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Sustained attention and serotonin: a pharmaco-fMRI study

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Objective Evidence suggests that stimulation of serotonergic function in healthy humans causes an impairment of sustained attention. The present study assessed the influence of increased serotonin levels on brain areas involved in sustained attention.
Methods Ten healthy volunteers (5♀, 5♂) received the selective serotonin reuptake inhibitor (SSRI) escitalopram (20 mg) and placebo in a balanced, double blind, two-way crossover design. Participants performed the Mackworth Clock Test to measure sustained attention during functional MRI measurements at 3 Tesla. Subjective measurements after pharmacological manipulation were conducted with the Bond and Lader Questionnaire.
Results Independent of treatment, brain areas associated with task performance on a sustained attention task were activated, including right prefrontal and parietal areas. After escitalopram administration, less activation was shown in the caudate nucleus, thalamus, and frontal areas. No effect of escitalopram was shown on behavioral data although subjective measurements showed decreased alertness after escitalopram.
Conclusions The results of the current pharmaco-functional magnetic resonance imaging (fMRI) study give a first indication of involvement of serotonin in sustained attention through modulating activation of selective brain areas including the thalamus and caudate nucleus. Possibly, these areas are involved in a subcortical network for sustained attention, but further research is necessary. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — serotonin; sustained attention; pharmaco-fMRI

INTRODUCTION

The neurotransmitter serotonin is involved in different psychiatric disorders, including major depression. The majority of patients diagnosed with a depressive disorder have a malfunction of the serotonergic system (Meltzer, 1989). Depression and also other psychiatric disorders are associated with cognitive dysfunction such as impaired memory performance and attention or planning deficits (Elliott *et al*., 1997; Austin *et al*., 2001; Landro *et al*., 2001). There is evidence that serotonin plays a role in the etiology of depression and is associated with cognitive problems (reviewed by Schmitt *et al*., 2006). Some studies in depressed patients showed that serotonin stimulation by antidepressants such as fluoxetine, fluvoxamine, and trazodone directly improves cognitive functions independent of elevation of depressive symptoms (Doraiswamy *et al*., 2003; Koetsier *et al*., 2002; Riedel *et al*., 1999b). Studies in healthy volunteers have also provided evidence for a possible role of serotonin in cognitive performance. Both low serotonin levels following tryptophan depletion and high serotonin levels following selective serotonin reuptake inhibitor (SSRI) administration have been shown to reduce memory performance (Riedel *et al*., 1999a; Riedel *et al*., 2002; Wingen *et al*., 2006b). It has been suggested that the relation between serotonin and memory reflects an inverted U-curve (Meeter *et al*., 2006) which entails that both overstimulation and understimulation of serotonin levels worsen memory performance. Other cognitive functions such as cognitive flexibility and attention are possibly also modulated by serotonin (Schmitt *et al*., 2006).

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Particularly, sustained attention, often called vigilance, is one aspect of the general construct of attention that is associated with serotonergic functioning (Schmitt *et al*., 2002). Single and repeated doses of SSRIs have been shown to reduce sustained attention in healthy volunteers in a number of studies using the Mackworth Clock Test paradigm (Ramaekers *et al*., 1995; O'Hanlon *et al*., 1998; Schmitt *et al*., 2002; Riedel *et al*., 2005). While performing the Mackworth Clock Test, participants have to sustain their attention for 45 min while monitoring a circular arrangement of sequentially illuminating dots. A button press is required when a rare event occurs (signal detection) (Mackworth, 1950). As time passes, it becomes more difficult to sustain attention and more misses occur. This is called the vigilance decrement (Teichner, 1974).

