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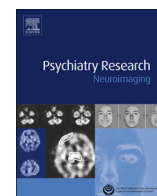
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Articles

The influence of age-of-onset of antidepressant use on the acute CBF response to a citalopram challenge; a pharmacological MRI study

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ABSTRACT

Preclinical studies have demonstrated that antidepressant treatment in juvenile rodents affect the ontogeny of the serotonin system. However, whether early antidepressant use has similar effects on the development of the serotonin system in humans remains unknown. Therefore, we investigated whether effects of selective serotonin reuptake inhibitor (SSRI) treatment on the serotonin system are modulated by age. With pharmacological Magnetic Resonance Imaging the cerebral blood flow (CBF) response to an acute citalopram challenge was measured, as a proxy for serotonin function. Fifty-one females with major depressive disorder or anxiety disorder were stratified into three groups: 1) those treated with SSRIs <23 years of age, 2) those treated with SSRIs >23 years of age, and 3) those that were never treated with SSRIs. Additionally, a group of 14 healthy controls was included. CBF decreased after a citalopram challenge in the amygdala, hippocampus and orbitofrontal cortex across the whole sample. However, in contrast to preclinical studies, we did not find any age-dependent effect of SSRI exposure on the CBF response. In view of recent concerns on potential adverse effects of SSRIs administered to children, future studies are needed to replicate our negative findings in larger samples sizes and potentially in a prospective design.

1. Introduction

Selective serotonin (5HT) reuptake inhibitors (SSRIs) are currently the first line of treatment for major depressive disorder (MDD) and anxiety disorder (AD) for adult patients in many countries. However, SSRIs are also increasingly prescribed to children and adolescents with these disorders. While MDD prevalence is low in prepubertal children (1-2%) (Egger and Angold, 2006), it ranges from 4 to 5% in the adolescent population (Thapar et al., 2012). It is important to note that although SSRIs are effective in treating childhood depression (Hetrick et al., 2012), they are prescribed at an age that the brain is still in development (Sowell et al., 1999). This is an important issue, as it has not been well studied as to whether SSRIs affect brain development or the serotonin system.

Despite this lack of knowledge, prescription rates of antidepressants to children and adolescents are increasing; with 17.6% in The Netherlands alone in the period from 2005 to 2012 (Bachmann et al.,

2016). Earlier evidence suggested that antidepressant exposure in adulthood is safe and does not have any long-term consequences (Mourilhe and Stokes, 1998), and it is therefore generally assumed that the same applies for children and adolescents. However, this assumption has been challenged as evidence accumulated that the developing brain responds differently to psychotropic drugs than the adult brain (Andersen and Navalta, 2004). For example, an increased risk for suicide was reported after SSRI use specifically in children (Hammad et al., 2006), after which the US Food and Drug Administration (FDA) decided to issue a warning regarding the use of SSRIs in children.

Despite the paucity in human studies, many preclinical studies have studied effects of long-term SSRI treatment on the developing brain. For instance, early, but transient, exposure to SSRIs in young rats resulted in an increased 5HT transporter (SERT) density when adulthood is reached (Wegerer et al., 1999). Shresta et al. further showed that the SERT is upregulated in young adulthood when rhesus monkeys were treated with SSRIs during the juvenile period (Shrestha et al., 2014).

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Moreover, acute antidepressant treatment in juvenile rats was found to impact dendritic length and spine density (Dávila-Hernández et al., 2018). Behaviorally, we have reported that fluoxetine treatment in rats exerts adverse, age-dependent effects on depressive behavior and wakefulness (Homborg et al., 2011). Finally, using pharmacological MRI (phMRI) as a proxy for serotonin functioning, we showed that treatment with fluoxetine results in an increased brain response to an acute SSRI challenge in juvenile rats, with opposite effects in adult rats (Klomp et al., 2012b). Together, these findings suggest that SSRI treatment at an early age could affect brain development and function (Norrholm and Ouimet, 2000), including long-term alterations in the 5HT system.

