

# Reduced Serotonin-1A Receptor Binding in Social Anxiety Disorder

Rupert R. Lanzenberger, Markus Mitterhauser, Christoph Spindelegger, Wolfgang Wadsak, Nikolas Klein, Leonhard-Key Mien, Alexander Holik, Trawat Attarbaschi, Nilufar Mossaheb, Julia Sacher, Thomas Geiss-Granadia, Kurt Kletter, Siegfried Kasper, and Johannes Tauscher

**Background:** Results from studies in serotonin-1A (5-HT<sub>1A</sub>) knockout mice and previous positron emission tomography (PET) studies in humans imply a role for 5-HT<sub>1A</sub> receptors in normal state anxiety as well as in certain anxiety disorders. The objective of this study was to investigate 5-HT<sub>1A</sub> receptor binding potential (BP) in social anxiety disorder (SAD).

**Methods:** Using PET and [carbonyl-<sup>11</sup>C]WAY-100635, we compared a homogeneous group of 12 unmedicated, male SAD patients with 18 healthy control subjects (HC). A multivariate ANOVA with all regional BP values as dependent variables, age and four radiochemical variables as covariates was performed.

**Results:** We found a significantly lower 5-HT<sub>1A</sub> BP in several limbic and paralimbic areas but not in the hippocampus ( $p = .234$ ) of SAD patients. The difference in 5-HT<sub>1A</sub> binding was most significant in the amygdala ( $-21.4\%$ ;  $p = .003$ ). There was also a more than 20% lower 5-HT<sub>1A</sub> BP of SAD patients in the anterior cingulate cortex ( $p = .004$ ), insula ( $p = .003$ ), and dorsal raphe nuclei ( $p = .030$ ).

**Conclusions:** The lower 5-HT<sub>1A</sub> binding in the amygdala and mesiofrontal areas of SAD patients is consistent with 1) preclinical findings of elevated anxiety in 5-HT<sub>1A</sub> knockout mice, 2) a previous PET study in healthy volunteers showing an inverse correlation between 5-HT<sub>1A</sub> BP and state anxiety, and 3) another human PET study in patients with panic disorder showing reduced 5-HT<sub>1A</sub> binding, thus corroborating the potential validity of 5-HT<sub>1A</sub> receptors as targets in the treatment of human anxiety disorders.

**Key Words:** Affective disorders, anxiety, PET, serotonin, social phobia, STAI, 5-HT<sub>1A</sub>

The neurotransmitter serotonin (5-HT) is involved in the regulation of mood and affective states. Drugs modulating the serotonergic system are widely used in the treatment of anxiety disorders and depression, and agonists of the serotonin-1A (5-HT<sub>1A</sub>) receptor have modest anxiolytic properties (Blier and de Montigny 1999). Recent studies in humans (Neumeister *et al.* 2004; Tauscher *et al.* 2001a), nonhuman primates (Bethea *et al.* 2005), and rodents (Gross and Hen 2004; Gross *et al.* 2002) suggest a central role for the 5-HT<sub>1A</sub> receptor in the modulation of anxiety and fear. Neumeister *et al.* (2004) showed a significant reduction of 5-HT<sub>1A</sub> receptor binding in the cingulate cortex and midbrain raphe nuclei in patients with panic disorder, indicating both lower presynaptic and postsynaptic 5-HT<sub>1A</sub> receptor densities. The same research group reported no significant change in patients with posttraumatic stress disorder compared with healthy subjects (Bonne *et al.* 2005). Sullivan *et al.* (2005) reported an association between lower 5-HT<sub>1A</sub> binding potential (BP) in patients suffering from major depression and comorbid panic disorder. In patients with chronic fatigue syndrome and elevated Spielberger State-Trait Anxiety Inventory (STAI) scores, a widespread reduction in 5-HT<sub>1A</sub> receptor binding has been observed (Bailer *et al.* 2005).

From the Departments of General Psychiatry (RRL, CS, NK, AH, TA, NM, JS, TG-G, SK, JT) and Nuclear Medicine (MM, WW, L-KM, KK), Medical University of Vienna, Vienna, Austria; the Department of Pharmaceutical Technology and Biopharmaceutics (MM, L-KM), University of Vienna, Vienna, Austria; and Eli Lilly and Co. (JT), Lilly Research Laboratories, Indianapolis, Indiana.

Address reprint requests to Rupert Lanzenberger, M.D., Department of General Psychiatry, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria; E-mail: rupert.lanzenberger@meduniwien.ac.at. Received March 29, 2006; revised May 27, 2006; accepted May 31, 2006.

