Modulation of functional networks related to the serotonin neurotransmitter system by citalopram: evidence from a multimodal neuroimaging study

Daphne Boucherie[1], Liesbeth Reneman[1], Jan Booij[1], Daniel Martins[2], Ottavia Dipasquale[2]*, Anouk Schrantee[1]*

[1] Department of Radiology and Nuclear Medicine, Amsterdam UMC, location Amsterdam Medical Center, Amsterdam, the Netherlands

[2] Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK

* These authors contributed equally

Abstract

Selective serotonin reuptake inhibitors (SSRIs) potentiate serotonergic neurotransmission primarily by blocking the serotonin transporter (5-HTT), but the functional brain response to SSRIs involves neural circuits beyond regions with high 5-HTT expression. Currently, it is unclear whether and how changes in 5-HTT availability after SSRI administration modulate brain function of key serotoninergic circuits, including those characterized by high availability of serotonin 1A receptors (5-HT1AR). Here, we investigated the association between 5-HTT availability and 5-HTT- and 5-HT1AR-enriched functional connectivity (FC) after an acute citalopram challenge. We analyzed multimodal data from a dose-response, placebo-controlled, double-blind study, in which 45 healthy women were randomized into three groups receiving placebo, a low (4 mg), or high (16 mg) oral dose of citalopram. Receptor-Enhanced Analysis of functional Connectivity by Targets was used to estimate 5-HTT- and 5-HT1AR-enriched FC from resting-state fMRI and task-based fMRI data. 5-HTT availability was determined using [^{123}I]FP-CIT single-photon emission computerized tomography. 5-HTT availability was negatively correlated with resting-state 5-HTT-enriched FC, and with task-dependent 5-HT1AR-enriched FC. We also found lower 5-HT1AR-enriched FC in the low dose group compared to the high dose group at rest and to the placebo group during the task. Our findings provide evidence for differential links between 5-HTT availability and brain function within 5-HTT and 5-HT1AR pathways that are likely context- and dose-dependent, supporting a potential pivotal role of the 5-HT1AR in the effects of citalopram on the human brain and adding to the interest in this target as a potential therapeutic avenue for mood and anxiety disturbances.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs), such as citalopram, are frequently used to treat depression and anxiety disorders [1,2]. Despite their frequent prescription, approximately one third of patients with depression [3] and anxiety [4] fails to respond to this treatment. Uncovering mechanisms that could account for interindividual differences in treatment response is thus paramount to inform the development of new and more effective therapeutic approaches. However, the current lack of a full mechanistic understanding of the effects of SSRIs on the human brain has marred substantial advances in this direction.

While the exact mechanisms underlying the therapeutic effects of SSRIs remain contentious, the primary pharmacological mode of action entails blocking the serotonin (5-HT) transporter (5-HTT) [5], resulting in increased extracellular levels of 5-HT. Acute SSRI administration thereby results in activation of both the postsynaptic serotonin-1A receptor (5-HT1AR) in projection sites and 5-HT1A autoreceptors of the dorsal raphe nucleus (DRN) [6]. Activation of the latter results in hyperpolarization, thereby diminishing 5-HT release from the 5-HT nerve terminals in the synapses and inhibiting neuronal activity [7]. Consecutively, chronic SSRI treatment is thought to induce desensitization of DRN 5-HT1A autoreceptors, thereby decreasing their inhibitory effect and allowing increased 5-HT binding to postsynaptic 5-HT receptors [8-10]. As such, 5-HT1ARs are thought to play a pivotal role in controlling 5-HT neuromodulation in the brain and have been suggested to contribute to the antidepressant properties of SSRIs [7,11].

Our current knowledge of the effects of SSRIs on the human brain has greatly benefited from the use of non-invasive neuroimaging techniques. Particularly, the combination of functional magnetic resonance imaging (fMRI) with an acute pharmacological challenge, i.e. pharmacological MRI (phMRI), has offered an unprecedented opportunity to investigate acute drug effects on brain function at the systems level, which is essential for understanding the psychopharmacological effects of drugs affecting widespread neuromodulatory systems such as 5-HT. Studies employing phMRI at rest have demonstrated that SSRIs modulate activity and connectivity both in brain regions with high 5-HTT density and in their projection areas, likely reflecting downstream effects via 5-HT (and other neurotransmitter) receptors. For example, SSRI administration has been found to alter connectivity within and with the Default Mode Network (DMN) [12-16], which has

frequently been associated with rumination in depression [17]. Furthermore, task-based studies using emotion recognition paradigms have frequently reported altered activation in (networks encompassing) the limbic system in response to SSRIs [18-22], which has been implicated in aberrant emotion regulation in depressed patients [23]. However, how engagement of 5-HT molecular targets, including the 5-HTT and 5-HT1AR, might contribute to these circuit-level effects remains poorly understood.

Multimodal studies combining phMRI with molecular imaging have shed further light on the involvement of specific 5-HT receptors in modulating FC and SSRI-induced changes in brain function. For instance, one study found that 5-HTT availability following citalopram administration was associated with FC between the DMN and cortical regions [15], highlighting a prominent role of 5-HTT in modulating the effects of SSRIs on DMN connectivity. Moreover, Hahn *et al.* [24] emphasized the importance of 5-HT1AR in regulating DMN connectivity by demonstrating diverse and area-specific patterns of associations between the FC of the posterior cingulate cortex (PCC), a core node of the DMN, and 5-HT1A autoreceptor and local heteroreceptor binding. Noteworthy, 5-HT1AR has also been hypothesized to be a key modulator of neural circuits underlying emotional processing in task-based fMRI studies, as evidenced by associations between DRN 5-HT1AR binding and (changes in) amygdala activation during emotion processing [21,25]. Given the prominent role of 5-HT1AR in regulating the 5-HT system, imaging studies dissecting the pharmacodynamics of SSRIs in the brain and its correspondent interindividual variability should incorporate concomitant investigations of the relation between post-drug increases in 5-HT and modulations of neural circuits associated with specific 5-HT receptor subtypes, such as 5-HT1AR. Crucially, both resting-state and task-based paradigms seem to capture interindividual variation in molecular target bioavailability on brain function, and could provide new insights on the context-dependent modulation of 5-HT brain networks by SSRIs.

