The functional circuits related to serotonin transporter and serotonin 1A receptor are differentially associated to serotonin transporter availability after an acute citalopram challenge

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

[1]Amsterdam UMC, location Amsterdam Medical Centre, Department of Radiology and Nuclear Medicine, Amsterdam, the Netherlands
[2]Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK

* These authors contributed equally

Introduction: The primary target of selective serotonin reuptake inhibitors (SSRIs) is the serotonin transporter (5-HTT). However, the functional brain response to SSRIs involves neural circuits beyond brain regions with high 5-HTT expression and has been shown to vary between rest and task-related conditions. Secondary effects of SSRIs on the serotonin 1A receptor (5-HT1AR) have been implicated in the therapeutic response to this class of drugs, but to date the independent role of the 5-HTT and 5-HT1AR in modulating the functional brain response to SSRIs is unknown. Here, we administered different doses of acute citalopram to investigate the association between 5-HTT availability and 5-HTT- and 5-HT1AR-enriched functional connectivity (FC).
Methods: We analyzed multi-modal data from a dose-response, placebo-controlled, double-blind study, in which 45 young healthy female controls were randomized into three treatment groups receiving either placebo, a low (4 mg), or a high (16 mg) oral dose of citalopram. Resting-state fMRI and task-based fMRI scans (during an emotional face matching task) were analyzed using Receptor-Enhanced Analysis of functional Connectivity by Targets (REACT) to determine 5-HTT- and 5-HT1AR-enriched FC maps. In-vivo 5-HTT availability by placebo or citalopram was determined with [^{123}I]FP-CIT single-photon emission computerized tomography (SPECT). We assessed the association between 5-HTT availability and 5-HTT- and 5-HT1AR-enriched FC, and conducted exploratory analyses for group differences in 5-HTT- and 5-HT1AR-enriched FC.
Results: 5-HTT availability was negatively correlated with 5-HTT-enriched FC at rest, and with 5-HT1A-enriched FC during the task. For resting-state 5-HT1AR-enriched FC, we observed a dose-dependent effect in the anterior and posterior cingulate and thalamus, where the low dose group showed lower 5-HT1AR-enriched FC than the high dose group. For task-based fMRI, we observed a lower 5-HT1AR-enriched task-dependent FC with the precuneus in the low dose group, compared to the placebo group.
Conclusions: Using multimodal data, our findings highlight a direct link between pharmacokinetic and pharmacodynamic response of the healthy brain to an acute citalopram challenge, relating 5-HTT availability to differential modulations of its functional pathway at rest and during an emotional face matching task. Our data suggests a potential pivotal role for the 5-HT1AR in a complex dose-response pattern, which highlights the need of further studies examining how dose, context and their interaction might moderate the effects of serotonergic drugs on the brain. Longitudinal assessments of changes in the 5-HTT- and 5-HT1AR-enriched FC over the course of treatment and its relation with clinical response would be crucial next steps.

KEY WORDS: SSRI, serotonin transporter, serotonin 1A receptor, phMRI, SPECT, functional connectivity

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 29-46% of depressed patients [3] and 25-40% of patients with anxiety [4] fail to respond to this pharmacological treatment. Uncovering mechanisms that could account for these interindividual differences in treatment response is thus paramount to inform the development of new and more effective therapeutic approached and alternatives. However, the current lack of full understanding of the mechanisms of action of SSRIs on the human brain have marred substantial advances in this direction.