So far, it is unknown whether the effects of SSRIs are also modulated by age in humans. Therefore, we used phMRI in an exploratory study to investigate whether the acute response of the 5HT system to citalopram, a commonly prescribed SSRI, is modulated by the age of first SSRI exposure. PhMRI is a non-invasive imaging technique that can be used as a proxy for 5HT function (Chen et al., 2011). This technique has amongst others, been successfully used to study the age-dependent effects of SSRIs in rats (Klomp et al., 2012b). To this purpose, young adult females with a life-time MDD or AD diagnosis were stratified into three groups: 1) patients who were unexposed (UN) and had never received antidepressant treatment; 2) early exposed (EARLY) patients, who received their first SSRI treatment before the age of 23 years; and 3) late exposed (LATE) patients, who received their first antidepressant treatment after the age of 23 years. The cut off criteria of these ages are based upon the fact that brain maturation is considered not complete until early adulthood (Paus et al., 1999). In addition, a group of healthy young females with no life-time MDD diagnosis (HC) were added in order to compare the phMRI response to citalopram between patients and healthy controls.

2. Methods

2.1. Participants

Sixty-five female participants were recruited through online advertisements and via collaborations with general practitioners and pharmacies, via the Triversum Center for Child and Adolescent Psychiatry (Alkmaar, The Netherlands) and the PHARMO Institute for Drug Outcomes research (Utrecht, The Netherlands). After a complete description of the study, written informed consent was obtained from the participants. The Medical Ethical Committee of the Academic Medical Center Amsterdam approved the study procedures.

Inclusion criteria for the participants were a life-time diagnosis of MDD and/or AD. Participants were stratified into groups based on their age of first SSRI exposure: 1) EARLY: the group that first received SSRIs before the age of 23, 2) LATE: the group that first received SSRIs after the age of 23, 3) UN: the group that was not treated with SSRIs. To exclude acute pharmacological effects of SSRIs, a medication free interval of at least three weeks before scanning was maintained. In addition, a group of healthy female controls (HC) was included.

All subjects were screened for current Axis-I psychiatric disorders using a shortened version of the Mini International Neuropsychiatric Interview 6.0 Plus (M.I.N.I. Plus) (Sheehan et al., 1998). Exclusion criteria were current psychotropic medication use, a history of chronic or neurological disorder, family history of sudden heart failure or epileptic attacks, pregnancy (tested via urine sampling prior to the assessment), breast feeding, alcohol or nicotine dependence and contraindications for an MRI scan (e.g., ferromagnetic fragments). Participants agreed to abstain from smoking, caffeine and alcohol use for 24 hours prior to the assessments.

2.2. Procedure and behavioral measures

Participants first completed a neuropsychological test battery and

questionnaires, including a screening for depressive symptoms (MDD/AD group: the Inventory Depressive Symptoms (IDS) (Rush et al., 1996); HC group: the Beck Depression Inventory (BDI) (Beck et al., 1988a), the Beck Anxiety Inventory (BAI) (Beck et al., 1988b) and the Dutch Adult Reading Test (Schmand et al., 1991). Additionally, M.I.N.I. Plus (Sheehan et al., 1998) was used to determine whether the subjects were currently suffering or suffered from depression and/or anxiety in the past.

Salivary samples were collected from the MDD/AD groups for DNA analyses to determine the triallelic 5HT-transporter-linked polymorphic region (5-HTTLPR) polymorphism, i.e. the gene that encodes for the SERT. This polymorphism was measured in order to preclude effects of genotype on CBF, as it could mediate the effects of SSRIs. Genotyping of the 5-HTTLPR polymorphism was performed using a simple sequence length analysis in a polymerase chain reaction, comparable to previous methods (van Strien et al., 2016). 5-HTTLPR genotypes were coded as s/s, s/l and l/l genotypes (for analysis and results, see Supplements).

Following these procedures, an intravenous line was placed and all subjects underwent the phMRI scan. During the phMRI scan, a bolus of 7.5 mg citalopram (dissolved in 45 ml saline) was infused over 7.5 minutes, followed by 15 ml saline flush over 2.5 minutes, similar to a previous study (McKie et al., 2005).

