Validation of [^{123}I]β-CIT SPECT to Assess Serotonin Transporters *In Vivo* in Humans: a Double-Blind, Placebo-Controlled, Crossover Study with the Selective Serotonin Reuptake Inhibitor Citalopram

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Disturbances in the serotonin (5-HT) system are associated with various neuropsychiatric disorders. The 5-HT system can be studied *in vivo* by measuring 5-HT transporter (SERT) densities using ^{123}iodine-labeled 2β-carbomethoxy-3β(4-iodophenyl)tropane ([^{123}I]β-CIT) and single photon emission computed tomography (SPECT). Validation of this technique is important because [^{123}I]β-CIT does not bind selectively to SERTs. Some studies have validated this technique *in vivo* in the human brain in SERT-rich areas, but the technique has not been validated yet in SERT-low cortical areas. The aim of this study was to further validate [^{123}I]β-CIT SPECT in assessing SERTs *in vivo* in humans in both SERT-rich and SERT-low areas. A double-blind, placebo-controlled, crossover design was used with the selective 5-HT reuptake inhibitor (SSRI) citalopram. Six male subjects underwent two [^{123}I]β-CIT SPECT sessions: one after pretreatment with citalopram and one after placebo. Scans were acquired 4 h and 22–27 h p.i., and both region-of-interest and voxel-by-voxel analyses were performed. Citalopram reduced [^{123}I]β-CIT binding ratios in SERT-rich midbrain and (hypo)thalamus. Binding ratios were also lower after citalopram in SERT-low cortical areas, but statistical significance was only reached in several cortical areas using voxel-by-voxel analysis. In addition, citalopram increased binding ratios in the DAT-rich striatum and increased absolute uptake in the cerebellum. The results show that [^{123}I]β-CIT SPECT is a valid technique to study SERT binding *in vivo* in human brain in SERT-rich areas. Although we provide some evidence that [^{123}I]β-CIT SPECT may be used to measure SERTs in SERT-low cortical areas, these measurements must be interpreted with caution.
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INTRODUCTION

Nowadays, it is possible to study the serotonergic system in the living human brain by visualizing the serotonin (5-HT) transporter (SERT) at the terminus of the 5-HT axon. The SERT is a plasma membrane and a structural element of the presynaptic membrane of the 5-HT axon. Therefore, it is used as a reliable marker for the 5-HT axon (Scheffel *et al*, 1992). SERT density can be assessed using a radioligand that binds to SERT in combination with positron emission tomography (PET) or single photon emission computed tomography (SPECT).

The serotonergic system modulates many neuropsychological processes, such as mood and memory, through the neurotransmitter 5-HT. Disturbances in the 5-HT system are associated with neuropsychiatric disorders including depression, eating disorders, and Alzheimer's disease (Lucki, 1998; Wenk, 2003). There is also evidence that the popular recreational drug ecstasy (3,4-methelenedioxy-methamphetamine, MDMA) causes damage to the 5-HT axons both in animals (eg Hatzidimitriou *et al*, 1999; Ricaurte *et al*, 2000) and humans (Gerra *et al*, 1998; Kish *et al*, 2000). The use of ecstasy is associated with decreased memory function and increased symptoms of depression (Parrott, 2000; Verbaten, 2003; Thomasius *et al*, 2003).

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SERT is also the primary site of action for selective 5-HT reuptake inhibitors (SSRIs), the most frequently prescribed antidepressants. Therefore, visualization and quantification of SERT is important for studying neuro-psychiatric disorders in which the serotonergic system is involved and for studying occupancy of SERTs by SSRIs *in vivo*.

One of the SPECT ligands that is used for studying SERTs is ^{123}iodine-labeled 2β-carbomethoxy-3β(4-iodophenyl)tropane ([^{123}I]β-CIT), a cocaine derivate that binds with high affinity to SERTs and dopamine transporters (DATs) (Laruelle *et al*, 1993). The use of [$^{123/125}$I]β-CIT in visualizing SERTs and DATs has been validated *in vitro* and *ex vivo* in rodents (Boja *et al*, 1992; Scheffel *et al*, 1992; Lew *et al*, 1996; Reneman *et al*, 2002) and in human brain slices (Staley *et al*, 1994). In addition, *in vivo* displacement studies in monkeys demonstrated that binding in the hypothalamus and midbrain was mainly associated with binding to SERTs, while binding in the striatum was mainly associated with binding to DATs (Laruelle *et al*, 1993). Therefore, it may be possible with [^{123}I]β-CIT SPECT to study both SERT and DAT densities in anatomical separated areas.

