Citalopram challenge functional magnetic resonance imaging for investigating the role of serotonin neurotransmission in healthy brain and in migraine

PhD thesis

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List of Abbreviations

5-HIAA	5-hydoxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
5-HTT	serotonin transporter
5-HTTLPR	serotonin transporter promoter polymorphism
ACC	anterior cingulate cortex
aMCC	anterior middle cingulate cortex
ANOVA	analysis of variance
AUC	area under the curve
BOLD	blood oxygen level dependent
CNS	central nervous system
fMRI	functional magnetic resonance imaging
FWE	Family-Wise Error
GABA	gamma-aminobutyric acid
GWAS	genome-wide association study, whole genome association study
iv	intravenous
LASSO	least absolute shrinkage and selection operator
MCG	middle cingulate cortex
MNI	Montreal Neurological Institute
MTG	middle temporal gyrus
PET	positron emission tomography
PCC	posterior cingulate cortex
pgACC	pregenual anterior cingulate cortex
phMRI	pharmacological magnetic resonance imaging
RB	robustness
RDoc	Research Domain Criteria
ROI	region of interest
SPET	single photon emission tomography
SPM	Statistical Parametric Mapping

SSRI	selective serotonin reuptake inhibitor

1. Introduction

1.1 The serotonergic system and its role in the human brain

Serotonergic nuclei in the central nervous system (CNS) are mostly localized in the Raphe nuclei in the midbrain and medulla oblongata and their axons are widely distributed in the brain (Takeutchi, 1988), which is in line with the diverse role of serotonin (5-hydroxytryptamine, 5-HT) in complex brain mechanisms and several aspects of human behaviour.

Up to this day 14 serotonergic receptor subtypes in 7 receptor families are known. The 7 families or classes (5-HT1-7) are splitted into subclasses or suptypes based on their structural and pharmacological characteristics. The subtypes are denoted by letters. (Hoyer et al, 1994, Sharp and Barnes, 2020).

5-HT1A and 5-HT1B receptor subtypes are functioning as autoreceptors and they are located pre- and postsynaptically. 5-HT1A receptors regulate serotonergic tone of the CNS by inhibiting neural firing and reducing the release of 5-HT. In terms of its central nervous system role 5-HT1A receptors are involved in controlling cognition, mood, thermoregulation and motor activity and based on growing evidence its agonists have anxiolytic and antidepressant effects (e.g. buspiron). 5-HT1B receptors are located on the soma and inhibit 5-HT release and they are involved in regulation of mood, anxiety, behaviour and based on animal models its antagonists exert antidepressant and anxiolytic effects. 5-HT1B and 5-HT1D receptor agonists, the triptans are commonly used for acute treatment of migraine and 5-HT1F agonists might be also effective in acute migraine pain. (Sharp and Barnes, 2020) 5-HT2 receptors also have diverse role: regulation of body temperature, sleep, mood, anxiety, sexual behaviour and eating. (Hoyer et al, 1994) 5-HT2A receptor agonists have hallucinogen and antidepressant effects, while its antagonists are linked to improved efficacy and better profile of side effects of antipsychotic drugs (e.g. clozapine, aripiprazole). (Sharp and Barnes, 2020) 5-HT2B and 5-HT2C receptors exert NO synthesis and vasodilatation and their agonists have migraine-inducing effect. (Johnson et al, 1998) 5-HT2C receptor subtype also plays a role in appetite, motor functions, sleep and thermoregulation. 5-HT3 receptors play a role in, appetite, nausea, vomiting and mood and anxiety, their antagonist, the so-called setrons

exert antiemetic and possibly anxiolytic and procognitive effects. 5-HT4 receptors play a role in motor activity, eating, cognition and emotions and its agonists possibly have antidepressant effects based on preclinical research. (Hoyer et al, 1994, Sharp and Barnes, 2020).

The impact of 5-HT5, 5-HT6 and 5-HT7 receptor subtypes is still under discovery, presumably they play a role in cognition, learning and memory. (Sharp and Barnes, 2020)

Based on the above shortly summarized roles in various behavioural and physiological functions of different 5-HT receptor subtypes it can be admitted that it is essential to investigate the serotonergic system to understand the mechanism of these functions in the healthy functioning brain and to deepen our understanding in the pathomechanism of mental and neurological disorders that are related to 5-HT neurotransmission such as migraine. In the next chapters I shortly provide insight into the neuroimaging methods that allow us to investigate the serotonergic neurotransmission in the living human brain with special focus on functional MRI (fMRI) and summarize the role of this neurotransmitter in neuroticism personality trait and in migraine, which both are associated with altered 5-HT.

1.2 Neuroimaging 5-HT neurotransmission

Neuroimaging methods can be used to obtain information, directly or indirectly, about the structure or functioning of the human brain. The most commonly used methods for neuroimaging neurotransmission in human brain are functional magnetic resonance imaging, positron emission tomography (PET) and single photon emission tomography (SPET) (Edes et al, 2014).

Pharmacological magnetic resonance imaging (phMRI or pharmacoMRI) is a suitable method for visually examining the effect of drugs with fMRI in the living human brain, and analysing brain activation pattern during or after the administration of a drug. By administration of a molecule with known pharmacokinetic and pharmacodynamic profile, it might be possible to observe which areas of the brain are affected by the drug and the obtained data is also able to show us the time course of changes in blood oxygen level dependent (BOLD) signal (Edes et al, 2014). BOLD signal comes from the different magnetic characteristic of oxy- and deoxyhaemoglobin. Due to increased neuronal

activation blood flow and oxyhaemoglobin amount increases and deoxyhaemoglobin concentration decreases locally which leads to increased BOLD signal in the given brain area (Anderson et al, 2008). With choosing the appropriate serotonergic drug it is feasible to investigate the acute or chronic effects of changed 5-HT neurotransmission in different brain areas and on different brain functions during specific tasks (modulation phMRI) or on resting brain activation (challenge phMRI) (Anderson et al, 2008; Edes et al, 2014).

1.2.1 Modulation phMRI methods to investigate the 5-HT neurotransmission

Modulation phMRI is widely used to investigate the modulating effects of drugs during information processing (Anderson et al, 2008; Khalili-Mahani et al, 2017).

Selective serotonin reuptake inhibitor (SSRI) administration has been used to acutely increase synaptic 5-HT content and to investigate modulatory effects of increased 5-HT on neuronal responses induced by emotional and cognitive processing tasks with phMRI (Anderson et al, 2008). Following the study by Del-Ben in 2005 (Del-Ben et al, 2005), numerous studies have shown decreased amygdala activation in healthy subjects after one dose of SSRI during viewing fearful facial expressions.

"In depressed patients, suppression of amygdala responses to fearful faces occurs early in treatment and predicts subsequent therapeutic benefit (Anderson et al, 2011; Godlewska et al, 2012, 2016; Harmer et al, 2006; Klomp et al, 2013; Murphy et al, 2009). A number of studies have reported modulatory phMRI effects not only on emotion processing (Anderson et al, 2007, 2011; Harmer et al, 2003; Pringle and Harmer, 2015), but also on reward and punishment sensitivity (Macoveanu, 2014), attention, memory and response inhibition (Anderson et al, 2008). More recently a number of studies have described modulation of connectivity in resting-state networks by SSRIs and other drugs in humans (Klaassens et al, 2015, 2017, 2018) and rodents (Schaefer et al, 2014; Schwarz et al, 2009)." (Edes et al, 2020, p. 2)

1.2.2 Challenge phMRI methods to investigate the 5-HT neurotransmission

Some studies used phMRI method to investigate the direct effects of increased extracellular 5-HT level on the resting brain activation, induced by SSRIs.

“McKie and colleagues reported increasing BOLD signal following i.v. citalopram in the caudate, amygdala, hippocampus, striatum and thalamus in 12 healthy male volunteers compared to i.v. saline (McKie et al, 2005). Similar results have been observed in rodents (Schwarz et al, 2007; Sekar et al, 2011). Although this method has the potential to quantify the dynamism of serotonergic neurotransmission in disorders such as depression, this has not yet been realised.” (Edes et al, 2020, p. 2)

In my work I used the paradigm developed by McKie et al and aimed to characterise and investigate the time course of brain activation and its dose responsiveness.

1.3 Traits and disorders related to 5-HT neurotransmission

As discussed above, in terms of its central nervous system role, 5-HT is involved in several aspects of behavior, such as appetite (Blundell, 1977), sexual activity (Meston and Gorzalka, 1992), mood (Maes and Meltzer, 1995), learning and memory (McEntee and Crook, 1991), aggression (Coccaro, 1992), anxiety (Soubrie, 1988) and pain (Le Bars, 1994). For this reason, in addition to depression, alterations of serotonergic system have been associated with a number of psychiatric and neurologic illnesses, such as anxiety and eating disorders (Murphy et al, 1989), and migraine (Sicuteri et al, 1961; Curran et al, 1965).

1.3.1 5-HT neurotransmission in neuroticism and emotion control

One potential common risk factor for the above mentioned neurologic and psychiatric disorders is neuroticism, a personality trait that indicates emotional lability and sensitivity to stressors. “In early life central serotonergic tone modulates development of the sensory system and the corticolimbic circuits (Booij et al, 2015; Gaspar et al, 2003) that may contribute to behavioural traits such as neuroticism which acts as a vulnerability factor for several neuropsychiatric disorders (Gaspar et al, 2003; Hariri and Holmes, 2006; Lesch et al, 1996). Neuroticism is an endophenotype and major risk factor for disorders treated by SSRIs such as anxiety, depression and chronic pain (Kendler et al, 2006; Kendler et al, 2007; Ligthart et al, 2012). Previous human studies suggested that neuroticism and alterations in emotion processing were associated with genetically less active serotonin transporter gene variants (5-HTTLPR) (Hariri and Holmes, 2006; Lesch et al, 1996; Munafo et al, 2009), although some studies (Middeldorp et al, 2007;

Terraccino et al, 2009; Willis-Owen et al, 2005) and a large whole genome association study (GWAS) have failed to support this hypothesis (Smith et al, 2016)

One explanation for the inconsistent genetic results is that the less active serotonin transporter, putatively associated with increased synaptic serotonin content, might amplify processing of both positive and stressful environmental stimuli as reported in gene-environment interaction studies (Belsky et al.,2009) and in a human experimental SSRI study (Fox et al., 2011).

Similarly, it has been hypothesised that SSRIs acutely facilitate positive sensory information processing that may be related to their therapeutic effect (Browning et al, 2007; Castren, 2005, 2013; Harmer et al, 2003; Harmer and Cowen, 2013). Furthermore, according to the neuroplasticity theory, the therapeutic effect of long-term SSRI treatment is mediated by changes in synaptic plasticity in interaction with environmental factors (Kraus et al, 2017; Umemori et al, 2018).” (Edes et al, 2020, p. 2)

Based on the above summarised observations the aim of my work was also to determine whether the citalopram challenge-elicited brain activation changes, measured by local BOLD signal changes, relates to neuroticism.

1.3.2 5-HT neurotransmission in migraine

During my work I was specifically interested in migraine pathophysiology and its relationship to 5-HT neurotransmission. Migraine is a primary headache with recurring moderate or severe headache attacks characterised by usually unilateral, throbbing pain, accompanied with photo- and phonophobia, nausea and vomiting. “5-HT has been implicated in migraine pathophysiology for a long time. Several studies have found increased urinary 5-HT metabolite 5-hydoxyindoleacetic acid (5-HIAA) levels in migraine patients ictally (Sicuteri et al, 1961; Curran et al, 1965). One study showed increased levels of 5-HIAA in cerebrospinal fluid in migraineurs during attacks (Kangasniemi et al, 2005). In addition, interictally decreased plasma 5-HT levels were also observed in migraine patients (Ferrari et al, 1989; Juhasz et al, 2003a, 2003b, 2004, 2005). These early observations of the altered serotonergic neurotransmission were the basis of the idea that migraine is characterized with chronically low 5-HT levels and with a temporary increase during headache. However, PET studies previously reported

increased 5-HT brain levels indexed by 5-HT4 receptor binding in episodic (Deen et al, 2018, 2019) and chronic (Deen et al, 2019) migraine patients, therefore the theory of low 5-HT levels in migraine has been questioned.

