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Gray matter and intrinsic network changes in the posterior cingulate cortex after selective serotonin reuptake inhibitor intake

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ABSTRACT

Preclinical studies have demonstrated that serotonin (5-HT) challenge changes neuronal circuitries and microarchitecture. However, evidence in human subjects is missing. Pharmacologic magnetic resonance imaging (phMRI) applying selective 5-HT reuptake inhibitors (SSRIs) and high-resolution structural and functional brain assessment is able to demonstrate the impact of 5-HT challenge on neuronal network morphology and functional activity. To determine how SSRIs induce changes in gray matter and neuronal activity, we conducted a longitudinal study using citalopram and escitalopram. Seventeen healthy subjects completed a structural and functional phMRI study with randomized, cross-over, placebo-controlled, double-blind design. Significant gray matter increases were observed (among other regions) in the posterior cingulate cortex (PCC) and the ventral precuneus after SSRI intake of 10 days, while decreases were observed within the pre- and postcentral gyri (all $P < 0.05$, family-wise error [FWE] corrected). Furthermore, enhanced resting functional connectivity (rFC) within the ventral precuneus and PCC was associated with gray matter increases in the PCC (all FWE $P_{corr} < 0.05$). Corroborating these results, whole-brain connectivity density, measuring the brain's functional network hubs, was significantly increased after SSRI-intake in the ventral precuneus and PCC (all FWE $P_{corr} < 0.05$). Short-term administration of SSRIs changes gray matter structures, consistent with previous work reporting enhancement of neuroplasticity by serotonergic neurotransmission. Furthermore, increased gray matter in the PCC is associated with increased functional connectivity in one of the brain's metabolically most active regions. Our novel findings provide convergent evidence for dynamic alterations of brain structure and function associated with SSRI pharmacotherapy.

Introduction

Magnetic resonance imaging (MRI) and voxel-based morphometry (VBM) studies in patients with depression and obsessive–compulsive disorder (OCD) showed gray matter enhancements after treatment with selective serotonin reuptake inhibitors (SSRIs) (Hoexter et al., 2012; Smith et al., 2012). Moreover, depressive patients homozygous for the L_A-allele in the SERT gene (rs25531) seem to be more susceptible to gray matter atrophy (Frodl et al., 2008), this polymorphism also seems to affect gray matter in healthy subjects (Frodl et al., 2008). Remarkably, at least 3 of the 16 known 5-HT receptors (5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A}) (Gaspar et al., 2003) and the 5-HT transporter (SERT) (Benninghoff et al., 2012) are involved in neuroplasticity processes (Gould, 1999; Mogha et al., 2012; Vitalis et al., 2002). Tight links between the neurotrophin system and 5-HT have previously been shown (Castrén and Rantamäki, 2010) demonstrating the role of 5-HT in regulating neuronal morphology and circuitry (Daubert and Condron, 2010).

Selective 5-HT reuptake inhibitors represent the first line medication for depression (Bauer et al., 2007), anxiety disorder, OCD, posttraumatic stress disorder and eating disorders (Aigner et al., 2011; Bandelow et al., 2008). At the serotonergic synapse, SSRIs bind to a binding site at the SERT and block reuptake of 5-HT (Kasper et al., 2009; Stahl, 1998). Treatment with SSRIs results in rapid 20-fold increase in 5-HT levels within the midbrain raphe nuclei (Tao et al., 2000) that increases 5-HT binding at 5-HT_{1A} autoreceptors there, which subsequently alters neuronal firing rates and promotes desensitization of 5-HT_{1A} receptors (Stahl, 1998; Zimmer et al., 2004). The resulting lack of autoinhibition triggers 5-HT release at axon terminals (Gibbons et al., 2012).

Most of the existing studies using phMRI applied functional MRI and investigated task related blood oxygen dependent level (BOLD)

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responses. Evidence from phMRI and VBM, which addresses the impact of pharmaceuticals on gray matter structure, is scarce. Yet, this technique is a powerful tool that is able to detect morphological alterations in vivo at high resolution. Recent work has confirmed gray matter alterations detected by MRI with ex vivo MRI scans as well as post-mortem volumetric analysis (Vernon et al., 2011), and structural MRI exhibits an excellent test–retest reliability (Wonderlick et al., 2009). In vivo gray matter changes found by MRI and VBM were validated by post-mortem findings (Hornberger et al., 2012).

Taken together, a convergent line of evidence demonstrates that 5-HT is involved in development and regulation of gray matter morphology through a series of mechanisms associated with neuroplasticity. Treatment with SSRIs might thus trigger gray matter changes, yet confirmation in healthy subjects is missing and the impact of regionally altered gray matter on neuronal functionality is hardly known. Hence, the aim of this study was to (1) investigate the influence of 5-HT on gray matter and (2) to elucidate the associated functional neuronal network changes. This was accomplished by administration of SSRIs to healthy subjects followed by structural and functional phMRI with quantification of gray matter changes through VBM and assessment of neuronal networks through resting functional connectivity (rFC) analyses.