In humans, *in vivo* [^{123}I]β-CIT SPECT imaging of the SERT has been used, among others, to study SERT availability in depression and alcoholism and to study the neurotoxic effects of ecstasy (Malison *et al*, 1998; Heinz *et al*, 1998; Reneman *et al*, 2001a, b). However, because [^{123}I]β-CIT does not bind selectively to SERTs, but also to DATs and norepinephrine transporters, there are some difficulties in using [^{123}I]β-CIT SPECT for studying SERT binding *in vivo*. Therefore, it is important to validate in which brain areas [^{123}I]β-CIT can be accurately used to study SERTs. Although several studies used [^{123}I]β-CIT SPECT to assess SERTs *in vivo* in humans, validation of this technique has been limited.

First, a limited number of studies have been performed to validate the use of [^{123}I]β-CIT SPECT for studying SERTs *in vivo* in humans (Pirker *et al*, 1995, 2000) or to study the effects of [^{123}I]β-CIT binding to SERTs and DATs *in vivo* in humans after blocking of SERTs with a SSRI (Pirker *et al*, 1995; Tauscher *et al*, 1999; Kugaya *et al*, 2003). One study validated the use of *in vivo* [^{123}I]β-CIT SPECT in detecting MDMA-induced serotonergic neurotoxicity in SERT-rich brain areas in a monkey (Reneman *et al*, 2002).

Second, previous SSRI displacement studies did not validate the *in vivo* [^{123}I]β-CIT binding to SERTs in cortical regions, probably because the interpretation in these areas is hampered by low SERT and DAT densities (Cortés *et al*, 1988; Bäckström *et al*, 1989; Heinz and Jones, 2000). Since studies in non-human primates showed long-term and possible irreversible damage of serotonergic axons in the cerebral cortex induced by MDMA (Hatzidimitriou *et al*, 1999), assessment of SERT density in the cerebral cortex is especially important for studying the neurotoxic effects of ecstasy. Reductions of SERT densities in cortical brain regions were reported in male (Semple *et al*, 1999) and female (Reneman *et al*, 2001b) ecstasy users. However, others discussed these results because of the presumed limited sensitivity of [^{123}I]β-CIT SPECT imaging to measure SERT density in the cerebral cortex (Heinz and Jones, 2000; Ricaurte and McCann, 2001; Kish, 2002).

Third, previous validation studies have been limited by some methodological weaknesses. Some of these studies were performed in depressed patients, whereas depression may be an important confounder. For example, lower [^{123}I]β-CIT binding ratios were reported in SERT-rich areas in depressed patients treated with SSRIs compared to healthy volunteers (Pirker *et al*, 1995; Tauscher *et al*, 1999), whereas also in untreated depressive patients decreased SERT binding has been described (Malison *et al*, 1998). Only Kugaya and co-workers (2003) studied the *in vivo* alterations of [^{123}I]β-CIT binding during SSRI treatment in both depressive patients and in healthy volunteers. To our knowledge, no validation studies have been performed yet in the same healthy subjects before and after SSRI treatment.

The purpose of the present study was to further validate the technique of [^{123}I]β-CIT SPECT to assess SERTs *in vivo* in humans in both SERT-rich and SERT-low brain areas using the most optimal study design. Therefore, a double-blind, placebo-controlled, crossover design with the most selective SSRI citalopram was used to study the binding of [^{123}I]β-CIT to SERTs and DATs in SERT-rich, DAT-rich and in SERT-low cortical brain regions. As citalopram selectively blocks SERTs, we hypothesized that [^{123}I]β-CIT binding ratios would decrease in both SERT-rich areas and in SERT-low cortical areas after pretreatment with citalopram.

MATERIALS AND METHODS

Subjects

Six healthy male volunteers were included in the present study between November 2002 and May 2003. They were recruited by means of posters at the Faculty of Medicine of the University of Amsterdam, The Netherlands. Exclusion criteria were age below 18 or above 35 years, major mental or physical problems, use of psychopharmaceuticals such as SSRIs, and use of hard drugs such as ecstasy, cocaine, amphetamine, or heroine in the past. All volunteers had to complete the Beck Depression Inventory (BDI; Beck *et al*, 1961), a validated depression questionnaire, before each imaging session to exclude depression. Participant with a BDI $\geqslant$ 10 were excluded. Written informed consent was obtained from all subjects and the study was approved by the local medical ethics committee.

Study Procedure

We used a double-blind, placebo-controlled, crossover design to study the effect of the SSRI citalopram on binding of [^{123}I]β-CIT to SERTs and DATs. Therefore, all six subjects participated in two different sessions with an interval of 4–6 weeks between both sessions. At 3 h before injection with [^{123}I]β-CIT, the subjects were given a capsule with either 20 mg of the most selective SSRI citalopram (Johnson, 1991) or a placebo that had to be taken orally under supervision of the examiner. Both volunteer and examiner were blinded for the content of the capsule. At 3 h after intake of the capsule, [^{123}I]β-CIT was injected. Per session two SPECT scans were acquired, 4 h post-injection (p.i.) as well as 22–27 h p.i., when stable uptake to the SERTs

and DATs is expected to be reached, respectively (Laruelle *et al*, 1994; Pirker *et al*, 2000). After 4–6 weeks, the procedure was repeated and subjects who were given citalopram in the first session got placebo in the second session, and *vice versa*.

