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Chapter 10

Test-retest reliability of task-related pharmacological MRI with a single-dose oral citalopram challenge

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Abstract

Non-invasive assessment of human neurotransmitter function is a highly valuable tool in clinical research. Despite the current interest in task-based pharmacological MRI (phMRI) for the assessment of neural correlates of serotonin (5-HT) function, test-retest reliability of this technique has not yet been established. Using a placebo-controlled crossover design, we aimed to examine the repeatability of task-related phMRI with a single dose of oral citalopram in twelve healthy female subjects. Since we were interested in the drug’s effect on neural correlates of 5-HT related cognitive processes, a sensorimotor and an emotional face processing paradigm were used. For both paradigms, we found no significant effects of the oral citalopram challenge on task-positive brain activity with whole-brain analysis. With ROI-based analysis, there was a small effect of the challenge related to emotional processing in the amygdala, but this effect could not be reproduced between sessions. We did however find reproducible effects of the challenge on task-negative BOLD-responses for both paradigms, particularly in the medial frontal cortex and paracingulate gyrus. In conclusion, our data shows that a single oral dose of citalopram does not reliably affect emotional processing and sensorimotor activity, but does influence task-negative processes in the frontal cortex. This latter finding validates previous studies indicating a role for 5-HT in suppression of the task-negative network during goal-directed behavior.
Introduction

The measurement of serotonin (5-HT) function in the human brain is an important tool to improve our understanding of the underlying neurobiological mechanism of mood-, anxiety- and impulse control disorders (Deakin, 1991; Meltzer, 1990; Taylor, 1990). For this reason, there is currently much interest in the application of imaging techniques to assess neurotransmitter function. With pharmacological magnetic resonance imaging (phMRI), 5-HT function can be modulated pharmacologically with selective 5-HT reuptake inhibitors (SSRIs) while brain functional MR images (fMRI) are simultaneously collected. In this way, the neural activity related to the 5-HT response can be visualized (for a recent review on this topic, please refer to Anderson et al. (2008)). In comparison with other imaging techniques, such as positron emission tomography (PET) (Aznavour et al., 2006) and single photon emission computed tomography (SPECT) (Hwang et al., 2007), fMRI is pre-eminently suitable for measuring (changes in) neural activity and offers both excellent spatial as well as good temporal resolution. It is also less invasive due to the lack of involved radiation exposure. This allows for use in longitudinal studies to follow disease progression and/or treatment efficacy.

In order for a research tool to be readily used for multiple assessments within the same patient, its repeatability (test-retest reliability) should be established. Until now, this is lacking for most phMRI applications. Recently, our group has published one of the first repeatability studies for 5-HT phMRI using an arterial spin labelling (ASL)-based technique in the same subjects as in this current study in which we concluded that the test-retest reliability of pulsed ASL phMRI with an oral citalopram challenge is low (Klomp et al., 2012a). While with techniques such as ASL the direct effects of the challenge on cerebral blood flow can be measured, it may be of even greater interest to assess the effects of the drug on cognitive processes. 5-HT is known to play a role in a broad range of cognitive processes, especially in emotional processing, behavioral inhibition, reward behavior, memory, and sensorimotor function. Functional MRI studies have shown previously that SSRIs can influence these processes in both healthy controls and patients (Anderson et al., 2008). Although most of these 5-HT phMRI studies looked at the effects of an intravenous drug challenge or at the effects of chronic drug pre-treatment, there are also studies looking at the effect of a single oral dosage of the challenging drug, which is less invasive for the participants and more practical in use, especially in case of repeated measurements. However, inter-individual differences in drug response and the long Tmax (time to maximal plasma concentrations) oral drugs typically have, give rise to additional sources of variation (e.g. getting subjects in and out of the scanner), making the detection of significant drug-effects more difficult.
Still, several studies have shown that administration of a single oral dosage of an SSRI can affect 5-HT related neural processes in the human brain (Loubinoux et al., 1999; Murphy et al., 2009; Takahashi et al., 2005; Vollm et al., 2006). Nevertheless, the repeatability of this technique has never been assessed. Also, most studies did not account for within-day variations in the MR signal, did not include a placebo-condition, and are in some cases not corrected for multiple comparisons. There is thus a clear need to assess the reliability of this specific MR method.