To this end, we conducted a multimodal study combining single-photon emission computerized tomography (SPECT) with Receptor-Enriched Analysis of functional Connectivity by Targets (REACT) [26] to investigate how acute citalopram-induced variations in 5-HTT availability relate to FC of 5-HTT- and 5-HT1AR-enriched functional networks at rest and during an emotional face-matching task. Forty-five healthy women were randomized into three treatment groups receiving placebo, a low (4mg) or high (16mg) oral dose of

citalopram; SPECT, resting state fMRI (rs-fMRI), and task-based fMRI (tb-fMRI) were acquired within the same participants to allow between-modality associations across treatment. In line with previous findings, we hypothesized an inverse relationship between 5-HTT availability and 5-HTT- and 5-HT1AR-enriched FC both at rest and during the task. In accordance with previous studies showing that amygdala activation during emotion recognition is associated with DRN 5-HT1AR binding [21,25], we expected a stronger association between 5-HTT availability in task-dependent FC and the 5-HT1AR-enriched functional network compared to the 5-HTT-enriched functional network.

MATERIALS AND METHODS

Participants

Forty-five healthy female volunteers (HC, mean age=21.6 years, range=18–28) were enrolled in the study. Exclusion criteria included a history of a chronic neurological/psychiatric disorder, family history of sudden heart failure, current use of psychostimulant medication, abnormal electrocardiogram, excessive consumption of alcohol (>21 units/week), caffeine (>8 cups/day), or nicotine (>15 cigarettes/day), and neuroimaging contraindications. The Mini-International Neuropsychiatric Interview Plus was used to screen for psychiatric illnesses and drug abuse. To avoid confounding effects of the hormonal cycle, participants were required to use hormonal contraception. The study protocol was approved by the medical ethics committee of the Academic Medical Center in Amsterdam and in accordance with the Declaration of Helsinki. All subjects gave written informed consent. Prior analyses on the same sample assessed the relation between 5-HTT availability and DMN FC [15] and dose-dependent effects on 5-HTT availability and arterial spin labeling phMRI [27].

Study design

We used a placebo-controlled, dose-response, double-blind design (Figure 1). Following a baseline SPECT scan two hours post-injection of [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4iodophenyl)nortropane ([^{123}I]FP-CIT; data not shown), participants were randomly assigned to one of three treatment groups: placebo (n=15), low dose (4mg; n=15), or high (clinical) dose (16mg; n=15) of citalopram (oral solution, 16mg equivalent to 20mg in tablet form, Lundbeck). These doses correspond to 5-HTT occupancy levels of 0%, 40%, and

80%, respectively [28]. Participants underwent a second SPECT scan to assess 5-HTT occupancy by placebo or citalopram three hours after drug intake, followed by an MRI scan one hour later, which included a rs-fMRI and a tb-fMRI scan during an emotional face-matching task.

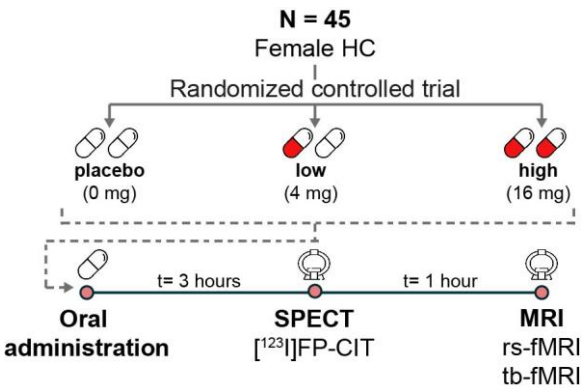

Figure 1. Experimental design

HC: healthy controls; mg: milligram; t: time; SPECT: single-photon emission computerized tomography; [^{123}I]FP-CIT: [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane; BP: binding potential; fMRI: functional Magnetic Resonance Imaging, rs-fMRI: resting-state fMRI; tb-fMRI: task-based fMRI

SPECT acquisition and analysis

SPECT scans were acquired using a brain-dedicated InSPiraHD SPECT camera (Neurologica, Boston, USA), as described previously [15,27]. 3D images were reconstructed and a region-of-interest (ROI) analysis was performed. As the radioligand [^{123}I]FP-CIT has been shown to bind with high affinity primarily to the dopamine transporter in the striatum, and primarily to the 5-HTT in extrastriatal areas such as the thalamus [29], we determined 5-HTT binding in the thalamus with the cerebellum as a reference region reflecting non-specific binding. Specific to non-specific binding ratios (binding potential; BP) after drug administration were calculated, reflecting 5-HTT availability (here, a lower BP reflects a higher occupancy and thus a lower availability of the 5-HTT).

MRI acquisition and preprocessing

MRI data was acquired using a 3.0T Ingenia scanner (Philips, Best, The Netherlands) with a 32-channel receive-only head coil. A high-resolution 3D T1w scan was obtained (TR/TE=3195/7ms; FOV=256×256×180; voxel size=1x1x1mm; flip angle=9°). Rs-fMRI data were acquired using a gradient-echo echo-planar imaging sequence: TR/TE=2150/27ms; FOV=240×240×131mm, voxel size=3×3×3mm; gap=0.3mm; flip angle=76.2°; dynamics=240(~9min). Participants were instructed to keep their eyes open, focus on a fixation cross, and let their mind wander. Tb-fMRI data were acquired with the following

parameters: TR/TE=2300/30ms, FOV=220x220x117mm; resolution=2.3×2.3×3mm, 39 sequential slices, flip angle=80°, dynamics=70(~3min). The emotional face-matching fMRI paradigm (adapted from [30]), consisted of a blocked design with emotional stimuli displaying angry and fearful faces (*faces*) and neutral stimuli displaying ellipses assembled from scrambled faces (*shapes*). Two blocks of emotional stimuli were interleaved with three neutral blocks, with each 30-s block containing six 5-s trials. Three stimuli were displayed simultaneously, and individuals had to choose which of the lower two stimuli showed the same emotion or orientation as the target stimulus above, for the emotional and neutral trials respectively.

