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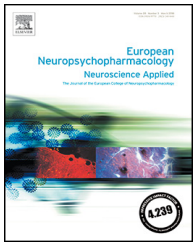

Modeling the acute pharmacological response to selective serotonin reuptake inhibitors in human brain using simultaneous PET/MR imaging

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Abstract
Pharmacological imaging of the effects of selective serotonin reuptake inhibitors (SSRI) may aid the clarification of their mechanism of action and influence treatment of highly prevalent neuropsychiatric conditions if the detected effects could be related to patient outcomes.

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Selective serotonin reuptake inhibitors; serotonin transporter; resting-state fMRI

In a randomized double-blind design, 38 healthy participants received a constant infusion of 8 mg citalopram or saline during either their first or second of two PET/MR scans. Resting-state functional MRI (fMRI) was acquired simultaneously with PET data on the binding of serotonin transporters (5-HTT) using [^{11}C]DASB. Three different approaches for modeling of pharmacological fMRI response were tested separately. These relied on the use of regressors corresponding to (1) the drug infusion paradigm, (2) time courses of citalopram plasma concentrations and (3) changes in 5-HTT binding measured in each individual, respectively. Furthermore, the replication of results of a widely used model-free analysis method was attempted which assesses the deviation of signal in discrete time bins of fMRI data acquired after start of drug infusion. Following drug challenge, average 5-HTT occupancy was 69±7% and peak citalopram plasma levels were 111.8 ± 21.1 ng/ml. None of the applied methods could detect significant differences in the pharmacological response between SSRI and placebo scans. The failed replication of SSRI effects reported in the literature despite a threefold larger sample size highlights the importance of appropriate correction for family-wise error in order to avoid spurious results in pharmacological imaging. This calls for the development of analysis methods which take regional specialization and the dynamics of brain activity into account.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) constitute the first-line pharmacological treatment in several psychiatric conditions, including major depression, anxiety disorders and obsessive compulsive disorder, to name those with the highest impact on the global burden of disease (Bauer et al., 2015). Pharmacological neuroimaging promises to advance the understanding of the mechanisms of action of psychopharmaceuticals and to influence treatment algorithms. The presented work investigated the acute effects of SSRIs on brain activation using resting-state functional magnetic resonance imaging (fMRI). This focus was particularly enticing due to the potential to transfer the results into clinical practice with reasonable effort, that is if fMRI responses to acute SSRI challenge could be identified and in a next step, associated with therapy response in patients which occurs weeks later. While the majority of pharmacological fMRI studies rely on T2*-weighted sequences, the resulting of blood-oxygen-level dependent (BOLD) signal data imposes a number of peculiarities with regard to data analysis and interpretation, for which a number of different approaches has been proposed (Khalili-Mahani et al., 2017). In the context of imaging acute pharmacological effects, the most relevant challenges are that resting-state BOLD signal is dynamic and cannot be treated as an absolute measure, such that it is difficult to establish a baseline to which to compare data acquired after drug application, and that the timing, localization and size of effects is rarely known a priori. This is in contrast to task-based fMRI, where timing is dictated by the hemodynamic-response function and regions of interest may be indicated by the nature of the task performed (Rischka et al., 2018; Sladky et al., 2015). Therefore, in order to increase the sensitivity for detection of pharmacological fMRI (phMRI) responses, investigators have relied on external information regarding the timing of the expected effects in case these were available. Common approaches were the use of drug plasma concentrations measured at several time points during imaging which e.g., aided the detection of brain activation changes after ketamine challenge (Höflich et al., 2017b), and the use of linear ramp functions for modeling the duration of drug infusion which e.g., allowed for characterization of the phMRI response to buprenorphine infusion (Becerra et al., 2013). On the other hand, integration of phMRI with data on the distribution of drug targets derived from positron emission tomography, post-mortem autoradiography or gene expression data may explain regionally differential phMRI responses (Dukart et al., 2018; Gryglewski et al., 2018). The recent development and dissemination of hybrid PET/MR systems promises to advance the understanding of drug effects by allowing for the simultaneous assessment of phMRI responses with information on the distribution and engagement of molecular targets. This methodology has hitherto aided elucidating the effects of drugs acting on dopamine and the serotonin 1A and 1B receptors in animal studies (Hansen et al., 2017; Mandeville et al., 2013; Vidal et al., 2018).

