF) response to different levels of SERT occupancy. Repeated measures ANOVA was used to assess time x group interactions and correlational analyses were used to determine associations between SPECT and phMRI measurements.

Result: Citalopram displaced thalamic [123I]FP-CIT binding in the low and high citalopram group compared to placebo ($F=11.22$; $p<0.001$). SERT occupancy also affected the phMRI response to intravenous citalopram ($F=3.46$; $p=0.04$). The percentage change in thalamic binding showed a non-significant correlation with percentage change in CBF ($r=-0.28$ $p=0.07$).

Conclusion: In addition to replicating dose-dependent effects of SERT occupancy with SPECT, we here demonstrate that also phMRI can detect such differences. The phMRI signal changes do not strongly correlate with SPECT as these techniques likely assess different functional aspects of the serotonergic synapse.

**Introduction**

The serotonin transporter (SERT) plays a key role in regulating extracellular levels of serotonin. It has been implicated in a number of psychiatric disorders and is an important target for many antidepressants and psychotropic medications, such as selective serotonin reuptake inhibitors (SSRIs)[1]. Molecular imaging techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are invaluable imaging tools to assess the occupancy of the SERT by SSRIs, thereby blocking reuptake of serotonin from the synapse and increasing extracellular serotonin levels[2]. In addition, PET and SPECT studies have demonstrated that the treatment response to SSRIs in depressed patients is associated with SERT occupancy. For instance, a minimum therapeutic dose of SSRIs coincided with approximately 80% SERT occupancy. For SSRIs like paroxetine or citalopram, a curve-linear relationship was observed, with subclinical doses showing less SERT occupancy, whereas higher doses quickly reached a plateau[3,4].

PET and SPECT imaging techniques are not ideal, however, to monitor changes in the serotonergic system repeatedly, particularly in children, or over longer periods of time, due to their use of radioactivity. Yet, longitudinal and prospective measurements and drug response monitoring are important to facilitate personalized treatment strategies for patients with neuropsychiatric disorders like major depressive disorder. These considerations have prompted researchers to develop non-ionizing alternatives to study neurotransmitters in vivo. One such alternative, that can be used repeatedly and is easily accessible, is pharmacological magnetic resonance imaging (phMRI). PhMRI is thought to provide an index of neurotransmitter function based on changes in brain cerebral hemodynamics after administration of a specific pharmacological challenge. This approach takes advantage of a neurotransmitter-specific response to drugs that elicits a specific pattern of neural activation and alterations in cerebral hemodynamics, such as the blood oxygen level-dependent (BOLD) signal or cerebral blood flow (CBF)[5].

A number of preclinical and clinical studies have demonstrated the potential of phMRI to examine the serotonin system in living brain[6]. For instance, a preclinical study has shown that an increase in serotonin release induced by the serotonin-releaser fenfluramine, evokes region-specific changes in the BOLD response in rats[7]. Conversely, a depletion of the serotonin pool with p-chlorophenylalanine attenuated the phMRI response to fenfluramine[7], showing the sensitivity of phMRI to detect extracellular serotonin fluctuations in the brain.

PhMRI signal changes following a serotonin challenge also corresponded with results from autoradiographic measures of CBF and glucose metabolism[8] and c-fos mapping[9]. Finally, persistent changes in serotonergic function could be assessed following prolonged SSRI exposure with phMRI in rodents[10]. A few research groups have further observed similar region-specific changes in humans either using BOLD[11] or arterial spin labeling (ASL)[12,13].

These findings suggest that phMRI can be used as a non-invasive tool to study serotonin function. This novel technique may also yield a wealth of information on how serotonin-related drugs, like SSRIs, modulate brain function, and how drug responses are altered in neuropsychiatric or developmental disorders. So far however, the relationship between neurovascular responses and SERT occupancy has not been studied in humans. The ability to measure differences in serotonin function with phMRI is of particular interest, as it enables us to non-invasively monitor treatment effects of serotonergic medication in patients. This is highly relevant, because treatment efficacy of SSRIs in e.g. major depressive disorder is dependent on the percentage of SERT occupancy. However, the required dose is also dependent on interindividual differences in available SERT (e.g., SERT gene polymorphism), as well as interpatient pharmacokinetic differences[14]. A non-invasive tool to monitor treatment effects would thus enable to optimize dose regimens on an individual basis and in doing so also reduce side effects. Thus, in the present study we set out to assess whether phMRI is sensitive enough to detect a dose-related hemodynamic response to SSRIs.

