Psychotropic medications and the developing brain

Solleveld, M.M.

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Chapter 6

A pharmacological MRI study on the effects of selective serotonin reuptake inhibitors on the human serotonergic system: modulation by age of first use.

Michelle M. Solleveld

Anouk Schrantee

Henk-Jan J.M. Mutsaerts

Judith R. Homberg

Paul J. Lucassen

Liesbeth Reneman

In preparation

Abstract

Although studies in young animals have shown lasting effects of early antidepressant treatment on the development of the serotonin system, it is still unknown whether similar changes occur in humans. In this first exploratory study, we investigated in female depressed patients, whether the responsiveness of the human serotonin system to a selective serotonin reuptake inhibitor (SSRI) is modulated by the age of first SSRI treatment.

To this end, we used pharmacological Magnetic Resonance Imaging (phMRI) to measure cerebral blood flow (CBF) response to an acute citalopram challenge. Fifty-two females were stratified into three groups of patients: one for whom the first SSRI treatment took place before the age of 23, one with first SSRI treatment after age 23 and one with subjects who were never treated with SSRIs. Results were compared to a group of 14 healthy control subjects.

The citalopram challenge resulted in a significant overall decrease in median CBF in three regions studied: amygdala, hippocampus and orbitofrontal cortex. However, linear mixed model analyses failed to reveal any age-dependent effects of SSRI exposure on the CBF response.

While these first human data are in contrast to earlier preclinical studies that suggested chemical imprinting effects occur after early SSRI treatment, we conclude it is too early to assume that similar effects do not occur in the developing human serotonergic system. This awaits follow-up studies with a longitudinal design and larger, more homogeneous groups. Such studies are still lacking but urgently needed, given the earlier concerns, raised by the FDA a.o, regarding potential adverse, lasting effects of SSRI administration to children.

Introduction

Major Depressive Disorder (MDD) is a highly prevalent and severe psychiatric disorder that is characterized by feelings of worthlessness or excessive guilt, a depressed mood, fatigue and anhedonia[1] and affects more than 350 million people worldwide[2]. Selective serotonin (5HT) reuptake inhibitors (SSRIs) are commonly prescribed to treat MDD and/or anxiety disorder (AD) patients. Although it is still unclear how exactly SSRIs alleviate depressive symptoms[3], these drugs are thought to bind the serotonin transporter (SERT) and thereby reverse the reductions in serotonin (5HT) levels in the synaptic cleft in MDD patients[4,5]. Also drugs or approaches that deplete or reduce 5HT can trigger depressive symptoms[6] or alter emotional processing[7,8].

With a well-characterized safety profile[9] and a response rate of about 60% (compared to 47% in placebo)[10], SSRIs are currently the first line of treatment for adult MDD patients in many countries. Recently, however, SSRIs are increasingly prescribed to children and adolescents suffering from mood problems and MDD. While MDD prevalence is low in prepubertal children (1-2%), it ranges from 10 to 17% in the adolescent population[11]. It is important to note that although SSRIs decrease symptoms in childhood depression[12], they are often given at an age when the brain is still developing. Whether these drugs also affect brain development per se, or the serotonin system in particular, is still unknown.

Despite this lack of knowledge, prescription rates of antidepressants in children and adolescents have increased with 17.6% in the Netherlands alone in the period from 2005 to 2012[13]. Current evidence suggests that antidepressant exposure in adulthood is safe and does not induce long-term consequences[14], and it is therefore generally assumed that the same applies for children and adolescents. However, this assumption has recently been challenged as evidence accumulated that the developing brain responds differently to psychotropic drugs than the adult brain[15]. Also, the US Food and Drug Administration (FDA) issued a warning on SSRI treatment in children, because an increased risk for suicide was found after SSRI use in this age group[16].