Antidepressants are suitable means for challenging the serotonergic system while measuring cognitive functions. Moreover, combining (psycho)pharmacological studies with functional magnetic resonance imaging (fMRI) might give more insight into the underlying neuroanatomical substrates of task performance and changes in functioning of these brain areas associated with cognitive performance. Previous pharmaco-fMRI studies for establishing the association between serotonin and cognition have used several antidepressants. For instance, one study used the noradrenergic and specific serotonergic antidepressant (NaSSa) mirtazapine to examine the effects of serotonin on behavioral inhibition in a parallel group design. The results showed a modulatory role for serotonin of brain responses in a Go/No-Go and a Reward/No-Reward task in various brain areas including orbitofrontal cortex and parietal cortex (Vollm *et al*., 2006). Unfortunately, mirtazapine has additional noradrenergic and histaminergic affinity, which can influence the results on the behavior level as well as on the neuroanatomic level. Ideally, a more selective serotonergic antidepressant is required. Another study used the SSRI citalopram in a within-subject fMRI design and demonstrated effects of serotonin on brain areas involved in several neuropsychological tasks, despite little behavioral changes due to a ceiling effect (Del Ben *et al*., 2005). Another previous functional MRI study also demonstrated that a single dose of citalopram changes the serotonergic system (McKie *et al*., 2005). A very recent study demonstrated a role for serotonin in modulating amygdala activation to aversive faces, tested in an fMRI-setting after citalopram administration (Anderson *et al*., 2007). No prior pharmaco-fMRI studies have yet been undertaken to assess the effects of serotonin on sustained attention.

The aim of the present study was to define the brain regions that are involved in performance during the Mackworth Clock Test. In addition, the effects of increased serotonin levels in healthy human volunteers were assessed on sustained attention and on brain areas underlying sustained attention. Serotonin stimulation was obtained by blocking serotonin reuptake through administration of the most selective SSRI, escitalopram. It was expected that acutely increased serotonin levels would impair sustained attention performance measured by the Mackworth Clock Test. In addition, this impaired performance was expected to be reflected in activation changes in the brain areas that underlie sustained attention. Previous studies using sustained attention tasks and fMRI showed involvement of the right parietal and frontal brain areas (Lewin *et al*., 1996; Coull *et al*., 1998; O'Conner *et al*., 2004). It was expected that the same brain areas would be involved while performing the Mackworth Clock Test.

EXPERIMENT AND METHODS

Subjects

Ten healthy volunteers (5♀, 5♂), mean age (s.e.) 26.3 (2.46) were recruited. All participants underwent a screening procedure, which consisted of a telephone interview, health questionnaire, 12-lead electrocardiogram, laboratory testing (hematology and blood chemistry, urinalysis, drug- and pregnancy screening) and a routine medical examination. Volunteers were included when they were 21 to 45 years of age, healthy, had a normal static binocular acuity (corrected or uncorrected), a body mass index between 19 and 30, and were willing to sign an informed consent. Excluded were those volunteers who suffered from, or had a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological, or psychiatric illness. History of psychiatric illness or current psychiatric illness was assessed by the health questionnaire which was approved by a medical doctor. Other exclusion criteria were excessive drinking (>20 glasses of alcohol containing beverages a week), pregnancy or lactation, menstrual disorder, use of medication other than oral contraceptives, smoking tobacco, or use of illicit drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. Those volunteers who had a first-degree relative with a psychiatric disorder or a history of a psychiatric disorder were also excluded. The study was approved by the standing medical ethics committee of Maastricht University and the Maastricht Academic

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Hospital's Board of Directors. It was carried out in accordance with the World Medical Association's *Declaration of Helsinki* (Edinburgh, 2000). Written informed consent was obtained from each volunteer prior to participation to the study.

Design and treatment

The study was conducted according to a double-blind, placebo controlled, two-way crossover design. Complete balancing of the treatments led to two treatment orders that were randomly assigned to the participants. Treatments consisted of escitalopram (20 mg) and placebo administered at two different test days separated by a wash-out period of at least 7 days. The treatments were randomly assigned resulting in a balanced treatment design.

Testing procedure

Participants underwent a short training session of the Mackworth Clock Test on the day of the medical examination in order to minimize learning effects. On the two separate test days, participants arrived at 9.00 a.m. at the laboratory, filled out an informed consent concerning scanning procedures, received a standard breakfast, and completed a sleep quality questionnaire. They received the treatment capsule containing either escitalopram or placebo at 9.30 a.m. Participants were then seated for the next few hours in a secluded waiting room in order to wait for escitalopram to reach the maximum concentration in blood (C_{max}). At noon participants received a standard light lunch. Mood assessments were conducted at 13.00 p.m. followed by an anatomical scan. Scanning and testing took place at 13.30 p.m., that is, 4 h after drug intake, till 14.15 p.m. Both test days were identical in design. Participants were not allowed to consume alcohol 24 h prior to testing and caffeine-containing beverages 4 h prior to the start of the test day.