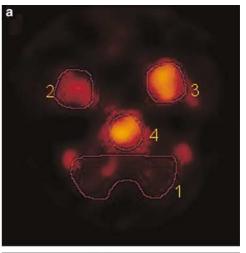## [^{123}I]β-CIT SPECT Imaging

Subjects were examined using SPECT with the radioligand [^{123}I]β-CIT that binds to SERTs, DATs, and, in a lesser extent, also to norepinephrine transporters. Radiosynthesis of [^{123}I]β-CIT was performed by electrophilic radioiodo-destannylation of the trimethyltin precursor (RadioNuclide Center, Vrije Universiteit Amsterdam, The Netherlands), with carrier-free [^{123}I] as NaI (BV Cyclotron, Vrije Universiteit) in the presence of peracetic acid as oxidant. After labeling, the product was further purified with HPLC separation, diluted, and sterile filtrated to an injectable solution with a radioactive concentration of 55–60 MBq/ml. The radiochemical purity of the solution was higher than 99.5%. At both sessions, 112 MBq (3.02 mCi) [^{123}I]β-CIT was injected intravenously as a bolus. Subjects received a potassium iodide solution to block thyroid uptake of free radioactive iodide.
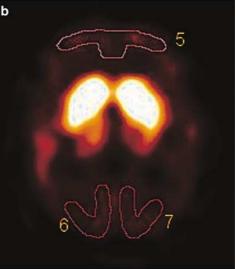
SPECT studies were performed using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum resolution of approximately 6.5 mm, throughout the 20 cm field-of-view (http://www.neurophysics.com). After positioning of the subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps. Each acquisition consisted of approximately 15 slices with 3 min scanning time per slice, acquired in a 64×64 matrix. The energy window was set at 135–190 keV.

Image Reconstruction and Analysis

Attenuation correction of all images was performed as described earlier (Booij *et al*, 1997). Images were reconstructed in 3D mode (http://www.neurophysics.com). For quantification, both region-of-interest (ROI) and voxel-by-voxel analyses were performed.

Standardized templates of 2D ROIs were drawn with the help of a high-resolution MRI and a brain atlas. For the SPECT scans 4 h p.i., ROIs for the midbrain, (hypo)thalamus, frontal cortex, temporal cortex, occipital cortex, and cerebellum were used. For the SPECT scan 22–27 h p.i., ROIs for the striatum and cerebellum were used. The ROIs were positioned on the SPECT slices by the same examiner who was blinded for the content of the pretreatment (Figure 1). For the right and left striatum, a template with irregular ROIs, according to the contour of the putamen and caudate nucleus, was positioned on four consecutive axial slices with highest striatal activity. Individual variation required movement of the fixed ROIs, without changing size and shape, within the template for optimal fitting. For the frontal, right occipital, and left occipital cortices, irregular ROIs were drawn in one template. The template was positioned on four consecutive slices: on the three superior slices where the striatal ROIs were positioned and on one
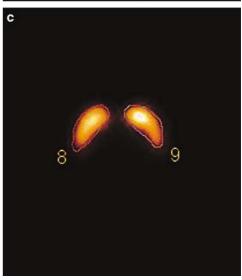

Figure 1 [^{123}I]β-CIT SPECT images with ROI templates of cerebellum (1), right temporal cortex (2), left temporal cortex (3), midbrain (4), frontal cortex (5), right occipital cortex (6), left occipital cortex (7), right striatum (8), and left striatum (9). Images a and b are 4 h p.i. and image c is 24 h p.i. The level of radioactivity is color encoded from low (black) through medium (yellow) to high (white) and scaled to the maximum of the study. To visualize low specific binding to SERTs in images a and b, the upper threshold was set at approximately 25% of the maximum of the study.

slice above. We defined the inferior level of the (hypo)thalamus to correspond with the inferior level of the striatum. For the (hypo)thalamic area, a template with an oval-shaped ROI was placed on four SPECT slices with the highest (hypo)thalamic activity. The superior level of the midbrain was defined to coincide with the most superior slice without visible striatal activity. For this midbrain area, a template with a round-shaped ROI was placed on four SPECT slices with the highest activity of the midbrain. For the cerebellum, a template with an irregular-shaped ROI was positioned on three SPECT slices with highest cerebellar activity. A template with irregular ROIs for right and left temporal cortex was placed on the two most superior slices where the cerebellum template was positioned. Mean striatal, mean temporal, and mean occipital binding densities were averaged from right and left ROIs. Mean cortical binding was calculated as the mean counts per voxel from the frontal, temporal, and occipital cortex together. Absolute uptake in the cerebellum was defined as the mean activity within the ROI of the cerebellum divided by the injected dose (Bq/ml/MBq). Activity in the cerebellum was assumed to represent nondisplaceable activity (nonspecific binding and free radioactivity). Specific to nonspecific binding ratios were calculated as (activity in ROI−activity in cerebellum)/activity in cerebellum.