2.3. Data acquisition

We assessed changes in cerebral blood flow (CBF) induced by citalopram using pseudo-continuous ASL (pCASL). pCASL was acquired by means of a 3.0T Philips Ingenia MR scanner (Philips Medical Systems, Best, the Netherlands) using a receive-only head coil (MDD/AD groups: 16 channels; HC: 32 channels) with the following parameters: 2D EPI readout; TR/TE=4000/14 ms; post-label delay=1525ms; label duration=1650ms; FOV=240 × 240mm; 17 7mm slices; voxel size=3 × 3 × 7mm; number of ASL volumes=183. Citalopram was administered as a bolus injection after 5 minutes of baseline imaging (37 ASL volumes). In addition, M0 and T1-weighted scans were obtained.

ASL post-processing was performed with the ExploreASL toolbox (Mutsaerts et al., 2016), to obtain pre- and post-citalopram cerebral blood flow (CBF) images. In short, T1w images were segmented into gray matter (pGM) and white matter (pWM) probability maps. Motion was estimated and motion spikes were excluded. Perfusion-weighted images were rigid-body registered to the pGM images. CBF was quantified using a single compartment model (Alsop et al., 2014). The pGM and pWM maps were spatially normalized using diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) (Ashburner, 2007), and all transformations were combined into a single interpolation to transform the CBF maps to the Montreal Neurological Institute (MNI) template (see Fig. 1 for an example of a representative CBF map and mean signal-to-noise ratio over scans). The first and last 37 ASL volumes were averaged to obtain the pre- and post-citalopram CBF maps respectively. Median CBF values were extracted for our four regions of interest (ROIs): orbitofrontal cortex (OFC), thalamus, amygdala and hippocampus (Fig. 2), in addition to overall gray matter (GM) CBF. These ROIs were chosen because they demonstrated the strongest age-dependent effects in the preclinical study by Klomp et al. (2012b). Subsequently, change scores of median CBF were calculated to represent the phMRI response. Heart-rate was recorded during the scan using a photoplethysmogram. A phase-contrast scan was used to obtain a measure of total blood flow to the brain pre- and post-citalopram.

2.4. Statistical analyses

SPSS version 22.0 (IBM) was used for statistical testing. Descriptive data of the sample were compared between groups using the appropriate (non)parametric tests. The other data were first assessed for