Subjective measurements

Subjects filled out the Groninger Sleep Quality Scale (Mulder-Hajonides van der Meulen, 1981) on each test day to assess sleep quality during the preceding night. The total score consisted of 14 yes/no questions to score the number of sleep complaints (ranging from good sleep (score 0) to worst possible sleep (score 14)). In addition, specific questions on time needed to fall asleep, number of awakenings during the night and sleep duration in hours were included. The Bond and Lader Questionnaire was also assessed using different scales for alertness, contentedness and calmness (Bond and Lader, 1974).

FMRI data acquisition

A 3T Siemens Allegra MR scanner, situated at the Faculty of Psychology (University Maastricht, The Netherlands) was used for the anatomical and functional measurements. A T1-weighted anatomical scan was acquired for each subject using a 3-D Modified Driven Equilibrium Fourier Transform (MDEFT) sequence with an isotropic spatial resolution of 1 mm. During task performance 896 whole-brain volumes of $T2^*$-weighted functional measurements were acquired, each comprising 32 slices (slice thickness 3.5 mm; no slice gap; flip angle 90°) using a blood oxygenation level dependent (BOLD) measurement and echo planar image (EPI) pulse sequence (TR: 2 s; TE: 30 ms; resolution: $3.5 \times 3.5 \times 3.5\,mm^3$, matrix size 64×64) and interleaved slice sampling. Stimulus presentation and scanning were synchronized at the beginning of the task.

Sustained attention—Mackworth Clock Test

Participants were scanned on two occasions (placebo condition and escitalopram condition). FMRI scans were obtained while subjects performed the Mackworth Clock Test for 30 min. Participants laid supine in the scanner with the head fixated with foam pads. Headphone and earplugs were provided. Participants looked at the projection of a computer screen displaying a circular arrangement of 60 gray dots, via a mirror in the head volume coil. The dots were briefly illuminated in clockwise rotation at a rate of one per 500 ms, moving 6° from dot to dot. Volunteers were instructed that occasionally the dot would move 12°. This ''skipping of one of the dots'' was the signal participants had to detect throughout the task by pressing a response button as fast as possible with the right index finger. A response within 3 s of the signal was registered as a correct detection. A total of 20 signals were randomly presented with 10 per 15 min period. Behavioral measures were total correct detections (hits) and corresponding reaction times. For objectives of a different study a resting condition (fixation) was included prior to as well as proceeding to the task block. These additional time points were modeled as covariates in the current study.

Statistical analyses

Behavioral data were analyzed by means of a repeated measures general linear model (GLM) analysis (SPSS

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11.5) with Treatment (2 levels) and Time on task (2 levels) as the main factors. The alpha criterion significance level was set at $p = 0.05$.

Preprocessing of the functional images was done using BrainVoyager QX version 1.6 (www.brainvoyager.com), and contained slice scan correction, 3-D motion correction (trilinear interpolation) and linear trend removal and high pass temporal filtering (cut-off = 5 cycles per time course), no spatial smooting. Individual anatomical datasets were spatially normalized to a standardized 3-D space (Talairach and Tournoux, 1988). Individual scans were realigned using the first scan as a reference and coregistered and normalized to the anatomical data, and resampled to a voxel size of $3 \times 3 \times 3\,mm^3$.

Analysis of the brain activations was performed using a whole-brain, fixed effects ANCOVA that included 12 predictors based on the task design. A protocol specific for each subject and each condition entailed the predictors. These predictors included correct detections, misses, false alarms, task block, time before correct detections, fixation (resting condition), and 6 motion correction preprocessing parameters. The predictor correct response (corresponding to two time scans or volumes) was used as a main measure for sustained attention as previous studies showed an effect of increased serotonin levels on this variable (Ramaekers *et al*., 1995; O'Hanlon *et al*., 1998; Schmitt *et al*., 2002; Riedel *et al*., 2005). The implication was that as a participant responds to the event (signal detection), it is also sustaining attention. A maximum of 20 correct detections per subject per condition were included in the analysis.