For the voxel-by-voxel analysis, Statistical Parametric Mapping software (SPM 99, welcome of Cognitive Neurology, Institute of Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm) implemented in matlab (Mathworks, Sherborn, MA, USA) was used (Friston *et al*, 1995). All SPECT images were spatially normalized by a linear coregistration to a standard stereotaxic space (Talairach and Tournoux, 1988). Subsequently, the images were intensity scaled to the corresponding mean cerebellar nonspecific counts per voxel. The mean cerebellar counts were obtained from the ROI analysis.

Statistical Analysis

Differences in binding densities in ROIs between citalopram and placebo pretreatment were analyzed using a two-sided Student's *t*-test for paired samples. The Kolmogorov–Smirnov test was used to analyze whether data were normally distributed. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS 11.5.

Based on the coregistered images, statistical parametric maps of the paired t-statistics SPM{T} were calculated and transformed to the unit normal distribution SPM{Z}. Only clusters of connected voxels above an extent threshold of 20 voxels (voxel threshold was set at $p < 0.01$, Z-score = 2.33, uncorrected for multiple comparisons) were tested for significance by means of spatial extent statistical theory (Worsley *et al*, 1992; Friston *et al*, 1996). Clusters of voxels surviving the thresholds were color coded and superimposed on reference images.

RESULTS

Mean age of the subjects was 24.0 ± 4.3 years (range 18.7–31.2 years). Mean BDI scores were 1.3 ± 2.3 (range 0–6) at placebo session and 1.3 ± 1.9 (range 0–5) at citalopram session. As BDI scores between 0 and 9 are regarded within the normal range, none of the subjects had clinical depression.

ROI Analysis

Data from the ROI analysis were normally distributed. The individual and mean absolute uptake in the cerebellum and the individual and mean uptake ratios within ROIs are described in Table 1. Figure 2 shows a representative sagittal slice of the activity uptake in the midline 4 h p.i. after placebo pretreatment (a) and after citalopram pretreatment (b). Absolute nonspecific cerebellar activity was significantly higher after citalopram pretreatment than after placebo intake 4 h, but not 24 h p.i. Binding ratios of [^{123}I]β-CIT 4 h p.i. were significantly lower after citalopram intake than after placebo intake in the SERT-rich midbrain ($p = 0.009$) and (hypo)thalamus ($p = 0.001$). [^{123}I]β-CIT binding ratios 4 h p.i. were also lower after citalopram intake than after placebo intake in the SERT-low temporal, frontal, and occipital cortical areas, although these findings did not reach statistical significance ($p = 0.055$, 0.088, and 0.340, respectively). The calculated mean cortical binding ratio was also not significantly different between the two sessions ($p = 0.124$). The binding ratio in the DAT-rich striatum 22–27 h p.i. was significantly higher ($p = 0.009$) after citalopram intake than after placebo intake.

Voxel-by-Voxel Analysis

Voxel-by-voxel analysis of the scans made 4 h p.i. showed clusters of significantly lower [^{123}I]β-CIT binding ratios in the SERT-rich midbrain and thalamus ($Z_{max} = 4.45$, $p_{cluster\ level} = 0.000$) after pretreatment with citalopram. Clusters of significantly decreased binding ratios after citalopram pretreatment were also found in several SERT-low (mainly) cortical areas, for example, subcortical insula, left and right middle- and superior temporal gyrus, inferior frontal gyrus, and postcentral gyrus ($Z_{max} = 4.20$, $p_{cluster\ level} = 0.000$), right middle- and superior frontal gyrus ($Z_{max} = 3.74$, $p_{cluster\ level} = 0.047$) and anterior cingulate ($Z_{max} = 4.88$, $p_{cluster\ level} = 0.004$) (Figure 3a). Analysis of the scans 22–27 h p.i. showed a cluster of significantly lower binding ratios in the midbrain/thalamus area ($Z_{max} = 3.53$, $p_{cluster\ level} = 0.000$) after citalopram pretreatment (Figure 3b) and increased binding ratios in the DAT-rich left caudate nucleus ($Z_{max} = 3.32$, $p_{cluster\ level} = 0.000$) (Figure 3c).