Several preclinical studies have investigated effects of long-term SSRI treatment on the developing brain. For instance, Klomp et al. used pharmacological magnetic resonance imaging (phMRI) in juvenile rats and found that treatment with the SSRI fluoxetine resulted in an increased brain response to an acute SSRI challenge, whereas adult rats showed opposite effects[17]. Additionally, exposure to SSRIs in rats at a young age resulted in an increased 5HT transporter density when adulthood was reached[18]. Shrestha et al. further showed

that the SERT is upregulated in young adulthood when monkeys were treated with SSRIs during their juvenile period[19]. Moreover, Homberg et al. reported that fluoxetine treatment in rats exerts adverse, age-dependent effects on depressive behavior and wakefulness[20]. Finally, administration of a single injection of fluoxetine in juvenile rats was found to impact dendritic length and spine density[21]. Taken together, this raises the possibility that SSRI treatments at an early age may exert lasting effects and alter brain structure and function in the 'mature' brain.

So far, it is unknown whether the effects of SSRIs are also modulated by age in humans. Therefore, we here used phMRI to investigate in a first exploratory study in humans 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 is used as a proxy for 5HT function[22] and is as such ideally suited to study the age-dependent effects of SSRIs in the brain, at least in rats[17]. To this purpose, here we stratified young adult females with a life-time diagnosis of MDD and/or AD 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. In addition, a group of healthy young females without a MDD diagnosis (HC) was added in order to compare the phMRI response to citalopram in patients and healthy controls.

Methods

Participants

Fifty-two female participants were recruited through online advertisement and via collaborations with general practitioners and pharmacies, 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 anxiety disorder (AD). Participants were stratified into groups based on their age of first SSRI exposure (EARLY: before 23 years of age, LATE: after 23

years of age, and UN: no SSRI received). The cut-off criteria of these ages are based upon the fact that overall human brain maturation is considered to be incomplete until 18-20 years of age[23]. To exclude acute pharmacological effects, a medication-free interval of at least 3 weeks before scanning was maintained. In addition, 14 healthy female volunteers were included, whom were recruited through online advertisement and participated in a different study[24].

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)[25]. 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 dependence and contra-indications 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.

Procedure and behavioral measures

Participants first completed a neuropsychological test battery and subjective questionnaires, including the Inventory of Depressive Symptomatology (IDS, patients)[26], the Beck Depression Inventory (BDI, control subjects)[27], the Beck Anxiety Inventory (BAI)[28] and the Dutch Adult Reading Test[29]. Additionally, M.I.N.I. Plus[25] was used to determine whether the subjects were currently suffering or had suffered from depression and/or anxiety in the past.

Prior to the phMRI scan, salivary samples were collected from the previously depressed subjects for DNA analyses to determine the triallelic 5HTTLPR polymorphism, i.e. the gene that encodes for the SERT, and that is associated with MDD[30]. Genotyping of the 5HTTLPR polymorphism was performed using a simple sequence length analysis in a polymerase chain reaction, comparable to the methods in Van Strien et al.[31]. 5HTTLPR genotypes were coded as s/s, s/l and l/l genotypes.

Following these procedures, an intravenous line was placed and all subjects underwent the phMRI scan. During the phMRI ASL scan, a bolus of 7.5 mg (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[32].

Data acquisition

We assed changes in cerebral blood flow (CBF) induced by citalopram using pseudo continuous ASL (pCASL). pCASL was acquired on a 3.0T Philips Ingenia MR scanner (Philips Medical Systems, Best, the Netherlands) using a 16-channel receive-only head coil with the following parameters: 2D EPI readout; TR/TE=4000/14 ms; post-label delay=1525 ms; label duration =1650 ms; FOV=240x240 mm; 17 7 mm slices; voxel size=3x3x7 mm; number of dynamics=183. Citalopram was administered as a bolus injection after 5 minutes of baseline imaging (37 dynamics). In addition, M0 and 3D-TFE T1 scans were obtained, and a phase-contrast scan was used to obtain 2D-flow to the brain pre- and post-citalopram.