While the exact mechanisms underlying the therapeutic effects of SSRIs remain contentious, it is generally accepted that the primary pharmacological mode of action of SSRIs entails blocking the serotonin (5-HT) transporter (5-HTT) [5], which results in increased extracellular levels of 5-HT. After acute SSRI administration, this increase in 5-HT 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 and reduces 5-HT neuronal activity, thereby decreasing the 5-HT release from the 5-HT nerve terminals in the synapses and inhibiting neuronal activity [7]. In turn, chronic SSRI treatment is thought to result in desensitization of DRN 5-HT1A autoreceptors, decreasing their inhibitory effect [8-10], which subsequently allows increased 5-HT binding to postsynaptic 5-HT receptors. 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 mechanisms of action of SSRIs on the human brain has greatly benefited from the use of non-invasive neuroimaging measures, such as magnetic resonance imaging (MRI). Particularly, the combination of functional MRI (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 whole systems level, which is essential for understanding how drugs affecting widespread neuromodulatory systems, such as 5-HT, might produce their psychopharmacological effects. 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 found altered activation in the limbic system in response to SSRIs [18-21], which has been implicated in aberrant emotion regulation in depressed patients [22]. 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 attempted to bridge this gap and have shed further light on the involvement of specific 5-HT receptors in modulating intrinsic aspects of FC, as well as their contribution to SSRI-induced changes in brain function. For instance, one study found the level of 5-HTT availability following citalopram administration to be associated with FC strength between the DMN and a number of cortical regions [15], highlighting a prominent role of 5-HTT in modulating the SSRI's effects on DMN connectivity. Moreover, Hahn *et al.* [23] emphasized the importance of 5-HT1AR in regulating DMN connectivity by demonstrating diverse and area-specific patterns of associations between the FC of the 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 the engagement 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 facial emotion processing [20, 24]. However, given the prominent role of 5-HT1AR in regulating the 5-HT system, dissecting the pharmacodynamics of SSRIs in the human brain and its correspondent interindividual variability should incorporate concomitant investigations of how post-drug increases in 5-HT might relate to subsequent modulations of neural circuits associated with specific 5-HT receptor subtypes, such as 5-HT1AR. Ideally, these investigations should encompass data acquired both at rest and during emotional processing tasks, as both paradigms seem to capture the effects of interindividual variation in molecular target bioavailability on brain function, and could provide new insights on the extent to which SSRI modulation of 5-HT brain networks might be context-dependent.

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) [25] to investigate how acute citalopram-induced variations in 5-HTT availability relate to the functional connectivity of 5-HTT- and 5-HT1AR-enriched functional networks both at rest and during an emotional face matching task. Forty-five healthy female subjects were randomized into three treatment groups receiving placebo, a low (4 mg) or high (16 mg) oral dose of citalopram; all three modalities [SPECT, resting state fMRI (rs-fMRI), and task-based fMRI (tb-fMRI)] were acquired within the same participants after treatment to allow for investigations of between-modality associations across treatment effects. In line with previous findings on FC changes after citalopram, we hypothesized an inverse relationship between 5-HTT availability and 5-HTT- and 5-HT1AR-enriched FC post-treatment 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 [20, 24], we expected 5-HTT availability in task-dependent FC to be mostly subserved by 5-HT1AR.

MATERIALS AND METHODS

Participants

The study protocol was approved by the medical ethics committee of the Academic Medical Center in Amsterdam, and all subjects gave written informed consent, which was obtained in accordance with the Declaration of Helsinki. Participants were recruited via internet advertisements and brochures distributed at local universities and colleges. A total of 45 healthy female volunteers (HC, average age 21.6 years, range 18–28) were enrolled in the study. A history of a chronic neurological/psychiatric disorder, a family history of sudden heart failure, current use of psychostimulant medication, an abnormal electrocardiogram, excessive consumption of alcohol (>21 units/week), caffeine (>8 cups/day), or nicotine (>15 cigarettes/day), and contraindications for MRI or SPECT were all used as exclusion criteria. The Mini-International Neuropsychiatric Interview Plus was used to screen for psychiatric illnesses and drug abuse. To avoid confounding effects of the hormonal cycle, all participants were required to be on hormonal contraception (for results of prior analyses on the same sample see [15, 26]).

Study design

We used a double-dose, placebo-controlled double-blind design (Figure 1). To prevent thyroid uptake of free radioactive iodide, participants were given potassium-iodide tablets prior to receiving [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4iodophenyl)nortropane ([^{123}I]FP-CIT). To measure baseline 5-

HTT availability, the first SPECT scan was performed 2 hours after the injection (data not shown). Following this SPECT scan, participants were randomly assigned to one of three treatment groups: placebo ('placebo' n=15), low dose (4 mg; 'low' n=15), or clinical dose (16 mg; 'high' n=15) of citalopram (oral solution administration, 16 mg equivalent to 20 mg in tablet form, Lundbeck). These doses have been demonstrated to correspond to 5-HTT occupancy levels of 0%, 40%, and 80%, respectively [27]. 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 1 hour later, which included a resting-state fMRI scan and task-based fMRI during an emotional face matching task.