Serotonergic challenge is a method widely used to investigate the effect of acute increases in 5-HT level or serotonergic receptor activation due to administration of a serotonergic agent (Panconesi et al, 2008; Anderson et al, 2008). Reserpine, fenfluramine and m-chlorophenylpiperazine (mCPP) are widely used to investigate serotonergic neurotransmission, however, these drugs often provoke headache in migraine patients (Panconesi et al, 2008). However, SSRIs are able to increase brain 5-HT levels, have little affinity to other receptors (Baumann et al, 1996) and rarely provoke headache in migraine (Juhasz et al, 2004)." (Edes et al, 2019, p. 2)

1.3.3 The effect of 5-HT neurotransmission on ACC function in pain modulation

Migraine patients repeatedly suffer from debilitating pain which activates an extensive system of brain areas that are involved in pain processing. An important brain region of this system is the anterior cingulate cortex (ACC) (Edes et. a, 2016). ACC is "a key structure of the pain processing network (May, 2009) as it is involved in descending pain modulation, attention to pain (Tracey and Mantyh, 2007), emotional dimensions of pain and has also been implicated in the pathophysiology of pain related disorders (Baliki et al, 2006). Important structural and functional alterations have been found in this brain area in case of experimental headache, medication-overuse headache, tension-type headache and migraine (May, 2013). A recent meta-analysis of grey matter changes in migraine showed a grey matter volume decrease in ACC associated with headache frequency (Jia and Yu, 2017). However, the specificity of grey matter changes in this region is questionable as this phenomenon has been observed in several other psychiatric and neurologic conditions (Hougaard et al, 2016). Previous whole brain functional MRI (fMRI) analysis showed increased brain activation during noxious trigeminal heat stimulation specifically in the ACC in migraine patients compared to healthy controls (Russo et al, 2012). However, another study with similar method did not find any differences between the two groups (Stankewitz et al, 2011)." (Edes et al, 2019, p. 2)

“Animal studies have demonstrated that activation of excitatory synapsis in the ACC facilitate pain perception and increase the sensitivity to unpleasantness of pain (Bliss et al, 2016), specifically, glutamatergic projections from the ACC enhance spinal sensory transmission which have been shown to amplify pain (Zhuo et al, 2017).” (Edes et al, 2019, p. 2) 5-HT modulates the excitatory neurotransmission in this brain area, more precisely 5-HT inhibits glutamate release in the ACC (Tian et al, 2017).

In line with these results, study in healthy humans showed that subchronic administration of SSRI escitalopram decreased the ventral ACC activation during aversive vs. pleasant image anticipation (Simmons et al, 2009). “In addition, 7 days of fluvoxamine treatment in healthy individuals led to decreased regional cerebral blood flow in the ACC during painful stimuli (Nemoto et al, 2003). These observations show the impact of serotonergic neurotransmission on the ACC functions and on corresponding pain modulation.” (Edes et al, 2019, p. 2)

As the citalopram challenge has not been used in migraine research my aim was to investigate the effect of acutely increased serotonin neurotransmission in migraine patients focusing on the BOLD signal changes in the ACC.

1.4 Short overview of the research

In summary, during my PhD I used the novel citalopram challenge phMRI method, first time in Hungary, to investigate the 5-HT neurotransmission in living human brain. My aim was to determine the effect of acute increase of serotonergic neurotransmission on brain activation in healthy controls and in migraine patients and to provide further data on the relationship between 5-HT neurotransmission and neuroticism. My objectives are listed in the next paragraph.

2. Objectives

In my work I applied challenge phMRI to investigate acute brain activation pattern during and after SSRI administration in resting brain, in healthy subjects and migraine patients. In study I, 32 healthy participants (aged between 18 and 50, mean ± SD= 25.8 ± 4.16 years; 19 women) received normal saline or 7.5 mg citalopram, and 9 of them (mean ± SD= 25.9 ± 5.21 years; 6 women) received 11.25 mg citalopram in a third session, infused over 7.5 min during separate 30-minute lasting phMRI. In study II, 27 healthy (mean ± SD= 25.8 ± 4.33 years; 15 women) and 6 migraine participants (mean ± SD= 24.3 ± 4.42 years; 5 females) received normal saline and 7.5 mg citalopram and the potential differences in activation specifically in the ACC were investigated between the two groups.

Exclusion criteria were:

- left-handedness
- history of serious medical, neurological and psychiatric disorders
- long-term daily medication use (except oral contraceptives)
- history of psychotropic medication use or excessive consumption of alcohol

Participants refrained from any medication (for at least 2 days, except oral contraceptives), alcohol (24 h) and caffeine (4 h) before the fMRI sessions. Migraine without aura patients were diagnosed by neurologist researchers.

Citalopram was chosen as the serotonergic agent since it is the only SSRI available for intravenous (iv) use and it is also well tolerated in this dosage form, and as even a low dose of this drug leads to increased plasma prolactin and cortisol level as a neuroendocrine response to increased serotonergic tone (Attenburrow et al, 2001; Lotrich et al, 2005). Both studies had randomized, double-blind, balanced-order design.

My work includes two studies that were published recently (Edes et al., 2020, 2019) and in my thesis I summarise these publications. My aims were:

1. To determine the effect of citalopram challenge on human brain using phMRI in healthy subjects (Study I), including:

2.1.1. To determine if acute iv citalopram administration would lead to increased BOLD signal in brain areas involved in sensory and emotional information processing.

2.1.2. To test whether the BOLD response of amygdala to citalopram challenge would show increased activation compared to placebo.

2.1.3. To test whether citalopram related brain activation would show differences between male and female subjects.

2.1.4. To test whether regional BOLD responses relate to neuroticism and its component traits.

2. To compare ACC activation during citalopram challenge phMRI in migraine patients compared to healthy controls (Study II), including:

2.2.1. To investigate whether citalopram induced BOLD signal changes in ACC would show differences between healthy subjects and migraine patients.

3. Results

3.1 Study I: Citalopram challenge phMRI in healthy participants

3.1.1 Behavioural and physiological data

Subjects were asked to decide in every 5 minutes whether they felt lightheaded, restless, anxious, nauseous, drowsy and uncomfortable with pressing a yes/no button. Based on Wilcoxon test the number of 'yes' button presses did not differ significantly ($p>0.05$) between lower dose (7.5 mg) of citalopram and placebo sessions ($n=32$) and between higher dose of (11.25 mg) citalopram and placebo sessions ($n=9$). No subjective feelings showed sex differences ($p>0.05$).

We did not find any sex differences ($t=1.684$, $p=0.103$) in NEO-PI-R neuroticism scores (mean± SD = 81.7 ± 25.34) in our participants.

Pulse rate and blood pressure were recorded 2 h before and 1 h after the MRI sessions. We found no significant difference in changes of heart rate and blood pressure between the 7.5 mg citalopram and placebo sessions (heart rate: mean (SD)=2.9 (10.85), $t=1.516$, $p=0.140$); systolic blood pressure: mean (SD)=3.9 (14.77), $t=1.449$, $p=0.158$; diastolic blood pressure: mean (SD)=1.6 (12.83), $t=0.716$, $p=0.479$), and between the 11.25 mg citalopram and placebo sessions (heart rate: mean (SD)=-1.1 (7.18), $t=-0.464$, $p=0.655$; systolic blood pressure: mean (SD)=-2.8 (12.88), $t=-0.647$, $p=0.536$; diastolic blood pressure: mean (SD)=-2.0 (12.81), $t=-0.469$, $p=0.652$).

3.1.2 7.5 mg citalopram minus placebo analysis

Individual phMRI scans were split into 1-minute time bins, then these time bins were normalised by subtraction of first time bin. After this step placebo time bins were subtracted from the corresponding citalopram time bins. One baseline and twenty post-infusion time bins were used for second level analyses. All phMRI analyses was performed with second level repeated measure ANOVA using the flexible factorial model in Statistical Parametric Mapping (SPM 12, Friston, The Welcome Department of Cognitive Neurology, London, UK).Results of all whole-brain analyses are reported by $p<0.001$ primary and $p(FWE)<0.05$ Family-Wise Error corrected threshold ($k\geq10$) to control the number of false positive activations. „Analysis of 7.5 mg citalopram data led

to significant drug x time interaction in several brain areas in three clusters with the most extensive activation in the lingual gyrus, posterior cingulate, precuneus and parahippocampal gyrus. In addition, significant activation appeared in two clusters including the right postcentral gyrus, left middle temporal and middle occipital areas." „There was no significant difference between males and females in the effect of 7.5 mg citalopram on whole-brain BOLD signal changes. We found no significant difference in BOLD signal responses to 7.5 mg citalopram between the n=9 participants who later received the higher dose of citalopram, and the other n=23 participants." (Edes et al, 2020, p. 4)

For details please see Table 1 and Figure 1.

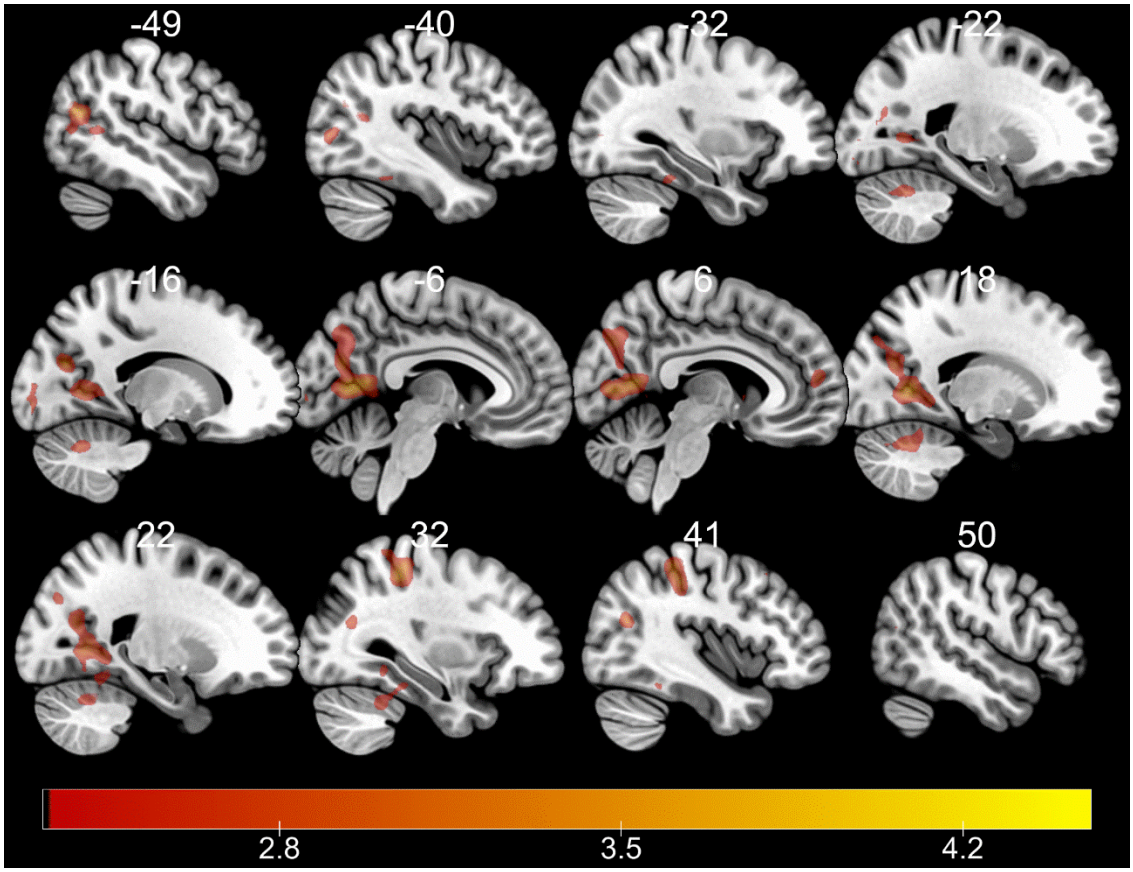

Figure 1. Sagittal view of 7.5 mg citalopram minus placebo significant time x treatment interaction (p<0.001 uncorrected threshold). (Edes et al, 2020, p. 5)

Table 1. Brain regions with significant time x treatment interaction in 7.5 mg citalopram minus placebo analysis at p(FWE)<0.05 secondary extent threshold.