To this end, the objective of this study was to examine the test-retest reliability of task-related phMRI with a single dose of an oral SSRI (citalopram), in order to assess the effects of altered cerebral 5-HT function on neural correlates of 5-HT related cognitive processes. Since emotional processing is known to be strongly regulated by 5-HT and also to be affected in mood disorder (Surguladze et al., 2004), we chose an emotional face processing paradigm. This is one of the most used paradigms in task-related 5-HT phMRI studies. fMRI studies have consistently shown that emotional stimuli tend to primarily activate the amygdala, especially in case of emotional face processing (Anderson et al., 2008; Fusar-Poli et al., 2009). In both healthy volunteers and MDD patients, acute SSRI treatment was found to reduce amygdala responses to fearful facial expressions (Anderson et al., 2007; Anderson et al., 2011a; Del-Ben et al., 2005; Murphy et al., 2009). We also assessed 5-HT modulation of sensorimotor activity, as it has been shown that 5-HT can improve motor function in both animals (Geyer, 1996; Jacobs and Fornal, 1999) and humans (Hindmarch, 1995). Furthermore, sensorimotor task paradigms are known for their robustness, good repeatability and ease-of-use (Havel et al., 2006; Ramsey et al., 1996; Yetkin et al., 1996), and are consequently very suitable paradigms for test-retest reliability studies.

We hypothesized that, presuming the method to be reliable, a single oral dosage of the SSRI citalopram would affect the amygdala response to emotionally laden facial stimuli, when compared to the baseline and the placebo scan sessions in both citalopram sessions. In accordance with Murphy et al. (2009) and Takahashi et al. (2005) and with findings after an intravenous or chronic SSRI challenge (Anderson et al., 2007; Anderson et al., 2011a; Del-Ben et al., 2005; Harmer et al., 2006; Windischberger et al., 2010), we expect this effect to be a decrease, although increased amygdala activation has been described as well (Bigos et al., 2008; Murphy et al., 2009). For the sensorimotor task, based upon previous studies (Loubinoux et al., 1999; Loubinoux et al., 2002), we expected a repeatable enhancing effect of citalopram on the controlateral sensorimotor cortex (S1M1).
Methods

Participants

Twelve healthy right-handed female volunteers were recruited through advertisement. Participants and in- and exclusion criteria have been described previously (Klomp et al., 2012a). In short, the inclusion criteria were: female gender, right-handed, between 20 and 30 years of age, and currently taking oral contraceptives. Exclusion criteria included any serious general medical condition or one that could interfere with the interpretation of results, contraindications to MR imaging, current or past known brain disease, psychiatric or neurological disorders (according to the Mini-International Neuropsychiatric Interview (M.I.N.I.) Plus (van Vliet and de Beurs, 2007)), any current medication that could affect 5-HT function, habitual drug use and excessive consumption of alcohol (>21 U/week), caffeine (>8 cups of coffee/day), or nicotine (>10 cigarettes/day). All subjects were instructed to withhold consumption of substances that could directly influence CBF (e.g. caffeine, alcohol, nicotine; (Mathew and Wilson, 1991)) before each scan session and between scans. Also, subjects were asked to refrain from any form of drug use during the entire study. Subjects were all on oral contraception during the study and were not scanned at menstruation in order to assure stable gonadal hormone levels at all scans, as fluctuations in gonadal hormones across the menstrual cycle are known to influence amygdala reactivity (Mareckova et al., 2012; Ossewaarde et al., 2010). The study was approved by a local Research Ethics Committee and written informed consent was obtained from each participant.