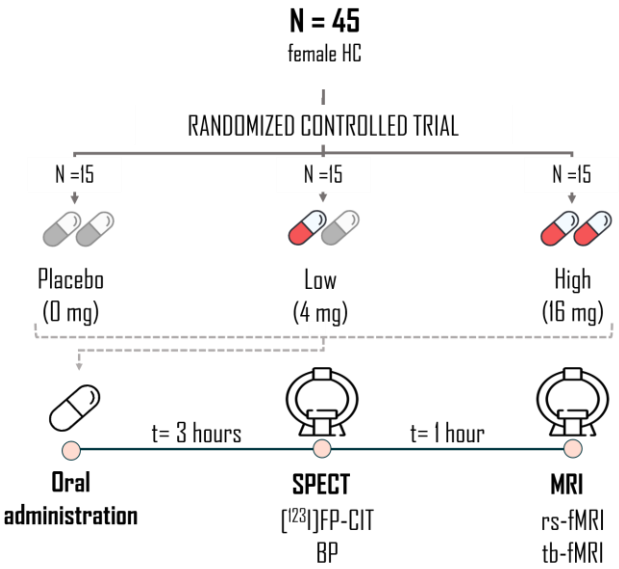

Figure 1. Experimental design.
HC: healthy controls; mg: milligram; t: time; SPECT: single-photon emission computerized tomography; [^{123}I]FP-CIT: [^{123}I]N-ω-fluoropropyl-2β- carbomethoxy-3β-(4-iodophenyl) nortropane; BP: binding potential; fMRI: functional Magnetic Resonance Imaging, rs-fMRI: resting-state fMRI; tb-fMRI: task-based fMRI

SPECT acquisition and analysis

SPECT scans were acquired using a brain-dedicated InSPiraHD SPECT camera (Neurologica, Boston, USA). 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 to the dopamine transporter primarily in the striatum, and primarily to the 5-HTT in extrastriatal areas such as the thalamus [28], 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, which reflects 5-HTT availability (such that a lower BP reflects a higher occupancy and, thus, a lower availability of the 5-HTT). For details on acquisition and analysis see [15, 26].

MRI acquisition and preprocessing

MRI data were acquired using a 3.0T Ingenia scanner (Philips, Best, The Netherlands) with a 32-channel receive-only head coil. For anatomical reference, a high-resolution 3D T1w scan was obtained (TR/TE = 3195/7 ms; FOV = 256 × 256 × 180; voxel size = 1 x 1 x 1 mm; gap = 0 mm; flip angle = 9°). Rs-fMRI data were acquired using a T2*-weighted gradient-echo echo-planar imaging sequence using the following parameters: TR/TE = 2150/27 ms; FOV = 240 × 240 × 131 mm, voxel size = 3 × 3 × 3 mm; gap = 0.3 mm; flip angle = 76.2°; dynamics = 240 (approximately 9 mins). The participants were instructed to keep their eyes open, focus on a fixation cross, and let their mind wander. Task fMRI data were acquired with the following parameters: TR/TE = 2300/30 ms, FOV = 221 x 221 x 90 mm; resolution = 2.3 × 2.3 × 2.3 mm; gap = 0.7 mm, 39 sequential slices, flip angle = 80°, dynamics = 70 (approximately 3 mins). For this task, we used an emotional face-matching fMRI paradigm adapted from Hariri *et al.* [29], consisting of a blocked design with emotional stimuli

displaying angry and fearful faces (*faces*) and the 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 for each emotional trial, and individuals had to choose which of the lower two stimuli showed the same emotion as the target stimulus presented above. Similarly, three stimuli were provided for each neutral trial, but subjects had to choose which of the bottom two ellipses was aligned identically to the target ellipse.

All fMRI data were preprocessed using fMRIPREP v.20.0.6 [30]; RRID:SCR_016216), which is based on *Nipype* 1.4.2 [31]; RRID:SCR_002502). 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 (ICA-AROMA) was employed. Data were spatially smoothed (6mm FWHM) (FSL/FEAT v.6.00; RRID: SCR 002 823, see Supplementary material for further detail). Following preprocessing with fMRIprep, white matter (WM) and cerebrospinal fluid (CSF) (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 and three subjects exhibiting high motion (mean framewise displacement (FD) >0.25 mm) were excluded from the rs-fMRI analysis.

5-HT1AR-receptor- and 5-HTT-enriched functional connectivity at rest

A high-resolution in vivo atlas of the distribution density of 5-HTT and 5-HT1AR [32] was used for the REACT analysis. The 5-HTT map is characterized by a high intensity in the entorhinal and insular cortices, subcortical regions and the DRN. The 5-HT1AR expression is relatively high in the DRN, hippocampus, septum, amygdala and corticolimbic areas (Figure 2). In a two-step multiple linear regression employing the REACT approach [25] 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 of the analysis as it was used as a reference region in the kinetic model for both systems [32]. 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 the 5-HTT- and 5-HT1AR-enriched FC.