ASL post-processing was performed with the ExploreASL toolbox[33], 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[34]. The pGM and pWM maps were spatially normalized using DARTEL[35], and all transformations were combined into a single interpolation to transform the CBF maps to the Montreal Neurological Institute (MNI) template (see Figure 1 for an example of a representative perfusion-weighted image). The first and last 37 dynamics were averaged to obtain the pre- and post-citalopram CBF map respectively. Mean CBF values were calculated for our four regions of interest (ROIs): orbitofrontal cortex (OFC), thalamus, amygdala and hippocampus (Figure 2), in addition to overall gray matter (GM) CBF. We chose these ROIs as they demonstrated the strongest age-dependent effects in our earlier preclinical study by Klomp et al.[17]. Heart-rate was recorded during the scan using a photoplethysmogram.

Figure 1. CBF image
Representative CBF image masked by the individual gray matter mask

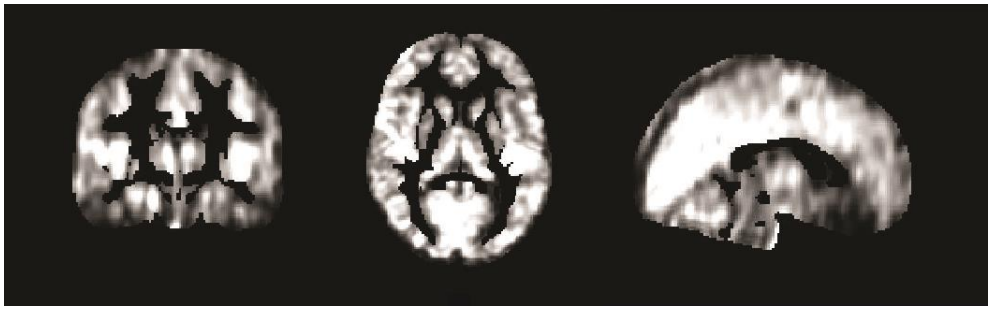
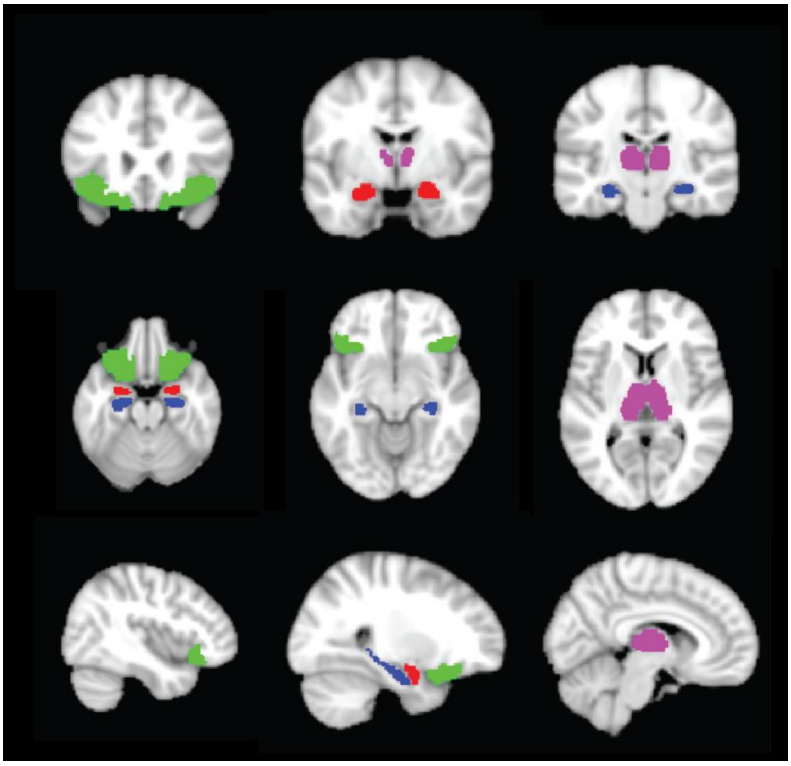


Figure 2. Masks
Masks of the ROIs superimposed on a standard MNI152 brain. Green represents the orbitofrontal cortex; red represents the amygdala; blue represents the hippocampus; purple represents the thalamus.