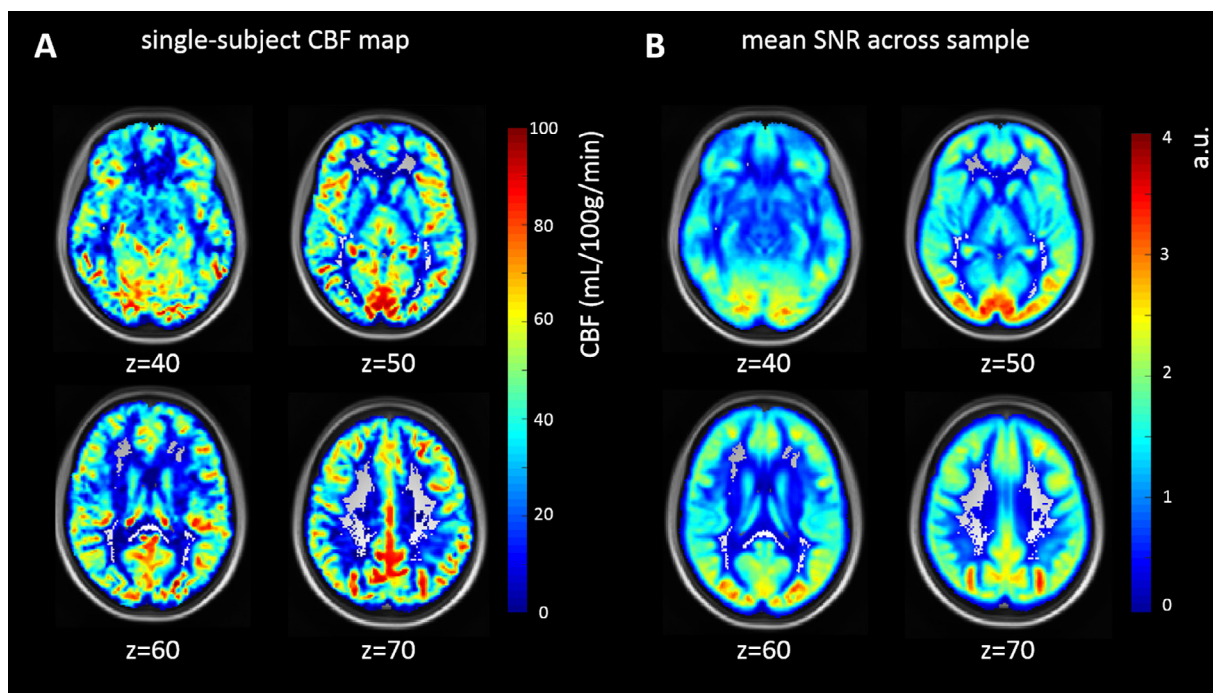


Fig. 1. CBF A) Representative CBF image of one subject B) mean SNR of CBF across the sample at 4 different transversal positions (z indicates the slice number), overlaid on the mean T1-weighted scan of all subjects, and masked with a mean grey matter probability mask.

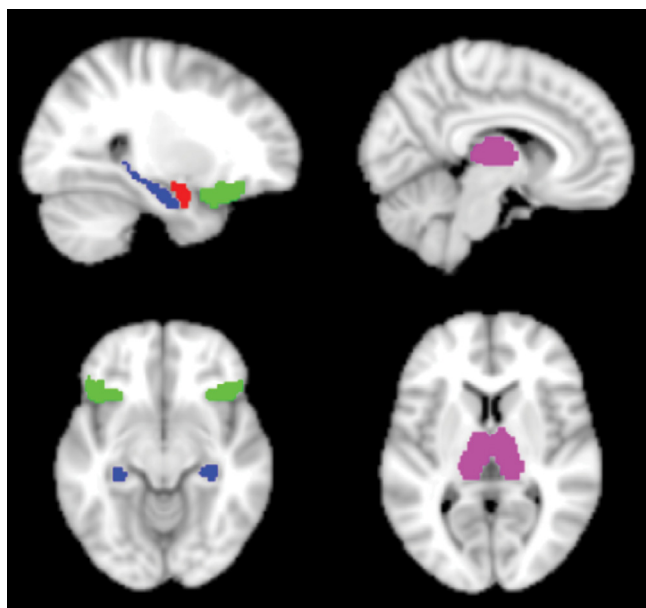


Fig. 2. Masks of the ROIs superimposed on a standard MNI152 brain. Green represents the orbitofrontal cortex, red represents the amygdala, blue represents the hippocampus and purple represents the thalamus.

normality and outliers and log-transformed when non-normally distributed. To assess the effect of age-of-first-exposure on the CBF response to the citalopram challenge (time), linear mixed models were performed for the four ROIs separately with change in median CBF as dependent variable. A compound symmetry covariance matrix was assumed, with a fixed intercept. The model was estimated using maximum likelihood. Significance for the linear mixed models was set at $p=0.025$ after Sidak's correction for the 4 ROIs. Follow-up pairwise comparisons were corrected for multiple testing using Sidak's correction. The variables heart-rate, age, and GM CBF were tested as possible confounders and if necessary, added to the mixed model analysis for

correction. In addition, we conducted an exploratory analysis to investigate the effect genotype on CBF (see Supplements).

3. Results

3.1. Sample characteristics

Sixty-five subjects were included in the study; $N=14$ in the UN group, $N=19$ in the EARLY, $N=18$ in the LATE group and $N=14$ in the HC group. One subject was not included in the analysis because she used sedative medication prior to the assessment and therefore could not receive the challenge medication. For 3 subjects, the scan protocol could not be completed and they were removed from the analyses. This resulted in 61 subjects who could be included in the statistical analyses (UN $N=14$, EARLY $N=17$, LATE $N=16$, HC $N=14$). Mean age of the subjects was 29.40 years (SD 4.84). Based on diagnosis history and the M.I.N.I. Plus, 26 subjects were diagnosed with only MDD, 5 with only AD and 16 with both MDD and AD.