DISCUSSION

To our knowledge, this is the first study that validated the use of [^{123}I]β-CIT SPECT to assess SERTs *in vivo* in the human brain using a double-blind and placebo-controlled design in which the same healthy volunteers were examined with and without pretreatment with the most selective SSRI citalopram. The potential confounders age, gender, and depression were excluded because we only included men within a small age range without depression. Although [^{123}I]β-CIT SPECT is currently not used as a diagnostic tool to assess SERT densities, validation of this technique is important because a valid technique would make it possible

Table I Results of the ROI Analysis of the *In Vivo* Binding of [^{123}I]β-CIT in the Midbrain, (Hypo)thalamus, Striatum, and Cortex (Temporal, Frontal, Occipital, and Mean Cortex)

	Cerebellum 4 h p.i.		Cerebellum 24 h p.i.	
	Placebo	Citalopram	Placebo	Citalopram
Absolute uptake (Bq/ml/MBq)[a]				
Subject 1	20.27	24.16	4.10	5.87
Subject 2	17.29	20.82	5.32	3.83
Subject 3	15.11	22.44	3.33	4.39
Subject 4	14.55	19.40	4.92	3.75
Subject 5	18.19	17.36	5.17	3.34
Subject 6	22.79	26.96	5.18	3.83
Mean	18.03±3.13	21.85±3.44[b]	4.67±0.79	4.17±0.90

	Midbrain		(Hypo)thalamus		Striatum (24 h p.i.)	
	Placebo	Citalopram	Placebo	Citalopram	Placebo	Citalopram
Ratios[c]						
Subject 1	0.54	0.27	1.15	0.56	9.53	10.87
Subject 2	0.70	0.47	1.31	0.45	9.23	13.86
Subject 3	0.62	−0.00	1.35	0.34	9.56	11.84
Subject 4	1.08	0.07	1.70	0.64	9.40	12.74
Subject 5	0.72	0.42	1.29	0.87	9.93	12.87
Subject 6	1.05	0.23	1.15	0.44	9.60	10.04
Mean	0.88±0.23	0.24±0.19[b]	1.32±0.20	0.55±0.19[b]	9.54±0.23	12.04±1.41[b]

	Temporal cortex		Frontal cortex		Occipital cortex		Mean cortex	
	Placebo	Citalopram	Placebo	Citalopram	Placebo	Citalopram	Placebo	Citalopram
Ratios[c]								
Subject 1	0.28	0.19	−0.01	−0.05	−0.06	−0.03	−0.04	−0.05
Subject 2	0.29	0.11	0.15	−0.10	0.10	−0.08	0.07	−0.11
Subject 3	0.39	−0.23	0.10	−0.19	0.04	−0.11	0.01	−0.21
Subject 4	0.22	−0.01	0.17	−0.19	0.22	−0.06	0.12	−0.14
Subject 5	0.34	0.40	−0.06	0.10	−0.02	0.15	−0.05	0.10
Subject 6	0.22	−0.08	0.10	−0.17	0.05	−0.10	−0.01	−0.17
Mean	0.29±0.07	0.06±0.22	0.07±0.09	−0.10±0.11	0.05±0.10	−0.04±0.10	0.02±0.06	−0.10±0.11

[^{123}I]β-CIT uptake was measured 4 h p.i., except for striatal uptake which was measured 24 h p.i., and cerebellar uptake which was measured 4 and 24 h p.i.
[a]Absolute uptake (expressed as mean uptake (Bq)/ml within the ROI)/injected dose in MBq.
[b]Uptake significant different from placebo (paired Student's *t*-test).
[c]Uptake ratios expressed as (uptake in the region of interest−uptake in cerebellum)/uptake in the cerebellum.

to study adequately the serotonergic system *in vivo* in several diseases such as mood disorders, Parkinson's disease, eating disorders, alcoholism, and MDMA neurotoxicity. Moreover, [^{123}I]β-CIT SPECT could be used to evaluate the occupancy of SERTs by medication such as SSRIs.

In the present study, we measured (with ROI analyses) [^{123}I]β-CIT uptake ratios for SERT binding 4 h p.i., although kinetic studies showed that in SERT-rich areas binding ratios were closer to a state of transient equilibrium between 20 and 24 h p.i. than 4 h p.i. (increase in binding ratios in the midbrain of 5.5±3.9%/h *vs* 2.5±5.3%/h at 4–10 h p.i. and 20–24 h p.i., respectively) (Pirker *et al*, 2000). As the differences were small and because several studies reported stable uptake in the (hypo)thalamus, midbrain, cerebral cortex, and cerebellum at 4 h p.i. (Brücke *et al*, 1993; Pirker *et al*, 1995), it was suggested that both time points are reliably for measurements of SERTs with [^{123}I]β-CIT (Pirker

et al, 2000). Moreover, as the count statistics are better 4 h than 24 h p.i., leading to better visibility especially of the SERT-low cortical areas, it would be likely that measurements of SERT binding 4 h p.i. have better test–retest reliability than measurements of SERT binding 24 h p.i., although we did not study this.