Cluster size	Region	Side	Peak F-value	Peak MNI Coordinates		
				x	y	z
1205	Lingual Gyrus	-	4.46	0	-67	5
	Lingual Gyrus	R	3.56	15	-61	5
	Lingual Gyrus	L	3.53	-15	-73	23
	Posterior Cingulate	L	3.40	-9	-55	8
	Middle Temporal Gyrus	R	3.35	39	-64	20
	Parahippocampal Gyrus	R	3.14	15	-46	2
	Precuneus	R	3.10	9	-73	35
	Precuneus	-	3.07	0	-70	41
	Lingual Gyrus	L	3.06	-18	-64	5
	Lingual Gyrus	R	3.02	21	-67	20
	Posterior Cingulate	R	2.96	24	-64	17
	Cerebellum	R	2.90	18	-61	-28
	Parahippocampal Gyrus	R	2.87	30	-46	-10
	Parahippocampal Gyrus	R	2.85	24	-49	-1
	Precuneus	R	2.81	24	-79	35
	Fusiform Gyrus	R	2.60	36	-46	-19
175	Postcentral Gyrus	R	3.97	36	-34	53
	Postcentral Gyrus	R	2.91	33	-43	62
177	Middle Temporal Gyrus	L	3.72	-48	-64	20
	Middle Temporal Gyrus	L	3.63	-45	-70	23
	Middle Occipital Gyrus	L	3.02	-39	-79	5
	Middle Temporal Gyrus	L	2.97	-54	-52	8
	Middle Occipital Gyrus	L	2.75	-48	-70	8
	Middle Temporal Gyrus	L	2.41	-48	-55	2

R = right; L = left; MNI = Montreal Neurological Institute

3.1.3 11.25 mg citalopram minus placebo analysis

„Analysis of 11.25 mg citalopram minus saline revealed widespread activation in cortical and subcortical structures including clusters extending to the middle cingulate cortex (MCG), inferior frontal gyrus, thalamus and midbrain." „The caudate, which showed significantly increased activation only at uncorrected p<0.001 threshold in 7.5 mg citalopram minus saline analysis (MNI coordinates=9,11, 2; F=2.72) became significant even after FWE correction in case of higher dose". (Edes et al, 2020, p. 4) For details please see Table 2 and Figure 2.

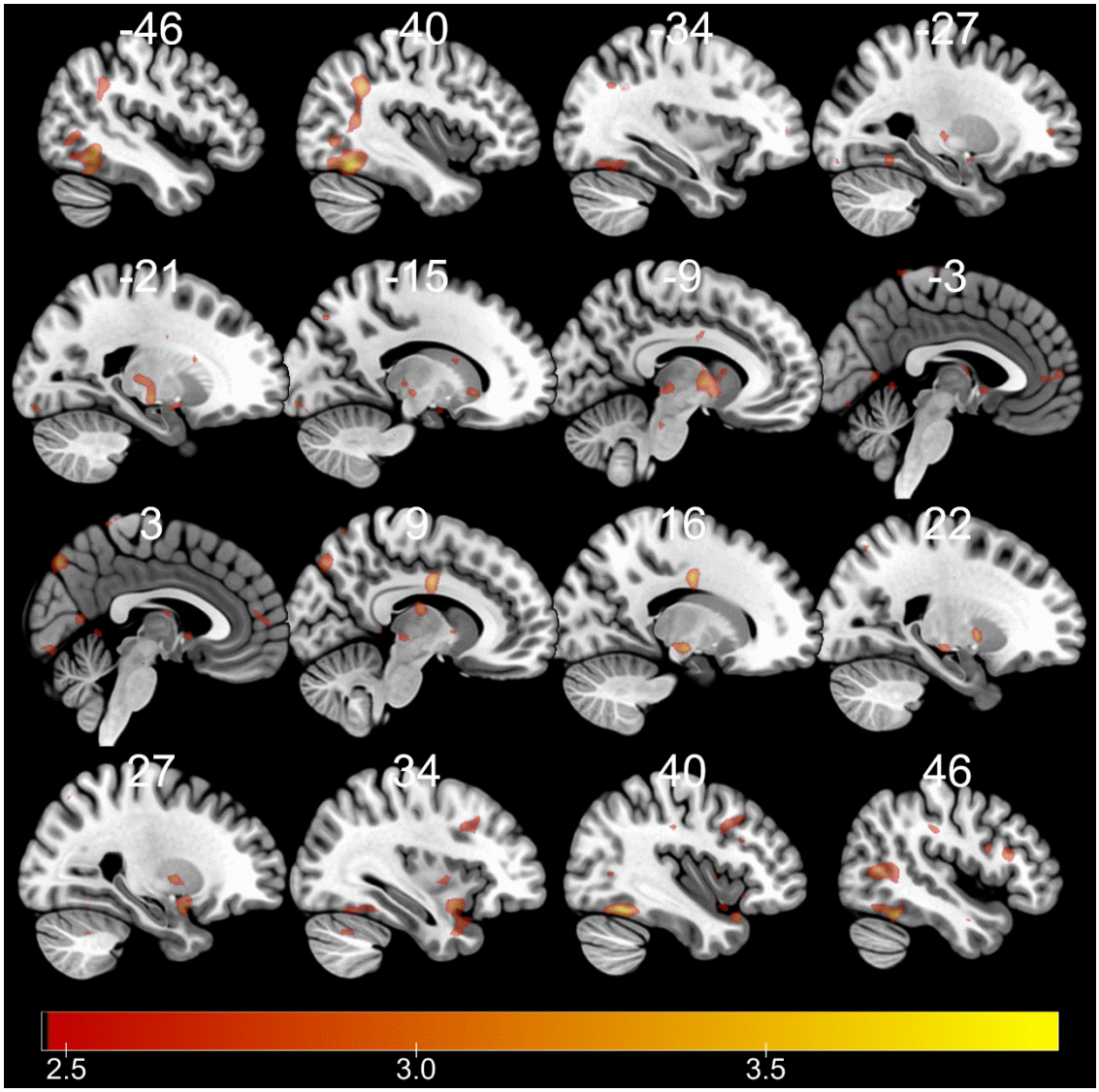

Figure 2. Sagittal view of 11.25 mg citalopram vs. placebo significant time x treatment interaction at p<0.001 uncorrected threshold.

Table 2. Brain regions with significant time x treatment interaction in 11.25 mg citalopram vs. placebo analysis at p(FWE)<0.05 secondary extent threshold.

Cluster size	Region	Side	Peak f-value	Peak MNI coordinates		
				x	y	z
59	Middle Cingulate Gyrus	R	4.88	15	-10	35
	Middle Cingulate Gyrus	R	4.76	12	-7	38
101	Fusiform Gyrus	R	4.86	45	-49	-22
	Fusiform Gyrus	R	4.77	39	-55	-19
	Fusiform Gyrus	R	3.44	33	-43	-19
	Cerebellum	R	3.19	27	-58	-16
327	Fusiform Gyrus	L	4.79	-39	-61	-16
	Cerebellum	L	4.56	-45	-52	-7
	Supramarginal Gyrus	L	4.56	-39	-52	32
104	Parahippocampal gyrus	R	4.31	30	5	-16
	Superior Temporal Gyrus	R	3.95	39	20	-22
	Superior Temporal Gyrus	R	3.84	36	11	-31
	Superior Temporal Gyrus	R	3.78	33	8	-34
119	Extra-nuclear	L	4.29	-9	-4	2
	Caudate	L	4.19	-15	20	-4
	Caudate	L	4.18	-6	5	-4
	Thalamus	R	3.89	6	8	2
	Caudate	R	3.85	-6	11	8
	Caudate	L	3.60	9	-1	11
	Caudate	R	3.21	9	-13	17
75	Extra-nuclear	L	4.26	-21	-16	-7
	Extra-nuclear	L	4.02	-24	-19	2
	Thalamus	L	3.98	-18	-25	5
	Midbrain	L	3.88	-9	-25	-1
	Thalamus	L	3.71	-18	-19	-4
	Midbrain	L	3.55	-12	-31	-4
	Extra-nuclear	L	3.14	-30	-25	2
121	Middle Temporal Gyrus	R	4.19	54	-52	8
	Middle Temporal Gyrus	R	4.01	48	-61	8
	Superior Temporal Gyrus	R	3.92	57	-55	23
74	Inferior Frontal Gyrus	R	3.92	45	23	17
	Inferior Frontal Gyrus	R	3.90	39	17	38
	Middle Frontal Gyrus	R	3.80	39	11	35
	Precentral gyrus	R	3.72	39	20	26
	Middle Frontal Gyrus	R	3.29	51	17	17

R = right; L = left; MNI = Montreal Neurological Institute

3.1.4 11.25 mg citalopram minus 7.5 mg citalopram analysis

The dose-effect analysis after subtracting activation changes to 7.5 mg citalopram from changes to 11.25 mg citalopram showed significant BOLD signal changes therefore dose-dependent activation in one cluster extending to the MCG (size=103 voxels, MNI coordinates= 24,-16, 35, Peak F=3.84). MCG activation changes over time in 7.5 mg citalopram minus placebo and 11.25 mg citalopram minus placebo analysis are shown in Figure 3.

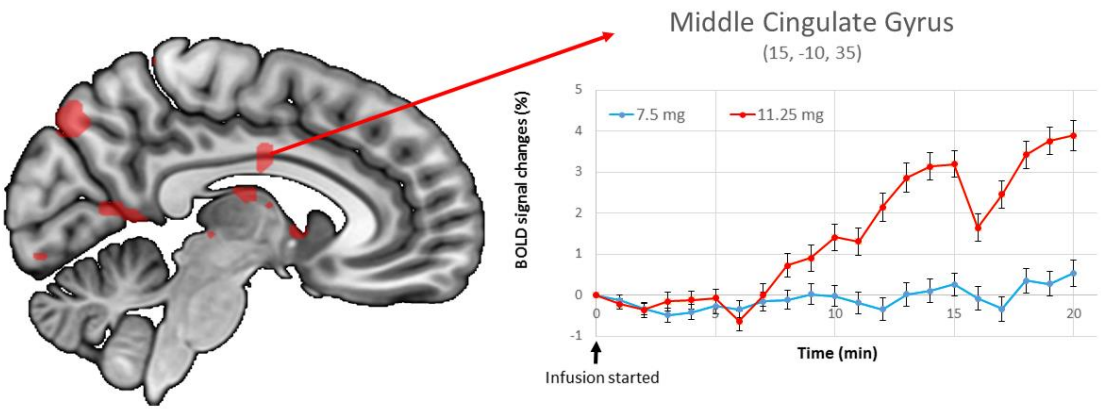

Figure 3. „BOLD signal changes in MCG after the infusion started in 7.5 mg citalopram minus saline (blue) and 11.25 mg citalopram minus saline (red) comparison showing dose-dependent activation changes over time with error bars indicating standard error (SE).” (Edes et al, 2020, p. 6)

“An exploratory analysis of the results with a more lenient threshold of p<0.05 with p(FWE)<0.05 revealed that activation changes between the two different doses involve extensive brain regions including the caudate, thalamus and middle frontal gyrus, suggesting that the brain activation pattern is dose-dependent during citalopram challenge.” (Edes et al, 2020, p. 4) For detailed results please see Table 3.

Table 3. Brain regions with significant time x treatment interaction in 11.25 mg citalopram vs. 7.5 mg citalopram analysis by p(FWE)<0.05 secondary threshold.