Materials and methods

Subjects

A longitudinal, crossover, double-blind, placebo-controlled study design was used. The study sample is part of a previously published fMRI study (Windischberger et al., 2010), yet all analyses were previously not considered. Twenty-four healthy adult subjects were recruited by advertisement at community boards at the General Hospital in Vienna, four subjects did not meet inclusion criteria or refused to participate, 20 subjects were randomized, two subjects dropped out (not related illness, non-compliance) and structural data from one subject was not available for all three points. Hence, structural MRI datasets were available from 17 healthy Caucasian subjects (6 female, 11 male 26.5 ± 6.1 years, mean ± SD, see Table 1). All subjects provided written informed consent and received reimbursement after participation. All subjects underwent a medical examination at the screening visit that included medical history, electrocardiogram and routine blood tests. Exclusion criteria were history of severe disease, any psychiatric (according to assessment by Structured Clinical Interview for DSM-IV Axis I + II Disorders, SCID I + II) or neurological disorder, drug abuse including anabolic steroids, psychiatric medication, use of hormonal contraceptives for the past 6 months, and a positive urine pregnancy test. All subjects were naïve to SSRIs and psychotropic medication. No particular menstrual phase for scanning of female subjects was defined. The interventions ended with a final check-up visit for each participant. All study related procedures were approved by the Ethics Committee of the Medical University of Vienna.

Table 1
Demographic data of the study sample. Data are given as means ± SD. Alcohol units per week = alcohol consumption (liter) × alcohol by volume ratio. BMI = body mass index. tGMD = total gray matter density (placebo condition). P compares males and females with independent sample t-test or Mann–Whitney U test ($^+$) where normal distribution was not obtained by Levene's test.

	All subjects	Males	Females	P
N	17	11	6	
Age (years)	26.5 ± 6.1	28 ± 7.1	23.8 ± 2.1	0.185
BMI (kg/m^2)	22.3 ± 2.3	22 ± 2.7	23 ± 1.3	0.382
Cigarettes/day	1.8 ± 4	0.8 ± 2.2	3.6 ± 5.7	0.533$^+$
Alcohol/week	5.7 ± 6.5	3.9 ± 4.9	9 ± 8.2	0.128
tGMD	919.3	918.6 ± 19.9	920.6 ± 14.8	0.746

Study design and medication

All study subjects received 10 mg escitalopram (S-citalopram), an equivalent dosage of 20 mg citalopram (the 1:1 racemic mixture of R-citalopram and S-citalopram) and placebo, in randomized order respectively, for 10 days prior to MRI scanning. This period of medication intake was chosen to reach the plasma steady-state condition (Kasper et al., 2009; Klein et al., 2007). Study subjects consecutively underwent three MRI scanning sessions (one after citalopram, escitalopram and placebo) with an average interval of 21.8 ± 13.0 (mean, SD) between screening visit and MRT1 (no wash-out period), 33.8 ± 6.5 days between MRT1 and MRT2 and 33.1 ± 4.9 between MRT2 and MRT3. According to the half-lives of citalopram and escitalopram (Bezchlibnyk-Butler et al., 2000; Rao, 2007), visit intervals have provided enough time to ensure previous drug/placebo washout. Treatment adherence was ascertained by announcing control of medication intake through measurements of plasma-levels at any given time point during study duration. Color-matched dextrose tablets were used as placebo. In order to blind all study personnel and participants to medication group assignment, independent pharmacists prepared the medication in accordance with a computer generated randomization list and each blister was encoded with a unique number to prevent inferences on treatment type and subject. For quantification of plasma levels, blood samples were taken from each subject approximately 10 min before each fMRI session. Plasma was frozen at −20 °C and shipped for analysis (Quintiles Analytical Services, Sweden).

MRI measurements and image analyses

Structural MRI measurements were performed on 3 Tesla (T) whole-body MEDSPEC S300 MR-scanner (Bruker BioSpin, Ettlingen, Germany) using a standard quadrature single-loop transmit/receive birdcage head coil at the MR Center of Excellence at the Medical University of Vienna, Austria. The imaging protocol comprised a magnetization-prepared rapid gradient echo (MPRAGE, T1-weighted) sequence yielding 128 slices, a 256 × 256 matrix, at a slice thickness of 1.56 mm and a voxel size of 0.78 × 0.86 mm.

Additionally, all study subjects underwent fMRI with a facial expression task (described below), of which the fMRI results were published previously (Hahn et al., 2009). In the same MRI session a single-shot gradient-recalled echo planar imaging (GR-EPI) sequence was applied, optimized for imaging blood oxygen dependent (BOLD) contrast. This EPI-sequence was done at a TE = 31 ms, TR = 1000 ms and a matrix size = 128 × 91, which resulted in a total slab width of 34.5 mm with 10 axial slices of 3 mm thickness aligned to the AC–PC line (0.5 mm slice gap).

Voxel-based morphometry

In order to test the main hypothesis of this study, which was to detect alterations of gray matter after 5-HT reuptake inhibition, we used VBM for structural brain assessment. All analyses of images were performed with statistical parametric mapping (SPM8, Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, London, United Kingdom, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and MATLAB 7.10 (MathWorks, Natick, MA). An optimized VBM protocol was used, applying the DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) algorithm (Ashburner, 2007). The images were segmented into gray matter, white matter, and cerebrospinal fluid (CSF) compartments and successfully passed visually checking for major artifacts. Subsequently, the gray matter maps obtained by this procedure were separately normalized to a gray matter template representing the stereotactic standardized Montreal Neurological Institute (MNI) space at a voxel size of 1.5 × 1.5 × 1.5 mm. Based on deformation fields calculated during segmentation, a template was generated by the DARTEL algorithm. The Jacobian determinants derived