To this purpose, we measured both the hemodynamic response with phMRI and acute SERT occupancy with SPECT, to different doses of the SSRI citalopram. In view of literature, we expected that phMRI could detect dose-dependent changes in hemodynamic response. More specifically, we expected that the phMRI response would be greatest when SERT is unoccupied by citalopram, and increased SERT binding by citalopram would be associated with decreased phMRI signal.

## Methods

### *Participants*

The study was approved by the local Institutional Review Board and all participants had provided written informed consent. Forty-five healthy female volunteers (mean age = 21.6 years, age range 18-28) were included. Exclusion

criteria for all participants were a history of a chronic neurological or psychiatric disorder, family history of sudden heart failure, current use of psychostimulant medication, abnormal electrocardiogram (ECG), excessive consumption of alcohol (>21 units/week), caffeine (more than eight cups of coffee per day) or nicotine (more than 15 cigarettes per day), and standard contra-indications for the MRI or SPECT exam. The absence of psychiatric disorders and drug abuse was checked with the Mini-International Neuropsychiatric Interview (M.I.N.I) Plus[15]. All participants were required to be on hormonal contraceptives to minimize confounding effects of hormonal cycle. An ECG was made prior to study inclusion to preclude cardiac abnormalities (as SSRI use has been associated with QT prolongation[16]).

*Study design and procedures*

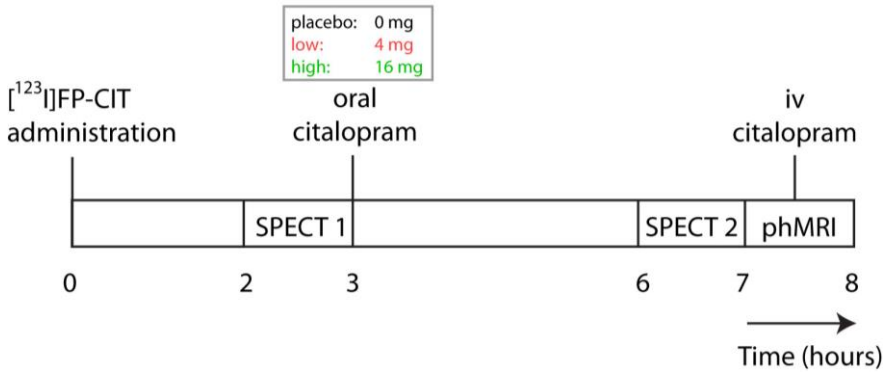
To study the dose-dependent effects of citalopram on phMRI response and SPECT occupancy, a double-blind, dose-response design was used. Participants received potassium iodide tablets prior to the [123I]FP-CIT administration to block thyroid uptake of free radioactive iodide. The first SPECT scan was conducted 2 h post-injection to assess baseline SERT availability. Following the first SPECT scan, participants were randomized into one of the three groups: those receiving placebo ('placebo' N=15), those receiving a low dose (4 mg; 'low group' N=15) and those receiving a clinical dose (16 mg; 'high group' N=15) of oral citalopram (solution, 16 mg equivalent to 20 mg in tablet form, Lundbeck). These doses have been shown to correspond to 0%, ~40% and ~80% SERT occupancy, respectively[17]. The citalopram was dissolved in lemonade and administered immediately after the first SPECT scan. The placebo condition consisted of lemonade only. After three hours, participants underwent a second SPECT scan. Then, a MRI scan was made in which all participants received a 7.5 mg intravenous challenge with citalopram in line with previous studies[11,13]. Blood samples were obtained at baseline, 3 h after citalopram administration, and following the MRI scan. For a schematic illustration of the study design, please see Figure 1.