Experimental design

In this study, a balanced within-subject cross-over design was used. Participants visited the research centre on three occasions, with an interval of two weeks to assure complete washout of the drug challenge before the next session (Seifritz et al., 1996). On each occasion, subjects received a series of baseline scans, directly followed by an oral drug challenge. On two occasions, this challenge consisted of citalopram (16 mg citalopram solution (equivalent to one 20 mg tablet; Lundbeck, Amsterdam) dissolved in lemonade) and on one occasion a placebo was given (lemonade only). The order in which the different challenges were given was counterbalanced. Half of the subjects were randomly allocated to receive citalopram on the first and third research day and the other half received citalopram on the second and third research day. Subjects were blind to the type of challenge received. Exactly 2h after the oral challenge (= Tmax of oral citalopram solution; http://www.medicines.org.uk/EMC/medicine/6153/SPC), a second series of scans was performed, which were identical to the first. For a detailed overview of the experimental design, we refer to (Klomp et al., 2012a). This
way, it was possible to both assess effect of a placebo challenge as well as the reproducibility of the citalopram effect. Each of the six scan sessions took about 45 minutes and consisted of an emotional processing task, a sensorimotor task, a behavioral inhibition task (data not shown; full data set was available for only 6 subjects due to technical difficulties and poor task performance) and a resting-state ASL scan (data described elsewhere (Klomp et al., 2012a)). Also, during one of the sessions, a structural MRI scan was made for registration and segmentation purposes. Following the last MRI scan session, subjects performed an emotional face recognition task outside of the scanner to confirm their ability to correctly recognize the emotional expression of unfamiliar faces.

**fMRI paradigms**

All tasks were programmed using ePrime programming software (v2.0, Psychology Software Tools, Inc., www.pstnet.com). Task images were displayed onto a rear-projection screen placed in front of the MRI bed and were viewed via a mirror attached to the MRI head coil. Before start of the first MRI scan session, subjects shortly practiced each task outside the scanner behind a computer screen to verify that each subject understood the tasks and was able to perform them correctly. In case of the sensorimotor task, it was verified that participants complied with the auditory pacing.

**Sensorimotor activity**  A finger tapping task was used to elicit sensorimotor activity. The paced finger tapping task followed a block design and consisted of ten 25-s epochs alternating between rest (R) and activation (A), with a total task length of ± 4 min. Subjects were asked to sequentially touch the thumb with each of the four digits of their right hand during the ‘activation’ period, which was presented on the screen. During activation periods, participants heard a 120 Hz auditory cue, which they had to use for pacing. During the ‘rest’ condition, subjects were asked to relax and not to move at all.

**Emotional processing**  To assess emotional processing, a covert emotional face processing task was used. Subjects were presented with pictures of faces adapted from the NimStim Face Stimulus Set (MacArthur Foundation Research Network on Early Experience and Brain Development, http://www.macbrain.org). The faces included different genders and ethnicities and were presented in black and white on a black background, with hair and clothing removed (See Figure 1 for examples). Included face models of the NimStim set were 08, 10, 11, 14, 28, 34, 39, and 40. The following types of affect were presented: happy (H), fear (F) and neutral (N). The emotionally loaded pictures (H and F) were presented at two different intensities; either very (100%) happy or fearful (H/F100) or slightly (50%) happy or
fearful (H/F50) (Perlman et al., 2012). As control condition (C), pictures of distorted faces were used. Care was taken to ensure that the colors, size, shape and contrast of the control stimuli were equal those of the experimental stimuli. For each of these six conditions (N, H50, H100, F50, F100, and C), four blocks of four stimuli were presented, making a total of 24 blocks. Stimulus presentation time was 3.5 s, with an interstimulus interval of 0.5 s and a 4.0 s fixation between blocks. Thus, each block lasted 20 s, with total task duration of approximately 8 min. Volunteers were instructed to identify the gender of the faces (“press left button for women and right button for men”) or indicate in which direction the arrow was pointing in case of the control stimuli. In order to overcome learning effects, there were four different versions of this task, which were randomly assigned over the six different scan sessions per subject. The different versions consisted of the exact same stimuli, only the order in which the stimuli and the different conditions were presented was changed. The practice session that was conducted 30 minutes prior to the first series of MRI scans consisted of three blocks of four stimuli (N, H, F, and C), presented in random order and was the same for all participants.

*Emotional face recognition task*

At the end of the study and outside of the scanner, participants were shown similar faces as during the emotional processing task (NimStim Face Stimulus Set), displaying the same types of affect (happy (H), fear (F) and neutral (N)). Subjects were asked to identify which of these three types of affect the face was expressing. Again, the emotionally loaded pictures (H and F) were presented at two different intensities. Stimuli were presented for either a long time (2000 ms) or a short time (100 ms). In total, there were 80 stimuli to assess (8 pictures per affect (H50, H100, F50, F100 and N) and per presentation time). Hits, misses and omissions were recorded.