Results were color-coded and superimposed onto a standardized template of a single brain. Main task effects of placebo were visualized using a p (bonf) < 0.01 corrected, and a cluster threshold of >100 voxels. Activation corresponding to the main measure (correct responses) is relative to baseline activity meaning all other (mean) activity. The differential effect of escitalopram was analyzed using whole-brain analyses within the placebo-escitalopram contrast on the correct responses of the task using a q (FDR) < 0.001 (Genovese *et al*., 2002) (which corresponded to $p < 0.000008$, uncorrected) and a cluster threshold of >50 voxels. Activation within the contrast is the activation corresponding to the main measure after escitalopram administration relative to the activation corresponding to the main measure after placebo administration. Subsequent event-related averaging on the significant voxels of the ROI of the cluster time courses, corresponding to the correct response trials, was performed. Events were selected through the protocols which were based on the subjects' performance and an average was calculated per condition (escitalopram or placebo).

RESULTS

Missing data

In total, 12 participants were included of which two ended their participation due to claustrophobic reactions in the fMRI scanner. Ten participants completed the study, there were no missing data. Escitalopram was well tolerated in all subjects.

Behavioral data

Means and standard errors (s.e.) of the sustained attention task performance are shown in Table 1.

Treatment did not significantly affect correct detections or reaction time ($F_{1,9} = 0.91$, $p = 0.366$). There was a trend for a Time on task effect for correct detections ($F_{1,9} = 3.41$, $p = 0.098$) and a significant Time on task effect for reaction time ($F_{1,9} = 9.61$, $p = 0.013$), which indicates an overall tendency for a vigilance decrement during the 30 min performance. There was no Treatment by Time on task interaction effect.

Subjective measurements

Mean (s.e.) of subjective evaluations are shown in Table 1. The outcome measures of the Groninger Sleep Quality Scale were not different for the two treatments. Treatment did significantly affect the alertness ratings ($F_{1,9} = 21.5$, $p = 0.001$) but not the contentedness or calmness ratings. Escitalopram reduced alertness as compared to placebo.

Table 1. Mean (s.e.) outcome variables of the Mackworth Clock Test and the Bond and Lader Questionnaire

Treatment $N = 10$	Escitalopram	Placebo
Mackworth Clock Test		
Total correct responses	15.8 (1.25)	15.0 (1.69)
Total reaction time (ms)	629 (32.4)	662 (38.3)
Correct responses 0–15 min	8.4 (0.50)	7.9 (0.92)
Reaction time 0–15 min (ms)	589 (25.4)	635 (36.5)
Correct responses 15–30 min	7.4 (0.82)	7.1 (0.91)
Reaction time 15–30 min	664 (43.2)	680 (42.3)
Bond and Lader Questionnaire		
Alertness	67.0 (6.37)[a]	81.4 (3.96)
Contentedness	79.0 (4.41)	83.4 (4.05)
Calmness	79.3 (4.28)	80.7 (5.21)

[a] $p = 0.001$.

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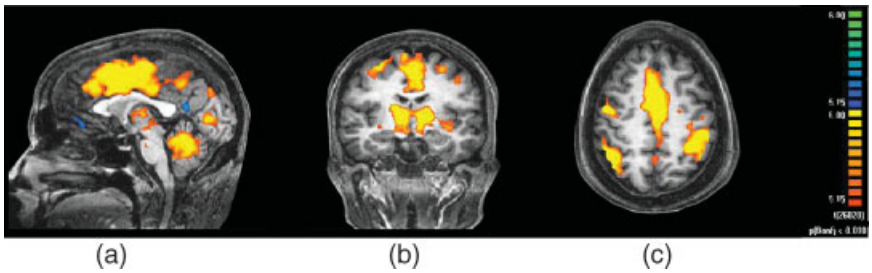