All fMRI data were preprocessed using fMRIPREP v.20.0.6 [31], which is based on *Nipype* 1.4.2 [32]. T1-weighted scans were normalized to MNI space. Motion correction (FLIRT), distortion correction (*fieldmap-less*), and T1-weighted coregistration were performed as part of the functional data preprocessing. To generate non-aggressively denoised data, Independent Component Analysis-based Automatic Removal Of Motion Artifacts was employed. Data were spatially smoothed (6mm FWHM; FSL/FEAT v.6.0.0). See Supplementary Methods for further details. Following preprocessing with fMRIPREP, white matter and cerebrospinal fluid (obtained from fMRIPREP) were regressed out of the main signal using FSL v6.0.0, followed by high pass-filtering (100s). One subject with insufficient scan quality was removed from all analyses and three subjects exhibiting high motion (mean framewise displacement>0.25mm) were excluded from the rs-fMRI analysis.

Target-enriched FC at rest

A high-resolution in vivo atlas of the distribution density of 5-HTT and 5-HT1AR [33] was used for the REACT analysis (Figure 2). In a two-step multiple linear regression employing REACT [26] and FSL, the atlas was used as a molecular template to estimate the 5-HTT- and 5-HT1AR-enriched FC at rest. In the first step, the fMRI signal in the gray matter was weighted using the molecular templates as a spatial regressors to estimate the dominant blood-oxygen-level-dependent (BOLD) fluctuations of the 5-HTT- and 5-HT1AR-enriched functional systems at the subject level. The cerebellum was excluded at this stage as it was used as a reference region in the kinetic model for both systems [33]. In the second step, the subject-specific time series estimated in the first step were used as temporal regressors to estimate the subject-specific spatial maps of 5-HTT- and 5-HT1AR-enriched FC.

Target-enriched functional response during the emotional face-matching task

For the tb-fMRI analysis, the dominant BOLD time series of the target-enriched functional systems were estimated at the subject level as described previously. In the second step, the resulting subject-specific time series were used to estimate the 5-HTT- and 5-HT1AR-enriched faces>shapes functional response using a generalized psychological-physiological interaction design with five regressors, namely the convolved faces>shapes regressor, combining both *faces* and *shapes* blocks (task effect), the BOLD time series of the 5-HTT- and 5-HT1AR-enriched functional systems (task-independent target-enriched FC), and the regressors of the interaction between the convolved faces>shapes regressor and these BOLD time series (task-dependent target-enriched FC).

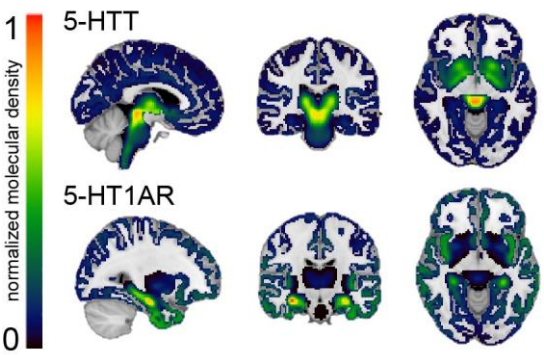

Figure 2. Normalized high-resolution in vivo maps of the distribution density of the serotonin transporter (5-HTT) and serotonin 1A receptor (5-HT1AR)

5-HTT and 5-HT1AR density distributions are normalized between 0 and 1 intensity (with 0 indicating a low density and 1 a high density) and overlaid onto a 1 mm Montreal Neurological Institution (MNI) template brain. The normalized distribution density of the 5-HTT is characterized by high density in the entorhinal and insular cortices, subcortical regions and the raphe nucleus (top). The normalized distribution density of the 5-HT1AR shows high density in the raphe nucleus, hippocampus, septum, amygdala and corticolimbic areas (bottom).

Statistical analyses

All voxelwise analyses were conducted using permutation tests in Randomise (5000 permutations; threshold-free cluster enhancement [34]; significance inferred when family wise error corrected $p<0.05$).

For the rs-fMRI analysis, we evaluated the voxel-wise correlation of the target-enriched FC maps with the individual thalamic [^{123}I]FP-CIT BP. Subsequently, we conducted exploratory analyses to assess differences in the target-enriched FC maps between treatment groups using two-sample t-tests, applying a Bonferroni correction for multiple comparisons.

For the tb-fMRI analysis, we investigated the voxel-wise correlation of the task activation map and target-enriched task-dependent FC maps with the individual thalamic [^{123}I]FP-CIT

BP. We also conducted exploratory analyses to assess group differences in task (de)activation and task-dependent FC. Here, we first identified clusters showing significant activity/connectivity in the placebo group using a one-sample t-test (to exclude drug-induced effects on the target-enriched FC). Using a small volume correction within these clusters, we subsequently assessed group differences using two-sample t-tests applying a Bonferroni correction for multiple comparisons. To assess the influence of our decision to use a small volume correction mask defined from the placebo group only, we conducted a sensitivity analysis where we first identified clusters showing significant activity/connectivity in all groups combined using a one-sample t-test, and then repeated the between-group comparisons as described above (Supplementary Results).

RESULTS

Target-enriched FC at rest

The mean 5-HT1AR- and 5-HTT-enriched FC maps are visualized in Figure S1.

The correlation analyses revealed a significant negative relationship between the thalamic [^{123}I]FP-CIT BP and 5-HTT-enriched FC, such that subjects with lower thalamic 5-HTT availability (high occupancy by citalopram) showed higher FC in some regions of the 5-HTT-enriched FC maps, including in the planum polare, central opercular cortex, temporal and occipital fusiform gyrus, temporal gyrus, and opercular cortex (Figure 3A). No significant relationship was found between thalamic [^{123}I]FP-CIT BP and 5-HT1AR-enriched FC at rest.