Statistical analyses

SPSS version 22.0 (IBM) was used for statistical testing. Data were first assessed for normality and outliers and log-transformed when non-normally distributed. To assess the interaction of age-of-first-exposure (group) with the CBF response to the citalopram challenge (session), linear mixed models were performed for the four ROIs separately. A compound symmetry covariance matrix was assumed, with a fixed intercept and the model was estimated using maximum likelihood. Significance for the linear mixed models was set at $p=0.0125$ after Bonferroni correction for the 4 ROIs. Follow-up pairwise comparisons were corrected for multiple testing using Sidak's correction. The variables motion, heart-rate, current depressive score, age, 5HTTLPR genotype and GM CBF were tested as possible confounders and if necessary, added to the mixed model analysis for correction.

Results

Sample characteristics

Sixty-six subjects were included in the study; N=14 in the UN group, N=19 in the EARLY, N=19 in the LATE group and N=14 in the control group (HC). 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 62 subjects who could be included in the statistical analyses (UN N=14, EARLY N=17, LATE N=17, HC N=14). According to the M.I.N.I. Plus[25], 15 subjects were diagnosed with only MDD, 3 with only AD and 22 with both MDD and AD (8 subjects did not receive a diagnosis due to incomplete M.I.N.I. Plus assessment). According to the M.I.N.I. Plus, none of the HC subjects were ever diagnosed with MDD or AD.

As shown in Table I, age differed significantly between the four groups ($F(3,58)=38.96$, $p<0.001$). Current depressive score as measured with IDS differed between the three patient groups ($F(2,45)=5.70$, $p=0.006$). As a result of the stratification, patient groups differed significantly in their age of first symptoms ($F(2,43)=30.32$, $p<0.001$), age of first medication ($F(2,31)=58.27$, $p<0.001$), as well as their total duration of medication use ($F(2,43)=7.55$, $p=0.002$), but also on time since last medication ($F(1,30)=11.29$, $p=0.002$). Furthermore, based on cut-off scores that were calculated for the IDS (score of 18 or above)[36] and BDI (score of 10 or above)[37], the patient groups had a significantly higher number of current depressive subjects compared to the healthy control group ($\chi^2=13.06$, $p<0.001$).

CBF

Baseline CBF values did not differ between the three patient groups in any of the four ROIs: amygdala ($F(2,45)=1.39$, $p=0.260$), thalamus ($F(2,45)=0.80$, $p=0.454$), hippocampus ($F(2,45)=0.98$, $p=0.382$) and OFC ($F(2,45)=0.83$, $p=0.442$) (Table II), nor was there a difference in median total GM CBF between the three patient groups ($F(2,45)=1.01$, $p=0.374$). Also no baseline differences were found between the patient groups for motion ($F(2,45)=0.27$, $p=0.765$) or genotype (5HT-transporter-linked polymorphic region (5HTTLPR) (chi-square $p=0.338$, Table I). Heart-rate, GM CBF and age had either a high correlation with CBF in the ROIs, or a significant difference between the groups. They were therefore added as possible