Figure 1. Main task effect of the Mackworth Clock Test (correct detections) in the placebo condition, increased and decreased activations are both presented at a p (bonf) < 0.01 and a cluster threshold of >100 voxels. (a) Sagittal plane; (b) coronal plane; (c) transaxial plane

Functional MRI during task performance

Figure 1 shows significantly activated brain areas in the placebo condition during the Mackworth Clock Test: activation corresponding to the correct responses relative to baseline (all other) task performance. The main effect of task showed increased activation of motor areas including the left post central gyrus, the right precentral gyrus, the middle cingulate gyrus, and the supplemental motor area. In addition there was increased activation in brain areas associated with an attentional network including the right inferior parietal gyrus, the right angular gyrus, the insula, the thalamus, and the caudate nucleus. Other areas which showed increased activation were the middle frontal gyrus, the right middle temporal gyrus, the parietal-occipital fissure, the precuneus, and the vermis. Decreased activation was seen in the posterior and anterior cingulate gyrus.

Figure 2 shows significantly activated brain areas after a contrast between escitalopram and placebo two conditions with corresponding event-related averaging plots. Comparing activation corresponding to the correct responses in the escitalopram condition to activation corresponding to the correct responses in the placebo condition resulted in decreased activation of the left supplementary motor area, parts of the thalamus, the left caudate nucleus, the left precentral sulcus, the right middle frontal gyrus, the left inferior frontal gyrus, and the left superior frontal gyrus. In addition, after escitalopram administration there was increased activation in the right superior temporal

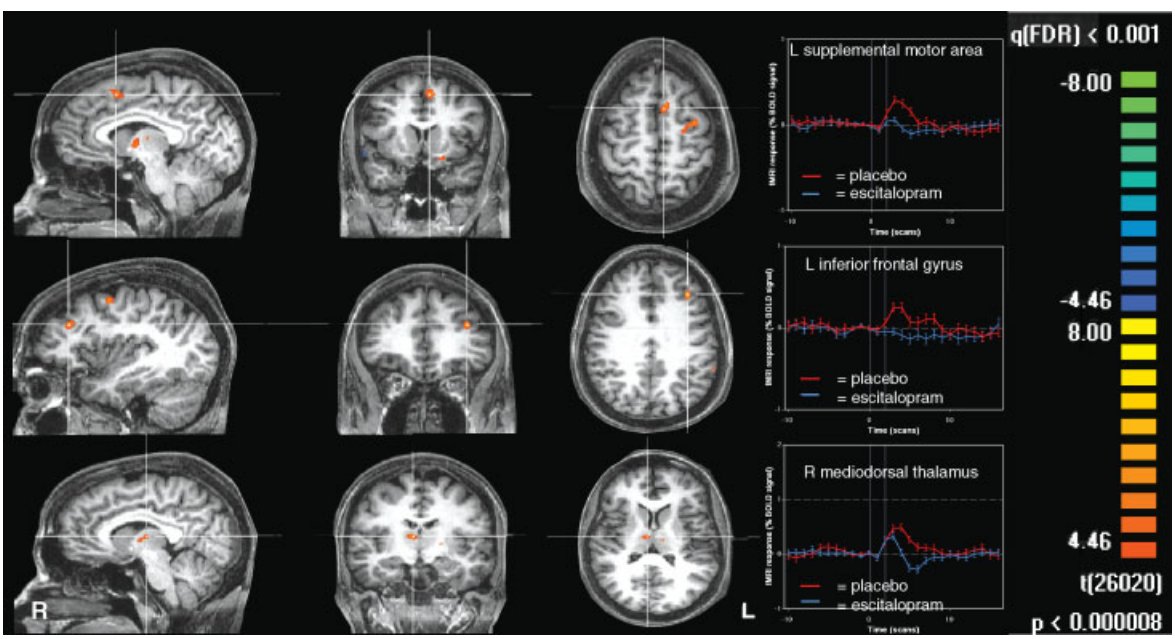

Figure 2. Decreased activation after the placebo-escitalopram contrast at q (FDR) < 0.001 and a cluster threshold of >50 voxels