Age differed significantly between the four groups ($H=40.24$, $p<0.001$) (Table 1). Also, current depressive score as measured with IDS differed between the three patient groups $F_{(2,44)}=5.29$, $p=0.009$. Based on cut-off scores that were calculated for the IDS (score of 18 or above (Rush et al., 1996) and BDI (score of 10 or above (Beck et al., 1988)), none of the HC scored as currently depressed, whereas 26 out of 47 patients scored as currently having depressive symptoms. As a result of the stratification, patient groups differed in age of first symptoms ($H=26.46$, $p<0.001$), age of first medication ($U=254.5$, $p<0.001$), as well as time since last medication ($U=36.5$, $p=0.001$). The patient groups did not differ significantly in total length of medication ($U=111.5$, $p=0.74$) or 5-HTTLPR genotype ($\chi^2=4.46$ $p=0.35$).

3.2. phMRI response

No group differences were found for head motion ($F_{(3,61)}=0.85$, $p=0.47$). For all ROIs, GM CBF, but not heart-rate or age, was a significant co-variate and was therefore added to the model. For amygdala CBF, change scores did not significantly differ between groups ($F_{(3,54)}$

Table 1
Demographics

	UNTREATED			EARLY			LATE			HC			Statistics
	M	SD	N	M	SD	N	M	SD	N	M	SD	N	
Age (y)	26.7	2.6	14	27.2	3.0	17	33.5	4.8	16	20.9	1.7	14	H = 40.24, p < 0.001
BMI	21.6	2.0	11	24.7	5.9	14	25.0	4.7	14	22.2	3.0	13	F(2,48) = 2.013, p = 0.13
IQ	108	4.6	14	104	7.1	16	108	7.1	16	113	8.1	14	H = 13.13, p = 0.004
IDS	30.0	12.4	14	19.5	10.8	17	18.1	9.2	16	-	-	-	F(2,44) = 5.29, p = 0.009
BDI	-	-	-	-	-	-	-	-	-	2.5	1.9	14	-
BAI	13.7	8.4	13	9.7	9.9	15	11.8	10.0	16	4.5	4.8	13	F(3,53) = 2.712, p = 0.054
First symptoms, y	19.0	5.0	12	16.9	3.0	17	28.6	5.2	16	NA	-	-	H = 26.46, p < 0.001
First medication, y	-	-	-	17.3	2.9	16	28.9	5.2	16	NA	-	-	U = 254.5, p < 0.001
Last medication, m	-	-	-	84.8	38.8	15	34.9	34.6	16	NA	-	-	U = 36.5, p = 0.001
Time treatment, m	-	-	-	32.0	35.5	15	20.6	14.8	16	NA	-	-	U = 111.5, p = 0.74
Genotype			N			N			N			N	
SS			3			1			2			2	$\chi^2 = 4.46, p = 0.35$
SL			9			8			9			9	
LL			2			8			5			5	

Abbreviations: BAI, Beck Anxiety Inventory; BMI, Body Mass Index; IDS, Inventory for Depressive Symptomatology; IQ, Intelligence Quotient; m, months; SD, standard deviation; y, years