*Imaging data acquisition*

All MR imaging was conducted on a 3.0 Tesla Philips MR scanner with an SENSE 8-channel head coil and body coil transmission (Philips Intera; Philips Medical Systems, Best, The Netherlands). Imaging parameters of the task-related functional MRI scans were: TR/TE 2300/30 ms; FOV 220×220×120 mm²; matrix size 96×95; 40 contiguous transversal slices; ascending acquisition, thickness 3 mm; gradient echo single shot echo-planar EPI pulse sequence. Number of dynamics for the emotional processing task was 215 with a total scanning time of 8:23 min, and 100 dynamics with a total scanning time of 4:22 min for the sensorimotor task. For the structural scan imaging parameters were as follows:
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high-resolution 3D T1-weighted anatomical image; TR, 9.8 ms; TE, 4.6 ms; 120 contiguous transversal slices.

Figure 1. Examples of stimuli of the covert emotional face processing paradigm. Stimuli used in this task paradigm comprised of faces expressing either happiness (A) or fear (B) or a neutral face expression. Baseline stimuli consisted of a distorted face with a black arrow on top (C), pointing either to the left or to the right.

Data analysis

Imaging data was analyzed using FEAT (FMRI Expert Analysis Tool) v5.90, part of FSL v4.1.6. (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl). Images were skull stripped, corrected for motion artifacts, spatially smoothed with a full width half maximum Gaussian kernel of 5 mm and registered to the Talairach and Tournoux stereotactic space (‘standard space’, Talairach and Tournoux, 1988) using Montreal Neurological Institute (MNI) templates to facilitate intersubject averaging. Imaging data were high-pass filtered with a cutoff of 50 s in the emotion processing task and 25 s in the sensorimotor task. Subsequently, first-level analysis was performed on each scan using a task-specific general linear model (GLM) to model task-related blood oxygenation level-dependent (BOLD) signal changes. For the emotional processing task, each facial expression (N, H, F) was contrasted to either the control condition (C) or the neutral face expression (N). Also, all face expression together (N+H+F) were contrasted to the control (C) and all emotional expressions (H+F) to the neutral expression (N), resulting in seven first-level contrasts. For the sensorimotor task, BOLD signal change was modeled for ‘tapping’ vs. ‘rest’ only. For every contrast, both the positive as well as the negative contrast was modeled (representing respectively task-related activation and deactivation).
Voxel-based higher-level analysis  First-level contrasts were entered into higher level mixed-effects models within FEAT. Mean task-related brain activity was assessed by taking the average activation over all sessions per subject and subsequently averaging these subject-averages to a group mean. The within-day and between-days variability were determined with either a paired t-test (placebo vs. its baseline) or a repeated measures ANOVA analysis (all three baseline sessions). To determine the effects of citalopram on task-related activity, a 2-by-2 ANOVA with time (pre- & post-challenge) and drug (citalopram & placebo) as factors was performed. Only in case of significant time-by-drug interaction effects, paired t-tests were performed as post-hoc analyses to determine which scan sessions were driving the observed effects. For the emotion processing task, the different emotion conditions were only retained in the case of significant drug-by-emotion interactions, which were evaluated using factorial ANOVAs. All resulting statistical parametric maps were thresholded at a Z-value > 1.8 with a cluster-based FWE correction at $p < 0.05$.

ROI-based higher level analysis  For each first-level contrast of interest, the average percentage signal change in a predefined region of interest (ROI) was extracted. For the emotional processing task, this was the bilateral amygdala. For the sensorimotor task, this was the left motor area, consisting of the left primary motor cortex, premotor cortex and primary somatosensory cortex. All masks were made based upon Juelich Histological atlas templates (incorporated within FSL v4.1.6). Effects of citalopram within the ROIs were determined by a 2-by-2 ANOVA with time (pre- & post-challenge) and drug (citalopram & placebo) as factors, analogous to the voxel-based analysis. In analogy to previous studies in which no placebo session was used, post-hoc analyses (paired t-tests) were exploratively performed regardless the significance of time-by-drug interaction effects. ROI-based analyses were performed in SPSS (IBM SPSS Statistics v19.0).