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Table 2. Areas in which voxels were activated corresponding to correct detections of the Mackworth Clock Test

		Main effect (placebo) Talairach coordinates						Different activation after escitalopram administration Talairach coordinates				
Region	Left/ Right	*X*	*Y*	*Z*	Number of voxels	Mean *t*-value	Left/ Right	*X*	*Y*	*Z*	Number of voxels	Mean *t*-value
Postcentral gyrus/motor cortex[a]	L	−30	−30	50	945	7.725						
Middle cingulate gyrus[a]	L/R	1	3	36	1331	10.248						
Supplementary motor area[ad]	L/R	2	12	51	1321	9.043	L	−6	11	48	333	−4.972
Inferior parietal gyrus[a]	R	40	−47	42	1045	8.771						
Precentral gyrus[a]	R	41	−6	41	963	7.494						
	R	46	3	24	1274	7.946						
Angular gyrus[a]	R	49	−43	31	1211	9.330						
Middle temporal gyrus[a]	R	53	−43	9	1160	8.336						
Insula[a]	L/R	35	18	7	1331	11.139						
Thalamus[a]	L/R	9	−16	10	1070	8.015						
Ventral nucleus left[d]							L	−8	−18	9	54	−4.649
Mediodorsal nucleus left[d]							L	−5	−7	9	209	−4.991
Mediodorsal nucleus right[d]							R	7	−13	11	176	−4.847
Anterior nucleus[d]							L/R	0	−5	7	372	−5.021
Caudate nucleus[ad]	L/R	9	5	9	1133	7.247	L	−13	4	14	262	−4.796
Middle frontal gyrus[a]	L/R	37	37	24	835	6.064						
Parietal-occipital fissure[a]	L/R	10	−70	28	735	6.349						
Precuneus[a]	L/R	1	−53	35	645	6.234						
Vermis[a]	L/R	2	−53	−19	1317	8.565						
Posterior cingulate gyrus[b]	L/R	−3	−54	17	652	−6.055						
Anterior cingulate gyrus[b]	L/R	−3	31	−7	157	−5.455						
Precentral sulcus[d]							L	−24	−8	51	299	−4.975
Middle frontal gyrus[d]							R	32	16	43	79	−4.824
Inferior frontal gyrus							L	−34	31	28	183	−5.072
Superior frontal gyrus[d]							L	−27	53	15	67	−4.731
Superior temporal gyrus[c]							R	53	4	4	76	4.763
Lateral fissure[c]							R	61	−19	15	77	4.856

[a]Increased activation.
[b]Decreased activation.
[c]Significant increase of activation compared to placebo.
[d]Significant decrease of activation compared to placebo.

gyrus and the right lateral fissure. The event-related plots of the specific brain areas in the contrast between the two conditions showed less or no response (BOLD signal) after escitalopram administration. In Table 2, significantly activated brain areas during Mackworth Clock Test performance are presented in the placebo condition and after a contrast between the two conditions (placebo-escitalopram) including mean *t*-value and number of voxels. Figure 3 shows the event-related averaging plot of the primary motor cortex, which shows no difference between the two conditions.

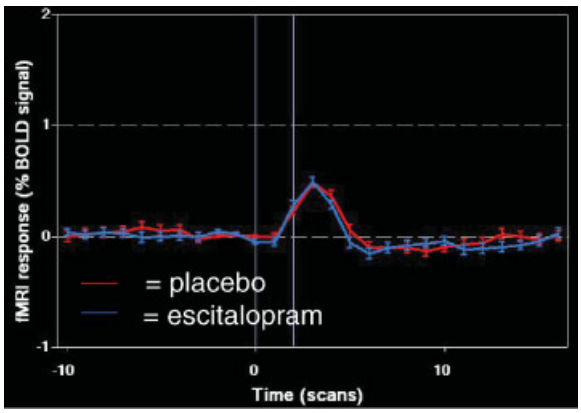

Figure 3. Event-related plot in the primary motor cortex which shows no difference between escitalopram and placebo administration

DISCUSSION

The present study examined the effects of increased serotonin levels on sustained attention and underlying

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brain activation. Similar to previous studies that measured sustained attention (Lewin *et al*., 1996; Coull *et al*., 1998; O'Conner *et al*., 2004) the current study showed activation of right dorsal and prefrontal areas. The brain areas of the present study involved in sustained attention show some remarkable overlap with brain areas associated with visual attention (Corbetta *et al*., 2002). In addition, escitalopram and consequently the neurotransmitter serotonin modulated activity of several brain areas including the thalamus and prefrontal areas during a sustained attention task, although no effect was found on behavioral measurements of sustained attention.