5-HT1AR- and 5-HTT-enriched functional response during emotion recognition

For task-based analysis, the atlas was used to estimate the 5-HTT- and 5-HT1AR-enriched functional response during an emotional face matching task. In the first step of this analysis, the fMRI signal in the gray matter was weighted using the molecular templates as spatial regressors, and the dominant blood-oxygen-level-dependent (BOLD) time series of the 5-HTT- and 5-HT1AR-enriched functional systems were estimated at the subject level. As before, the cerebellum was excluded at this stage [32]. 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 of the brain using a generalized psychological-physiological interaction (gPPI) design. The gPPI design included five regressors, namely the convolved Faces>shapes regressor, combining both *faces* and *shapes* blocks, 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).

Statistical analyses

All voxelwise analyses were conducted using permutation tests in Randomise (5000 permutations; threshold-free cluster enhancement [33]; significance was inferred when family wise error (FWE) 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 also conducted exploratory analyses to assess differences in the target-enriched FC maps between treatment groups using two-sample t-tests (placebo vs low group, placebo vs high group, low group vs high group), applying a Bonferroni correction for multiple comparisons.

For the tb-fMRI analysis, we investigated the voxel-wise correlation of the target-enriched task-dependent FC maps with the individual thalamic [^{123}I]FP-CIT BP. For the task activation map and the task-dependent FC maps, 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 in order to exclude drug-induced effects on the target-enriched FC. Subsequently, using a small volume correction within these clusters, we assessed group effects using two-sample t-tests, using 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 significant clusters showing significant activity/connectivity in all treatment groups combined using a one-sample t-test, and then repeated the between-group comparisons as described above (Supplementary Material).

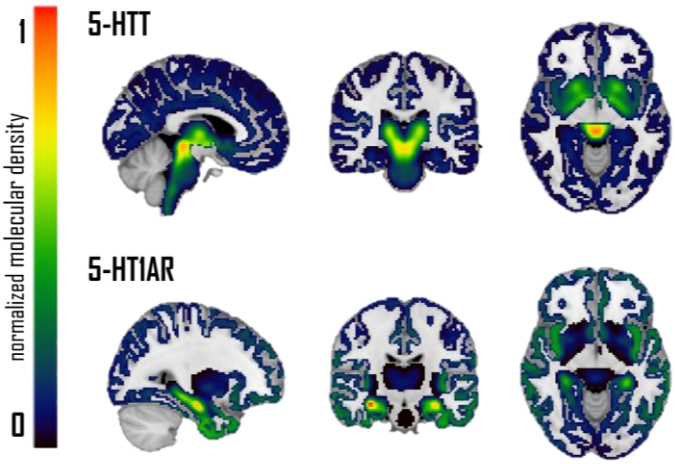

Figure 2. Normalized high-resolution in vivo maps of the distribution density of the serotonin transporter (5-HTT) and serotonin 1A receptor (5-HT1AR)

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 1 mm 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).

RESULTS

5-HTT- and 5-HT1AR-enriched functional response at rest

The mean 5-HT1AR- and 5-HTT-enriched FC maps across participants are visualized in Figure 3. The 5-HTT-enriched functional circuit involved the anterior cingulate, medial prefrontal cortex, juxtapositional lobule cortex, insular cortex, amygdala, thalamus, and the striatum (Figure 3A). The 5-HT1AR-enriched functional network comprised the temporal gyrus, hippocampus, anterior cingulate and paracingulate gyri, posterior cingulate and precuneus, inferior frontal gyrus, brain stem, and the angular gyrus (Figure 3B).

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 (i.e. high occupancy by citalopram) showed higher FC in some regions of the 5-HTT-enriched functional network, including in the planum polare, central opercular cortex, temporal and occipital fusiform gyrus, temporal gyrus, and opercular cortex (Figure 3C). No significant relationship was found between the 5-HT1AR-enriched FC and thalamic [^{123}I]FP-CIT BP.

The exploratory analysis 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 3D). All cluster information can be found in Table S1. The same analysis on the 5-HTT-enriched functional network did not show any between-group FC differences.