*SPECT acquisition and analysis*

Subjects underwent SPECT imaging 2 h and 6 h after intravenous administration of approximately 110 MBq [123I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4iodophenyl)nortropane ([123I]FP-CIT, specific activity > 750

MBq/nmol; radiochemical purity > 98%, produced according to GMP criteria at GE Healthcare, Eindhoven, the Netherlands). The radioligand [123I]FP-CIT binds with high affinity to the dopamine transporter (DAT) primarily in the striatum, and to the SERT primarily in extrastriatal brain areas, such as the thalamus and midbrain18. In a previous study, we showed that 2 h after injection is the ideal time-point to assess SERT binding with this radioligand19. SPECT scans were acquired using a brain-dedicated InSPira-HD SPECT camera (Neurologica, Boston, USA) with the following parameters: matrix = 121x121; slice thickness = 4 mm; acquisition time per slice = 180 s; energy window = 159 keV (with 20% lower and upper boundaries). 3D images were reconstructed (using an iterative expectation maximization algorithm, correction using a CT template and spatial smoothing (3mm)). SPECT images were co-registered with the individual 3D T1-weighted (T1w) MR image using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). A region of interest (ROI) analysis was performed to determine SERT binding in the thalamus. Thalamic masks were extracted from individual T1w scans using Freesurfer. The cerebellum was used as a reference region to assess non-specific binding. Specific relative to non-specific binding ratios (binding potential: BPND) were calculated as follows: (mean thalamic binding - mean cerebellum binding / mean cerebellum binding). The reduction in BPND following citalopram administration (representing occupancy) was expressed normalized to the placebo group.

**Figure 1.** Study day outline.
Two hours after the radioligand [$^{123}$I]FP-CIT was administered, the first SPECT scan was made. Subsequently, the subjects randomly received 0, 4 or 16 mg oral citalopram (dissolved in a drink) and were thereby assigned to the placebo, low or high group, respectively. Then, 3 hours after the oral citalopram (at peak plasma levels), the second SPECT scan was made, followed by the phMRI scan. After 37 baseline volumes (5 min), all subjects received 7.5 mg citalopram intravenously (diluted in 45 mL of saline, injected over 7.5 minutes of infusion and flushed with 15 mL of saline).



*MRI acquisition and analysis*

MRI data were acquired using a 3.0T Ingenia (Philips, Best, the Netherlands) with a 32-channel receive-only head coil. A 3D anatomical MRI was obtained using a T1-weighted sequence. Pseudo-continuous arterial spin labeling (pCASL) data were acquired with a 2D echo-planar imaging readout and the following parameters: TR/TE = 4100/14 ms; post-label delay = 1525 ms; label duration = 1650 ms; FOV = 240x240 mm; 17 7 mm slices, voxel size= 3x3x7 mm; dynamics = 183. In addition, an M0 scan was obtained for quantification purposes. Citalopram was administered as a bolus injection after 5 minutes of baseline imaging (37 dynamics). The bolus of 7.5 mg (dissolved in 45 ml saline) was infused over 7.5 minutes, followed by 15 ml saline flush (2.5 minutes). The first and last 37 dynamics were averaged to obtain the pre- and post-citalopram CBF map respectively. ASL post-processing was performed with the ExploreASL toolbox[20]. In short, T1w images were segmented into gray matter (pGM) and white matter (pWM) probability maps. Motion was estimated and motion spikes were excluded[21]. Perfusion-weighted images were rigid-body registered to the pGM images. CBF was quantified using a single compartment model[22]. The pGM and pWM maps were spatially normalized using DARTEL[23], and all transformations were mathematically combined into a single interpolation to transform the CBF maps to Montreal Neurological Institute (MNI) space. Individual thalamic masks used in the SPECT analyses were transformed to MNI space and used to extract mean CBF in this ROI. In addition, GM CBF was obtained to assess the global effect of citalopram. Heart rate (HR) was measured during the scan using a peripheral pulse unit and phase-contrast MRI was used to obtain blood flow (2D-flow) to the brain both before and after intravenous (iv) citalopram administration.