The most important results of our study are that [^{123}I]β-CIT binding ratios were lower in SERT-rich as well as in SERT-low areas (although only statistically significant with voxel-by-voxel analysis and not with ROI analysis) and higher in the DAT-rich striatum after pretreatment with citalopram compared to placebo. Moreover, we found that citalopram increased the absolute [^{123}I]β-CIT in the SERT-

and DAT-low cerebellum. These results will be discussed in detail in the following paragraphs.

First, in the SERT-rich midbrain and (hypo)thalamus, [^{123}I]β-CIT binding significantly decreased with 72 and
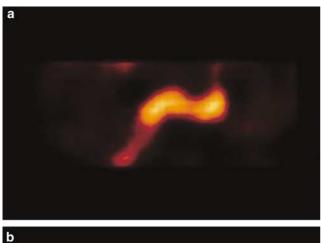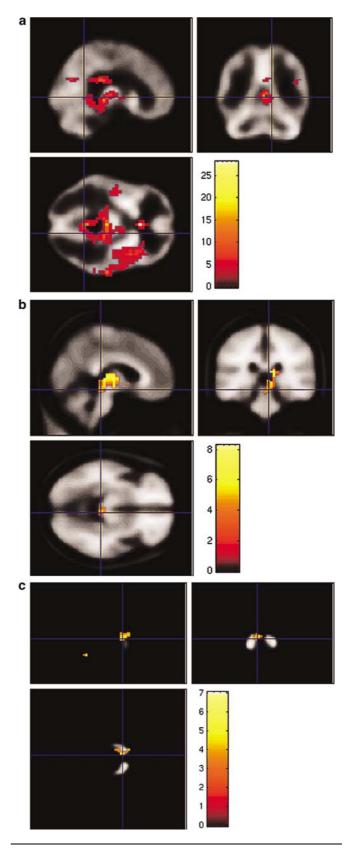

Figure 2 Representative sagittal images of the [^{123}I]β-CIT uptake in the midline 4 h p.i. after placebo pretreatment (a) and after citalopram pretreatment (b). The level of radioactivity is color encoded from low (black) through medium (yellow) to high (white) and scaled to the maximum of the study.

Figure 3 Clusters of significant different binding ratios after voxel-by-voxel analysis with citalopram pretreatment compared to placebo pretreatment, superimposed on reference images. Image a shows 4 h p.i lower binding ratios in subcortical (insula, midbrain, and thalamus) and in several cortical areas (eg middle- and superior temporal gyrus, inferior-, middle-, and superior frontal gyrus, anterior cingulate, and postcentral gyrus). The clusters high on the sagittal slice are related to the upper boundary of the field of view and are likely to represent artifacts rather than real significant differences in [^{123}I]β-CIT binding ratios. Image b shows lower binding ratios in the midbrain/(hypo)thalamus area and image c shows increased binding ratio in the left caudate nucleus 22–27 h p.i.

58%, respectively (ROI analysis), after pretreatment with citalopram. This corroborates previous findings of *in vivo* studies in humans, which showed that citalopram significantly inhibited [^{123}I]β-CIT binding to SERTs in the brainstem and diencephalon (Pirker *et al*, 1995; Kugaya *et al*, 2003). Human PET studies using the SERT-selective radiotracer [^{11}C]DASB also showed high occupancy of SERTs in the midbrain and thalamus after (sub)therapeutic doses of various SSRIs (Meyer *et al*, 2004). In addition, previous studies in animals showed that [^{123}I]β-CIT binds to SERTs in these brain areas (Laruelle *et al*, 1993). Decreased binding of [^{123}I]β-CIT to SERTs after pretreatment with citalopram could be caused by decreased availability of SERTs due to blockage of SERTs by citalopram, by downregulation of the SERT protein, by a change in affinity of [^{123}I]β-CIT for the SERT, or by a combination of these factors. Benmansour *et al* (2002) showed that marked loss of SERT binding sites due to downregulation occurred in rats only after 10–15 days of SSRI treatment. Therefore, we think it is not likely that SERT downregulation in our subjects occurred within one day after only one dose of citalopram. However, downregulation of SERT proteins may play a role in subjects who have taken SSRI treatment for a longer period in other studies. Although we cannot exclude that SSRIs induce fast changes in the affinity of [^{123}I]β-CIT for SERTs, the presently observed decreased binding of [^{123}I]β-CIT in SERT-rich brain areas after citalopram pretreatment is most likely explained by acute blockage of SERT binding sites.