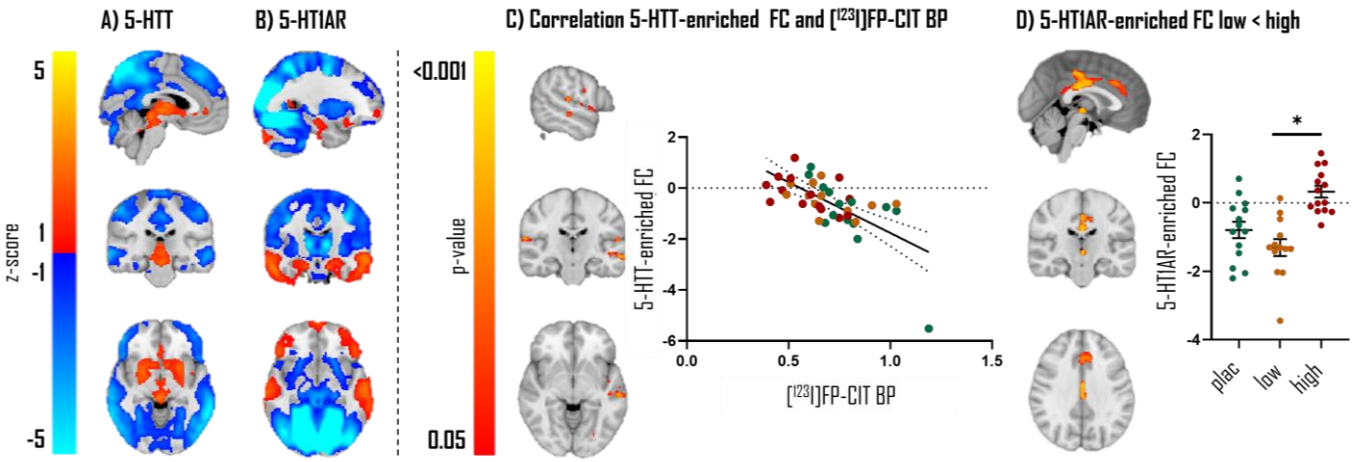

Figure 3. Serotonin transporter (5-HTT)- and serotonin 1A receptor (5-HT1AR)-enriched functional connectivity (FC) at rest and its relationship with thalamic [^{123}I]FP-CIT binding potential (BP)

*The mean **A)** 5-HTT- and **B)** 5-HT1AR-enriched FC at rest was calculated by averaging spatial z-score maps for all subjects. Positive FC is depicted in red/yellow, whereas negative FC is depicted in blue ($p < 0.05$; FWE corrected). **C)** Clusters showing significant negative correlation between thalamic [^{123}I]FP-CIT BP and 5-HTT-enriched FC (left) and scatter dot plot illustrating this correlation (right). **D)** 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).*

[^{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).

5-HTT- and 5-HT1AR-enriched functional response during emotion recognition

The mean z-score maps of the generalized Psychological-Physiological Interaction (gPPI) are reported in Figure S1.

Task-dependent 5-HTT- and 5-HT1AR-enriched functional connectivity

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. This effect was localized in the precuneus and lateral occipital cortex (Figure 4B). A detailed description of all cluster sizes and locations can be found in Table S1. No differences were found between the placebo and the high dose group, and between the low and the high dose groups. No between-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 S2).