*Statistical analysis*

All data were analyzed with SPSS version 22 (IBM Corp., Armonk, USA), with the significance level set at $p<0.05$ (2-sided). Baseline differences between groups were assessed using univariate analysis of variance (ANOVA). Repeated measures ANOVA were used for the dependent variables thalamic binding ratio, thalamic CBF, blood plasma, HR and 2D-flow, with time as within-subjects variable and citalopram dose as a between-subjects variable. Post-hoc tests are reported with a Sidak's correction. Linear contrasts were used to assess the dose-dependency of this effect. In addition, Spearman's correlation analyses were used to assess the association between the change in SPECT, change in phMRI and blood plasma levels.

*Sample size estimation*

A previous [123I]β-CIT SPECT study observed statistically significant reductions in thalamic SERT binding of about 72% following an oral pre-treatment with 20 mg citalopram even in 6 subjects[24]. Furthermore, statistically significant citalopram-induced changes were detected using ASL-based phMRI in 10 MDMA users when compared to 7 control subjects [13]. Furthermore, the sample size needed to detect a reproducible effect of 16 mg oral citalopram with phMRI in the thalamus (which is also the region of interest in this study) was 11 subjects[25]. In the current study, we also want to assess smaller effects of 40% SERT occupancy. Therefore, we have set our sample size to N = 15 per group.

**Results**

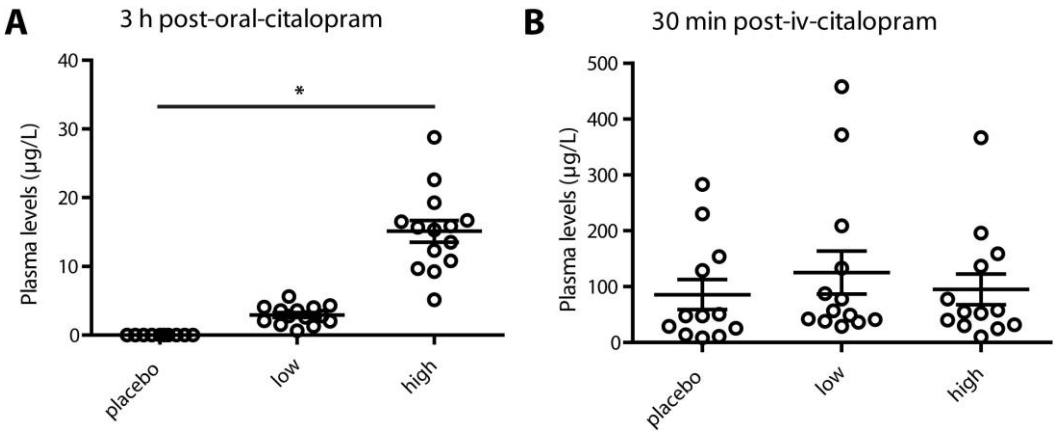
*Citalopram plasma levels*

The oral dosage of citalopram was well tolerated by all subjects, and positively associated with blood plasma levels of citalopram (Figure 2). Three hours after oral citalopram, a Kruskall-Wallis test showed a difference between the groups ($H=33.03$; $p<0.001$) and post-hoc Mann-Whitney-U tests showed that all groups differed from each other ($p<0.001$). A Kruskall-Wallis test showed no difference between groups in citalopram plasma levels after iv citalopram ($H=1.48$; $p=0.48$).

*SPECT*

SPECT data were missing for three subjects (N=2 from the low group and N=1 from the high group) due to technical problems (data could not be reconstructed). At baseline, no significant differences in thalamic binding between the groups was observed ($F(2,39)=0.42$; $p=0.66$). We observed a significant time x group interaction ($F(2,39)=11.22$; $p<0.001$) (Figure 3) as a result of SERT displacement by citalopram. The post-hoc tests showed that the high group showed significantly higher displacement (representing SERT occupancy) compared to the placebo group (-40.5% $p<0.001$), as well as a clear trend for a significant displacement in the low group compared to the placebo group (-24.5% $p=0.05$). No difference was found between the low and high condition ($p=0.12$), but the dose-dependent effect was confirmed by a linear contrast ($p<0.001$).