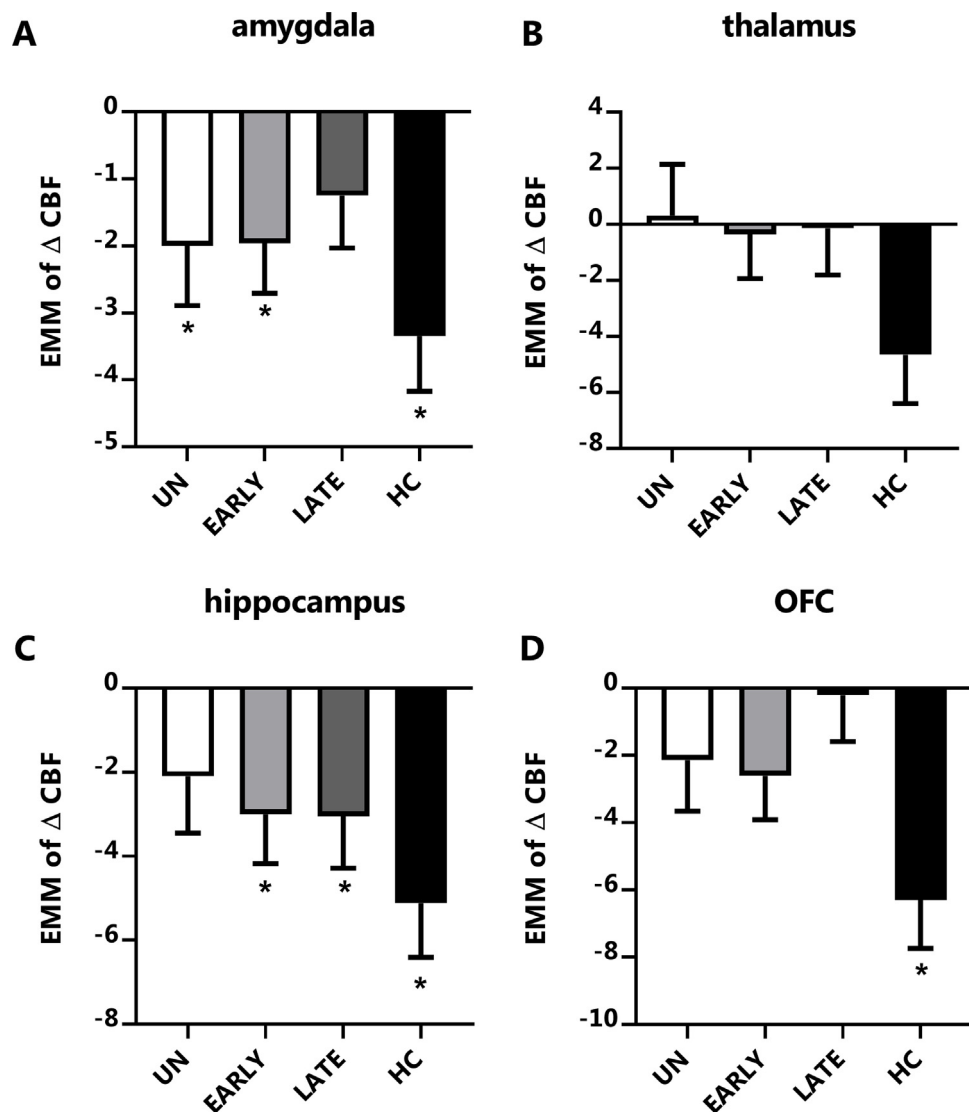


Fig. 3. Changes in CBF after the intravenous citalopram challenge. Delta median CBF values are depicted for the four groups (UN (white), EARLY (light grey), LATE (dark grey) and HC (black)). Bars represent estimated marginal means (EMM) (corrected for delta GM CBF). A) Amygdala, B) Thalamus, C) Hippocampus, D) Orbitofrontal cortex (OFC). * Indicates a significant difference between pre- and post-citalopram (Sidak's post-hoc correction).

= 1.15, $p = 0.34$), but change in amygdala CBF differed significantly from zero ($F_{(1,57)} = 27.45$, $p < 0.001$). Post-hoc tests indicated a significant decrease in mean CBF controlled for GM CBF in the UN ($p = 0.02$), EARLY ($p = 0.008$), and HC group ($p < 0.001$) after the citalopram challenge, but not for the LATE group ($p = 0.11$) (Fig. 3). For thalamus CBF, no group differences ($F_{(3,55)} = 1.77$, $p = 0.16$) nor a main effect of citalopram administration was found ($F_{(1,58)} = 1.15$, $p = 0.29$). In the hippocampus, no group differences in median CBF were found ($F_{(3,55)} = 0.97$, $p = 0.41$). However, a main effect of citalopram administration was present for median hippocampus CBF ($F_{(1,58)} = 24.18$, $p < 0.001$). Post-hoc tests revealed a decrease in median CBF in the EARLY ($p = 0.01$), LATE ($p = 0.01$) and HC group ($p < 0.001$), but not significantly for the UN group ($p = 0.12$) post citalopram challenge. In the OFC, we found a significant interaction effect between time and group ($F_{(3,55)} = 3.19$, $p = 0.03$), but this did not survive multiple comparison correction for the four ROIs. Subsequent (exploratory post-hoc tests show that the HC group showed a significantly stronger decrease in median CBF in the OFC compared to the late group ($p = 0.02$). Only the HC ($p < 0.001$) differed significantly from zero in terms of change in median OFC CBF, but not the UN ($p = 0.16$), EARLY ($p = 0.046$) or LATE group ($p = 0.89$).