In this work, different methods to detect phMRI response to an infusion of a frequently prescribed SSRI, citalopram were evaluated. These included the widely used models of drug plasma concentrations and infusion timing, as well as a novel approach which employs the simultaneously measured time course of serotonin transporter binding. The randomized, double-blind placebo-controlled study design and drug challenge paradigm were approximated to a pioneering phMRI study of citalopram effects (McKie et al., 2005). This study has first introduced a model-free analysis method which relies on separating phMRI data into time bins and comparing the deviations from baseline between placebo and drug challenge scans. As this model-free method has had a high impact on the pharmacological imaging field where it has been applied in numerous studies, e.g., to investigate ketamine effects (Deakin et al., 2008; Hodkinson et al., 2012) or challenges in the serotonin system (Canese et al., 2011; Newman-Tancredi et al., 2016; Vidal et al., 2018), we attempted to replicate the original results.

2. Experimental procedures

2.1. Study design and subjects

38 healthy subjects aged 18–50 (average=29.1 ± 9.4) years (21 female) underwent two PET/MR scans with the radioligand [^{11}C]DASB during which double-blind pharmacological challenge with the SSRI citalopram or placebo (saline) was performed in a randomized cross-over study design. Scans were separated by a minimum of 48 h. During an initial screening visit, general and mental health were ascertained by a psychiatrist based on medical history, physical examination, structured clinical interviews for DSM-IV (SCID I and II), electrocardiography, a routine laboratory profile (hematology, clinical chemistry, coagulation, thyroid hormones) and urine pregnancy tests. All subjects were non-smoking, had no history of psychiatric or severe somatic diagnoses, and did not take any medication on a daily basis (excepting hormonal contraception in 3 female subjects). Illicit drug use was ruled out based on clinical interviews and urine drug screening before inclusion and on scanning days. All study procedures were carried out according to good clinical practice guidelines and the Declaration of Helsinki of 1975, as revised in 2008 and approved by the Ethics Committee of the Medical University of Vienna. Subjects provided written informed consent and were reimbursed for their participation. The study was registered before the start of recruitment at clinicaltrials.gov (NCT02711215).

2.2. PET/MR scanning procedures

On scanning days, arterial and venous cannulas were inserted in the radial artery and a forearm vein of opposite arms for blood sampling and administration of tracer and study drug, respectively. Synthesis and quality control of the 5-HTT specific tracer ([^{11}C]-N,N-dimethyl-2-(2-amino-4-cyanophenylthio)-benzylamine ([^{11}C]DASB) was performed following a published protocol (Haeusler et al., 2009). Application of [^{11}C]DASB was initiated outside of the scanner and administered as bolus plus constant infusion with $K_{bol} = 162$ min using a tungsten-shielded syringe pump according to an optimized protocol for quantification of serotonin transporter binding (Gryglewski et al., 2017). After approximately 30 min, subjects were transferred into the PET/MR scanner (3T, Siemens mMR, Erlangen, Germany) and PET data was acquired continuously in list-mode. 70 min after initiation of tracer application double-blind challenge with citalopram 8 mg or saline was performed as a continuous infusion over 8 min. Resting-state fMRI data was acquired for 40 min starting ten minutes (median=9.2 min; min/max=2.2/14.5 min) before drug application with an echo-planar imaging sequence (TE/TR= 30/2440 ms, 2.1 × 2.1 mm in-plane resolution, 3 mm slice thickness). Subsequently, a T1-weighted sequence was acquired (MPRAGE, TE/TR=4.21/2000 ms, 1 × 1 mm in-plane resolution, 1 mm slice thickness, 0.1 mm gap). Arterial blood samples were drawn at 2, 5, 8, 12, 18, 30, 50, 60 and 70 min after start of study drug application using 1.5 m long tubing in order to minimize interference with the MR field. Concentration of [^{11}C]DASB and radioactive metabolites in plasma was determined in the last three arterial blood samples collected in tracer equilibrium using solid-phase extraction cartridges and a gamma-counter calibrated with the PET/MR scanner (Ginovart et al., 2001).