**Figure 2.** Blood plasma levels.
Blood samples were collected before the second SPECT scan and after the phMRI scan. Citalopram plasma levels (µg/L) were determined using mass spectrometry. A) Citalopram plasma levels prior to the second SPECT scan at 3 hours post-oral-citalopram. B) Citalopram plasma levels after the phMRI scan at 30 min post-iv-citalopram. * ANOVA: $p<0.05$




*phMRI*

For one subject ASL data were missing due to nausea (N=1 from the placebo group). Before the intravenous citalopram challenge, pre-treatment conditions did not affect thalamic CBF ($F(2,41)=2.57$; $p=0.09$). However, pre-treatment conditions did significantly affect the phMRI response to the intravenous citalopram (time x group interaction: $F(2,41)=3.46$; $p=0.04$). Post-hoc tests showed non-significant differences between the groups (high vs placebo: $p=0.05$; high vs low: $p=0.15$, low vs placebo=0.95). Compared to baseline (i.e. prior to the intravenous citalopram challenge), the high group did not show a significant decrease in thalamic CBF (+4.1% $p=0.67$), but CBF was reduced in the low group (-6.71% $p=0.03$) and in the placebo group (-11.92% $p=0.005$) and this dose-dependent effect was confirmed by a linear contrast ($p=0.02$).

*Cardiovascular effects*

No differences in HR were observed prior to the ASL scan (i.e. before the iv citalopram, but after the oral citalopram) ($F(2,36)=0.41$; $p=0.66$). A main effect of citalopram on HR was found ($F(1,36)=11.47$; $p=0.002$), but no interaction was found with group ($F(2,36=0.23$; $p=0.80$) (Figure 5a). There was no baseline effect of group on 2D-flow ($F(2,41)=1.31$; $p=0.28$), nor a main effect of citalopram on 2D-flow

(F(1,39)=0.15; p=0.70), nor an interaction with group was observed (F(2,39)=1.01; p=0.37) (Figure 5b). Likewise, no main effect or interaction effect was found on GM CBF (main effect of time: F(1,41)=0.01; p=0.91, interaction effect F(2,41)=1.14; p=0.33).

*Correlational analyses*

Citalopram plasma levels prior to the second SPECT scan correlated significantly with the percent change in thalamic BPND (r=-0.48 p=0.002) and non-significantly with thalamic CBF (r=0.32 p=0.052). The percentage displacement in thalamic BPND also showed a non-significant correlation with percentage change in CBF (r=-0.28 p=0.07).

**Figure 3.** SPECT results.
A) Representative T1w image with coregistered SPECT data (red/yellow) and the individual thalamic mask (green) superimposed. B) Scatter-dot plot of individual data points from the second SPECT scan and C) the percentage change in BP, normalized to the placebo group, from pre- to post oral citalopram.
* ANOVA: p<0.05