Cluster size	Region	Side	Peak F-value	Peak MNI Coordinates		
				x	y	z
746	Extra-nuclear	R	3.84	24	-16	35
	Middle Cingulate Gyrus	R	3.77	18	-13	35
	Putamen	R	3.34	33	-19	-4
	Putamen	R	3.11	30	-22	-1
	Middle Cingulate Gyrus	R	2.92	6	-7	29
	Extra-nuclear	L	2.88	-3	-7	26
	Middle Cingulate Gyrus	L	2.78	-6	-10	29
	Putamen	R	2.69	27	-1	5
	Extra-nuclear	R	2.59	30	-28	-1
	Caudate	L	2.39	-6	8	11
	Extra-Nuclear	L	2.36	-6	2	-7
	Olfactory Cortex	-	2.23	0	14	-4
	Putamen	R	2.22	30	-1	11
	Caudate	R	2.20	12	-4	20
	Middle Cingulate Gyrus	L	2.11	-6	-19	35
	Extra-Nuclear	R	2.10	6	2	-7
1490	Thalamus	L	3.16	-18	-31	5
	Cerebellum	R	3.07	3	-55	2
	Parahippocampal Gyrus	L	2.94	-27	-19	-22
	Thalamus	L	2.86	-9	-28	-1
	Fusiform Gyrus	R	2.81	36	-58	-16
	Inferior Temporal Gyrus	L	2.73	-54	-4	-31
	Middle Temporal Gyrus	L	2.71	-66	-16	-4
	Cerebellum	R	2.57	27	-55	-19
	Cerebellum	L	2.45	-9	-61	-10
	Cerebellum	L	2.43	-3	-73	-10
	Fusiform Gyrus	L	2.43	-33	-55	-13
	Lingual Gyrus	L	2.42	-15	-55	-7
	Inferior Occipital Gyrus	L	2.37	-30	-82	-1
	Lingual Gyrus	L	2.36	-15	-70	-7
	Middle Temporal Gyrus	R	2.34	48	-52	-1
	Lingual Gyrus	L	2.32	-15	-64	-4
854	Anterior Cingulate Gyrus	L	2.90	-9	23	26
	Middle Frontal Gyrus	L	2.89	-30	50	8
	Superior Frontal Gyrus	L	2.83	-21	56	17
	Sub-Gyral	L	2.76	-18	23	26
	Inferior Frontal Gyrus	L	2.72	-54	29	2
	Middle Frontal Gyrus	L	2.64	-36	41	26
	Superior Frontal Gyrus	L	2.56	-9	59	26
	Anterior Cingulate Gyrus	L	2.55	-6	44	5
	Inferior Frontal Gyrus	L	2.54	-57	26	5
	Region	Side	Peak F-value	Peak MNI Coordinates		

Cluster size				x	y	z
	Superior Frontal Gyrus	L	2.44	-21	59	29
	Superior Frontal Gyrus	L	2.42	-9	53	17
	Superior Frontal Gyrus	L	2.30	-24	56	26
	Middle Frontal Gyrus	L	2.24	-36	20	41
	Anterior Cingulate Gyrus	R	2.15	9	38	5
	Superior Frontal Gyrus	L	2.10	-15	38	41
	Anterior Cingulate Gyrus	L	2.10	-3	53	11

R = right; L = left; MNI = Montreal Neurological Institute

3.1.5 Post hoc test of amygdala activation

Results of amygdala region of interest (ROI) analyses are reported by $p<0.001$ uncorrected primary threshold and $p(FWE)<0.05$ peak level secondary threshold ($k\geq5$) to control the number of false positive activations.

„No significant effect of 7.5 mg citalopram was found in the a priori bilateral amygdala ROI but 11.25 mg evoked significant BOLD signal changes bilaterally compared to saline (right: MNI coordinates = 30, 5,-19, F = 4.13, 9 voxels; left: -24, -4, -13, F = 3.59, 11 voxels). Direct comparison of the larger and smaller dose of citalopram showed no significant effect." (Edes et al, 2020, pp. 4-5)

3.1.6 Time-series analysis

„To determine and visualise the order of the spatiotemporal changes during the 7.5 mg citalopram challenge paired t-tests were applied to compare successive time bins to the baseline using the flexible factorial repeated measure ANOVA model. The first significant activation change occurred in the occipital cortex 7 minutes after citalopram infusion started (12,-76, 2; T = 4.56, 1919 voxels). The activation spread in a posterior-anterior direction, significant frontal activation first emerging in the medial frontal gyrus (-12, 59, 26; T=4.55, 165 voxels) in the 11th minute as the occipital activation intensified and spread (-12,-97,-4; T=5.61, 4340 voxels). The Supplementary video shows the statistically significant BOLD signal changes (t-tests) emerging over 1 min time bins from the onset of the infusion at $p<0.001$ primary height threshold without correction." (Edes et al, 2020, p. 5)

3.1.7 Neuroticism-associated changes in brain activity

3.1.7.1 Correlation between neuroticism and citalopram-evoked brain activation

Area under the curve (AUC) values were calculated using significant peak MNI coordinates of 7.5 mg citalopram minus placebo results.

"Significant peak coordinates with MNI coordinates were as follows: lingual gyrus (0,-67, 5), postcentral gyrus (36,-34, 53), left middle temporal gyrus (MTG; -48,-64, 20) based on significant results in the 7.5 mg citalopram minus placebo analysis; MCG (15,-10, 35), right fusiform gyrus (45,-49,-22), left fusiform gyrus (-39,-61,-16), parahippocampal gyrus (30, 5,-16), caudate (-9,-4, 2), thalamus (-21,-16,-7), right MTG (54,-52, 8), inferior frontal gyrus (IFG; 45, 23, 17) and midbrain (-12,-31,-4) based on significant results in the 11.25 mg minus placebo analysis; and right amygdala (30, 5,-19) and left amygdala (-24,-4,-13) based on the 11.25 mg minus placebo ROI analysis." (Edes et al, 2020, Supplementary Material, p. 3)

I determined the relationship between activation changes to 7.5 mg citalopram and neuroticism and its subscales (anxiety, angry-hostility, self-consciousness, impulsiveness, vulnerability and depression) with Spearmann correlation. $P = 0.05$ level was accepted as nominal significance.

Neuroticism scores correlated with activation changes in thalamus ($r=0.49$, $p=0.005$) and midbrain ($r=0.41$, $p=0.021$). The most significant correlation was between thalamus activation and anxiety facet ($r=0.63$, $p=0.0002$) and right MTG and self-consciousness facet ($r=0.48$, $p=0.006$)." Only the correlation between thalamus and anxiety would survive using Bonferroni correction (7 neuroticism facets, 14 regions, $p<0.0005$).

For more details please see Figure 4 and Table 4.

Due to the interdependencies between the used variables we applied least absolute shrinkage and selection operator (LASSO) method for correction of multiple testing and modelling our data (see next paragraph).

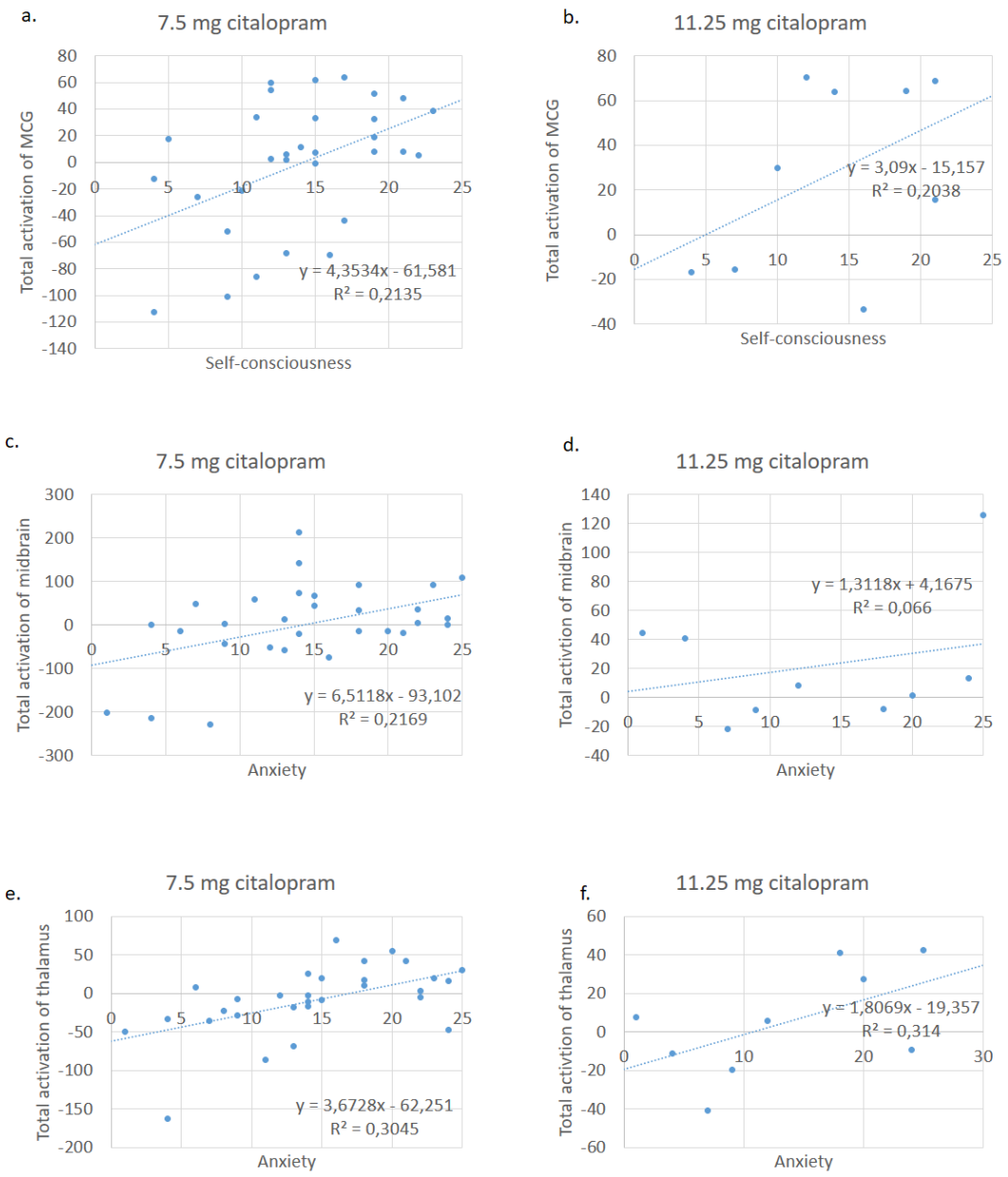

Figure 4.”Scatter plots showing total BOLD signal changes (AUC) of 7.5 mg citalopram minus placebo contrasts in relation to scores of neuroticism that survived Bonferroni corrections (panel e) or showed direct relationships in the LASSO model (panel a, c, e). Scatter plots showing total BOLD signal changes (AUC) of 11.25 mg citalopram minus placebo contrasts in relation to scores of neuroticism demonstrate similar trends to our main correlation analysis (panel b, d, f).

a. Self-consciousness facet's in correlation with MCG AUC - 7.5 mg citalopram (n=31 subjects, p=0.011, RB=0.37). b. Self-consciousness facet's in correlation with MCG AUC – 11.25 mg citalopram (n=9 subjects, similar trend for demonstration purposes only). c. Anxiety facet's in correlation with midbrain AUC - 7.5 mg citalopram (n=31 subjects, p=0.027, RB=0.10). d. Anxiety facet's in correlation with midbrain AUC – 11.25 mg citalopram (n=9 subjects, similar trend for demonstration purposes only). e. Anxiety facet's in correlation with thalamus AUC - 7.5 mg citalopram (n=31 subjects, p=0.0002, RB=0.30). f. Anxiety facet's in correlation with thalamus AUC – 11.25 mg citalopram (n=9 subjects, similar trend for demonstration purposes only)." (Edes et al, 2020, Supplementary Material, pp. 12-13)

Table 4. Spearman's cross-correlation between neuroticism facets from NEO-PI-R and brain activation changes