2.3. Preprocessing of resting-state data

fMRI data was preprocessed using Statistical Parametric Mapping (SPM 12, www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB R2014a and ArtRepair version 5b (Mazaika et al., 2009). This included correction of transient slice artifacts, slice-timing correction, rigid body alignment, reslicing, smoothing at 4 mm full width at half maximum, movement regression (Grootoonk et al., 2000), despiking and exclusion of motion outliers after removal of linear trends and normalization to Montreal Neurological Institute (MNI) standard space with an isotropic resolution of 2 mm. Additional smoothing with a at 7 mm full width at half maximum was carried out after normalization. Subsequently, nuisance regressors for the first five principal components of white matter and cerebrospinal fluid signal defined using the Harvard-Oxford atlas (fsl.fmrib.ox.ac.uk/fsl/fslwiki) at a threshold of 95% were used.

2.4. Quantification of dynamic 5-HTT binding

Reconstruction of list mode PET data was carried out using an ordinary Poisson-ordered subset expectation maximization algorithm (OP-OSEM, 3 iterations, 21 subsets) into three 5 min frames before drug challenge, five 2 min and nine 5 min frames starting with the drug challenge and 10 min frames thereafter. Attenuation correction was performed using a low-dose CT scan (Siemens Biograph TruePoint PET/CT) which was recorded on a separate occasion, registered to T1 weighted MR data and bilinear scaled to attenuation coefficients for 511 keV (Carney et al., 2006). Motion correction was performed by aligning all frames to the mean image defined as the longest period without movement. PET data was registered to structural MR data which was normalized to MNI space in SPM12. Time activity curves were extracted for the thalamus delineated using the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002) and cerebellar gray matter which contains negligible concentrations of 5-HTT (Kish et al., 2005). The thalamus was chosen as it has a high density of 5-HTT and the tracer application protocol is optimized for rapid equilibration in this region (Gryglewski et al., 2017). Activity of brain regions was divided by metabolite-corrected activity in plasma to obtain distribution volumes. Distribution volume of cerebellar gray matter was subtracted from the distribution volume of the thalamus to obtain a time course of the specific volume of distribution (V_S=BP_P). BP_P time courses were scaled by setting average BP_P calculated for frames acquired before drug challenge equal between placebo and SSRI scans in order to remove deviations of occupancy from 0 due to variation of the baseline between scans. Finally, for each subject a regressor for changes in 5-HTT binding was obtained by fitting three exponentials to the relative difference in BP_P between conditions calculated for each frame (t) using the formula $\text{Occupancy}(t) = (BP_{\text{P-Placebo}(t)} - BP_{\text{P-SSRI}(t)})/BP_{\text{P-Placebo}(t)}$.

2.5. Quantification of citalopram plasma levels

Determination of citalopram concentrations was performed using liquid chromatography tandem-mass spectrometry (LC-MS/MS) for all samples collected after study drug application after storage of plasma at -80 °C. Plasma concentrations were fitted by two separate functions for the time during and after infusion, respectively. As too few samples were collected during drug infusion to allow for reliable fitting in each subject, the shape of the plasma concentration curve was fitted using one exponential function to plasma levels obtained from all participants. For each subject, this function was scaled to the maximum plasma concentration measured at the end of infusion. For the time after infusion, falling plasma concentrations were fitted by one exponential function in each individual separately.

2.6. Pharmacological modeling of the fMRI response

The ability of three different models to capture phMRI responses to SSRI challenge was tested: (1) A linear ramp function set equal

to zero before infusion, increasing linearly to 8 over 8 min and remaining stable thereafter. Thus, this model was corresponding to the cumulative dose applied at each time point. (2) Individual time courses of plasma drug concentrations obtained as described above. (3) Individual time courses of relative change in 5-HTT binding obtained as described above. Each model was tested independently by applying it as a regressor to fMRI data and calculating paired *t*-tests between regression coefficients obtained for placebo and SSRI scans, respectively. Identical regressors were used for placebo and verum scans.