Table I. Sample characteristics at baseline

	UN N=14	EARLY N=17	LATE N=17	HC N=14	p-value
Age (SD), y	26.71 (2.64)	27.24 (2.95)	33.76 (4.79)	20.93 (1.73)	<0.001[a]
BMI (SD)	21.67 (2.01)	24.72 (5.90)	25.35 (4.71)	22.22 (2.97)	0.084
IQ (SD)	107.64 (4.60)	103.94 (7.12)	107.00 (8.89)	113.00 (8.05)	0.015[b]
IDS (SD), score	30.00 (12.37)	19.53 (10.81)	17.71 (9.12)	NA	0.006[c]
BAI (SD), score	13.69 (8.42)	9.67 (9.91)	11.18 (9.98)	4.54 (4.82)	0.062
Age first symptoms (SD), y	19.00 (4.95)	16.94 (2.99)	28.24 (5.23)	NA	<0.001[d]
Age first medication (SD), y	NA	17.31 (2.87)	28.59 (1.26)	NA	<0.001[e]
Time since last medication (SD), m	NA	84.80 (38.67)	39.24 (37.93)	NA	0.002[f]
Total length of treatment (SD), m	0.00 (0.00)	32.00 (35.46)	22.88 (17.21)	NA	0.002[g]
5HTTLPR genotype					0.338[h]
SS	3	1	3	NA	
SL	9	8	9	NA	
LL	2	8	5	NA	

Abbreviations: BAI, Beck Anxiety Inventory; BMI, Body Mass Index; IDS, Inventory for Depressive Symptomatology; IQ, Intelligence Quotient; m, months; NA, not available; SD, standard deviation; y, years

[a] UN versus LATE, EARLY versus LATE, UN vs HC, EARLY vs HC, LATE vs HC p<0.001
[b] EARLY versus HC p=0.009
[c] UN versus EARLY p=0.029, UN versus LATE p=0.008
[d] LATE versus UN and LATE versus EARLY p<0.001
[e] EARLY versus LATE p<0.001
[f] EARLY versus LATE p=0.002
[g] EARLY versus UN p=0.001
[h] Pearson Chi-Square

Table II. CBF (mL/100g/min) pre- and post-citalopram challenges for the three patient groups. Due to scan parameter differences between the patient groups and the healthy control group, absolute CBF values could not be compared between patients and controls.

	UN		EARLY		LATE	
	pre	post	pre	post	pre	post
OFC (SD)	56.82 (22.20)	50.94 (16.60)	52.05 (18.97)	49.40 (16.66)	61.15 (20.74)	62.39 (23.74)
Amygdala (SD)	42.86 (18.56)	36.48 (14.49)	39.54 (14.66)	37.37 (13.89)	49.00 (17.16)	48.60 (17.53)
Thalamus (SD)	57.61 (21.61)	59.76 (19.26)	61.62 (22.72)	61.24 (21.38)	67.47 (21.14)	68.45 (22.28)
Hippocampus (SD)	55.17 (19.75)	48.71 (17.86)	55.53 (20.43)	52.49 (18.49)	64.01 (20.97)	62.04 (21.72)

Abbreviations: OFC, orbitofrontal cortex; SD, standard deviation

confounders to the mixed model analysis to correct for their possible confounding influences.