3.3. Cardiovascular effects

As SERTs are also present outside the brain, and they are known to have vasoconstrictor capabilities, heart-rate and intracranial blood-flow (2D-flow) were additionally measured. ANOVA showed a baseline difference in heart-rate between the four groups ($F_{(3,56)} = 2.85$, $p = 0.046$), although post-hoc tests did not show any differences in heart-rate between the groups. Linear mixed models showed no interaction effect of group and time on heart-rate ($F_{(3,60)} = 1.34$, $p = 0.27$). However, a main effect of time on heart-rate was observed ($F_{(1,60)} = 36.92$, $p < 0.001$), indicative of an overall increase of heart-rate after the citalopram challenge (Supplementary Figure 1).

There was no group effect at baseline for 2D-flow ($F_{(3,57)} = 1.09$, $p = 0.36$), nor was there an interaction between group and time on 2D-flow ($F_{(3,59)} = 1.16$, $p = 0.33$). There was also no main effect of time ($F_{(1,59)} = 0.006$, $p = 0.94$) (Supplementary Figure 1). Lastly, no interaction effect was found for GM CBF ($F_{(3,61)} = 1.29$, $p = 0.29$), nor a main effect time ($F_{(1,61)} = 0.31$, $p = 0.58$) (Supplementary Figure 1).

4. Discussion

To the best of our knowledge, this is the first study that investigated the modulating effects of age following SSRI treatment on the human serotonin system. We compared the CBF responses to an acute citalopram challenge in young adult females with a life-time MDD and/or AD diagnosis, who were stratified into three groups: never treated with SSRIs (UN), first SSRI exposure before 23 (EARLY), and first treatment after 23 years of age (LATE), and they were compared to an additional group of healthy female control subjects (HC). We found a decrease in CBF after citalopram in the amygdala, hippocampus and orbitofrontal cortex across the whole sample. However, we did not find an age-dependent effect of SSRI exposure on the CBF response.

The lack of age-dependent effects of SSRI exposure on the CBF response in the measured ROIs in humans in the present study is in contrast with preclinical studies in which age effects were studied (Klomp et al., 2012b; Shrestha et al., 2014; Wegner et al., 1999). In the preclinical studies, SSRI exposure during development induced changes in the serotonin system, e.g. changes in the SERT availability and changes in the functional MRI response to a subsequent SSRI challenge. Given that the present study is the first investigating the age-dependent effects of SSRIs in humans, it is difficult to pinpoint the exact reason for this discrepancy. One explanation could be that the preclinical studies administered SSRIs at a pre-pubertal developmental stage (post-natal day 25 for rats and 2 years of age in rhesus monkeys), whereas our

study included mostly subjects that started medication use post-puberty (mean age-of-onset 17 years). This suggests that specific developmental windows might be particularly vulnerable to exposure to psychotropic medications. Alternative explanations include the study design, the different doses used, the route of administration, prior exposure, and the washout period, as well as the facts that previous studies had used healthy animals and we included patients. Moreover, the heterogeneity that is generally higher, and the lower effect sizes in human studies likely play a role as well. Additionally, as a result of the cross-sectional nature of the study, pre-existing group differences in several variables, such as current depressive score and time since last treatment, were present, making it difficult to disentangle the variance attributable to age-of-onset of SSRI use and the variance resulting from other differences.

Administration of the citalopram challenge resulted in a decrease in CBF in three of our four ROIs; i.e. the amygdala, hippocampus and OFC. This is generally in line with previous literature; Chen et al. (2011) reported a decrease in CBF in the amygdala and OFC, in addition to the fusiform gyrus and insula in healthy adult subjects following a single oral dose of citalopram, as measured with ASL based phMRI (Chen et al., 2011). Moreover, six weeks of escitalopram treatment decreased left interior temporal gyrus and the middle- and interior frontal gyri CBF in MDD patients (Kaichi et al., 2016). Also, ten days of citalopram treatment in healthy subjects reduced the response in the amygdala using a blood-oxygen level dependent (BOLD) contrast (Windischberger et al., 2010). Interestingly, we did not observe a main effect of the citalopram challenge on the thalamus across the whole sample; whereas another study by our group in healthy controls did find a significant phMRI response to citalopram in the thalamus (Schranter et al., 2019). In addition, Klomp et al. (2012) reported a decrease in the frontal gyrus and thalamus after an oral citalopram challenge in healthy females (Klomp et al., 2012a). This suggests that the thalamus might be differently affected by citalopram for patients with MDD/AD, but we did not find statistical evidence for this hypothesis in the current study. Nevertheless, the thalamus is densely enriched in SERT and previous studies have demonstrated lower SERT binding in the thalamus in MDD patients, be it with a relatively small effect size (Gryglewski et al., 2014). This could explain the observed differences with other studies.