	Lingual Gyrus	**Postcentral Gyrus**	**MTG L**	**Amygdala R**	**Amygdala L**	**MCG**	**Fusiform Gyrus R**	**Fusiform Gyrus L**	**Parahippocampal gyrus**	**Caudate**	**Thalamus**	**MTG R**	**MFG**	**Midbrain**
Neuroticism	0.309	-0.106	0.344	0.220	-0.039	0.224	0.230	0.116	0.235	0.244	**0.491****	0.317	0.120	**0.412***
Anxiety	0.352	0.043	**0.358***	0.242	0.029	0.104	0.206	0.037	0.235	0.270	**0.628*****	0.280	0.191	**0.397***
Angry hostility	0.204	-0.126	0.172	0.234	0.050	-0.005	0.002	0.199	0.234	0.040	0.292	0.096	0.224	0.329
Self-consciousness	0.144	0.028	0.283	0.280	0.144	**0.451***	0.174	0.038	0.266	0.231	0.253	**0.480****	0.210	0.083
Impulsiveness	-0.172	-0.303	0.059	-0.162	-0.150	0.266	0.186	0.023	-0.087	0.002	-0.056	-0.034	-0.173	0.326
Vulnerability	0.354	-0.163	0.048	0.009	-0.201	-0.041	0.315	0.069	0.000	-0.084	**0.365***	0.095	-0.090	0.269
Depression	**0.388***	0.024	0.267	0.285	-0.183	0.135	0.277	0.112	0.237	0.258	**0.437***	0.288	-0.053	0.185

*** $p<0.001$. ** $p<0.01$. * $p<0.05$

3.1.7.2 LASSO regression analysis of citalopram-evoked brain activation and neuroticism scores

LASSO-based method was used with adaptive weights to identify LASSO coefficients i.e. the 'optimal set' of relationships between the variables using 'bootnet' R package (Epskamp et al., 2017). The Robustness (RB) of each coefficient is the number (%) of positive occurrences in 100 different models. "We modelled the 6 subscales of neuroticism with sex, age and the BOLD response (AUC) to increased serotonin of the significantly activated brain areas to achieve the 'optimal set' of relationships between investigated variables. Notable relationships between personality traits and brain areas detected with LASSO regression included self-consciousness–MCG (RB=0.37), anxiety–thalamus (RB=0.30) and anxiety–midbrain (RB=0.10)." (Edes et al, 2020, p. 6)

For detailed results of LASSO regression and relationships between brain areas and neuroticism please see Figure 5 and Table 5.

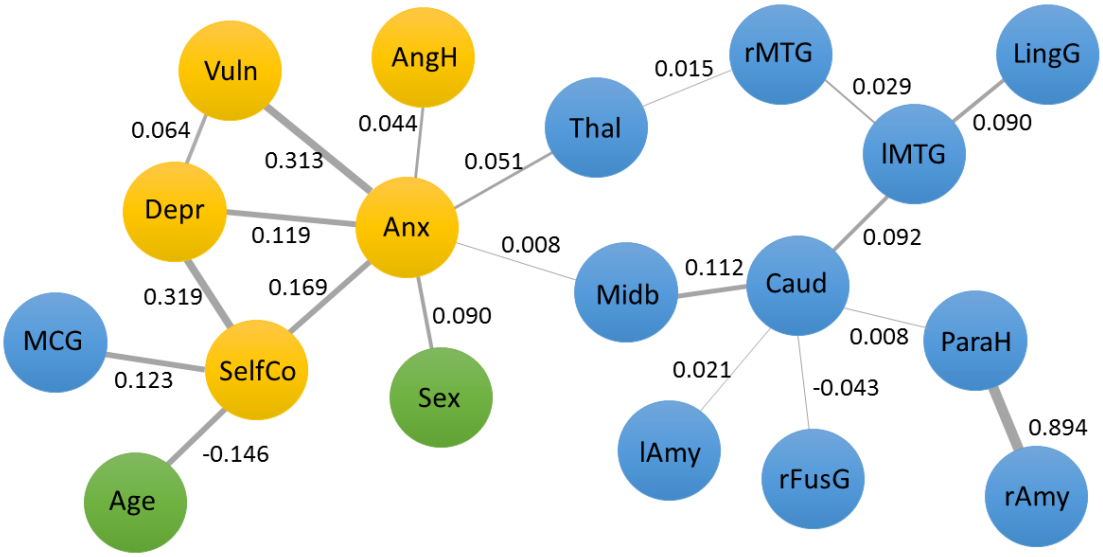

Figure 5. „Adaptive graphical lasso based model with the mean values of adaptive graphical LASSO coefficients, computed using 100 generated models. The thickness of lines represents the strength of relationships between variables. Colors: blue – peak regions, yellow – neuroticism facets from NEO-P-R, green – other covariates (sex and age). SelfCo – self-consciousness, Depr – depression, Vuln – vulnerability, Anx – anxiety, AngH – angry hostility, MCG – middle cingulate gyrus, Thal – thalamus, rMTG – right middle temporal gyrus, lMTG – left middle temporal gyrus, LingG – lingual gyrus, Midb – midbrain, Caud – caudate, ParaH – parahippocampal gyrus, rFusG – right fusiform gyrus, lAmy – left amygdala, rAmy – right amygdala" (Edes et al, 2020, p. 6)

Table 5. Adaptive graphical Lasso coefficients and their robustness given t=100 trials.

Relationship	Graphical Lasso coefficient						Robustness		
	Largest value			Mean value					
	k=2	k=3	k=4	k=2	k=3	k=4	k=2	k=3	k=4
MTG L -- Lingual gyrus	**0.42**	**0.42**	**0.40**	**0.027**	**0.107**	**0.090**	**0.16**	**0.39**	**0.35**
Fusiform gyrus R -- Lingual gyrus	0.00	0.00	0.09	0.000	0.000	0.001	0.00	0.00	0.01
MTG L -- Postcentral gyrus	0.12	0.14	0.00	0.002	0.002	0.000	0.02	0.02	0.00
Fusiform gyrus L -- MTG L	0.00	0.24	0.11	0.000	0.007	0.003	0.00	0.04	0.03
Caudate -- MTG L	**0.32**	**0.32**	**0.29**	**0.025**	**0.073**	**0.092**	**0.12**	**0.35**	**0.42**
Thalamus -- MTG L	0.00	0.09	0.00	0.000	0.001	0.000	0.00	0.01	0.00
MTG R -- MTG L	0.32	0.34	0.35	0.024	0.016	0.029	0.09	0.07	0.11
Parahippocampal gyrus -- Amygdala R	**0.94**	**0.94**	**0.94**	**0.824**	**0.886**	**0.894**	**0.95**	**1.00**	**1.00**
Caudate -- Amygdala R	0.00	0.00	-0.25	0.000	0.000	-0.004	0.00	0.00	0.02
Depression -- Amygdala R	0.00	0.00	0.08	0.000	0.000	0.001	0.00	0.00	0.01
Caudate -- Amygdala L	**0.20**	**0.18**	**0.18**	**0.007**	**0.020**	**0.021**	**0.05**	**0.18**	**0.19**
Caudate -- MCG	0.00	0.00	0.11	0.000	0.000	0.001	0.00	0.00	0.01
Thalamus -- MCG	0.00	0.00	-0.31	0.000	0.000	-0.010	0.00	0.00	0.04
age -- MCG	0.00	0.00	0.33	0.000	0.000	0.009	0.00	0.00	0.03
Self-consciousness -- MCG	*0.47*	*0.52*	*0.51*	*0.035*	*0.078*	*0.123*	*0.12*	*0.25*	*0.37*
Fusiform gyrus L -- Fusiform gyrus R	0.00	0.08	0.29	0.000	0.001	0.005	0.00	0.01	0.02
Caudate -- Fusiform gyrus R	**-0.54**	**-0.53**	**-0.44**	**-0.025**	**-0.034**	**-0.043**	**0.06**	**0.13**	**0.16**
Midbrain -- Fusiform gyrus R	0.00	0.00	0.21	0.000	0.000	0.002	0.00	0.00	0.01
Vulnerability -- Fusiform gyrus R	0.06	0.24	0.17	0.001	0.004	0.002	0.02	0.02	0.02
MTG R -- Fusiform gyrus L	0.05	0.00	0.00	0.001	0.000	0.000	0.01	0.00	0.00
Caudate -- Parahippocampal gyrus	0.08	0.39	0.36	0.001	0.008	0.008	0.02	0.08	0.08
Midbrain -- Parahippocampal gyrus	0.10	0.00	0.00	0.001	0.000	0.000	0.01	0.00	0.00

Relationship	Graphical Lasso coefficient						Robustness		
	Largest value			Mean value					
	k=2	k=3	k=4	k=2	k=3	k=4	k=2	k=3	k=4
Anxiety -- Parahippocampal gyrus	0.00	0.00	-0.04	0.000	0.000	0.000	0.00	0.00	0.01
Thalamus -- Caudate	0.20	0.24	0.25	0.002	0.012	0.007	0.01	0.07	0.04
Midbrain -- Caudate	**0.46**	**0.49**	**0.49**	**0.047**	**0.090**	**0.112**	**0.15**	**0.27**	**0.34**
Vulnerability -- Caudate	0.00	0.00	-0.15	0.000	0.000	-0.001	0.00	0.00	0.01
Midbrain -- Thalamus	0.00	0.00	0.14	0.000	0.000	0.001	0.00	0.00	0.01
MTG R -- Thalamus	0.32	0.39	0.42	0.007	0.007	0.015	0.03	0.02	0.06
Anxiety -- Thalamus	*0.30*	*0.27*	*0.27*	*0.037*	*0.047*	*0.051*	*0.19*	*0.30*	*0.30*
Anxiety -- Midbrain	*0.18*	*0.13*	*0.24*	*0.005*	*0.006*	*0.008*	*0.05*	*0.10*	*0.10*
Angry hostility -- Midbrain	0.00	0.04	0.00	0.000	0.000	0.000	0.00	0.01	0.00
Anxiety -- sex	**0.27**	**0.26**	**0.32**	**0.044**	**0.071**	**0.090**	**0.34**	**0.48**	**0.52**
Self-consciousness -- age	**-0.57**	**-0.59**	**-0.58**	**-0.028**	**-0.096**	**-0.146**	**0.10**	**0.30**	**0.42**
Angry hostility -- Anxiety	**0.25**	**0.27**	**0.28**	**0.014**	**0.045**	**0.044**	**0.09**	**0.24**	**0.25**
Self-consciousness -- Anxiety	**0.29**	**0.26**	**0.24**	**0.093**	**0.154**	**0.169**	**0.54**	**0.88**	**0.98**
Vulnerability -- Anxiety	**0.49**	**0.48**	**0.47**	**0.218**	**0.302**	**0.313**	**0.62**	**0.87**	**0.87**
Depression -- Anxiety	**0.26**	**0.19**	**0.21**	**0.063**	**0.098**	**0.119**	**0.43**	**0.69**	**0.80**
Vulnerability -- Angry hostility	0.00	0.22	0.00	0.000	0.002	0.000	0.00	0.01	0.00
Impulsiveness -- Self-consciousness	0.20	0.12	0.24	0.002	0.001	0.007	0.01	0.01	0.03
Depression -- Self-consciousness	**0.37**	**0.37**	**0.37**	**0.131**	**0.289**	**0.319**	**0.45**	**0.91**	**1.00**
Vulnerability -- Impulsiveness	0.07	0.09	0.08	0.001	0.002	0.001	0.01	0.02	0.02
Depression -- Vulnerability	**0.26**	**0.26**	**0.26**	**0.031**	**0.058**	**0.064**	**0.18**	**0.31**	**0.36**

k= number of crossvalidation folds (for each trial). Largest value= Largest non-zero adaptive Lasso weight. Mean value= Mean of adaptive Lasso weights for t=100 trials. Robustness= Percentage of trials with non-zero weight for t=100 trials (Bold= relevant relationship between personality traits or between brain areas. Italic= relevant relationship between a personality trait and a brain area)

3.2 Study II: Citalopram challenge phMRI effect on ACC in migraine patients vs healthy participants

3.2.1 Behavioral and migraine related data

The number of ‘yes’ button presses did not differ significantly between the placebo and citalopram sessions according to Wilcoxon test in 6 migraine patients and in 27 healthy subjects and (Table 6.).