Second, besides a decrease in [^{123}I]β-CIT binding in SERT-rich areas, we also found a decrease in [^{123}I]β-CIT binding ratios in SERT-low cortical areas after pretreatment with citalopram. Voxel-by-voxel analysis showed clusters of significant decreases within several cortical areas. Using ROI analysis, the decreases in cortical binding did not reach the level of statistical significance, possibly because of low statistical power. Reduction of [^{123}I]β-CIT binding after SSRI pretreatment has also been shown in the prefrontal cortex of rats (Scheffel *et al*, 1992). Moreover, autoradiographic studies of human brain sections showed that citalopram, but not the selective norepinephrine reuptake blocker desipramine or the selective DAT blocker GBR12909, was capable to displace cortical binding of the PET ligand [^{11}C]β-CIT (Farde *et al*, 1994). In addition, significant reductions of [^{123}I]β-CIT binding ratios were observed in various cortical areas of rats after pretreatment with MDMA, which is considered to be a selective neurotoxin for serotonergic neurons (Reneman *et al*, 2002). In line with this, [^{123}I]β-CIT SPECT studies in human ecstasy users showed reductions of SERTs in cortical brain regions (Semple *et al*, 1999; Reneman *et al*, 2001a, b). These findings suggest that [^{123}I]β-CIT binding in the cerebral cortex is predominantly caused by binding to SERTs. However, other studies were not able to show that [^{123}I]β-CIT binds to cortical SERTs (Laruelle *et al*, 1993) or did not study this issue (Pirker *et al*, 1995; Kugaya *et al*, 2003). In addition, a SSRI displacement study with the SERT-selective PET radioligand [^{11}C]McN 5652 in non-human primates also failed to show specific binding in the cerebral cortex (Parsey *et al*, 2000). Finally, studies showed in rats (Scheffel *et al*, 1992) and in post-mortem human brains (Günther *et al*, 1997) that cortical [^{123}I]β-CIT binding was mainly blocked by citalopram, and to a lesser extent also by selective DAT and norepinephrine reuptake inhibitors. Therefore, using the radiotracer [^{123}I]β-CIT, it will be very difficult to distinguish between SERT and DAT binding in the cortical areas and this would only be possible when selective blockers for DAT and norepinephrine transporter are used simultaneously. As expected, we found much lower [^{123}I]β-CIT binding ratios in the cortex than in the midbrain and (hypo)thalamus, because the SERT concentration is much lower in the cerebral cortex than in the midbrain or diencephalon (Cortés *et al*, 1988; Bäckström *et al*, 1989). These low binding densities and corresponding low signal-to-noise ratios cause poor visualization and delineation by which placement of ROI templates in the cerebral cortex is hampered. Owing to these arguments, we think that measurement of SERT in the cortical areas using [^{123}I]β-CIT must be interpreted with caution, although SPM data provide evidence that [^{123}I]β-CIT most likely binds to SERTs in specific SERT-low brain regions such as the temporal and frontal cortex. More selective SERT ligands for SPECT imaging, such as the recently developed [^{123}I]ADAM with a 1000-fold greater selectivity to SERTs than to norepinephrine transporters or DATs (Oya *et al*, 2000), will probably be more sensitive to the detection of decreased SERT availability in the cortical areas.

Third, in contrast to a decreased binding to SERTs, we observed an increased binding of [^{123}I]β-CIT in the DAT-rich striatum 22–27 h p.i. after pretreatment with citalopram compared to placebo. From other studies, it is known that SERTs are highly expressed in human lungs and that SSRIs accumulate in here (Suhara *et al*, 1998). Additionally, human biodistribution studies with [^{123}I]β-CIT showed intense binding in the lungs, liver, and lower large intestine (Seibyl *et al*, 1994). It is therefore likely that peripheral blockage of SERTs by citalopram has resulted in increased tracer availability in the brain. However, kinetic studies showed that the specific binding of [^{123}I]β-CIT to DATs in human brain is stable 20–30 h p.i. and the kinetic model predicts that both specific and nonspecific binding would increase in the same proportion (Laruelle *et al*, 1994). Therefore, it is not expected that a change in radiotracer availability would influence the striatal binding ratio. On the other hand, the finding of increased striatal activity after the administration of SSRIs was also observed in several other studies in animals (Scheffel *et al*, 1994; Szabo *et al*, 2002) and in humans (Tauscher *et al*, 1999; Kugaya *et al*, 2003). Kugaya *et al* (2003) suggested that the increase might be caused by interaction between 5-HT and dopamine systems. Scheffel *et al* (1994) suggested that pretreatment with SSRIs would cause displacement of the radiotracer from peripheral and central SERTs, and that this would lead to an increased availability of radiotracer for binding to DATs. In accordance with Scheffel *et al* (1994), we hypothesize that the combination of increased tracer availability (due to peripheral SERT blockage) and decreased SERT availability (due to central SERT blockage) in the brain might have led to increased availability of the tracer for binding to DATs, consequently leading to the observed increased uptake ratio in the DAT-rich striatum when compared to the DAT-devoid reference area of the cerebellum. Although the underlying mechanism is

debatable, the finding deserves attention in future studies. This is, for example, of importance because [^{123}I]β-CIT SPECT studies are performed frequently to measure striatal DATs in parkinsonian patients, and such patients quite often use SSRIs.