2.7. Analysis of pharmacological fMRI response using time bins

A widely used model-free approach to the detection of phMRI response was applied with minimal necessary modifications in order to provide the most accurate replication (McKie et al., 2005). To this end, fMRI resting-was separated into twelve 2 min time bins acquired during and after SSRI infusion. At the first level, each time bin was compared to a baseline time bin of 4 min. This timeframe was chosen, because it was available in most patients. At the second level, paired t-tests were calculated between SSRI and placebo scans for each 2 min time bin. Significance levels were set at $p<0.05$, corrected for family-wise error (FWE) at the cluster level.

3. Results

Statistical analysis was performed on a final dataset of (1) 38 subjects for the linear ramp model, (2) 37 subjects for the plasma level model, (3) 36 subjects for the 5-HTT binding model, and (4) 36 subjects for the time bin approach. Exclusion of subjects for individual analyses was necessary due to technical difficulties (delayed start or premature end of fMRI data acquisition in one subject each, delayed application of drug challenge in 2 subjects, and obstruction of the arterial line during one scan).

3.1. Pharmacological modeling of the fMRI response

Successful drug challenge could be verified by 5-HTT occupancy and citalopram plasma levels in all subjects. 5-HTT occupancy ranged from 48 to 81% (mean ± SD = 69 ± 7%) at the end of measurement, i.e., at least 55 min after start of drug challenge. Peak citalopram levels ranged from 69.7 to 152.9 ng/ml (mean ± SD = 111.8 ± 21.1 ng/ml) and decreased to 7.6–16.8 ng/ml (mean ± SD = 10.6 ± 1.9 ng/ml) at the end of measurement. Pharmacological regressors applied for modeling phMRI data are displayed in Fig. 1. After FWE correction, no significant differences in regression coefficients of any model could be detected between placebo and SSRI scans using paired *t*-tests.

3.2. Analysis of pharmacological fMRI response using time bins

No significant differences in deviation from baseline between SSRI and placebo scans could be shown for any time bin recorded after start of drug challenge. As the results of the first application of this method in the literature were described at an uncorrected significance level of $p<0.001$ (McKie et al., 2005), uncorrected results were also inspected. In this manner, from the first time bin recorded after start of challenge onwards, widely scattered differences were seen between conditions which were not consistent between time bins. The predominant valence of the differences was a reduced signal during SSRI scans.

4. Discussion

The applied approaches were not successful to detect a significant phMRI response to intravenous application of a subtherapeutic dose of the SSRI citalopram. These results are relevant as the methods applied are widely used in the field of pharmacological imaging and can therefore impact the interpretation of published data and the planning of future studies and analyses. The discussion starts by raising potential limitations of the study design which may have mitigated the detection of phMRI responses, then addresses the applied analysis methods in light of the literature on SSRI response, and finally appraises other methods with the potential to detect phMRI responses.

4.1. Limitations of the study

In the current study, a relatively large sample of 38 participants was enrolled which is seldom attained in phMRI studies. Given the repeated-measures design, a power of 85% for medium effect sizes of dz=0.5 could be expected at α=0.05 in two-tailed paired *t*-tests. This power may be insufficient to detect effects on brain activation elicited by interference with serotonergic neuromodulation which are likely to be more subtle than those elicited e.g., by blocking glutamatergic neurotransmission by anesthetics, especially when correcting for family-wise error in multidimensional imaging data. Subjective effects were seldom reported by participants and could not be differentiated from effects of prolonged imaging and blood sampling procedures. This precludes the secondary influence of drug experience on brain activation, which is in contrast to other drug challenges with pronounced effects on subjective experience such as ketamine (Höflich et al., 2017a). It is unlikely that carry-over effects of SSRIs between the two PET/MR scans mitigated results, as the interval between scans was long enough to guarantee wash-out of citalopram at a half-life of approximately 27 h after application of a single intravenous dose of the active enantiomer (Søgaard et al., 2005). The shortest interval between PET/MR scans in those subjects who received citalopram during the first measurement was five days. A potential confounder was the duration of imaging procedures, which may have resulted in movement artifacts. At the time of completion of fMRI scans, subjects were positioned in the scanner for approximately 60 min. This was partly compensated by motion correction during data preprocessing (Mazaika et al., 2009). As the start of drug challenge was fixed to 70 min after start of radio ligand infusion, delays in the initiation of imaging data acquisition (e.g., due to technical or procedural difficulties) resulted in a variable duration of fMRI