Results

Subjects

Citalopram administration was well tolerated by all subjects (mean age 22.9 ± 3.0 years) and no clinical side effects were reported during or after the experiment. The average time between the baseline scan session and the second scan session, i.e. time between challenge intake and the subsequent scan session, was 2 h and 3 min ± 4.2 min. The average time between the assessment days was 15 ± 2.5 days. One subject had to be excluded from
analysis, since no complete set of six scans could be obtained for this participant. In total eleven subjects were included in the analyses.

**Sensorimotor activity**

For the finger tapping task, highly robust task-related brain activity was found in the left primary somatosensory cortex, premotor cortex, visual cortex (V5), superior temporal gyrus (auditory cortex), and putamen, the bilateral thalamus, parietal cortices and cerebellum (Figure 2). Task-related deactivation was found in the left temporal cortex (temporal pole, temporal occipital fusiform cortex, and inferior temporal gyrus), in the precuneus, postcentral gyrus, cuneus and in the frontal region (superior frontal gyrus, paracingulate gyrus, anterior cingulate gyrus, inferior frontal gyrus, and frontal pole). No significant differences were found between the three baseline scan sessions. Analysis of variance revealed a significant time-by-drug interaction and subsequent post-hoc analyses revealed that these were predominantly driven by significant differences between the citalopram and placebo scans. There was a significant enhancing effect of the citalopram challenge (i.e. increased activity) in the anterior cingulate gyrus (ACC), paracingular gyrus (PCC) and frontal medial cortex (mPFC), and these findings were comparable between both citalopram sessions (Figure 3). These areas largely overlap with the earlier mentioned task-deactivated areas. ROI-based analysis showed no effect of citalopram compared to placebo (time-by-drug effect) on the left motor area during both citalopram sessions ($F(1,40) = 0.178$, $p = 0.675$, effect size ($\eta^2$) = 0.004 and $F(1,40) = 0.016$, $p = 0.900$, $\eta^2 < 0.001$, for Cit1 and Cit2 respectively).

![Figure 2. Statistical parametric map of task-related (de)activation of the sensorimotor paradigm. Task-related activity is shown in red, deactivation is shown in blue. Maps are thresholded at $Z > 1.8$ with a cluster-based multiple comparison correction (FWE; $p = 0.05$).](image)
Figure 3. Statistical parametric map of the citalopram effect related to the sensorimotor paradigm. Positive (i.e. enhancing) effect of the first citalopram challenge (Cit1) is shown in red, positive effects of the second citalopram challenge (Cit2) are shown in green. Maps are thresholded at $Z > 1.8$ with a cluster-based correction for multiple comparison (FWE; $p = 0.05$).

Figure 4. Statistical parametric map of task-related (de)activation of the emotional processing paradigms. A) Results of the ‘face stimuli vs. baseline stimuli’ contrast, and B) results of the ‘emotion vs. neutral faces’ contrast. Task-related activity is shown in red, deactivation is shown in blue. Maps are thresholded at $Z > 1.8$ with a cluster-based multiple comparison correction (FWE; $p = 0.05$).
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Figure 5. Statistical parametric map of the citalopram effect related to the emotional processing paradigm. Effect of the first citalopram challenge (Cit1) on emotional processing (‘faces vs. baseline stimuli’ contrast) is shown in red, effect of the second citalopram challenge (Cit2) is shown in green. Maps are thresholded at $Z > 1.8$ with a cluster-based multiple comparison correction (FWE; $p = 0.05$).