Task-effect

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

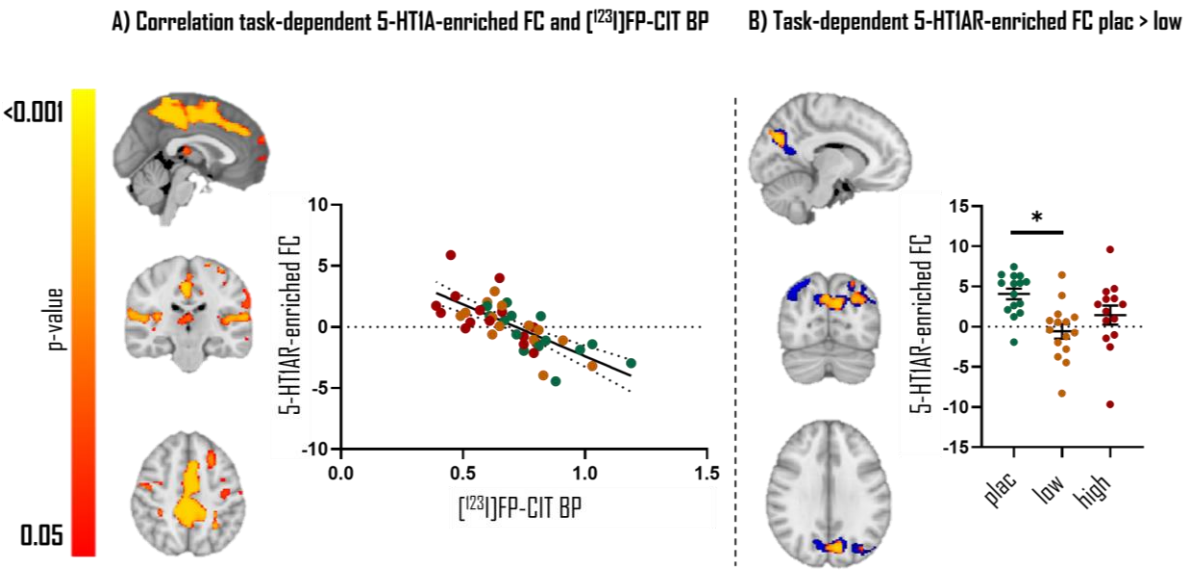

Figure 4. The relationship between citalopram-induced variations in thalamic [^{123}]FP-CIT binding potential (BP) and serotonin 1A receptor (5-HT1AR)-enriched task-dependent functional connectivity (FC) during an emotion face matching task

A) *Clusters showing a significant negative correlation between thalamic [^{123}I]FP-CIT BP and task-dependent 5-HT1AR-enriched FC and (left) and scatter dot plot illustrating this correlation (right).* ***B)*** *Blue: clusters showing significant 5-HT1A-enriched task-dependent 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) .*

[^{123}I]FP-CIT: [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)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 functional connectivity of 5-HTT- and 5-HT1AR-enriched networks at rest and during an emotion recognition task. We found thalamic [^{123}I]FP-CIT BP to be negatively associated with 5-HTT-enriched FC at rest, and with 5-HT1A-enriched FC during the emotional face matching task. Additionally, we conducted exploratory analyses to compare 5-HTT- and 5-HT1AR-enriched FC between treatment groups to investigate dose-dependent effects of citalopram. At rest, the low dose group showed lower 5-HT1AR-enriched FC than the high dose group, but no difference between the citalopram and the placebo groups was observed. During the task, we found task-dependent 5-HT1AR-enriched FC to be lower in the low dose group compared to the placebo group, but no significant differences were observed between the citalopram groups. We discuss each of these main findings in further detail below.

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 respective drug's main target in the healthy 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 [34, 35]. Here, the occipito-temporal cortex has been reported more frequently and is additionally shown to respond to acute (but not chronic) treatment with escitalopram [36]. Noteworthy though, we did not observe between-group differences in 5-HTT-enriched FC, which could reflect either lack of power for detecting smaller effects in the current study, or unexplained across-group variability in 5-HTT availability that could hinder the identification of average group effects. Indeed, previous studies have highlighted the influence of gene polymorphisms in the serotonin transporter-linked promoter region (5-HTTLPR), which is known to affect functionality of the transporter on the response to SSRIs (for a review, see [37]).

In contrast to our hypothesis, we did not find a relationship between thalamic 5-HTT availability and 5-HT1AR 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. We note though that we found preliminary evidence of between-group differences in the posterior and anterior cingulate cortices, which are compatible with a nonlinear dose-response pattern where FC in the low dose group appeared decreased in comparison with the placebo group but no differences between the high dose and placebo groups could be identified. While we present these findings to inform future larger studies dissecting this aspect of dose-response in well-powered designs, we note that our findings should be interpreted with caution since they arise from exploratory pairwise tests. Moreover, interpreting this dose-response pattern on the 5-HT1AR 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. For instance, it could be that a low dose, which should increase 5-HT to a smaller extent, might predominantly affect auto- or postsynaptic 5-HT1AR; while the larger 5-HT increases induced the high dose might lead to engagement of both types of receptors, leading to compensatory effects whose outcome is a null effect. Nevertheless, for now, this hypothesis remains speculatory. It is also worth

noting that the regions where we found this effect appear in line with previous literature and are highly relevant within the context of previous studies in depression. The posterior cingulate cortex, as part of the DMN, has frequently been implicated in the functional brain response to SSRIs [13, 16, 38, 39]. The anterior cingulate cortex is known to be densely innervated by serotonergic projections from the DRN and to have a relatively high density of 5-HT1AR [40]. Interestingly, a previous study suggested that FC of the ACC could serve as a predictor in escitalopram treatment outcome in patients [41], suggesting a crucial role for the ACC in the functional response to SSRIs. However, whether this is mediated by the 5-HT1AR system remains to be elucidated.