Table 6. Results of Wilcoxon test of ‘yes’ button presses between citalopram and placebo sessions in migraine patients (M) and controls (CO). A: anxious, D: drowsy, L: lightheaded, N: nauseous, R: restless, U: uncomfortable

	A		D		L		N		R		U	
	Z	p	Z	p	Z	p	Z	p	Z	p	Z	p
CO	-1.069	0.285	-1.212	0.226	-1.826	0.068	-1.633	0.102	-0.197	0.844	-0.104	0.917
M	0.000	1.000	-1.265	0.206	-1.342	0.180	-1.342	0.180	-1.272	0.785	0.000	1.000

The Mann-Whitney tests did not find any significant difference between groups in neither subjective states during the citalopram sessions (Table 7.).

Table 7. Results of Mann-Whitney tests showed no difference between the two groups in subjective states during citalopram measures. Diag: diagnoses, GV: grouping variable, A: anxious, D: drowsy, L: lightheaded, N: nauseous, R: restless, U: uncomfortable

GV	A		D		L		N		R		U	
	Z	p	Z	p	Z	p	Z	p	Z	p	Z	p
Diag.	-0.471	0.637	-0.688	0.492	-1.214	0.225	-1.347	0.178	-0.258	0.796	-1.553	0.121

“No migraine patients experienced headache during the scanning sessions. No subject reported headache after citalopram session in the following 72 hours. Two subjects reported migraine attack 24 and 48 hours after placebo session.” (Edes et al, 2019, p. 4)

3.2.2 Citalopram challenge phMRI

„We found significant difference in ACC activation between control and patient groups in two peaks in the right pregenual ACC (pgACC) during and after citalopram infusion compared with placebo. In addition, a trend of significant difference between groups was found in the left pregenual part of rostral ACC. The extracted time-series showed that the activation of ACC increased in migraine patients compared to controls, especially in the first 8-10 minutes after the beginning of the citalopram infusion." (Edes et al, 2019, p. 4) Difference in ACC activation between the two groups are shown on Table 8, while Figure 6 shows the BOLD signal changes over time for the peaks of the pgACC.

Table 8. Peak ACC activation differences in migraine patients compared to healthy subjects. (k = number of voxels, * peaks that survive p(FWE)<0.05 secondary correction)

MNI coordinates			side	k	p(FWE)	F value
x	y	z				
15	38	8	right	9	0.006	2.969*
9	41	-4	right	6	0.022	2.745*
-9	44	8	left	6	0.075	2.519

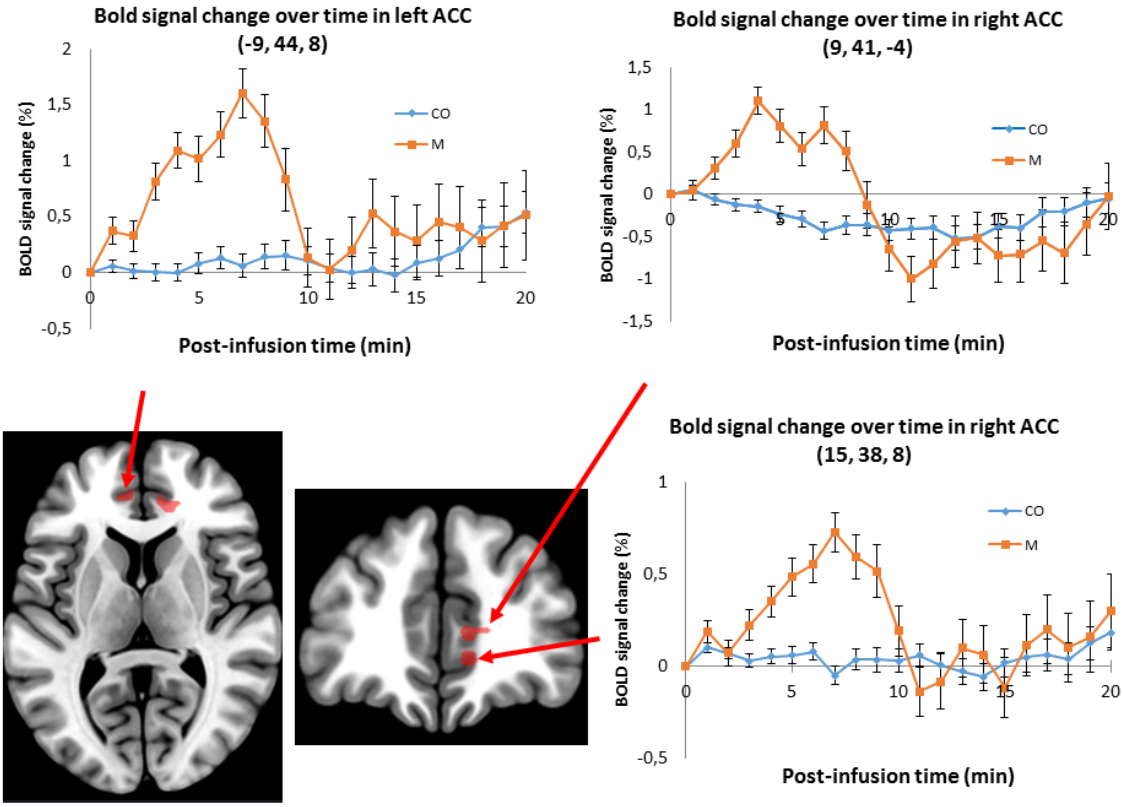

Figure 6. "BOLD signal changes over time after citalopram minus placebo data extraction in three clusters with error bars (standard error of mean), for control and patient groups, respectively. Coronal and sagittal view of ROI analysis results were made by p(FWE)<0.05 secondary threshold (with p<0.001 primary threshold, k≥5, CO = control group, M = migraine group)." (Edes et al, 2019, p. 5)

4. Discussion

4.1 Study I: Citalopram challenge phMRI in healthy participants

We found increased activation to 7.5 mg citalopram in occipital and temporal brain regions and post-hoc analysis of the time-dependent effect demonstrated that earliest activation occurred in posterior brain areas and spread forward in frontal direction (Supplementary video).

"PET studies reported low 5-HTT levels in most cortical areas except the limbic lobe and a diverse distribution of different 5-HT receptor types across the whole brain (Saulin et al, 2012; Savli et al, 2012)." (Edes et al, 2020, p. 6)

Previous PET/MR study showed that iv administration of low dose of citalopram (8 mg) led to 69±7% 5-HTT occupancy following the drug challenge (Gryglewski et al, 2019). Interestingly, despite the timeframe and the dose of citalopram administration is almost similar as in our research, no changes in BOLD activation were reported in this PET/MR study. The authors argued that the drift of the MRI scanner might have coupled the 5-HTT occupancy regressors and obscured the BOLD signal changes. In my work I applied drift correction to avoid this problem.

Regarding the mechanism of action, according to the most accepted theory SSRIs increase the extracellular 5-HT levels and activate high affinity inhibitory heteroreceptors, 5-HT1A receptors on inhibitory gamma-aminobutyric acid-ergic (GABA-ergic) neurons. Consequentially, activated 5HT1A receptors disinhibit the activity of glutamatergic pyramidal cells which is the mediator of BOLD response and neurovascular coupling (Chilmonczyk et al, 2015; Klaassens et al, 2015).

"It is likely that other 5-HT receptors and neurotransmitters in serotonergic projection areas take part in the observed activation changes, especially at higher doses. However, in this study we cannot draw a conclusion on the exact mechanisms behind the observed BOLD signal changes due to the diversity of 5-HT receptor and 5-HTT distribution in different brain regions and the complex relationships of 5-HT with other neurotransmitter systems. Nevertheless, the net cortical disinhibition could be the origin of extensive decreases in functional brain connectivity in resting state networks observed after single

oral doses of SSRIs in several studies (Klaassens et al, 2015, 2017, 2018; Schwarz et al, 2009; Schaefer et al, 2014).” (Edes et al, 2020, p. 7)

4.1.1 Dose effect of citalopram challenge

Previous microdialysis studies showed that SSRI elicited increase of 5-HT is dose dependent (Bel and Artigas, 1992).

“In addition, a previous phMRI study showed widespread and dose-dependent BOLD activation changes to acute citalopram treatment in rats (Sekar et al, 2011). In our study, we found similar widespread brain activation following the higher citalopram dose that additionally activated brain areas with dense serotonergic innervation including the midbrain, caudate, and thalamus. Activation of similar functional networks has been described in rat phMRI studies following acute i.v. fluoxetine (Schwarz et al, 2007, 2009) where responses correlated with raphe nuclei activation in the same subcortical structures as in our study, such as thalamus, caudate and amygdala (Schwarz et al, 2007).“ (Edes et al, 2020, p. 7)

The reason of the extended activation might be the more potent blockade of 5-HTT and increased extracellular 5-HT content by the higher dose of citalopram. However, it might be possible that some receptors which have less affinity for 5-HT, as 5-HT2A, contribute to the observed effect (Chilmonczyk et al, 2015; Marek et al, 2003).

“The MCG showed a clear significant dose-dependent increase in BOLD signal surviving whole-brain correction. The MCG is a key central site controlling rapid motoric body orientation response during sensory information processing and is primarily involved in Go/approach behaviour instead of noGo/avoidance response; a process which occurs before emotional/cognitive assessments of other brain areas (Vogt, 2014). Our study provides evidence of dose-dependent modulation of MCG activation by serotonergic innervation in the human brain, which may contribute to the mechanism of antidepressant action and represent a potential biomarker for depression and recovery in the light of previous evidence that this region is involved in the pathomechanism of unipolar depression and stress-related disorders (Vogt, 2014).” (Edes et al, 2020, p. 7)

4.1.2 Acutely increased serotonergic neurotransmission and heightened arousal

Our results show that the acute increase in ectracellular 5-HT during citalopram challenge enhances activation in several brain areas while none showing decreases in brain activation.

"The observed pattern of activation shows similarity with brain activation related to emotional arousal during threat processing. For example, Farrow and colleagues (Farrow et al, 2012) demonstrated that skin conductance changes during exposure to threatening vs harmless stimuli were significantly associated with brain activation in the lingual gyrus, precuneus, MCG, postcentral gyrus, bilateral precentral gyrus/supplementary motor area, medial prefrontal cortex and thalamus. Increased arousal increases emotion processing and involves extensive brain areas related to promotion of survival, such as facilitation of sensory information processing of environmental triggers, focus of attention to internal and external stimuli, and motoric orientation towards the source of information (LeDoux, 2012)." (Edes et al, 2020, p. 7)

The results suggest that acutely increased extracellular 5-HT produces a state in the brain which is similar to heightened arousal, although there were not any significant blood pressure and pulse rate changes or changes in arousal symptoms in this study.

"There is increasing interest in dysregulation of arousal as a transdiagnostic process (Hegerl and Hensch, 2014; Huang et al, 2015; Sander et al, 2015) contributing not only to the pathogenesis of depression (Hegerl et al, 2012) but also to other psychiatric disorders including anxiety (Domschke et al, 2010) and chronic pain disorders (Foo and Mason, 2003); indeed arousal has become a transdiagnostic research domain in the Research Domain Criteria project (RDoc) (Cuthbert, 2014; Morris and Cuthbert, 2012). Furthermore, increased arousal in depression (Hegerl et al, 2012; Schmidt et al, 2016, 2017) may be an important determinant of antidepressant treatment outcome, with studies showing that a subgroup of responders have heightened and faster reduction of arousal during antidepressant treatment compared to non-responders (Olbrich et al, 2016; Schmidt et al, 2017). Our findings suggest there could be a serotonergic basis to transdiagnostic dysfunctional arousal in psychiatric disorders. This could be a fruitful area of further investigation." (Edes et al, 2020, p. 7)

4.1.3 Neuroticism and 5-HT neurotransmission of the brain

„Several studies investigated neuroimaging biomarkers of personality traits such as neuroticism, an important endophenotype of depression, anxiety and chronic pain (Wade et al, 1992; Weinstock and Whisman, 2006). Association between neuroticism and decreased 5-HT1A receptor binding in several brain regions with the strongest negative correlation in the hippocampus, superior temporal gyrus and prefrontal cortex (Hirvonen et al, 2015), and increased 5-HT2A receptor binding in frontolimbic regions has been reported in previous PET studies (Frokjaer et al, 2008).