Emotional processing

Analysis of variance showed no main effect of emotion or an interaction effect of emotion with drug or time in the covert emotional face processing task. Therefore, no distinction was made between the different types of emotions displayed, and only the ‘faces vs. baseline’ and the ‘emotion vs. neutral’ contrasts were used for further analysis. For the ‘faces vs. baseline’ contrast, task-related brain activity was found mainly in the bilateral amygdala, and fusiform cortex (fusiform face area (FFA)) and further in the superior frontal gyrus, left inferior frontal gyrus, right frontal medial cortex and frontal orbital cortex, right primary somatosensory cortex, right premotor cortex, right superior temporal gyrus and the right precuneus (Figure 4a). Task-related activation was much less pronounced in case of the ‘emotion vs. neutral’ contrast, with only significant clusters of activation in the (bilateral) amygdala, left FFA, superior frontal gyrus / frontal pole, and bilateral inferior frontal gyrus (Figure 4b). Task-related deactivation was found in the bilateral lateral occipital cortex, precuneus, temporal occipital fusiform cortex, occipital fusiform gyrus, middle temporal gyrus, paracingulate gyrus, frontal pole, superior frontal gyrus, and inferior frontal gyrus for the ‘faces vs. baseline’ contrast (Figure 4a). Except for the frontal regions, regions of deactivation were again similar but less profound for the ‘emotion vs. neutral’ contrast (Figure 4b). For both contrasts, there were small but significant differences between the three baseline scans. Post-hoc analyses revealed that these differences depended mainly on differences between the second and third baseline scan. Effects of the citalopram challenge compared to the placebo challenge were only seen within the ‘faces vs. baseline’ contrast. The ANOVA analysis revealed small
but significant time-by-drug interaction effects during both citalopram sessions. However, post-hoc analyses showed that during the first citalopram session, the drug caused decreased activity in parts of the occipital and parietal lobe (precuneus, primary visual cortex, lingual cortex), while during the second citalopram session increased activity in the frontal cortex (frontal pole, frontal medial cortex, paracingulate gyrus) was seen (Figure 5). In both cases the drug-induced changes were located outside the task-activated areas, but did partially overlap with the task-deactivated areas.

ROI-based analyses showed no significant effect of citalopram compared to placebo (time-by-drug effect) in the amygdala for both contrasts and during both citalopram sessions (‘faces vs. baseline’: $F(1,40) = 0.031, p = 0.862, \eta^2 = 0.001$ and $F(1,40) = 2.794, p = 0.102, \eta^2 = 0.065$, for Cit1 and Cit2 respectively; ‘emotion vs. neutral’: $F(1,40) = 1.040, p = 0.314, \eta^2 = 0.025$ and $F(1,40) = 0.732, p = 0.397, \eta^2 = 0.018$, for Cit1 and Cit2 respectively). However, when directly comparing the citalopram scan and its preceding baseline scan (thus without taking the placebo session into account), there was a significant positive effect of citalopram in the amygdala for the ‘faces vs. baseline’ contrast, but only during the second citalopram session ($t(10) = -2.761, p = 0.020$).

**Emotional face recognition**

Subjects performed near ceiling on the emotional face recognition task that was applied at the end of the study. Subjects made very few incorrect responses or omission errors (on average, 87.6% of the targets were assessed correctly). Most of the errors made concerned a slightly happy or fearful face (H/F50) being mistaken for a neutral face (N). Almost all mistakes were made in the ‘short presentation time’ condition in which the stimuli were only shown for 100ms. All subjects were thus considered to be able to correctly recognize the shown emotions of unfamiliar faces.

**Discussion**

We aimed at verifying the test-retest reliability of BOLD-based phMRI with a single-dose oral SSRI challenge to assess neural correlates of 5-HT related cognitive processes. For this purpose, we chose fMRI paradigms for emotional face processing and sensorimotor function.

**Main task effects**

In accordance with previous studies (Loubinoux et al., 1999; Loubinoux et al., 2002), the sensorimotor task mainly activated the contralateral primary somatosensory cortex (S1M1)
and premotor cortex, bilateral thalamus, parietal cortices and cerebellum (See also Figure 2). Task-related deactivation was seen in the (pre)frontal regions, left medial temporal lobe and medial superior parietal lobe (precuneus cortex). For the emotional processing task, activation was mainly found in the bilateral amygdala, fusiform face area and (pre)frontal regions (Figure 4). The faces vs. baseline contrast resulted in larger and more robust patterns of activation than the emotional vs. neutral faces contrast. These results are in concordance with previous literature (Fusar-Poli et al., 2009). The patterns of task-related deactivation were similar for both contrasts and also similar to the areas of deactivation seen in the sensorimotor task. As expected, the sensorimotor task gave the most robust and reliable signal of the two task paradigms. For this task, there were no significant differences in activation pattern between the three baseline scans, each made two weeks apart. For the emotion processing task however, there were small but significant differences in activation pattern between the three baseline scans, showing more variable and thus less robust task activations with this particular paradigm.