The exploratory analyses highlighted significant group differences in 5-HT1AR-enriched FC, such that FC of the low dose group in the posterior cingulate gyrus, anterior cingulate gyrus and thalamus was lower compared to the high dose group (Figure 3B). Further details on significant clusters can be found in Table S1. No significant pairwise group differences in 5-HTT-enriched FC were observed.

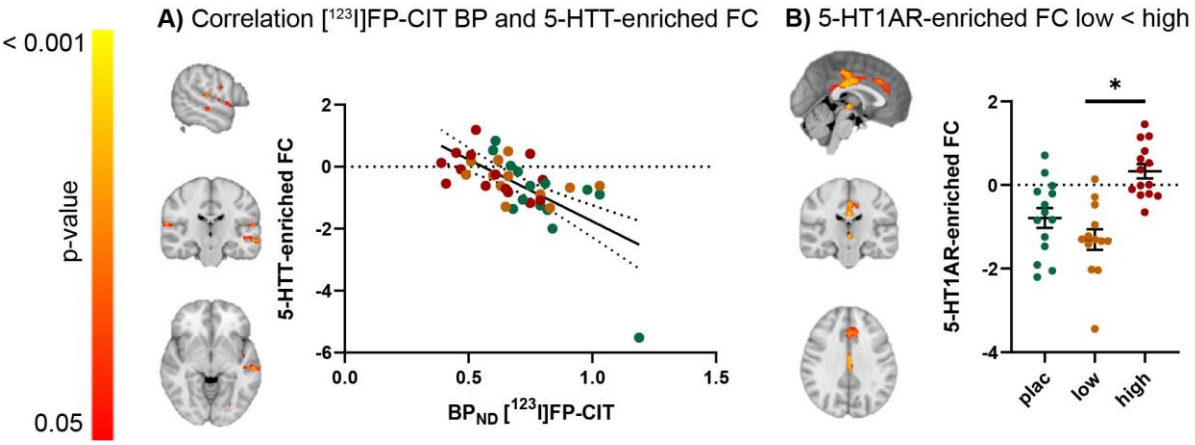

Figure 3. The relationship between thalamic [^{123}I]FP-CIT binding potential (BP) and serotonin transporter (5-HTT)- and serotonin 1A receptor (5-HT1AR)-enriched functional connectivity (FC) at rest

A) *Clusters showing a significant negative correlation between thalamic [^{123}I]FP-CIT BP and 5-HTT-enriched FC (left) and scatter dot plot illustrating this correlation (right).* ***B)*** *Clusters showing significantly lower FC for the 5-HT1AR-enriched FC in the low dose group compared to the high dose group (left) ($p < 0.05$; FWE corrected) and scatter dot plot illustrating these results per treatment group (right). Error bars indicate mean±SEM.*
[^{123}I]FP-CIT: [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane; low: low dose of citalopram (4 mg); high: high dose of citalopram (16 mg).

Target-enriched functional response during the emotional face-matching task

The mean z-score maps of all task-based regressors are depicted in Figure S2.

We observed a significant negative association between the thalamic [^{123}I]FP-CIT BP and task-dependent 5-HT1AR-enriched FC. Specifically, subjects with lower thalamic [^{123}I]FP-CIT BP (i.e. high occupancy by citalopram) had higher FC in specific areas of the task-dependent 5-HT1AR-enriched maps, including the precentral gyrus, posterior cingulate and precuneus, opercular cortex, superior and middle frontal gyrus, frontal and temporal pole, temporal gyrus, thalamus, and left putamen (Figure 4A). We did not find a relationship between thalamic [^{123}I]FP-CIT BP and task-dependent 5-HTT-enriched FC.

The exploratory analysis showed a significant dose-dependent decrease in 5-HT1AR-enriched FC in the low dose group compared to the placebo group in the precuneus and lateral occipital cortex (Figure 4B). Cluster sizes and locations can be found in Table S2. No differences were found between the placebo and the high dose group, and between the low and the high dose groups. No group differences were observed in the task-dependent 5-HTT-enriched FC maps. Of note, the results of this exploratory analysis did not change

significantly when using a small volume correction cluster derived from all groups combined (Figure S3 and Table S3).

No significant relationship was found between thalamic [^{123}I]FP-CIT BP and task activation. The exploratory analysis did not show group differences in task activation.

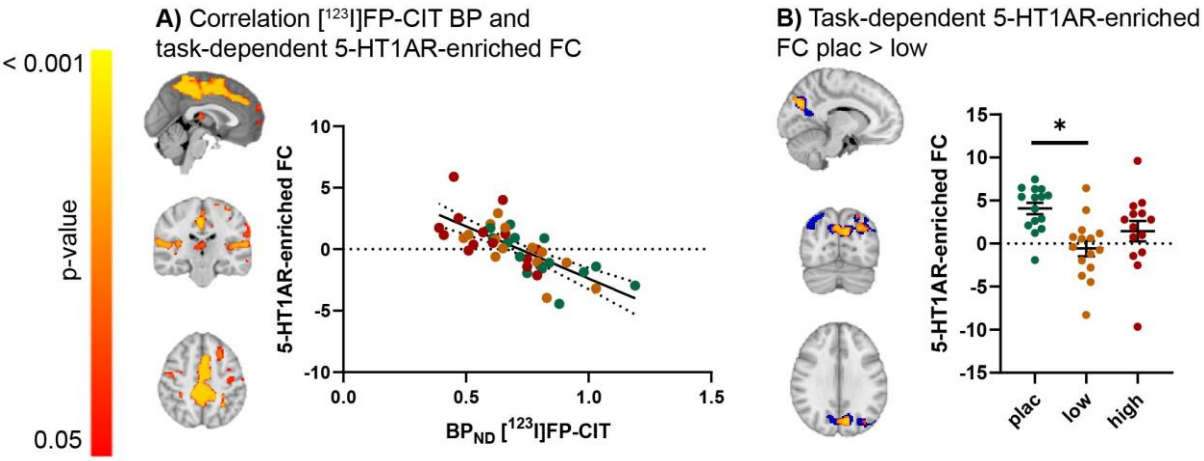

Figure 4. The relationship between thalamic [^{123}]FP-CIT binding potential (BP) and serotonin 1A receptor (5-HT1AR)-enriched task-dependent functional connectivity (FC) during an emotional face-matching task