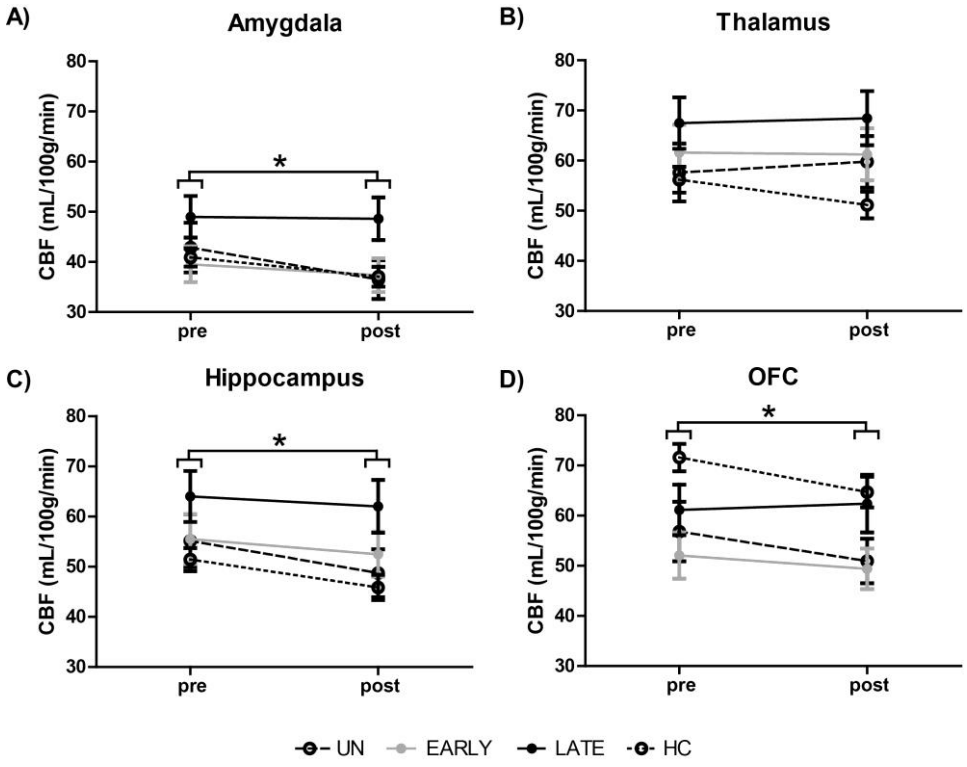
Linear mixed model analysis showed a main effect of session for median CBF in the amygdala ($F(1,62)=13.33$, $p=0.001$), but no significant interaction effect of session x group ($F(3,62)=1.87$, $p=0.145$). Addition of the covariates heart-rate, GM CBF and age did not alter this main effect of session ($F(1,76)=14.24$, $p<0.001$), although GM CBF also had a significant main effect ($F(1,73)=498.11$, $p<0.001$). Post-hoc tests indicated a significant decrease in mean CBF in all the groups after the citalopram challenge ($p<0.001$) (Figure 3).

For thalamus CBF, no interaction of group x session was found ($F(3,62)=2.67$, $p=0.055$), nor was there a main effect of group ($F(3,62)=1.35$, $p=0.265$) or session ($F(1,62)=0.17$, $p=0.682$). When the covariates were included in the model, a significant main effect of group was found ($F(3,60)=12.93$, $p<0.001$) (Figure 3). In addition, GM CBF showed a main effect ($F(1,65)=490.18$, $p<0.001$). Post-hoc tests showed significant differences between the HC group and the three patient groups in overall CBF of both sessions (HC vs. UN $p<0.001$, HC vs. EARLY $p<0.001$, HC vs. LATE $p=0.009$).

In the hippocampus, no interaction was found for group x session on median CBF ($F(3,62)=1.11$, $p=0.353$), nor a main effect of group ($F(3,62)=1.73$, $p=0.170$). A main effect of session was present for median hippocampus CBF ($F(1,62)=14.17$, $p<0.001$). After addition of the covariates to the model, the main effect of session on median hippocampus CBF remained significant ($F(1,76)=13.82$, $p<0.001$), in addition to a main effect of group ($F(3,62)=6.03$, $p=0.001$) (Figure 3). Also, a main effect of GM CBF ($F(1,73)=350.62$, $p<0.001$) was found. Post-hoc tests revealed a decrease in median CBF in all groups post citalopram challenge ($p<0.001$). Additionally, a difference in median CBF (mean of both sessions) was found between the HC subjects and the two of the three patients groups (HC vs. UN $p=0.006$, HC vs. EARLY $p=0.001$).

In the OFC, no interaction of group x session ($F(3,62)=2.02$, $p=0.121$) was found, but a significant main effect of group ($F(3,62)=3.79$, $p=0.015$) and session ($F(1,62)=8.53$, $p=0.005$) was found. After the covariates were added to the model, these main effect of sessions ($F(1,75)=6.86$, $p=0.011$) and group ($F(3,61)=4.29$, $p=0.008$) remained present. Furthermore, a significant main effect of GM CBF was found as well ($F(1,72)=705.70$, $p<0.001$). Again, post-hoc tests showed a significant decrease in median CBF over all subjects post citalopram ($p=0.011$). Furthermore, the HC groups differed in median CBF (mean of two sessions) from the EARLY