Fourth, we observed an increase of absolute cerebellar binding after pretreatment with citalopram, 4 h p.i. This increased binding was probably also caused by increased availability of radiotracer in the brain. Szabo *et al* (2002) reported a strong increase of brain uptake of the selective SERT tracers [^{11}C]DASB and [^{11}C]McN5652 in baboons treated with the SSRI paroxetine. The authors suggested that this finding was caused by a combination of a decreased peripheral binding to SERTs and a paroxetine-induced reduction of the metabolism of both radiotracers. In our study, we did not measure the influence of citalopram on the metabolism of [^{123}I]β-CIT. Although in literature there are no indications that SSRIs influence the metabolism of [^{123}I]β-CIT, we cannot exclude this as a cause of enhanced brain uptake. We believe it is more likely, however, that blockage of peripheral SERTs by citalopram has led to an increased absolute brain uptake (including increased nonspecific cerebellar uptake) of [^{123}I]β-CIT, although Farde *et al* (1994) did not observe a change in cerebellar activity of the PET ligand [^{11}C]β-CIT after the administration of citalopram. This discrepancy emphasizes the need for more studies on the influence of SSRIs on metabolism and on binding of SERT radiotracers to central as well as peripheral SERTs. It is not likely that binding ratios have been affected by the increased cerebellar uptake, because the binding ratios are considered to be stable 4 h p.i. (Brücke *et al*, 1993; Pirker *et al*, 1995, 2000). Therefore, increased tracer availability would increase both specific and nonspecific (cerebellar) binding in the same proportion. This means that the presently observed lower binding ratios in SERT-rich and cortical areas after citalopram presumably reflect blocking of specific binding to SERTs.

In contrast to the above-mentioned increased absolute cerebellar uptake of SERT radiotracers after pretreatment with SSRIs, pretreatment with the selective 5-HT neurotoxin MDMA significantly decreased cerebellar uptake of activity in the same study of Szabo *et al* (2002) and in a study with rats using the radioligand [^{123}I]β-CIT (De Win *et al*, 2004). Since the cerebellum is not devoid of SERTs (Cortés *et al*, 1988; Bäckström *et al*, 1989), this might be explained by an MDMA-induced decreased availability of SERTs in the cerebellum. Both SSRI and MDMA pretreatment could be used to evaluate the specificity of radiotracer binding to SERTs. However, the contrasting findings on cerebellar (nonspecific) binding emphasize that results from different studies should be interpreted with caution, because differences in binding may not solely reflect blockage of binding from specific central binding sites. In case of measuring MDMA-induced neurotoxicity, it has been advocated to use specific to nonspecific ratios, with the cerebellar binding as an adequate estimate of nonspecific binding. As specific binding ratios are calculated by the formula ((activity in ROI−activity in cerebellum)/activity in cerebellum), MDMA-induced loss of cerebellar SERT binding might lead to an overestimation of SERT density and thus an underestimation of the presumably neurotoxic

effects of MDMA. Therefore, it has been suggested that the specific to nonspecific SERT binding ratios may not be optimal (Szabo *et al*, 2002).

Finally, although the present study was performed using a crossover study design in which subjects were their own controls, the relatively small group of subjects may have resulted in a limited statistical power, especially with regard to the [^{123}I]β-CIT binding in SERT-low cortical areas with its limited signal-to-noise ratios. Moreover, due to the low specific to nonspecific binding ratios in the cortical areas, one may expect that the test reliability is lower in these areas than in areas with high SERT densities. However, because of the radiation exposure, we could not perform test–retest measurements to determine test reliability. The accuracy of this study would probably have been improved by coregistration of the SPECT images with MRI. Also, we did not check levels of citalopram in blood samples, although we supervised the intake of the citalopram or placebo capsule.

In conclusion, our results provide evidence that [^{123}I]β-CIT SPECT is a valid technique to study SERTs *in vivo* in humans in SERT-rich brain areas, as binding ratios significantly decreased by pretreatment with the selective SSRI citalopram. In addition, we show that [^{123}I]β-CIT also seems to bind to SERTs in SERT-low brain regions, such as the temporal and frontal cortex. Owing to the very low specific binding in these areas, statistical significant changes in specific binding ratios due to citalopram were only detectable with a voxel-by-voxel analysis, and, therefore, *in vivo* measurements with [^{123}I]β-CIT SPECT in cortical areas must be interpreted with caution. In addition, pretreatment with citalopram caused increased binding ratios in the DAT-rich striatum and increased absolute uptake in the cerebellum. More studies, especially in humans, are needed to evaluate the accuracy of the use of cerebellar binding to estimate nonspecific binding. Finally, in near future, this study needs to be repeated with SPECT radiotracers, which are more selective for SERTs than [^{123}I]β-CIT, to investigate whether the selective radiotracers are more sensitive to assess SERTs in, especially, SERT-low brain areas.

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