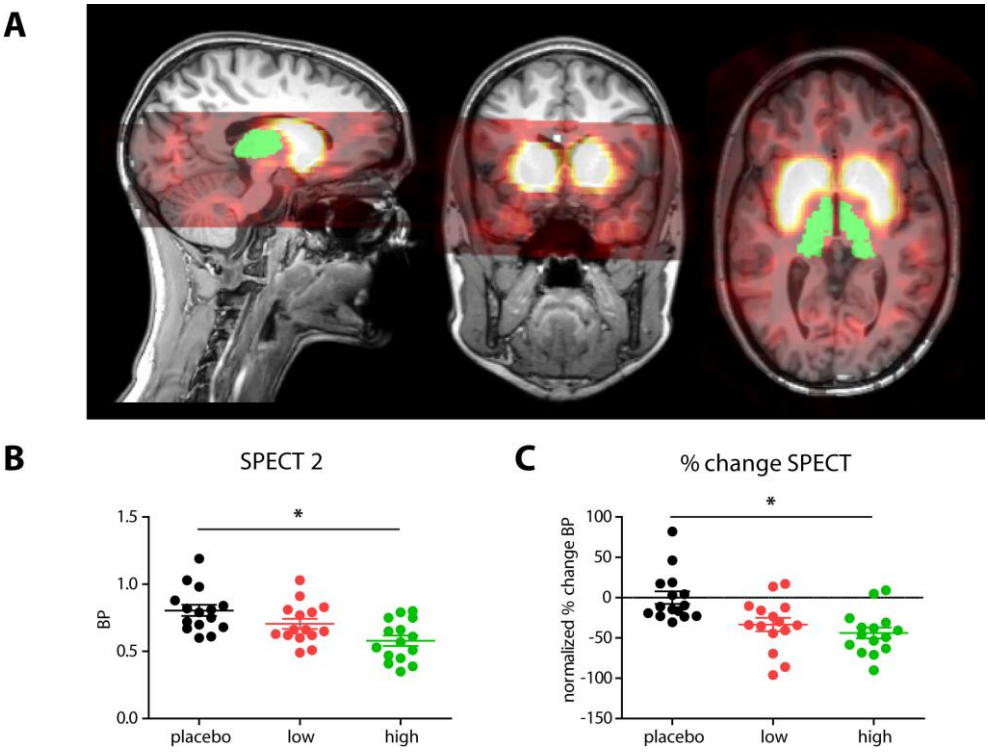


**Figure 4.** ASL results.
A) Representative CBF image masked by the individual gray matter mask with the individual thalamic mask (blue) superimposed. B) Spaghetti plot of the individual change in CBF and C) scatter-dot plot of the percentage change from pre to post intravenous citalopram. * ANOVA: p<0.05

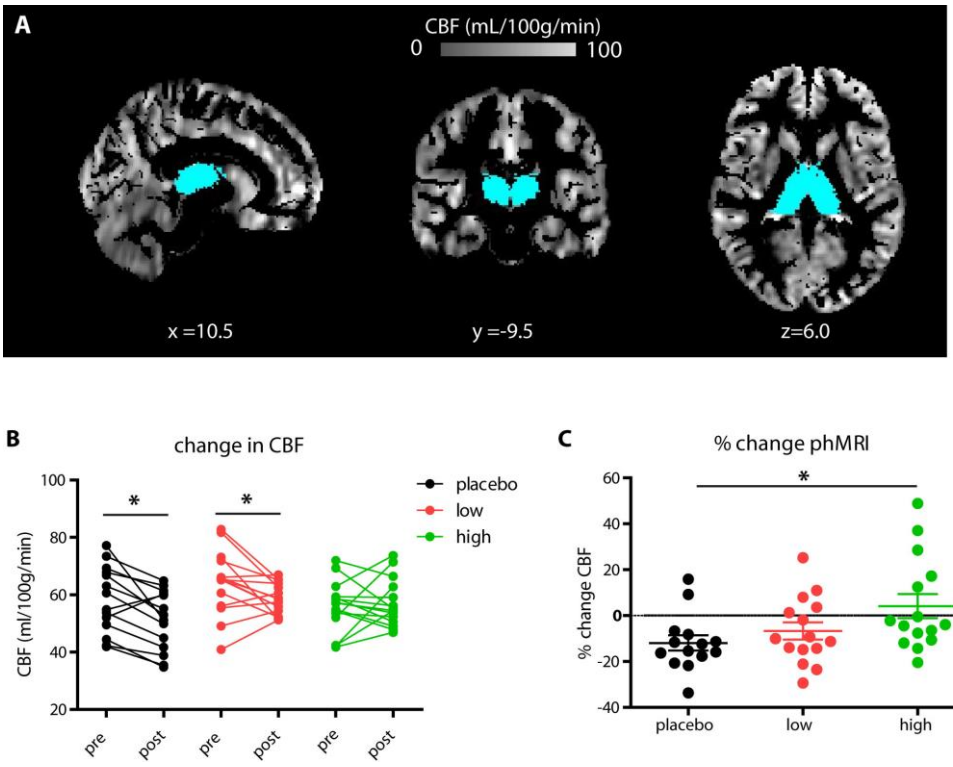
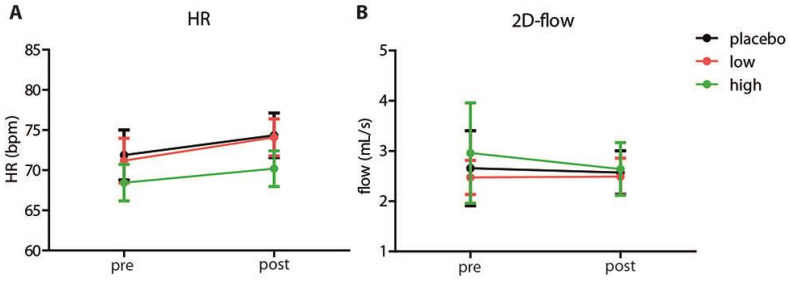