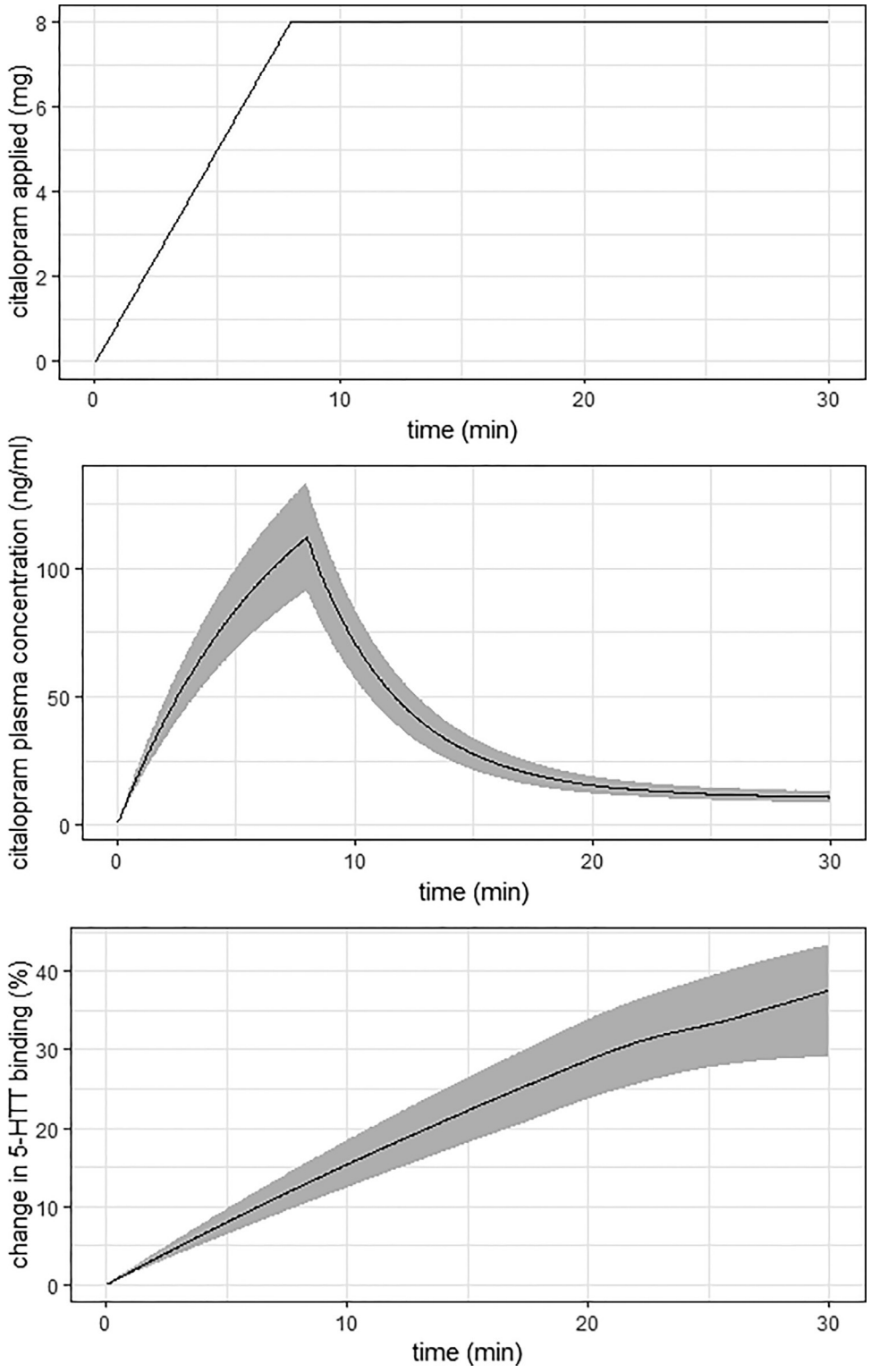

Fig. 1 Regressors used in phMRI analysis. Citalopram 8 mg was applied as constant infusion over 8 min. The first regressor was a ramp function which matches the time course of the pharmacological challenge (top). For each participant, a continuous time course of citalopram plasma levels was created by fitting exponential functions to measured concentrations. The resulting individual regressors were averaged for display purposes in this figure (middle). Relative changes in 5-HTT binding measured over time were fitted by three exponential functions to yield a regressor for each participant. The average 5-HTT binding regressor is shown (bottom). Grey ribbons correspond to the standard deviation.

baseline before drug challenge. Notably, baseline fMRI data of a minimum of five minutes was available in all subjects excepting one, who was consequently excluded from the time bin analysis which required a minimum of four minutes of baseline. The remaining analyses are based on modeling drug effects using regressors which are equal to zero only before drug challenge. Therefore, prolonged acquisition of baseline fMRI is expected to have a minimal effect on regression coefficients of pharmacological modeling as it does not capture activation changes induced by the drug. Although a replication of published estimates of effective concentrations relating 5-HTT occupancy to plasma levels of SSRIs would be valuable (Meyer et al., 2004), this could not be accomplished with the available data: given that a fixed dosage of citalopram was applied in all subjects, only a small fraction of the one-site binding model was explored as 5-HTT occupancy ranged from 48 to 81%. Furthermore, the citalopram plasma concentrations measured did not correspond to pre-dose trough levels, which reduces the comparability of any estimate of effective concentrations.

4.2. Pharmacological modeling of the fMRI response

Modeling of brain activation data was attempted using regressors corresponding to the time course of the applied dose, drug plasma levels and change in 5-HTT binding (Fig. 1). A significant result would indicate that the fMRI response followed the time course of a regressor to a greater extent in one condition than the other. It is not surprising that brain activation after citalopram did not follow the time course of plasma levels, i.e., rapidly increasing during infusion and rapidly falling thereafter, as this time course is reflecting the rapid distribution kinetics of citalopram rather than the concentration in the brain. While the rapid increase in plasma concentrations may actually correspond well to the increase in