A) *Clusters showing a significant negative correlation between thalamic [^{123}I]FP-CIT BP and task-dependent 5-HT1AR-enriched FC (left) and scatter dot plot illustrating this correlation (right).* ***B)*** *Blue: clusters showing significant task-dependent 5-HT1AR-enriched FC, corresponding to the interaction between the convolved faces>shapes regressor and the dominant blood-oxygen level-dependent (BOLD) fluctuation related to the 5-HT1AR-enriched functional network, for the placebo group only (One sample t-test; p <0.05; FWE corrected). These areas were used as a small volume correction to constrain between-group comparisons. Red/yellow: clusters showing significant citalopram-induced dose-dependent differences in the 5-HT1AR-enriched task-dependent FC (p <0.05; FWE corrected) (left) and scatter plot depicting these group differences (right). Error bars indicate mean±SEM.*

[^{123}I]FP-CIT:[^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4iodophenyl)nortropane; plac: placebo; low: low dose of citalopram (4 mg); high: high dose of citalopram (16 mg)

DISCUSSION

In this study, we aimed to investigate how 5-HTT availability during an acute citalopram challenge is differentially associated with FC of 5-HTT- and 5-HT1AR-enriched networks at rest and during an emotional face-matching task. We found thalamic [^{123}I]FP-CIT BP to be negatively associated with 5-HTT-enriched FC at rest, and with 5-HT1AR-enriched FC during an emotional face-matching task. Additionally, exploratory analyses revealed dose-dependent effects of citalopram on 5-HT1AR-enriched FC both at rest and during the task.

The (expected) negative relationship we observed between thalamic 5-HTT availability and 5-HTT-enriched FC showcases a direct link between citalopram-induced changes in neurotransmission and functional changes within a network defined by the canonical distribution of the drug's main target in the healthy human brain. Therefore, our results align well with predictions from the simplest pharmacodynamic models that posit regional variability in 5-HTT density at the core of pharmacological effects on the brain. Interestingly, we observed that 5-HTT-enriched FC was less strong for individuals with lower 5-HTT availability in clusters encompassing the occipito-temporal and opercular cortex, which have previously been shown to exhibit alterations in connectivity and/or activity in patients with depression compared to healthy controls [35,36]. The occipito-temporal cortex has been shown to respond to acute (but not chronic) treatment with escitalopram [37]. Contrastingly, we did not observe between-group differences in 5-HTT-enriched FC, which could reflect either a lack of power or unexplained across-group variability in 5-HTT availability that could hinder the identification of average group effects. Indeed, previous studies have highlighted gene polymorphisms in the 5-HTT-linked promoter region, which is known to affect functionality of the 5-HTT [38].

In contrast to our hypothesis, we found no relationship between thalamic 5-HTT availability and 5-HT1AR-enriched FC at rest, suggesting that the 5-HT1AR-enriched functional response to citalopram is at least not linearly dependent on 5-HTT occupancy by citalopram. Interestingly, we found preliminary evidence of group differences in the PCC and anterior cingulate cortex (ACC), which are compatible with a nonlinear dose-response pattern where FC in the low dose group appeared lower in comparison with the placebo group but no differences between the high dose and placebo groups could be identified. We note that these findings should be interpreted with caution since they arise from exploratory pairwise tests in the absence of a significant overarching main effect of group. Moreover, interpreting

this dose-response pattern on the 5-HT1AR-enriched functional network with our current understanding of the pharmacology of citalopram is admittedly challenging. One possible explanation could be that low and high doses of citalopram differently engage auto- and postsynaptic receptors. Here, a low dose could increase 5-HT to a smaller extent, and might predominantly affect auto- or postsynaptic 5-HT1AR; while the larger 5-HT increases induced by the high dose might engage both types of receptors, leading to compensatory effects summing to a null effect. Nevertheless, this hypothesis remains speculatory. It is worth noting that the regions where this effect occurred appear in line with previous literature and are highly relevant within the context of previous studies in depression. For instance, the PCC, as part of the DMN, has frequently been implicated in the functional response to SSRIs [13,16,39,40]. The ACC is known to be densely innervated by serotonergic projections from the DRN, to have a relatively high density of 5-HT1AR and to show FC with both dorsal and medial raphe nucleus [41,42]. Interestingly, a previous study suggested that ACC FC could serve as a predictor in escitalopram treatment outcome in patients [43], suggesting a crucial role for the ACC in the functional response to SSRIs. Whether this is mediated by the 5-HT1AR system remains to be elucidated.

In line with our hypothesis, results from the emotional face-matching task showed a negative relationship between 5-HTT availability and task-dependent 5-HT1AR-enriched FC. 5-HT1AR-enriched FC varied linearly with variations in 5-HTT availability in large clusters of cortical and subcortical areas, which show considerable overlap with the corticolimbic system. Interestingly, while previous studies have shown that limbic system activity during emotional processing is dependent on the 5-HT1AR [21,25], 5-HT1AR-enriched FC in this network is dependent on 5-HTT availability. This emphasizes the important role for the 5-HT1AR in regulating fluctuating levels of extracellular 5-HT in these regions, in which previous studies have shown increased activation and decreased FC in response to emotional stimuli in depressed patients, as well as a normalization to levels similar to HC following SSRIs treatment [44,45]. Outside of the limbic system, our group-level analyses revealed a more complex non-linear dose-response pattern. In the precuneus, an integral component of the DMN which has previously been described to show alterations in activation and FC during emotion processing and following citalopram administration [46,47], we observe positive 5-HT1AR-enriched FC in the placebo group, which seems reduced in the low dose group and, to a lesser extent, in the high dose group. Taken together, our findings support the involvement of 5-HT1AR in shaping how increased levels of 5-HT induced by SSRIs modulate neural circuits involved in emotion processing, adding to the

current enthusiasm around 5-HT1AR targeting compounds as promising therapeutic approaches.

