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Single-dose serotonergic stimulation shows widespread effects on functional brain connectivity

Bernadet L. Klaassens [a,b,c,d,*], Helene C. van Gorsel [d], Najmeh Khalili-Mahani [e], Jeroen van der Grond [b], Bradley T. Wyman [f], Brandon Whitcher [f], Serge A.R.B. Rombouts [a,b,c], Joop M.A. van Gerven [d]

[a] Leiden University, Institute of Psychology, Leiden, The Netherlands
[b] Leiden University Medical Center, Department of Radiology, Leiden, The Netherlands
[c] Leiden University, Leiden Institute for Brain and Cognition, Leiden, The Netherlands
[d] Centre for Human Drug Research, Leiden, The Netherlands
[e] McGill University, Montreal Neurological Institute, Montreal, QC, Canada
[f] Pfizer Inc., Cambridge, MA, USA

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ABSTRACT

The serotonergic system is widely distributed throughout the central nervous system. It is well known as a mood regulating system, although it also contributes to many other functions. With resting state functional magnetic resonance imaging (RS-fMRI) it is possible to investigate whole brain functional connectivity. We used this non-invasive neuroimaging technique to measure acute pharmacological effects of the selective serotonin reuptake inhibitor sertraline (75 mg) in 12 healthy volunteers. In this randomized, double blind, placebo-controlled, crossover study, RS-fMRI scans were repeatedly acquired during both visits (at baseline and 3, 5, 7 and 9 h after administering sertraline or placebo). Within-group comparisons of voxelwise functional connectivity with ten functional networks were examined ($p < 0.005$, corrected) using a mixed effects model with cerebrospinal fluid, white matter, motion parameters, heart rate and respiration as covariates. Sertraline induced widespread effects on functional connectivity with multiple networks; the default mode network, the executive control network, visual networks, the sensorimotor network and the auditory network. A common factor among these networks was the involvement of the precuneus and posterior cingulate cortex. Cognitive and subjective measures were taken as well, but yielded no significant treatment effects, emphasizing the sensitivity of RS-fMRI to pharmacological challenges. The results are consistent with the existence of an extensive serotonergic system relating to multiple brain functions with a possible key role for the precuneus and cingulate.

Introduction

The central serotonergic system plays an important modulatory role and affects diverse functions like cognition, mood, appetite, sleep and sensorimotor activity. Different classes of serotonin (5-hydroxytryptamine, 5-HT) receptors exist, and they are distributed throughout the whole brain, including the cortex, limbic areas, hypothalamus, basal ganglia, brain stem and cerebellum (Barnes and Sharp, 1999; Carr and Lucki, 2011; Hoyer et al., 2002; Jacobs and Azmitia, 1992; Nichols and Nichols, 2008). The selective serotonin reuptake inhibitor (SSRI) sertraline increases the concentration of synaptic serotonin and is commonly prescribed as a treatment for depression and anxiety disorders (McRae et al., 2001).

Functional brain imaging shows that SSRIs change brain activation and perfusion in limbic and prefrontal regions, which have been identified as important mediators in emotional processing. However, SSRIs also influence many other brain structures, including the precuneus, basal ganglia, brain stem, cerebellum, hypothalamus, temporal, parietal and occipital areas (Anderson et al., 2007; Bruhl et al., 2011; Geday et al., 2005; Klomp et al., 2012; McKie et al., 2005; Smith et al., 2002; Viviani et al., 2012).

Understanding the mechanism of action of the extensive system of serotonergic neurons requires a method for studying large-scale network interactions instead of isolated brain regions (Schaefer et al., 2014). The resting state functional magnetic resonance imaging (RS-fMRI) technique allows an integral non-invasive investigation of these network interactions, taking into account the brain's comprehensive neural circuit (Fox and Raichle, 2007; Lu and Stein, 2014).

The main focus of most RS-fMRI studies on SSRIs has been functional connectivity of the default mode network (DMN), which includes the posterior cingulate, precuneus and medial prefrontal, temporal and parietal regions. The DMN, one of the most consistent networks, is

* Corresponding author at: Leiden University, Institute of Psychology, Unit Methodology and Statistics, PO Box 9555, 2300 RB Leiden, The Netherlands. Fax: +31 71 527 3761.
E-mail address: b.klaassens@lumc.nl (B.L. Klaassens).

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affected in multiple mental disorders, including depression (Sundermann et al., 2014). SSRI administration shows a reduction in functional DMN connectivity, indicating normalization of patterns as seen in depression (Li et al., 2013; McCabe and Mishor, 2011; McCabe et al., 2011; van de Ven et al., 2013; van Wingen et al., 2014). Yet, SSRIs are expected to change functional connections in brain regions beyond the DMN too (Schaefer et al., 2014).

Here, we apply a technique of repeated measures RS-fMRI and an analysis of region-to-network connectivity to study whole brain treatment effects of an SSRI in healthy young volunteers. We have shown the sensitivity of this approach for various other pharmacological challenges (Cole et al., 2013; Khalili-Mahani et al., 2012, 2015; Klumpers et al., 2012; Niesters et al., 2012). Given the widespread distribution of serotonergic receptors in the brain and the involvement of serotonin in many brain functions, we hypothesize that a single-dose of the SSRI sertraline will not only affect the DMN but various resting state brain networks, related to emotional, sensory, motor, cognitive and executive functioning.