brain extracellular fluid, as shown in rats (Bundgaard et al., 2007) and evidenced by the almost immediate change in [^{11}C]DASB binding, the decrease in plasma concentrations after the end of infusion is rapid, while brain concentrations may still be increasing. Plasma concentrations might be an adequate reference if the aim is to detect the effect of SSRIs on cerebral vasculature (Hansen et al., 2017; Ofek et al., 2012).

The regressor corresponding to the cumulative dose at each time point is similar to the plasma regressor with respect to the rapid increase during infusion, but remains stable thereafter. This time course may correspond better to brain concentrations of citalopram which are expected to decrease to a small extent relative to peak levels in the time window of fMRI acquisition (Bundgaard et al., 2007). However, it ignores the fact that the putative mechanism of action of SSRIs is depending on the blockade of serotonin reuptake and therefore may gradually build up over time with increasing extracellular serotonin concentrations. While microdialysis in rats revealed a rapid increase of serotonin concentrations in rat hippocampus after systemic SSRI application (Bundgaard et al., 2007), the effect was dose-dependent in frontal cortex and slower in the raphe nuclei (Bel and Artigas, 1999). As average 5-HTT occupancy was 69% in the current study, the build-up of serotonin concentrations might have been low, occured at a later point in time, especially in the raphe nuclei, or masked by inhibitory regulation (Invernizzi et al., 1992).

Lastly, the regressor corresponding to relative change in 5-HTT binding was of particular interest, as in theory, occupancy of 5-HTT sites should be directly related to the action of SSRIs. In practice, a decrease in tracer binding can be measured, which is the result of the inability of free tracer molecules to bind to 5-HTT sites occupied by citalopram. The time course of relative changes in 5-HTT binding was dependent of the dissociation half-life of [^{11}C]DASB which is approximately 14 min in rat brain homogenates (Lundquist et al., 2005). Therefore, the relative change in 5-HTT binding used as a regressor is expected to lag behind the true occupancy of 5-HTT sites which can be assessed reliably in equilibrium (Gryglewski et al., 2017). Nevertheless, the regressor had the potential to align with the gradual build-up of serotonin concentrations and to capture inter-individual variation in the amplitude and timing of 5-HTT occupancy. However, as linear trends or scanner drifts could be of similar shape as this regressor, its application to capture phMRI responses in BOLD signal was limited.