We also investigated main effects of the task and treatment effect on BOLD responses, since this data has not been published previously. The main task effect we observed is in line with previous studies using a similar paradigm, showing increased BOLD signal mostly in key limbic areas [19] and decreased BOLD signal in DMN areas [48]. In contrast to prior literature, no effect of citalopram was observed on BOLD responses during the task contrast, adding to the mixed results from previous studies where an effect of citalopram has not been found consistently. For example, both decreased and increased amygdala activity following acute citalopram in HC was reported during fearful vs. neutral faces, respectively [21,49]. Another study reported that acute citalopram administration enhanced activation of fusiform gyri and thalamus and attenuated response in right lateral OFC and right amygdala for aversive vs neutral faces in HC [19]. However, comparing these studies with ours is challenging, given the differences in citalopram dose and route of administration as well as analysis approach. For instance, all these prior studies used intravenous administration of either 7.5mg or 10mg of citalopram and used a ROI approach. It is possible that we missed small effects of citalopram because of the relatively low power of our study or because the effects of the acute oral and acute intravenous dose used by the previous studies are not comparable.

Citalopram is considered a conventional antidepressant medication, but several other compounds binding to 5-HT receptors have gained a renewed interest for the treatment of depression, including 3,4-methylenedioxymethamphetamine (MDMA) [50] and the psychedelic lysergic acid diethylamide (LSD) [51]. While these compounds have different binding profiles compared to citalopram, target-enriched FC analyses have recently helped to unravel potential overlap in 5-HT receptors involved in the functional response to these compounds. MDMA predominantly binds to 5-HTT (in addition to other monoamine transporters), but is a releasing agent in addition to a reuptake blocker [52]. Using REACT, MDMA administration was found to significantly modulate networks informed by the distribution of both 5-HTT and 5-HT1AR [26]. LSD shows a different pattern of affinity, with high agonist activity at several 5-HT receptors, as well as at dopaminergic D1 and D2 receptors [53]. While not investigating the 5-HTT itself, Lawn *et al.* [54] showed that LSD significantly affected FC within serotonin 1A, 1B, 2A, and dopamine D1 and D2 receptor-

related networks. Interestingly, despite being a direct agonist to the 5-HT1AR, the 5-HT1AR-enriched maps showed only a very localized FC increase, while widespread effects in the serotonin 1B- and dopamine D1 receptor-enriched maps were observed. Therefore, alongside these previous studies, our data supports the idea that REACT has potential in aiding the dissection of brain pharmacodynamics of compounds targeting the 5-HT system. However, as the above-mentioned studies were conducted in healthy volunteers, future work including patients with depression would be of interest as suggested differences in the baseline function of the 5-HT system (including the 5-HT1A autoreceptor) between patients and controls might lead to different drug-related functional outcomes [55].

This study has some limitations. First, the sample included only a relatively small sample of healthy young women, which limits statistical power for detection of smaller effects and the possibility to extrapolate findings beyond women. Therefore, future larger studies attempting to replicate our findings would be welcome. Second, [^{123}I]FP-CIT is a non-selective radioligand that binds to both the dopamine transporter and 5-HTT. However, since the thalamus is mostly devoid of dopamine transporters, thalamic binding provides the most reliable summary estimate of 5-HTT availability [29]. Moreover, it should also be noted that the PET maps used to estimate the 5-HTT- and 5-HT1AR-enriched FC are average maps from PET images of healthy volunteers encompassing both men and women. It is unclear to what extent the distribution of the 5-HTT and 5-HT1AR is generalizable across genders. Finally, as this study investigated only the effects of acute doses of citalopram, future studies should measure the functional response to citalopram at different time points to unravel its long-term effects on the 5-HTT and 5-HT1AR functional circuits.

CONCLUSION

Building on the power of multimodal data acquired within the same participants, our study provides unprecedented empirical evidence linking 5-HTT availability and FC of 5-HTT-enriched (at rest) and 5-HT1AR-enriched (during an emotional face-matching task) functional networks. Moreover, our study highlights the potential pivotal contribution of 5-HT1AR, in addition to 5-HTT, to dose-dependent changes in the functional response after acute citalopram administration. We provide tentative evidence for a complex dose-response pattern in healthy women, which differs between resting-state and an emotional face-matching task, and calls for further studies examining how dose, context and their interaction

might moderate the effects of 5-HT-altering drugs on the brain. Our data supports the added value of REACT when studying the effects of citalopram and drugs alike on the brain. Given that the therapeutic effects of SSRIs are typically evaluated under chronic treatment, longitudinal studies examining 5-HTT- and 5-HT1AR-enriched FC changes over the course of treatment in patients, as well as its relationship with clinical response, would be interesting avenues to pursue in future research.

AUTHOR CONTRIBUTIONS

AS, LR and JB designed the study and AS collected the data. DB, AS and OD conducted the analyses. DB wrote the first draft of the manuscript. All authors contributed to the interpretation of findings and drafting the manuscript. All authors approved the final version of the manuscript.

FUNDING

Funding for this study was provided by ERA-NET PrioMed- Child (joined call of Priority Medicines for Children 40-41800-98-026). The brain-dedicated SPECT camera was funded by The Netherlands Organisation for Health Research and Development (ZonMw 91112002). AS is supported by an NWO ZonMw Veni 016.196.153.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

1 Baldwin DS, Asakura S, Koyama T, Hayano T, Hagino A, Reines E, et al. Efficacy of escitalopram in the treatment of social anxiety disorder: A meta-analysis versus placebo. Eur Neuropsychopharmacol. 2016;26(6):1062-9.

2 Hieronymus F, Emilsson JF, Nilsson S, Eriksson E. Consistent superiority of selective serotonin reuptake inhibitors over placebo in reducing depressed mood in patients with major depression. Mol Psychiatry. 2016;21(4):523-30.