Figure 3. Results
CBF changes after the intravenous citalopram challenge. Median CBF values are depicted for the four groups (UN (dashed), EARLY (light gray), LATE (dark gray) and HC (dotted)). Bars represent mean values, while the error bars represent the standard error of the mean. A) Amygdala, B) Thalamus, C) Hippocampus, D) Orbitofrontal cortex (OFC). * Indicates a significant reduction in CBF between pre- and post-citalopram, regardless of group (Sidak's post-hoc $p<0.05$).



patient group ($p=0.005$), but not from the UN ($p=0.306$) or LATE ($p=0.220$) group (Figure 3).

Cardiovascular effects

As SERTs are also present outside the brain, and they are known to have vasoconstrictive properties, heart-rate and intracranial blood-flow (2D-flow) were additionally measured, as this may have influenced our results. ANOVA showed a baseline difference in heart-rate between the four groups ($F(3,57)=3.02$, $p=0.037$, although post-hoc tests did not show any differences in this parameter between the

groups. Linear mixed models showed no interaction effect of group and session on heart-rate ($F(3,61)=1.27$, $p=0.294$). However, a main effect of session on heart-rate was observed ($F(1,61)=36.92$, $p<0.001$), indicative of an overall increase of heart-rate after the citalopram challenge ($p<0.001$) (Supplementary Figure 1).

There was no baseline effect of group on 2D-flow ($F(3,58)=1.60$, $p=0.200$), nor was there an interaction of group and session on 2D-flow ($F(3,60)=1.16$, $p=0.332$), nor a main effect of group ($F(3,61)=1.82$, $p=0.154$) or session ($F(1,60)=0.00$, $p=0.984$) (Supplementary Figure 1). Lastly, no interaction effect was found for GM CBF ($F(3,62)=1.03$, $p=0.386$), nor was a main effect of group ($F(3,62)=2.17$, $p=0.100$) or session ($F(1,62)=0.48$, $p=0.493$) on GM CBF observed (Supplementary Figure 1).

Discussion

To our knowledge, this is the first study that investigated possible modulatory effects of age of first SSRI treatment on the responsiveness of 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), who were compared to an additional group of healthy female control subjects.

Administration of the citalopram challenge resulted in a significant overall decrease in median CBF in three ROIs: amygdala, hippocampus and OFC. This result is generally in line with previous literature; also Chen et al. 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[22]. In addition, also using ASL based-phMRI, we found a decrease in thalamic CBF in healthy female subjects after an intravenous citalopram injection[24], while Klomp et al. reported a decrease in the frontal gyrus and thalamus after an oral citalopram challenge in healthy females[38]. Six weeks of escitalopram treatment decreased CBF in the left interior temporal gyrus and in the middle- and interior frontal gyri of MDD patients[39]. Also, ten days of citalopram treatment in healthy subjects reduced the blood-oxygen level dependent (BOLD) response in the amygdala[40].

Most preclinical studies, however, and one clinical study that also measured BOLD signal, reported an increased activation following an SSRI challenge[17,32,41,42]. This is interesting, as typically a positive linear correlation

between CBF and BOLD signal is found, mainly during functional MRI task activation[43,44]. However, when assessing drugs that affect both the vasculature and neural activity, interpretation of the BOLD signal is more difficult, as it depends on neurovascular coupling and changes in cerebral blood flow, which is influenced by oxygen or glucose consumption and neurotransmitter release[45]. The change in MRI signal in response to a 5HT challenge is known to be modified directly by the extracellular 5HT levels[46], and indirectly by the binding of 5HT to its receptors[47]. Also interactions of these receptors[48] and neurotransmitter release per se may modify this signal[45]. Together, this may explain the reported increases in BOLD signal following SSRI treatment[17,32,41,42], whereas decreases in ASL signal are reported in other studies[22,38–40]. Future research would benefit from more specific measures that can distinguish between neuronal activation and vascular effects.