Effects of citalopram on task-related (de)activation

For both paradigms, whole brain voxel-based analysis no significant effect of the oral citalopram challenge on task-related activation. In the emotional face processing paradigm, we found a positive effect of citalopram in the amygdala for the ‘face vs. baseline’ contrast during the second citalopram session only, when directly comparing the citalopram scan and its corresponding baseline scan. Although this is in concordance with findings of increased human amygdala reactivity to emotionally laden facial stimuli after intravenous administration (Bigos et al., 2008), this citalopram-related amygdala activation could not be reproduced between sessions and was no longer statistically significant when taking within-day variation (determined by the placebo session) into account. ROI-based analysis showed no robust effect of citalopram in the contralateral motor area in the sensorimotor paradigm.

However, for the sensorimotor paradigm, there was a significant and reproducible decrease of task-related deactivation in the mPFC and PCC after citalopram intake (Figure 3). With respect to the emotion processing paradigm, some effects of citalopram in task-deactivated areas were present as well (‘faces vs. baseline’ contrast only). However, the specific areas in which these effects were seen did not overlap between sessions: during the first citalopram session, a citalopram-induced decrease of activity was seen in the occipital and parietal regions (precuneus), whereas during the second citalopram session increased activity was observed in the frontal regions (mPFC and PCC) (Figure 5).

These results are only partially in agreement with our hypotheses and with previous work. While we had no prior hypotheses about the effects of the citalopram challenge on
task-deactivation processes, we had expected an effect of oral citalopram on the contralateral sensorimotor cortex (S1M1) for the sensorimotor task and on amygdala activation related to emotional faces, in accordance with previous studies using an oral challenge (Loubinoux et al., 1999; Loubinoux et al., 2002; Murphy et al., 2009; Takahashi et al., 2005). We were however not able to replicate these earlier findings and thus the repeatability of the effect of the oral challenge on sensorimotor activity and emotional processing could not be assessed.

Several possible explanations could underlie this negative finding. First, in the studies of Loubinoux et al. (1999; 2002) on sensorimotor activity, two different types of SSRI were used (fluoxetine and paroxetine), against citalopram in our case. Also, a much older subject population was assessed with a mean age of ± 50 years, against ± 23 years in our population. Both studies used both male and female subjects while we only included females. These methodological differences could have accounted for the fact that the results of Loubinoux et al. were not replicated. Also Murphy et al. (2009) used a different study design; a between-subjects design with one citalopram-treated group vs. a placebo-treated group. The design of Takahashi et al. (2005) was more in concordance with our design, but they used unpleasant vs. neutral pictures as stimuli instead of emotionally laden faces. Also, both studies did not include a non-challenged (baseline) scan prior to the challenge scan in order to account for within-day variation. The strength of the design chosen in the current study is that it enabled us to take variations in task-activation over time into account, in addition to possible placebo effects, and that we are able to assess the test-retest reliability of this technique, which no other study has done before. The importance of such a design is illustrated by the fact that we did find an effect of citalopram on amygdala activation during one of the citalopram sessions with a ROI-based approach, which was no longer statistically significant when taking within-day variation and which could not be replicated over sessions. Thus, although earlier studies have shown effects of citalopram on emotion processing in the amygdala and on motor activity in the S1M1, we were not able to replicate these findings with a single-dose oral challenge, most likely due to methodological differences between the studies.