4.3. Analysis of pharmacological fMRI response using time bins

The drawback common to all pharmacological modeling approaches described above was that a similar timing of phMRI responses was expected across all brain regions. This issue could potentially be overcome by model-free analysis methods. The method applied relied on the comparison of the deviation of discrete time bins acquired after drug challenge from a baseline time bin between placebo and SSRI scans. This classical method was applied widely in pharmacological imaging analysis, regardless of critical limitations of fMRI data: (1) BOLD signal is not an absolute measure and the subtraction of baseline data does not guarantee normalization. (2) Adequate correction for multiple comparisons is necessary to avoid spurious results. Appropriate applications of the method exist, e.g. in the analysis of phMRI responses of absolute cerebral blood flow (CBF) or volume (CBV) derived using arterial spin labeling (ASL) in brain regions selected a priori or with FWE correction, e.g., (Schouw et al., 2013). However, the method has often been applied without adequate correction for multiple comparisons, which may mislead research and hinder development of the field. To illustrate the effects of the lack of adequate correction of family-wise error, we aimed to replicate the original results (McKie et al., 2005) in a larger sample with state-of the art imaging equipment and processing pipelines. We could not find significant effects of SSRIs in any time bin when performing FWE correction. It could be argued, that even more conservative correction should be performed to correct for the number of time bins tested.

4.4. Future directions and concluding remarks

While the methods applied were not successful to detect a phMRI response to citalopram 8 mg, the question remains if

other analysis pipelines or study designs would be more successful. It could be shown that test–retest reliability of CBF assessed with ASL was higher compared to BOLD in certain applications (Holiga et al., 2018). A small study on seven healthy participants assessed the effects of an almost identical challenge with citalopram on CBF and did not find any effects in time bin analysis (Schouw et al., 2012). Despite high reliability of CBF, other authors reported small effects of an oral dose of 16 mg citalopram which were not consistent between two sessions with SSRI challenge (Klomp et al., 2012). Taken together, the current evidence casts some doubt if the detection of acute effects of SSRIs should be sought in absolute changes in resting-state fMRI signal. If such effects should be present, data driven methods relying e.g., on wavelet cluster analysis (Schwarz et al., 2007) may be able to capture them with higher sensitivity by allowing for diverse and regionally variable time courses and better interpretability of results than time bin analysis. Furthermore, outcomes relating to brain network properties were repeatedly shown to be influenced by a single dose of an SSRI (Klaassens et al., 2015; Schaefer et al., 2014; Schrantee et al., 2018). The full exploration of acute SSRI effects on resting-state phMRI is yet to be awaited.

Author disclosures

Role of funding source

The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

G.G. and L. R. designed the study. G.G. wrote the study protocol and the manuscript. G.G, V.T., K.A., H.M., S.L., G.G.M., U.J. and M.P. provided medical and administrative support. K.M., R.L., R.M.B., J.G.M. and H.A. were involved in data processing and analysis. B.N., B.T., P.V. and K.E. performed radio synthesis and analysis of radioactive metabolites under supervision of W.W. and M.M. Medical supervision of the study was carried out by H.M, W.E., H.M. and K.S. The scientific supervisor of the study and principal investigator was L.R. All authors read and approved the contents of the manuscript.

Conflict of interest

With relevance to this work there is no conflict of interest to declare. S. Kasper received grants/research support, consulting fees and/or honoraria within the last three years from Angelini, AOP Orphan Pharmaceuticals AG, AstraZeneca, Celegne GmbH, Eli Lilly, Janssen-Cilag Pharma GmbH, KRKA-Pharma, Lundbeck A/S, Neuraxpharm, Pfizer, Pierre Fabre, Schwabe and Servier. R. Lanzenberger received conference speaker honoraria from Shire within the last three years.

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