We did not find significant interaction effects between group and session, indicating that there are no age-dependent effects of SSRI exposure on the CBF response to citalopram, at least in the ROIs studied here. This is not in line with our earlier preclinical phMRI study in which we found a diminished BOLD response to an acute 5HT challenge, in several cortical and subcortical areas including the amygdala, in rats that were first treated with SSRIs at an adult age; whereas in rats first treated with SSRIs at an adolescent age, this response was increased[17]. Furthermore, other preclinical studies done by Wegerer et al. and Shrestha et al.[18,19], did also find age-dependent effects of SSRI treatment on the 5HT system, opposite to our results.

In the current study on age-dependent effects of SSRI exposure on the CBF response to citalopram, we did not find a significant effect of age of first SSRI exposure on this response (Figure 3). However, all studies in which significant age-dependent effects of SSRI exposure were reported before, were conducted in animals. Furthermore, these preclinical studies used the SSRI fluoxetine, whereas we used the SSRI citalopram. This response is here investigated for the first time in humans and perhaps differences in type of antidepressant and dosage (i.e. typically 5 mg/kg fluoxetine in rats versus in total 7.5 mg citalopram in humans), route of administration, and differences in type of prior SSRI exposure (all types of SSRI exposure in the patient subjects versus solemnly fluoxetine exposure in rats), may thus have contributed to the differences between studies. Furthermore, in our preclinical study, the chronic treatment with fluoxetine was followed by a one week washout period, whereas in our human cohort, the wash-out period varied strongly, on average 3.3 years, which could be compared to around one month in a rat's life[49]. An alternative explanation for these contradictions could be that in our

current study, depressed patients were included, whereas in preclinical studies, effects of SSRI exposure were investigated in healthy animals.

There are several limitations to our study. First, the retrospective design of this first exploratory approach, could have led to a recall bias. The inclusion of both healthy subjects and life-time depressed 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. Additionally, in the three patient groups, differences on several variables were present, such as time since last treatment and total length of treatment, that could not be controlled for in this retrospective design. Furthermore, the numbers of patients that were currently still depressed (based on IDS cut-off) differed between our three patient groups (Table I), resulting in 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, although this addition allowed us to compare the CBF response in the patient groups to a healthy control sample. However, in our analysis we tested these variables for their potential confounding effects and appropriate corrections were done where necessary. Lastly, both subjects with MDD and/or AD were included in this study. There were 3 subjects who suffered only from AD based on the M.I.N.I. Plus, but all other subjects either suffered from MDD or from both conditions. Although these two disorders are often comorbid and may share similar mechanisms and/or brain changes, this might in theory have contributed to our results. Hence, while this is to our knowledge the first study in humans, which shows discrepancies with earlier rodent work, it is too early to conclude that SSRIs do not affect development of the human serotonergic system, as future studies of a longitudinal nature, with a more homogeneous group need to be done to address this.

Conclusion

In conclusion, stimulation of the 5HT system using an acute citalopram challenge induced a general decrease in CBF in the amygdala, hippocampus and OFC. 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. Although these first human data differ from earlier preclinical studies, we find it too early to conclude that SSRIS do not affect the development of the human serotonergic system. For that, future follow-up studies with a longitudinal

design and a larger and more homogeneous group are needed. Such knowledge is still lacking but urgently needed, particularly given earlier concerns regarding potential adverse effects of SSRIs administered to children (e.g., black box warning issued by the FDA in 2004[51]).

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Supplementary Figure 1. Cardiovascular effects
Changes in cardiovascular variables after the intravenous citalopram challenge. Median values are depicted for the four groups (UN (dashed), EARLY (light gray), LATE (dark gray) and HC (dotted)). Bars represent mean values, error bars represent standard error of the mean. A) heart-rate, B) 2D-flow, C) GM CBF. * Indicates a significant difference between pre- and post-citalopram (Sidak's post-hoc $p<0.05$).