In contrast to the studies mentioned above, most preclinical studies, and one clinical study that also used BOLD signal measurements, reported an increased activation following an SSRI challenge (Bouet et al., 2012; Klomp et al., 2012b; McKie et al., 2005; Schwarz et al., 2007). This is interesting, as typically a positive linear correlation between CBF and BOLD signal is found, mainly during (cognitive) task activation (Raichle et al., 1976; Sokoloff, 1981). However, when assessing drugs that affect both the vasculature and neural activity, the interpretation of the BOLD signal is more difficult, as the BOLD signal depends on neurovascular coupling and changes in cerebral blood flow, which is influenced by oxygen or glucose consumption and neurotransmitter release (Logothetis and Wandell, 2004). The change in MRI signal as a response to a 5-HT challenge is known to be directly influenced by extracellular 5-HT levels (Preece et al., 2009), and indirectly by binding of 5-HT to its receptors (Sekar et al., 2011), and lastly by neurotransmitter release per se (Logothetis and Wandell, 2004). Together, these factors might explain the reported increase in BOLD signal following SSRI treatment (Bouet et al., 2012; Klomp et al., 2012b; McKie et al., 2005; Schwarz et al., 2007), whereas decreases in ASL signal are reported in other studies (Chen et al., 2011; Kaichi et al., 2016; Klomp et al., 2012a; Windischberger et al., 2010). Hence, future research would benefit from measures that can distinguish between neuronal activation and vascular effects.

There are several limitations to our study. The first is the cross-sectional design. Secondly, inclusion of both healthy subjects and MDD/AD patients in our study could have resulted in a more heterogeneous sample, which could have decreased the power of the group

variable in the linear mixed models, especially given the already limited group sizes. We have aimed to reduce the variability, e.g. by including only female subjects. However, this restricts extrapolation of these findings to the male sex. Third, the numbers of patients that were currently still depressed (based on IDS cut-off) differed between our three patient groups (Table 1), resulting in an unequal distribution of remitted and currently depressed patients between the groups.

Also, age differed significantly between our four groups, which was mainly the result of the inclusion of our HC group from a different study sample. Yet, a strength of this addition is that it allowed us to compare the CBF response in patient groups to a healthy control sample. In our analysis, we tested these variables for their potential confounding effects and appropriate corrections were applied where necessary. Finally, we cannot rule out that age-of-onset of SSRI use is related to different subtypes of MDD/AD or SERT polymorphism, which might follow different developmental courses in terms of neurobiology and behavior. Hence, while this is, to our knowledge, the first study in humans, it is too early to conclude that SSRIs do not affect development of the human serotonergic system, particularly because of the discrepancies with earlier rodent work that did demonstrate a strong modulating effect of age using the same technique. Future studies using a longitudinal design, with a more homogeneous group need to be done to confirm our current findings that in humans SSRIs do not seem to affect development of the 5-HT system.

In conclusion, as measured with phMRI, a citalopram challenge induced a general decrease in CBF in the amygdala, hippocampus and OFC across groups. We did not find an age-dependent effect of first time SSRI exposure on this CBF response in depressed female patients, suggesting that the human serotonin system has not undergone major alterations after an early first exposure to SSRI treatment. We feel it is too early to conclude that SSRIs do not affect the development of the human serotonergic system, particularly because these first human data differ from earlier preclinical studies and follow-up studies with a longitudinal design and more homogeneous, larger group are needed.

Contributors

MS and AS were responsible for the data collection. MS, AS and JH were responsible for the data processing. MS was responsible for the statistical analyses. MS, AS, JH, PL and LR interpreted the data. MS and AS wrote the manuscript. All authors reviewed and approved of the manuscript.

Declaration of Competing Interest

None of the authors declare a conflict of interest.

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Supplementary materials

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