**Figure 5.** Cardiovascular effects
A) Heart rate was measured during the ASL scan session. Pre and post intravenous citalopram averages (over the same 5 minutes over which the CBF was calculated) are shown in beats per minute. B) 2D-flow was measured just before and straight after the ASL scan. Pre and post blood flow to the brain in the intracranial arteries is shown in mL/s. Data represent mean ± standard error of the mean



**Discussion**

We investigated whether phMRI was sensitive enough to detect dose-related hemodynamic responses to the SSRI citalopram. In addition to replicating dose-dependent occupancy of the SERT using SPECT, we now demonstrate that also the phMRI response is dependent on SERT occupancy; the higher citalopram plasma levels and higher SERT occupancy were associated with reduced phMRI signal following an intravenous citalopram challenge. The fact that phMRI signal changes did not strongly correlate with changes in SPECT measurements, indicates that these techniques, at least partially, assess different functional aspects of the serotonergic neurotransmission.

*SPECT*

Previous PET and SPECT studies using the selective SERT radiotracers [11C]DASB and [123I]ADAM have shown dose-dependent associations of SERT occupancy with both dose and plasma levels of SSRIs[4,17,26]. Our results are comparable to these studies, because we also observed a time x group interaction and found an association between plasma levels and SERT occupancy. However, although typical therapeutic doses (16-32 mg for citalopram in liquid drops) are associated with an ~80% SERT occupancy, we only found a ~50% displacement of the [123I]FP-CIT radioligand at a citalopram dose of 16 mg. This could be due to various reasons, the most likely one being our administration of citalopram after the [123I]FP-CIT injection, implying that citalopram is required to displace the radioligand. As in-vivo [123I]FP-CIT binding showed a slow Koff[27], this probably resulted in lower than expected differences between the placebo and high dose groups. However, Ziebell et al.[28] also administered citalopram after [123I]FP-CIT and did obtain a 63% displacement, but they used a higher dose which was administered intravenously. Additionally, [123I]FP-CIT does not selectively bind to the SERT, but also binds to the DAT, and therefore at least some of the binding in the thalamus could result from DAT binding, which then cannot be displaced by citalopram. Nevertheless, when comparing plasma concentrations to a previous study[4], our mean citalopram concentration of 15.1 μg/L does correspond with an approximately 50% SERT occupancy. Although we found, similar to previous studies, a positive relationship between plasma levels and SERT displacement, there is much variability within the oral dosing groups. This could be a result of individual physiological variation in metabolism and SERT availability, as was also apparent in the baseline SERT measurements and has been shown previously for this radioligand[29].