Our study demonstrated an association between neuroticism and BOLD activation during the lower citalopram dose (7.5 mg) in the thalamus and midbrain with correlation between the anxiety component of neuroticism and BOLD activation changes in the thalamus and midbrain. This is in line with a previous neuroimaging study that showed a positive correlation between neuroticism and 5-HTT binding in the thalamus (Takano et al, 2007), but a similar study found no such association (Kalbitzer et al, 2009) while another showed an association between 5-HTT binding and neuroticism but with opposite directions in males and females (Tuominen et al, 2017). The positive association of anxiety with increased functional serotonergic function is compatible with the increased anxiety that commonly occurs early in SSRI therapy which is probably mediated by excessive 5-HT2C function with toleration occurring after repeated treatment (Deakin, 2013; Deakin and Graeff, 1991).

The region that showed a significant dose effect in the MCG in our study also showed a correlation with the self-consciousness facet of neuroticism. A previous study reported decreased functional coupling between the MCG and amygdala during emotion suppression associated with self-consciousness (Chen et al, 2017).” (Edes et al, 2020, p. 7)

The relationship between neuroticism and MCG function has been reported by several studies (DeYoung et al, 2010; Kano et al, 2014), however the interpretation is hidden since this region is often labelled as ACC or posterior cingulate cortex (PCC) (Kumari et al, 2007; Tzschoppe et al, 2014).

The results indicate the relationship between function of brain areas like the MCG, midbrain and thalamus and neuroticism which is mediated by the functional condition of serotonergic neurotransmission.

“This may also shed further light on the therapeutic effect of SSRIs, as previous studies have shown that neuroticism may have a role in the effect of SSRI treatment outcome in depressed patients beyond improved mood (Quilty et al, 2008; Tang et al, 2009). We speculate that SSRIs could bring about positive neuroplastic changes in neural circuits related to neuroticism that might be necessary to stabilise remission in neuropsychiatric disorders where neuroticism is an important risk factor.” (Edes et al, 2020, p. 8)

4.1.4 Limitations

Study I has some limitations. „We included only healthy participants and the findings need to be related to changes in patients with depression, anxiety and chronic pain disorders. In addition, the sample size for the dose-response is small and administration was not blinded for the 11.25 mg citalopram challenge. Nevertheless, we found highly significant activation changes with 9 participants in the 11.25 mg minus saline comparison occurring in regions with well-known serotonergic innervation, consistent with previous research. We did not measure physiological or performance measures of arousal and clearly the possible relationship to regional brain effects of citalopram challenge is speculative. However, the brain regions that responded with increased BOLD signal were similar to brain activation related to emotional arousal during threat processing. Nevertheless, our inferences need direct tests in future studies designed specifically to measure brain activation changes to external stressors and its relations to citalopram challenge.” (Edes et al, 2020, p. 8)

4.1.5 Summary of Study I.

„Direct pharmacological challenge study of acute citalopram as a probe of serotonergic neurotransmission revealed widespread and significant activation in several brain areas that are part of the default mode network (PCC, precuneus, angular gyrus, MTG and parahippocampal gyrus), the visual network (lingual gyrus, cuneus, fusiform gyrus and middle occipital gyrus), and the sensorimotor network (postcentral gyrus). Thus, we successfully applied and also extended to female participants the previously reported (McKie et al, 2005) citalopram phMRI method to follow direct regional BOLD response to serotonergic stimulation while also investigating the effect of a higher dose. Using a stricter significance threshold ($pFWE<0.05$) compared to the original study ($p < 0.001$) in

a larger sample we found a similar pattern of activation. However, some of these activations including well-known regions with dense serotonergic innervation, such as the amygdala, and clusters involving the thalamus and midbrain, showed significant activation only at a higher dose. The most significant dose effect of citalopram was detected in the MCG, a key hub of reflexive orientation toward sensory stimuli (Vogt, 2014).” (Edes et al, 2020, p. 6)

Analysis of time-series showed that the pattern of BOLD signal migrated from occipital to frontal direction which suggests that acute 5-HT reuptake inhibition and increased extracellular 5-HT level first facilitates the processing of visual information followed by the involvement of frontal executive and subcortical motor regions and threat processing in amygdala.

“We also demonstrated that the total BOLD response to citalopram is associated with neuroticism, a trait-like endophenotype for several neuropsychiatric disorders and a potent modulator of emotional arousal and threat processing (Goldstein and Klein, 2014; Kehoe et al, 2012). By modelling the subscales of neuroticism together with activated brain areas we demonstrated LASSO-relevant relationships between anxiety and thalamus, anxiety and midbrain, and self-consciousness and the MCG. These results further support serotonergic neurotransmission having an important role in determining neuroticism and therefore vulnerability to depression, anxiety and pain disorders.” (Edes et al, 2020, p. 6)

4.2 Study II: Migraine patients

4.2.1 Increased sensitivity of pgACC to citalopram challenge in migraine patients

„The altered functions of pgACC in migraine have been reported in previous fMRI studies. Increased activation was found in migraine patients to trigemino-nociceptive stimulation compared to controls also in the rostral part of the ACC (Aderjan et al, 2010). Emphasizing the importance of our results, previous fMRI studies using trigeminal heat stimulation to induce acute pain in migraine patients, found increased brain activation in the pgACC with almost identical MNI coordinates as reported here (Stankewitz et al, 2011; Russo et al, 2017). One of these studies showed that this enhanced activation to

noxious heat reduced after a 60 day successful treatment with external trigeminal neurostimulation (Russo et al, 2017). The authors suggested that the pgACC could be involved in the antinociceptive effect of the anti-migraine treatment (Russo et al, 2017). Our results are in line with these results as we found that this region is more active in migraine patients after a small increase of 5-HT levels compared to pain-free healthy controls. These observations together suggest that increased sensitivity of ACC in migraine patients might be related to altered serotonergic control of the incoming excitatory signals." (Edes et al, 2019, p. 4)

4.2.2 The role of pgACC in migraine

„According to literature the most consistently reported area of cingulate cortex to painful stimuli is the dorsal ACC or anterior middle cingulate cortex (aMCC). The activation of this area is one of the earliest responses to pain as this region has an impact in cortical nociception (Garcia-Larrea and Peyron; 2013). A recent meta-analysis showed a significant likelihood of activation to painful stimuli in this brain area in healthy subjects (Duerden and Albanese; 2013). In case of our study we did not find any activation of this area, however, we have not used any painful stimuli during the scanning sessions and we investigated the difference between migraine patients and healthy controls.

On the basis of the functional imaging studies of the last twenty years, the role of pgACC in many aspects of emotional processing seems to be established. In a PET study the pgACC was active during the assessment of internal emotional state induced by emotional pictures with different valences (Lane et al, 1997). Another study with fMRI showed that the subjective ratings of pleasantness or aversiveness of sensory stimuli correlated with the activation of this area during a decision making task (Grabenhorst et al, 2008). In addition, the pregenual part of ACC is a central node of the default mode network, a task-negative network that is consistently active during mind-wandering and it is implicated in the affective network (Yu et al, 2011). The pgACC is the only area in the cingulate gyrus that has connection with all other regions of the cingulate cortex therefore this ACC subregion could be considered as the anterior cingulate association area (Yu et al, 2011), which is involved – along with other brain areas – in integrating information across the brain (Barrett, 2017). These observations point to the essential role of pgACC in emotional processing, specifically in emotional awareness, e.g. the fundamental role of

this area for individuals to assess own emotional experience (Vogt, 2014). Thus, our results may suggest that migraine patients have exaggerated responses to interoceptive, e.g. emotional or visceroceptive stimuli that might be related to altered serotonergic neurotransmission in migraine. However, taking into account the rich brain network of the pgACC further studies are needed to determine whether the increased 5-HT level in the brain directly sensitizes the pgACC in migraineurs or the increased activation of the ACC is secondary to a complex interplay between the increased 5-HT level and those subcortical and other cortical areas that are important in processing different sensory and interoceptive signals.

Nevertheless, the pgACC is the ACC subregion with the highest opioid receptor density (Vogt et al, 1995) and it also plays an important role in opioid analgesia and opioid placebo effect (Bingel et al, 2006). In addition, the increased pgACC signal during noxious stimuli may reflect to the attention to unpleasantness of pain (Kulkarni et al, 2005, Vogt et al, 2005). The recurring headache attacks and the increased attention to unpleasantness of pain in migraine patients may cause altered pgACC functions. Our results extend this observation and suggest that this phenomenon might be related to altered serotonergic neurotransmission in migraine." (Edes et al, 2019, p. 6)

4.2.3 5-HT neurotransmission in pgACC may contribute to migraine attack

„A recent systematic review of electrophysiological and neuroimaging studies of serotonergic system in migraine confirmed the altered 5-HT neurotransmission which has been a main area of interest in migraine research for decades (Deen et al, 2017). The authors supported the notion of suddenly increasing 5-HT levels during migraine headache (Deen et al, 2017). Based on our results we speculate that during migraine attack the pgACC reacts to the spontaneously elevated 5-HT levels the same way as in our study by citalopram challenge i.e. with increased activation. If so, it may contribute to the development of migraine attack by facilitating the pain transmission at the trigeminal level (Tajti et al, 2011) similarly to that observed at the spinal cord (Zhuo et al, 2017).

In line with previous studies, we can conclude that migraine patients are more sensitive to acute increase in 5-HT levels and that this phenomenon can be observed in a subregion of the ACC which is involved in emotional aspects and suffering elements of pain and

may be involved in the modulation and/or chronification of migraine. However, the direction of the connection between migraine attacks and the steep increase of 5-HT levels during headache remains unclear." (Edes et al, 2019, p. 6)

In my previous case report seed-to-voxel analysis was performed using the PCC as seed, representing the default mode network in a 24-year old woman affected by migraine without aura at two times: during a spontaneous migraine headache attack and in a pain-free phase. The results showed increased connectivity during the spontaneous migraine attack between the DMN and pain processing areas such as the hypothalamus and pons. We might speculate that the changes of functional connectivity in the default mode network could be related to changes in endogenous 5-HT levels as well (Edes et al, 2017).

"Interestingly, a previous peripherial study of neurochemical changes in migraine showed that slight release of platelet 5-HT after a nitroglycerin test could be protective against migraine development (Juhasz et al, 2003). The authors reported that no migraine attack developed in migraine patients who responded with increased peripherial 5-HT level to nitroglycerin (Juhasz et al, 2003). However, this study investigated only the peripherial changes of 5-HT level. In addition, nitroglycerine induced migraine may develop through other mechanisms than migraine attacks caused by 5-HT releasing agents. As 5-HT releasing agents e.g. reserpine can provoke migraine (Panconesi et al, 2008), it is possible that migraine attacks caused by extensive increase in brain 5-HT levels in sensitive patients may be related to the increased activation and altered pain-modulation of the pgACC.

Nevertheless, it has to be mentioned that none of our 6 migraine patients developed headache during or immediately after the citalopram administration. As we used a relatively low dose of citalopram the increase in 5-HT levels probably did not reach the level that occurs during migraine attack." (Edes et al, 2019, p. 6)

4.2.4 Limitations

The major limitation in Study II is the low number of subjects in the migraine group. „Despite the low sample size, we found significant activation difference between the two groups. It would be fruitful however to investigate the acute effect of 5-HT level changes on the ACC activation in more migraine patients in the future to replicate and confirm

our results. It would be also important to investigate the activation differences between migraine patients and controls in other important pain processing areas or even in the whole brain with higher sample size.

Furthermore, it has to be mentioned that in contrast to PET phMRI is not suitable to detect specific receptor activation changes, therefore we were not able to determine which serotonergic receptor or receptors are responsible for the observed activation changes. However, our aim was not to detect changes in receptor activation, rather to investigate the general sensitivity to increased 5-HT levels in migraine.