In contrast to our findings at rest, but in line with our hypothesis, our 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 varies linearly with variations in 5-HTT availability in large clusters of cortical and subcortical areas (Figure 4A), which show considerable overlap with the corticolimbic system. It is interesting to observe that, while previous studies have shown that limbic system activity during emotional processing is dependent on the 5-HT1AR [20, 24], 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 those of healthy volunteers following SSRIs treatment [42, 43]. Outside of the limbic system, our group-level analyses revealed a more complicated non-linear dose-response pattern. That is, in the precuneus, which is an integral component of the DMN and has previously been described to show alterations in activation and connectivity during emotion processing and following citalopram administration [44, 45], 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 more selective and focused therapeutic approaches.

For completeness, we also investigated main effects of task and effects of citalopram on BOLD responses in our contrasts of interest since this data has not been published previously. The main task effect we observed is in line with previous studies (e.g. [19]), showing increased BOLD activation during emotion recognition mostly in key limbic areas and decreased BOLD activation in DMN areas [46]. In contrast to the previous literature, no effect of citalopram was observed on these task contrast maps, which is not surprising given the mixed results from previous studies. For example, [20, 47] found decreased and increased amygdala activity following acute citalopram in healthy volunteers during fearful vs. neutral faces, respectively. 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 healthy volunteers [19]. However, comparing these studies with ours is challenging, given the differences in citalopram dose and route of administration as well as the analysis approach. For instance, all these prior studies used intravenous administration of either 7.5 mg or 10 mg of citalopram and used a ROI approach. It is possible that, in our study, we missed small effects of citalopram because of the relatively low power of our study or because the effects of the acute oral dose are not comparable with the acute intravenous doses used by the previous studies.

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) [48] and the psychedelic lysergic acid diethylamide (LSD) [49]. While these compounds have different binding profiles compared to citalopram, receptor-enriched FC analyses have recently helped to shed light on potential overlap in the 5-HT receptors involved in the functional response of the brain to these compounds. MDMA predominantly binds to 5-HTT (in addition to other monoamine transporters), but it is a releasing agent in addition to a reuptake blocker [50]. Using REACT, MDMA administration was found to significantly modulate networks informed by the distribution density of both 5-HTT and 5-HT1AR [25]. LSD shows a different pattern of affinity, with high agonist activity at the 5-HT1AR, 5-HT1BR, 5-HT6R and 5-HT7R, as well as dopaminergic D1 and D2 receptors [51]. While not investigating the 5-HTT itself, Lawn *et al.* [52] showed that LSD compared to placebo significantly affected FC within 5-HT1AR-, 5-HT1BR-, 5-HT2AR-, D1R- and D2R-related networks. Interestingly, despite being a direct agonist to the 5-HT1AR, the 5-HT1AR-enriched maps showed a FC increase only in the right lingual gyrus, while having particularly widespread effects in the 5-HT1BR and dopamine D1R enriched maps. Therefore, alongside these previous studies, our data support the idea that REACT has potential in helping to dissect the brain pharmacodynamics of compounds targeting the 5-HT system. However, as no patients were included in any of the above-mentioned studies, 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 [53].

This study has some limitations. First, the sample included only healthy young females and was relatively small. Second, [^{123}I]FP-CIT is a non-selective radioligand that binds to both 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 [28]. 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 men and women, while our sample consisted of women only. It is not yet clear 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 administration at different time points to unravel its long-term effects on the functional circuits related to 5-HTT and 5-HT1AR and how they might differ from the acute effects.

CONCLUSION

Building on the power of a range of multimodal data acquired within the same participants, our study provides unprecedented empirical evidence linking 5-HTT availability and the functional connectivity of 5-HTT-enriched (at rest) and 5-HT1AR-enriched (in 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 brain response associated with 5-HT enhancement after acute administration of citalopram. Here, 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 the need of further studies examining how dose, context and their interaction might moderate the effects of serotonin altering drugs on the brain. Our data provides empirical evidence supporting the added value of REACT when studying the effects of citalopram and drugs alike on the brain, particularly in studies including clinical populations. 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.

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