3 Fava M, Davidson KG. Definition and Epidemiology of Treatment Resistant Depression. The Psychiatric Clinics of North America. 1996;19(2):179-200.

4 Reinhold JA, Rickels K. Pharmacological treatment for generalized anxiety disorder in adults: an update. Expert Opin Pharmacother. 2015;16(11):1669-81.

5 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 [^{11}C]DASB Positron Emission Tomography Study. American Journal of Psychiatry. 2004;161(5):826-35.

6 Barnes NB, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999;38:1083-152.

7 Artigas F. Serotonin receptors involved in antidepressant effects. Pharmacol Ther. 2013;137(1):119-31.

8 El Mansari M, Sanchez C, Chouvet G, Renaud B, Haddjeri N. Effects of acute and long-term administration of escitalopram and citalopram on serotonin neurotransmission: an in vivo electrophysiological study in rat brain. Neuropsychopharmacology. 2005;30(7):1269-77.

9 Hervas I, Vilaro MT, Romero L, Scorza MC, Mengod G, Artigas F. Desensitization of 5-HT 1A Autoreceptors by a Low Chronic Fluoxetine Dose Effect of the Concurrent Administration of WAY-100635. Neuropsychopharmacology. 2001;24(1):11-20.

10 Riad M, Watkins KC, Doucet E, Hamon M, Descarries L. Agonist-Induced Internalization of Serotonin-1A Receptors in the Dorsal Raphe Nucleus (Autoreceptors) But Not Hippocampus (Heteroreceptors). The Journal of Neuroscience. 2001;21(21):8378–86.

11 Carhart-Harris RL, Nutt DJ. Serotonin and brain function: a tale of two receptors. J Psychopharmacol. 2017;31(9):1091-120.

12 Arnone D. Functional MRI findings, pharmacological treatment in major depression and clinical response. Prog Neuropsychopharmacol Biol Psychiatry. 2019;91:28-37.

13 Klaassens BL, van Gorsel HC, Khalili-Mahani N, van der Grond J, Wyman BT, Whitcher B, et al. Single-dose serotonergic stimulation shows widespread effects on functional brain connectivity. Neuroimage. 2015;122:440-50.

14 McCabe C, Mishor Z, Filippini N, Cowen PJ, Taylor MJ, Harmer CJ. SSRI administration reduces resting state functional connectivity in dorso-medial prefrontal cortex. Mol Psychiatry. 2011;16(6):592-4.

15 Schrantee A, Lucassen PJ, Booij J, Reneman L. Serotonin transporter occupancy by the SSRI citalopram predicts default-mode network connectivity. Eur Neuropsychopharmacol. 2018;28(10):1173-79.

16 van de Ven V, Wingen M, Kuypers KP, Ramaekers JG, Formisano E. Escitalopram Decreases Cross-Regional Functional Connectivity within the Default-Mode Network. PLoS One. 2013;8(6):e68355.

17 Berman MG, Peltier S, Nee DE, Kross E, Deldin PJ, Jonides J. Depression, rumination and the default network. Soc Cogn Affect Neurosci. 2011;6(5):548-55.

18 Arce E, Simmons AN, Lovero KL, Stein MB, Paulus MP. Escitalopram effects on insula and amygdala BOLD activation during emotional processing. Psychopharmacology (Berl). 2008;196(4):661-72.

19 Del-Ben CM, Deakin JF, McKie S, Delvai NA, Williams SR, Elliott R, et al. The effect of citalopram pretreatment on neuronal responses to neuropsychological tasks in normal volunteers: an FMRI study. Neuropsychopharmacology. 2005;30(9):1724-34.

20 Grady CL, Siebner HR, Hornboll B, Macoveanu J, Paulson OB, Knudsen GM. Acute pharmacologically induced shifts in serotonin availability abolish emotion-selective responses to negative face emotions in distinct brain networks. Eur Neuropsychopharmacol. 2013;23(5):368-78.

21 Selvaraj S, Walker C, Arnone D, Cao B, Faulkner P, Cowen PJ, et al. Effect of Citalopram on Emotion Processing in Humans: A Combined 5-HT1A [(11)C]CUMI-101 PET and Functional MRI Study. Neuropsychopharmacology. 2018;43(3):655-64.

22 Windischberger C, Lanzenberger R, Holik A, Spindelegger C, Stein P, Moser U, et al. Area-specific modulation of neural activation comparing escitalopram and citalopram revealed by pharmaco-fMRI: a randomized cross-over study. Neuroimage. 2010;49(2):1161-70.

23 Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, et al. Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biol Psychiatry. 2005;57(10):1079-88.

24 Hahn A, Wadsak W, Windischberger C, Baldinger P, Hoflich AS, Losak J, et al. Differential modulation of the default mode network via serotonin-1A receptors. Proc Natl Acad Sci U S A. 2012;109(7):2619-24.

25 Fisher PM, Meltzer CC, Ziolko SK, Price JC, Moses-Kolko EL, Berga SL, et al. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. Nat Neurosci. 2006;9(11):1362-3.

26 Dipasquale O, Selvaggi P, Veronese M, Gabay AS, Turkheimer F, Mehta MA. Receptor-Enriched Analysis of functional connectivity by targets (REACT): A novel, multimodal analytical approach informed by PET to study the pharmacodynamic response of the brain under MDMA. NeuroImage. 2019;195(April):252-60.

27 Schrantee A, Solleveld MM, Schwantje H, Bruin WB, Mutsaerts HM, Adriaanse SM, et al. Dose-dependent effects of the selective serotonin reuptake inhibitor citalopram: A combined SPECT and phMRI study. J Psychopharmacol. 2019;33(6):660-69.

28 Klein N, Sacher J, Geiss-Granadia T, Attarbaschi T, Mossaheb N, Lanzenberger R, et al. In vivo imaging of serotonin transporter occupancy by means of SPECT and [^{123}I]ADAM in healthy subjects administered different doses of escitalopram or citalopram. Psychopharmacology (Berl). 2006;188(3):263-72.