Materials and methods

Subjects

Twelve healthy young volunteers (mean age 23.0 ± 2.8, range 19–28; gender ratio 1:1; BMI 19–24 kg/m^2) were recruited to participate in the study. All subjects underwent a thorough medical screening at the Centre for Human Drug Research (CHDR) to investigate whether they met the inclusion and exclusion criteria. They had a normal history of physical and mental health and were able to refrain from using nicotine and caffeine during study days. Other exclusion criteria included positive drug or alcohol screen on study days, regular excessive consumption of alcohol (>4 units/day), caffeine (>6 units/day) or cigarettes (>5 cigarettes/day), use of concomitant medication 2 weeks prior to study participation and involvement in an investigational drug trial 3 months prior to administration. The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and the scientific review board of the CHDR. Written informed consent was obtained from each subject prior to study participation.

Study design

This was a single center, double blind, placebo-controlled, crossover study with sertraline 75 mg. To cover the interval of maximum concentrations of sertraline (T_{max}: 5.5–9.5 h, $T_{1/2}$: 26 h), five RS-fMRI scans were acquired during study days, one at baseline and four after administering sertraline or placebo (at 3, 5, 7 and 9 h post dosing). Each scan was followed by performance of computerized cognitive tasks (taken twice at baseline) on the NeuroCart® test battery, developed by the CHDR for quantifying pharmacological effects on the central nervous system (CNS). By including multiple measurements during the T_{max} interval, this repeated measures profile increases the statistical power of the analysis. Currently, there are no formal power calculation methods that allow estimating the sample size for testing whole brain functional variations. Our sample size was selected based on previous studies (Khalili-Mahani et al., 2012, 2015; Klumpers et al., 2012; Niesters et al., 2012) that demonstrated sufficient power to detect significant effects in repeated measures designs with 12 subjects. Sertraline and placebo were administered orally as capsules, matched for size and weight. To reduce the most common side effect of sertraline (nausea and vomiting), drug treatment was combined with granisetron 2 mg tablets on both study days. Multiple blood samples were taken during the course of the day to define the pharmacokinetic (PK) profile of sertraline in serum, its active metabolite desmethylsertraline and concentrations of cortisol and prolactin. Washout period between the two study days was at least 10 days. An overview of the study design is provided in Fig. 1.

Blood sampling

Pharmacokinetics

Blood samples were collected in 4 mL serum tubes at baseline and 1.5, 3, 5, 6, 7 and 9 h post dosing, centrifuged (2000 *g* for 10 min) and stored at −40 °C until analysis with liquid chromatography–tandem mass spectrometry (LC–MS/MS). PK parameters for sertraline and its active metabolite desmethylsertraline were calculated using a non-compartmental analysis. Maximum serum concentrations (C_{max}) and time of C_{max} (T_{max}) were obtained directly from the serum concentration data. The area under the serum concentration versus time curve was calculated from time zero to the time of the last quantifiable serum measured concentration, which is equal to the last blood sample of the study day (AUC_{0-last}). The calculated PK parameters were not used for further analysis but investigated to validate the choice of time points of measurements.

Neuroendocrine variables

Blood samples were also obtained to determine cortisol and prolactin concentrations. Serum samples were taken in a 3.5 mL gel tube at baseline and 1.5, 3, 5, 6, 7 and 9 h post dosing, centrifuged (2000 *g* for 10 min) and stored at −40 °C until analysis. Serum concentrations were quantitatively determined with electrochemiluminescence immunoassay. Cortisol and prolactin concentrations were subsequently used for statistical analysis using a mixed effects model with treatment, time and treatment by time as fixed effects, subject, subject by treatment and subject by time as random effects and the average of the period baseline (pre-dose) values as covariate (SAS for Windows V9.1.3; SAS Institute, Inc., Cary, NC, USA). In the Results section, significant treatment effects (at $p < 0.05$) will be discussed.

NeuroCart® test battery

Each RS-fMRI scan was followed by functional CNS measures outside the scanner using the computerized NeuroCart® test battery measuring alertness, mood and calmness (Visual Analogue Scales (VAS) Bond & Lader), nausea (VAS Nausea), vigilance and visual motor performance (Adaptive Tracking task), reaction time (Simple Reaction Time task), attention, short-term memory, psychomotor speed, task switching and inhibition (Symbol Digit Substitution Test and Stroop task), working memory (N-back task) and memory imprinting and retrieval plus

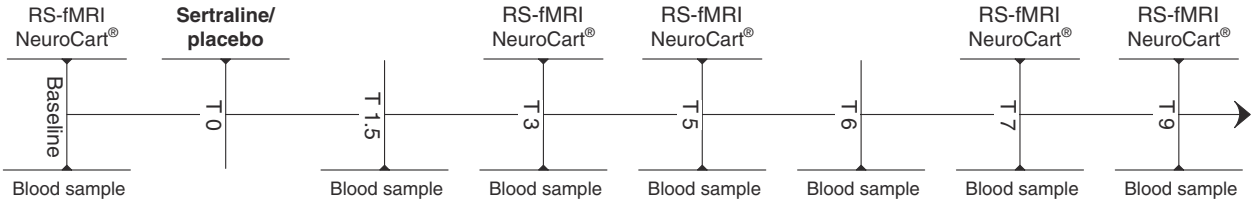

Fig. 1. Schematic presentation of a study day. At baseline, one RS-fMRI scan was acquired, followed by the NeuroCart® CNS test battery (performed twice at baseline). After drug administration, four more RS-fMRI scans were acquired at time points T = 3, 5, 7 and 9 h post dosing, each time followed the NeuroCart® test battery. During the day, seven blood samples were taken to measure the concentrations of (desmethyl)sertraline, cortisol and prolactin.