**Effects of citalopram on task-related deactivation**

In both paradigms, we observed a significant positive effect of the oral citalopram challenge in brain areas that were related to task-deactivation processes (mPFC, PCC and precuneus). These brain areas are known to be part of the so-called default mode network (DMN), also referred to as the task-negative network (Fox et al., 2005). This network is not only known to have high neuronal resting-state activity, but also to deactivate during goal-directed behavior (Buckner et al., 2008; Cavanna and Trimble, 2006). As is clear from previous studies, 5-HT
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plays a role in these processes, although the precise pathway(s) remain unclear (Greicius et al., 2007; Grimm et al., 2009; Kunisato et al., 2011). In our study, task-negative BOLD-responses diminished after the citalopram challenge. In case of the sensorimotor paradigm, this effect was reproducible over time and was mainly seen in the medial frontal region (mPFC and PCC). For the emotion processing task, an effect on task-deactivation was seen as well, but the effects were smaller and they were located in different task-negative brain areas over sessions (precuneus vs. medial frontal cortex). A possible explanation could be the difference in control conditions between the two paradigms. In the sensorimotor task, the control condition was ‘rest’, while in the emotional processing paradigm the control condition consisted of indicating the direction of an arrow. Although most probably still less cognitively demanding than indicating the gender of a face, this can still be considered goal-directed behavior. Thus, it seems reasonable that the deactivation of DMN brain regions during goal-directed behavior (and thus also the effect of an SSRI hereupon) was more difficult to detect in the emotional face processing paradigm. Reduced negative BOLD-response during emotion processing has been described in MDD patients (Anand et al., 2005; Grimm et al., 2009). Our findings are moreover in agreement with a recent meta-analysis from Delaveau et al. (2011), which concluded that antidepressants restore normal deactivation of the DMN during externally-oriented tasks in MDD patients. Future research investigating the role of 5-HT and the effects of SSRIs on the DMN is required and would be of great interest considering our findings.

Strengths and limitations

One of the main strengths of this study is its experimental design. By adding a baseline scan before each challenge scan and by using a placebo condition, we are able to take into account within-day and between-day variations in BOLD response related to the tasks and to assess possible placebo-effects. Order effects were ruled out by counterbalancing of the placebo and citalopram challenge and our within-subject design enables us to rule out most of the subjective differences in drug response. Last, but not least, by giving the oral citalopram challenge twice, we could evaluate the reproducibility of this specific technique. No previous study, using either an oral or intravenous challenge, has taken all these aspects into account. If phMRI is to become a reliable research tool readily usable in clinical practice, these methodological aspects need to be taken into account more routinely.

First onset of 5-HT-related disorders such as mood- and anxiety disorders usually occurs during (early) adolescence (Kessler et al., 2005; Merikangas et al., 2010). It has therefore not only been suggested that interventions aimed at prevention or early treatment need to focus more on youth, but also that research should lay more emphasis on the developing
brain instead of the adult brain. For this purpose we applied an oral administration, a less invasive research tool that is readily available for children and adolescents. Unfortunately, oral administration results in higher inter-subject variability in drug levels within the brain than for example intravenous administration. It might therefore have valued our study if the fMRI results were correlated to each individual’s plasma concentrations of citalopram on a given time after the challenge. Still, this variation is also taken into account with the placebo-controlled within-subject design that was used in this study. Related to this, another limitation could be that the current study was limited to a relatively small sample size of 12 subjects. Nevertheless, our group size is similar to related work (Loubinoux et al., 1999; Loubinoux et al., 2002; Murphy et al., 2009; Takahashi et al., 2005) and the within-subjects design that was used increases statistical power by reducing the error variance associated with individual differences, thus reducing the need for large sample sizes. Also, the effect sizes of our ROI analyses do not suggest that higher statistical power would have lead to significant and reproducible results in these areas.

Conclusions

Using a within-subject design, phMRI with a single-dose oral challenge of the SSRI citalopram in combination with 5-HT related fMRI paradigms did not result in reproducible effects on neuronal activation related to emotion processing and/or locomotor activity. However, we did find reproducible effects of the oral 5-HT challenge on task-related deactivation, particularly in the medial frontal cortex. The involved areas are known to be part of the task-negative network, which has been previously shown to be influenced by 5-HT as well. Our findings therefore suggest that the here described technique might be used to reliably assess 5-HT function via its effects on task-negative processes. Also, more care should be taken to assess the repeatability of drug effects on cognitive processes and to rule out effects of time and placebo treatment in future oral phMRI studies.

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