Performance of the Mackworth Clock Test in the placebo condition showed increased activation was found in the right inferior parietal gyrus, the right angular gyrus, the right precentral gyrus, the insula bilateral, the thalamus bilateral, and the bilateral middle frontal gyrus. These areas and particularly right parietal and prefrontal areas have been associated with sustained attention in previous research with fMRI (Cabeza and Nyberg, 2000; Sturm and Willmes, 2001) and Positron emission tomography (PET) (Mottaghy *et al*., 2006). Using the rapid visual information (RIVP) task to assess networks of sustained attention, Lawrence *et al*. (2003) demonstrated a network comprising frontal and parietal cortical areas and the thalamus and caudate nucleus, which is very comparable to the set of active brain areas found in the present study (Lawrence *et al*., 2003). Coull (1998) suggests that two different networks are involved in attention and arousal and interact with each other: a cortical network including the right frontal and inferior parietal cortex, which is associated with attention and a subcortical network including the thalamus, striatum, and the anterior cingulate, which is associated with arousal (Coull, 1998). Adequate levels of both arousal and attention are necessary to achieve good sustained attention performance (Coull, 1998). Other areas with increased activation in the present study were areas associated with the response by a button press including the left postcentral gyrus/motor cortex and the supplemental motor area. Activation of the parietal-occipital fissure is probably related to the seeing of the signal in the Mackworth Clock Test, as this area has been previously associated with the perception of visual motion (Richer *et al*., 1991).

After escitalopram administration, differences in activation were seen in several brain areas. Decreased activation was seen in parts of the basal ganglia, including the ventral lateral nucleus of the left thalamus, the mediodorsal nucleus of thalamus bilateral, and the left caudate nucleus. In addition, the left superior precentral sulcus, the left superior frontal gyrus, and the right middle frontal gyrus also showed decreased activation after acutely elevated serotonin levels. The event-related plots of these areas also showed lower amplitude of activity and/or a post-event dip in activation for the escitalopram administration condition in the above-mentioned areas. These decreased activations did not appear in the motor cortex, there were no differences between the two conditions in the primary motor cortex and also the event-related plot of the primary motor cortex showed an equal BOLD response after escitalopram and placebo. This suggests that the observed effects may be related to cognitive effects, possibly sustained attention.

Several nuclei of the thalamus including the ventrolateral and mediodorsal nuclei, in which decreased activity is found after escitalopram administration in the present study, are involved in mediating the interaction between attention and arousal in humans (Portas *et al*., 1998). The findings of the present study suggest that increments in serotonin levels may impair sustained attention through influencing the interaction between the cortical and subcortical networks involved in arousal and attention described previously by Coull *et al*. (1998). A candidate mechanism of the influence of serotonin on selective changes in brain activity may be through a serotonin–dopamine interaction mechanism. The basal ganglia (including the thalamus and the caudate nucleus) are often associated with the neurotransmitter dopamine (Garnett *et al*., 1983) and it has previously been proposed that serotonin has a general inhibitory effect on dopamine release (Soubrie, 1986; Spoont, 1992). Particularly serotonin projections from the raphe nucleus have an inhibitory influence over the mesocortical dopamine system and forebrain, which are involved in cognition (Kapur and Remington, 1996). It could be suggested that serotonin may impair sustained attention through inhibiting dopamine in the thalamus and the caudate nucleus. Consequently, a decrease in dopamine may lead to less activation of the mesocortical dopamine projections to the prefrontal cortex (Robbins, 1997). The results from the present study support this statement by showing decreased activity in the thalamus as well as in (pre)frontal areas after serotonin stimulation. In humans and in primates, connections between the mediodorsal nuclei of the thalamus and the prefrontal cortex are well demonstrated (Siwek and Pandya, 1991; Behrens *et al*., 2003) and these connections are involved in top-down modulation of attention (La Berge, 1995).