*phMRI*

In this study, we assessed for the first time whether the phMRI response is dependent on different doses of SERT blockade. We found a non-significant relation between plasma concentrations and CBF response. The phMRI literature has shown both in animal models and human studies that SSRI administration induced a hemodynamic response. For example, administration of both the SSRIs fluoxetine[10,30] and citalopram [31] elicited increased BOLD responses in rats. In humans, intravenous citalopram increased the BOLD response in the striatum, amygdala, hippocampus and thalamus[11]. Using ASL, both oral and iv administration of citalopram was assessed. Chen et al.[12] demonstrated decreased CBF in the amygdala, fusiform gyrus, insula, and orbitofrontal cortex after oral dosing, whereas Schouw et al.[13] showed decreased thalamic CBF in MDMA users (with presumed serotonergic depletion[32]) following an intravenous challenge. In this study, after oral dosing, i.e. at the baseline phMRI scan, we could not detect significant differences with the placebo group in the thalamus. However, significant group differences were found in the phMRI response to the intravenous citalopram challenge. As hypothesized, the group with the highest pre-dose of citalopram showed no change in CBF, whereas the placebo group showed the largest change. This indicates that phMRI can indeed detect a therapeutically relevant blockade of SERT. However, the subclinical dose (low group) did not result in a different phMRI response compared to placebo, although CBF was statistically significantly reduced from baseline. This either implies that such small changes in SERT binding do not influence the serotonin neurotransmission to a large extent, or that sensitivity of phMRI needs to be improved. In addition, we found a significant relation between plasma concentrations and CBF response, again confirming that phMRI can detect changes in SERT occupancy.

*Comparison between SPECT and phMRI*

Interestingly, we did not find a strong correlation between our SPECT and phMRI measurements. This a presumably because both techniques measure different aspects of the serotonergic system: SPECT measures transporter binding directly, whereas phMRI is an indirect measure. PhMRI measures the hemodynamic response in the whole synapse, including both pre- and postsynaptic transporters and receptors as well as serotonin release. The SPECT displacement explained a small portion of the variance of the phMRI response, implying that part of the response is indeed regulated by the SERT response. Some preclinical studies have shown that the phMRI response is indeed linked to

neurotransmitter release [33] while pre- and post-synaptic processes were shown to make up the lion's share of the synapse energy expenditure, therefore likely inducing the strongest changes in CBF.

Thus, the phMRI response is probably a sum of the intravenous citalopram binding to the SERT, which increases serotonin in the synaptic cleft, which can then bind to post-synaptic receptors and transmit the neuronal signal. Another explanation for the low correlation is that [123I]FP-CIT is not a selective SERT tracer and our measurements could have also included some DAT binding.

*Methodological and clinical considerations*

One of the limitations of our current study is that we only included female volunteers, and our results can thus not be extrapolated to males. Nevertheless, previous studies have shown considerable sex effects of [123I]FP-CIT on thalamic binding[29] and by only including women, variability in the current study was reduced. In addition, we chose to focus on the thalamus, as this area is rich in SERT (with 20 times more SERT than DAT[29]), making this ROI the ideal candidate to address our research question with the [123I]FP-CIT tracer. However, more SERT-selective PET and SPECT radiotracers, such as [11C]DASB and [123I]ADAM, may assess the raphe nuclei and a number of different serotonergic projections[34]. With regards to the phMRI, CBF was chosen as an outcome measure for drug-induced perfusion changes. As previous studies have also assessed the BOLD response to citalopram, it would be interesting for future research to apply a dual-echo ASL sequence to obtain both changes in CBF and BOLD. This could improve phMRI signal sensitivity and possibly provide a non-ionizing alternative to PET/SPECT for use in trials for treatment monitoring or drug development. However, with its current sensitivity ASL phMRI is not yet suitable to for patient-specific predictions.

**Conclusion**

This study demonstrated that SPECT and phMRI can detect changes in SERT occupancy. The phMRI response can only partially be explained by SERT occupancy. We show that phMRI is a promising technique, as it allows to study biologically relevant variations and abnormalities in the serotonergic system, e.g. in response to SSRI treatment. It can thereby complement common molecular imaging approaches like PET and SPECT.

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