29 Booij J, de Jong J, de Bruin K, Knol R, de Win MML, van Eck-Smit BLF. Quantification of Striatal Dopamine Transporters with ^{123}I-FP-CIT SPECT Is Influenced by the Selective Serotonin Reuptake Inhibitor Paroxetine: A Double-Blind,

Placebo-Controlled, Crossover Study in Healthy Control Subjects. Journal of Nuclear Medicine. 2007;48(3):359-66.

30 Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR. The amygdala response to emotional stimuli: a comparison of faces and scenes. Neuroimage. 2002;17(1):317-23.

31 Esteban O, Markiewicz CJ, Blair RW, Moodie CA, Isik AI, Erramuzpe A, et al. fMRIPrep: a robust preprocessing pipeline for functional MRI. Nat Methods. 2019;16(1):111-16.

32 Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, et al. Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python. Front Neuroinform. 2011;5:13.

33 Beliveau V, Ganz M, Feng L, Ozenne B, Hojgaard L, Fisher PM, et al. A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System. J Neurosci. 2017;37(1):120-28.

34 Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage. 2009;44(1):83-98.

35 Brown EC, Clark DL, Hassel S, MacQueen G, Ramasubbu R. Thalamocortical connectivity in major depressive disorder. J Affect Disord. 2017;217:125-31.

36 Liu J, Ren L, Womer FY, Wang J, Fan G, Jiang W, et al. Alterations in amplitude of low frequency fluctuation in treatment-naive major depressive disorder measured with resting-state fMRI. Hum Brain Mapp. 2014;35(10):4979-88.

37 Cheng Y, Xu J, Arnone D, Nie B, Yu H, Jiang H, et al. Resting-state brain alteration after a single dose of SSRI administration predicts 8-week remission of patients with major depressive disorder. Psychol Med. 2017;47(3):438-50.

38 Ruhé HG, Frokjaer VG, Haarman BCM, Jacobs GE, Booij J. Molecular Imaging of Depressive Disorders. In: Dierckx RAJO, Otte A, de Vries EFK, van Waarde A, Sommer IE, editors. PET and SPECT in Psychiatry. Springer Nature Switzerland; 2021.

39 Arnone D, Wise T, Walker C, Cowen PJ, Howes O, Selvaraj S. The effects of serotonin modulation on medial prefrontal connectivity strength and stability: A pharmacological fMRI study with citalopram. Prog Neuropsychopharmacol Biol Psychiatry. 2018;84(Pt A):152-59.

40 van Wingen GA, Tendolkar I, Urner M, van Marle HJ, Denys D, Verkes RJ, et al. Short-term antidepressant administration reduces default mode and task-positive network connectivity in healthy individuals during rest. Neuroimage. 2014;88:47-53.

41 Beliveau V, Svarer C, Frokjaer VG, Knudsen GM, Greve DN, Fisher PM. Functional connectivity of the dorsal and median raphe nuclei at rest. NeuroImage. 2015;116:187-95.

42 Celada P, Puig MV, Artigas F. Serotonin modulation of cortical neurons and networks. Frontiers in Integrative Neuroscience. 2013;7(25):1-20.

43 Tian S, Sun Y, Shao J, Zhang S, Mo Z, Liu X, et al. Predicting escitalopram monotherapy response in depression: The role of anterior cingulate cortex. Hum Brain Mapp. 2020;41(5):1249-60.

44 Delaveau P, Jabourian M, Lemogne C, Guionnet S, Bergouignan L, Fossati P. Brain effects of antidepressants in major depression: a meta-analysis of emotional processing studies. J Affect Disord. 2011;130(1-2):66-74.

45 Wang L, Hermens DF, Hickie IB, Lagopoulos J. A systematic review of resting-state functional-MRI studies in major depression. J Affect Disord. 2012;142(1-3):6-12.

46 Dutta A, McKie S, Downey D, Thomas E, Juhasz G, Arnone D, et al. Regional default mode network connectivity in major depressive disorder: modulation by acute intravenous citalopram. Transl Psychiatry. 2019;9(1):116.

47 Yang M, Tsai SJ, Li CR. Concurrent amygdalar and ventromedial prefrontal cortical responses during emotion processing: a meta-analysis of the effects of valence of emotion and passive exposure versus active regulation. Brain Struct Funct. 2020;225(1):345-63.

48 Fox MD, Snyder AZ, Vincent JL, Corbetta M, van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. The Proceedings of the National Academy of Sciences. 2005;102(27):9673-78.

49 Anderson IM, Juhasz G, Thomas E, Downey D, McKie S, Deakin JF, et al. The effect of acute citalopram on face emotion processing in remitted depression: a pharmacoMRI study. Eur Neuropsychopharmacol. 2011;21(1):140-8.

50 Gill H, Gill B, Chen-Li D, El-Halabi S, Rodrigues NB, Cha DS, et al. The emerging role of psilocybin and MDMA in the treatment of mental illness. Expert Rev Neurother. 2020;20(12):1263-73.

51 Rossi GN, Hallak JEC, Bouso Saiz JC, Dos Santos RG. Safety issues of psilocybin and LSD as potential rapid acting antidepressants and potential challenges. Expert Opin Drug Saf. 2022;21(6):761-76.

52 Oeri HE. Beyond ecstasy: Alternative entactogens to 3,4-methylenedioxymethamphetamine with potential applications in psychotherapy. J Psychopharmacol. 2021;35(5):512-36.

53 De Gregorio D, Comai S, Posa L, Gobbi G. d-Lysergic Acid Diethylamide (LSD) as a Model of Psychosis: Mechanism of Action and Pharmacology. Int J Mol Sci. 2016;17(11).

54 Lawn T, Dipasquale O, Vamvakas A, Tsougos I, Mehta MA, Howard MA. Differential contributions of serotonergic and dopaminergic functional connectivity to the phenomenology of LSD. Psychopharmacology (Berl). 2022;239(6):1797-808.

55 Boldrini M, Underwood MD, Mann JJ, Arango V. Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides. J Psychiatr Res. 2008;42(6):433-42.