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The present study did not show an effect of increased serotonin stimulation on behavioral measurements of attention. However, other studies did find an impairing effect of serotonin on sustained attention measured by the Mackworth Clock Test (Ramaekers *et al*., 1995; O'Hanlon *et al*., 1998; Schmitt *et al*., 2002; Riedel *et al*., 2005). The differences between the previous studies and the present study are probably due to a small sample size and a small effect size. In addition, in the present fMRI study, the task only lasted 30 min whereas it lasted 45 min in performance studies. Possibly, the task duration was too short to pick up any significant differences between the treatment conditions, which is supported by our finding of a non-significant trend of a vigilance decrement. Escitalopram did decrease alertness subjectively but this was not reflected in behavioral measures of sustained attention. In addition, the entire procedure of conducting the task in the scanner may have influenced performance in that both treatments affect sustained attention in a comparable way. The number of correct detections after 30 min in the placebo condition in other (not-fMRI) studies is around 17.4 (s.e. $\pm$ 2.0) (Ramaekers *et al*., 1995; Wingen *et al*., 2006a). In the present study the number of correct detections in the placebo condition is certainly lower, 15.0 (s.e. $\pm$ 1.8).

An important issue is the influence of drug administration on general blood flow. In fMRI, activations measured are an indirect reflection of neural activity but a direct reflection of the difference between the magnetic properties of oxygenated and deoxygenated hemoglobin or BOLD response. Theoretically, it is possible that serotonin influenced blood flow or BOLD changes in the present study. However, activations seen in the current study are task dependent and specific. This is in line with a previous study using intravenous administration of the SSRI citalopram that demonstrated that citalopram is a good tool to use in pharmacoMRI studies to manipulate the serotonergic system (McKie *et al*., 2005). Furthermore, the present study showed increased activations as well as decreased activations of BOLD response after increased serotonin levels in the same task manipulation. Activations in opposite direction are not possible in the context of a general effect on blood flow. Finally, SSRIs in general have few cerebrovascular effects (Ramasubbu, 2004) and particularly 20 mg of the parent compound of escitalopram (citalopram) did not alter heart rate in healthy males (Seifritz *et al*., 1996). Therefore, it is unlikely that general effects on the BOLD response caused by escitalopram influenced the results.

Presently, there are some limitations in the analysis of the fMRI data and the study design. Unfortunately, the small sample only permitted a fixed effect analysis of the fMRI data. In a future study with a bigger sample, random effect analysis would be preferable given the within-subject design. Furthermore, the analysis is driven by a model based on the task design which includes several predictors. The Mackworth Clock paradigm was used for the present study to be comparative to previous studies on sustained attention and SSRIs. The predictor ''correct responses'' and the brain activation corresponding to the correct responses relative to baseline task performance were used as a main measure for sustained attention. Perhaps, another model with other predictors may even better reflect a measure of sustained attention. For future studies it would be very interesting to use a data-driven method such as independent component analysis (ICA) (McKeown and Sejnowski, 1998) to indicate different components or functional networks related to sustained attention. The decreased activity demonstrated in the anterior and posterior cingulate cortex in the present study is a remarkable finding to further examine with ICA as these areas seem to be involved in a certain network concerning resting and are shown to decrease when the cognitive demand of a certain task increases (Raichle *et al*., 2001). Furthermore, the use of this method would allow us to better attribute the influence of increased serotonin on sustained attention to a certain neural network.

To conclude, the current study implies that serotonin stimulation may impair sustained attention through modulation of selective brain areas including (pre)frontal areas and parts of the basal ganglia, which are possibly involved in a subcortical network for sustained attention. The results presented have opened doors for future imaging studies on serotonin and sustained attention. More research is needed to draw stronger conclusions.

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