In addition, we have not corrected our analysis for potential individual differences of the grey matter density in ACC that might influence the BOLD signal changes.

Finally, in spite of the fact that pgACC is highly involved in emotional processing, we did not find any difference between citalopram and placebo sessions and between migraine and control groups in the subjective states. Thus, the difference between the two groups to citalopram could not be detected at subjective behavioral level, only at neural level. However, the lack of changes at behavioral level could be related to the dichotomous characteristic of the answers or to the relatively low dosage of citalopram." (Edes et al, 2019, p. 6)

4.2.5 Summary of Study II.

In Study II, using citalopram phMRI „we demonstrated a significant difference in temporal activation pattern of the ACC between healthy control participants and migraine without aura patients during the acutely increased extracellular 5-HT level in the brain. The iv citalopram elicited increased activation over time in two peaks within the pregenual part of the right ACC in migraine patients when compared to healthy controls. This increased activation in migraine subjects was more pronounced in the first 10 minutes after the start of the citalopram. In addition, increased activation was found in one cluster in the left rostral ACC in migraine patients. Although this latter activation did not survive the correction for multiple comparisons, our results suggests a bilateral effect of increased brain 5-HT level on the ACC in the migraine patients." (Edes et al, 2019, p. 4)

4.3 Synthesis of Study I. and II.

Altered 5-HT neurotransmission has been repeatedly implicated in the pathophysiology of both neurologic and mental disorders, such as depression and migraine. However, the exact mechanisms behind these disorders are not well understood. The main objective of this work was to investigate the effect of 5-HT-ergic challenge with low dose acute iv citalopram on brain activation in all areas of the brain in healthy subjects and on the specific activation of the important pain-processing area, the ACC in migraine patients during rest with functional MRI. Converging results suggest the crucial role of the cingulate cortex in the acute effect of citalopram.

My results suggest that citalopram challenge increases BOLD signal in areas with known serotonergic innervation and which are part of different functional networks and are similar to brain regions which are active during arousal. The activation in the posterior part of the brain was extended to the PCC, which is an important hub of the default mode network and it is also a key area of awareness and arousal (Leech and Sharp, 2014). These results suggest that acutely elevated 5-HT leads to increased brain activation in the PCC and other brain areas that are related to arousal and this might induce an arousal-like condition in the human brain even in healthy patients.

The migraine patients, i.e. the subjects who have been dealing with headache pain for years, showed increased activation in the ACC compared to controls. In healthy subjects I did not find any activation changes in the ACC during citalopram challenge. This lack of ACC activation in controls might be explained by the low dose of citalopram or by the fact that these subjects were both mentally and physically healthy. Interestingly, previous animal study in mice reported that ACC lesion can prevent the anxio-depressive effect of chronic pain, therefor it might be a central hub in pain-induced anxiety and depression (Barthas et al, 2015).

The 2 peaks in the ACC which showed increased BOLD signal in migraine patients are located in the pgACC, which area is implicated in pain-relief and in processing the emotional aspects of pain, including suffering. This altered pgACC functions might be caused by the elevated attention to pain and the recurring migraine attacks, and our new result suggests that this phenomenon might be related to altered serotonergic neurotransmission in migraine. This increased sensitivity of the pgACC in migraine to

acute elevation of synaptic 5-HT may contribute to increased sensitivity to stress in migraine and possibly to the comorbidity between migraine and mental disorders, such as depression and anxiety.

Another part of the cingulate cortex, the MCG, which is a central area of reflexive motor orientation to sensory stimuli (Vogt, 2014), showed significant dose effect to citalopram in healthy subjects. Interestingly, investigating the connection between neuroticism and citalopram-related brain activation in healthy subjects the MCG activation also showed a correlation with self-consciousness facet. Besides the MCG, the BOLD-signal in the thalamus and midbrain also associated with anxiety facet. Our results suggest that the relation between neuroticism and the functioning of these brain areas are mediated by the serotonergic system.

Based on the results in healthy subjects, altered PCC and MCG brain activity might be target areas of SSRI treatment to normalise heightened arousal and neuroticism in depression and anxiety. In addition, my results support that the serotonergic innervation of cingulate cortex is important in healthy functioning – mediating arousal by PCC and neuroticism by MCG – and it also might be relevant in migraine pathophysiology – due to increased sensitivity to 5-HT in ACC (Figure 7)

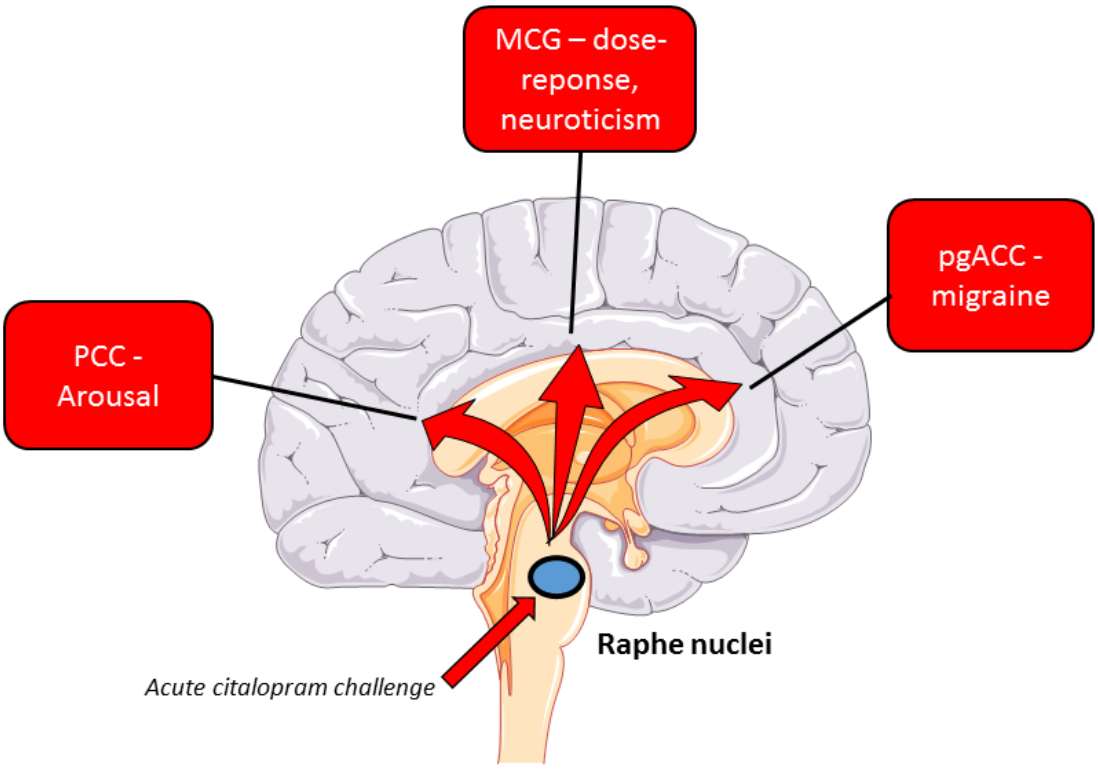

Figure 7. Different areas of cingulate cortex which functions are mediated by 5-HT neurotransmission based on this research. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

5. Conclusions

1. Acute iv citalopram administration led to dose dependent increase of brain activation in several brain areas that are involved in serotonergic neurotransmission and part of default mode, sensorimotor and visual networks. The higher, 11.25 mg dose of citalopram is quantitatively and qualitatively different from the 7.5 mg citalopram dose and it causes activation of the middle cingulate gyrus when compared to the lower dose.
2. Higher dose of citalopram led to increased activation in the amygdala ROI which is a well-known brain region involved in emotional processing and arousal.
3. Total BOLD response to acute citalopram in the thalamus, midbrain and MCG is associated with neuroticism which is an important endophenotype for several neuropsychiatric disorders and also a modulator of threat processing and emotional arousal. Based on our results the relationship between these brain regions and neuroticism is mediated by serotonergic neurotransmission.
4. Activated brain regions were similar to those that are involved in arousal, and because neuroticism is related to a tendency for increased reactivity to stress these results could reflect modulating effect of 5-HT to process environmental stressors.
5. The activation patterns following the 7.5 mg dose follow a posterior-anterior direction and the effects are sex-independent.
6. Citalopram challenge showed an increased pgACC activation in migraine patients compared to healthy controls. The findings confirmed the sensitivity to increased 5-HT and altered functions of ACC in migraine patients compared to controls.
7. Increased sensitivity of pgACC in migraine patients may contribute to the increased stress-sensitivity and to the recurring attacks in migraine and to the comorbidity between migraine and mental disorders.
8. Acute citalopram challenge phMRI is suitable to detect functional alterations not only in healthy subjects or patients with mood disorders but even in migraine. This method could deepen our understanding in the future about the pathophysiology of migraine and other neuropsychiatric disorders that are in connection with altered 5-HT neurotransmission.

9. Based on our research 5-HT mediates different functions in different parts of the cingulate cortex, as it modulates arousal in the PCC, associates with neuroticism in the MCG and sensitises the pgACC in migraine.

6. Summary

The aim of this work was to determine (I) the relationship between acute increase of 5-HT level due to iv SSRI administration and brain activation changes and (II) the differences of ACC activation to acute SSRI between healthy subjects and migraine patients.

In Study I, 7.5 mg iv. citalopram and normal saline was administered n=32 healthy participants and 11.25 mg citalopram was given to 9 subjects to determine the dose-response. Associations of the changing brain activation to citalopram with sex and neuroticism were investigated as well. Citalopram challenge led to significant activation changes in areas that are part of default mode, visual and sensorimotor networks, extending to midbrain and thalamus. Most effects showed dose-dependency which was significant in the MCG. Individual brain responses to citalopram were positively correlated with neuroticism and its subscales, including anxiety scores in thalamus and midbrain and self-consciousness in MCG. No sex differences were found in this study. As activated brain regions were similar to those that are involved in arousal our results demonstrate that SSRIs acutely induce an arousal-like state of cortical and subcortical systems and this state may be mediated by increased 5-HT neurotransmission. Brain response to acutely enhanced 5-HT neurotransmission, indexed by the level of neuroticism, may also underpin the trait sensitivity to stressors and environmental stimuli.

In study II, I found significant difference in BOLD signal activation in the right pgACC between control and patient groups following citalopram infusion compared to placebo. The pgACC activation increased in migraine subjects compared to healthy controls. This brain area is implicated in processing the emotional aspects of pain, including suffering. This increased sensitivity of the pgACC in migraine to elevation of 5-HT may contribute to recurring migraine attacks and increased sensitivity to stress in migraine and to the comorbidity between migraine and mental disorders.

Our results also demonstrate that 5-HT mediates different functions in distinct regions of the cingulate gyrus, as it modulates arousal in the PCC, associates with neuroticism in the MCG and sensitises the pgACC in migraine.

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8. Bibliography of the candidate's publications

8.1 Original publications related to the thesis

- **Edes A**, Gonda X, Bagdy G, Juhasz G. (2014) A farmakológiai funkcionális mágneses rezonancia vizsgálat (phMRI) felhasználásának lehetőségei a hangulatzavarok kutatásában. Neuropsychopharmacol. Hung. 16(2): 59–66.
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- **Edes AE**, McKie S, Szabo E, Kokonyei G, Pap D, Zsombok T, Magyar M, Csepany E, Hullam G, Szabo AG, Kozak LR, Bagdy G, Juhasz G. (2019) Increased activation of the pregenual anterior cingulate cortex to citalopram challenge in migraine: an fMRI study. BMC Neurol. 19: 237. **IF: 2.356**
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8.2 Original publications not related to the thesis

- Galambos A, Szabo E, Nagy Z, **Edes AE**, Kocsel N, Juhasz G, Kokonyei G. (2019) A systematic review of structural and functional MRI studies on pain catastrophizing. J Pain Res. 2019, 12: 1155–1178. **IF: 2.386**
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