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In a previous study, we demonstrated a negative correlation between 5-HT<sub>1A</sub> receptor BP and trait anxiety in healthy subjects (Tauscher *et al.* 2001a). This is in accordance with studies in 5-HT<sub>1A</sub> receptor knockout mice demonstrating increased anxiety-like behavior in several tests (Gross *et al.* 2000; Parks *et al.* 1998; Ramboz *et al.* 1998). To differentiate between state and trait effects of 5-HT<sub>1A</sub> receptor expression on the establishment of adult anxiety-like behavior, Gross *et al.* (2002) and Gross and Hen (2004) developed a transgenic mice model using time-dependent and tissue-specific regulation of 5-HT<sub>1A</sub> receptor expression. They revealed critical developmental periods for the establishment of adult anxiety behavior. These data suggest that early postnatal 5-HT<sub>1A</sub> receptor levels, especially in the frontal cortex and hippocampus, are important factors in the development of adult anxiety thresholds.

Using positron emission tomography (PET) and the highly specific and selective 5-HT<sub>1A</sub> receptor radioligand [carbonyl-<sup>11</sup>C]WAY-100635, we have investigated the regional 5-HT<sub>1A</sub> BP in symptomatic patients suffering from social anxiety disorder (SAD) compared with a group of matched healthy control subjects for the first time. This study was focused on the limbic and paralimbic system including the amygdala, hippocampus, insula, cingulate, and orbitofrontal cortices, which have been shown to be hyperactive or hypoactive in anxiety disorders (Schneider *et al.* 1999; Talbot 2004; Tillfors *et al.* 2001) and anxious states (Benkelfat *et al.* 1995; Chua *et al.* 1999; Liotti *et al.* 2000). Given the stimulus-specific hyperreactivity of the amygdala in social phobia (Stein *et al.* 2002; Tillfors *et al.* 2001; Veit *et al.* 2002; Wright *et al.* 2003) and the critical role of the amygdala in social fear conditioning (Morris *et al.* 2001; Pine *et al.* 2001; Robinson *et al.* 2005), we differentiated the hippocampal region from the amygdala in the mesiotemporal region. The anterior cingulate and orbitofrontal cortices were included in the pre-defined region of interest analysis because of the dense interconnections between it and the amygdala, the central role of the mesial frontal cortex in fear extinction (Amargos-Bosch *et al.*

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2004; Milad and Quirk 2002; Milad *et al.* 2004), and the integration of affective values of reinforcers (Kringelbach 2005).

## Methods and Materials

### Subjects

Fourteen medication-free male outpatients with social anxiety disorder and 18 healthy male control subjects were scanned with PET. Two patients were excluded from analysis: one because of drug consumption prior to the scan, discovered afterwards, and the second because of missing structural magnetic resonance imaging (MRI). Details of subject characteristics including psychometric assessment results are given in Table 1. All healthy subjects and most of the patients were recruited from the community via advertisements, with four patients being recruited from the outpatient clinic. Only male subjects were included in the study because of the influence of sex steroid hormones on the 5-HT<sub>1A</sub> receptor expression (Bethea *et al.* 2002; Bouali *et al.* 2003; Parsey *et al.* 2002) and the large between-subject 5-HT<sub>1A</sub> receptor variability that has been found in comprehensive investigations (Gunn *et al.* 1998; Rabiner *et al.* 2002). Consensus diagnoses according to DSM-IV-TR criteria were established by two psychiatrists performing a routine clinical interview, the Mini-International Neuropsychiatric Interview (MINI) (Sheehan *et al.* 1998), and the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger and Vagg 1984) on the day of the screening visit. The STAI was repeated on the day of the PET scan. All subjects underwent a medical examination including general physical and neurologic status, electrocardiogram, routine laboratory screening, and medical history at the screening visit to exclude subjects with relevant abnormalities, psychiatric comorbidities, and drug abuse. Six (50%) of 12 patients also fulfilled the criteria for agoraphobia. None of the patients had major depression, because comorbid depression was an exclusion criterion, given the reduced 5-HT<sub>1A</sub> receptor BP reported in several studies (Drevets *et al.* 1999; Parsey *et al.* 2006; Sullivan *et al.* 2005). To exclude treatment effects (Bhagwagar *et al.* 2004; Rabiner *et al.* 2000, 2002, 2004; Riad *et al.* 2004), it was ensured that patients were naïve to psychotropic drug treatment targeting the serotonergic system (except one who had ceased selective serotonin reuptake inhibitor [SSRI] medication 3 months prior to the PET

**Table 1.** Demographic and Clinical Characteristics, and Radiochemical Variables. Values Represent Mean ( $\pm$ SD) in Healthy Control Subjects and Social Anxiety Disorder (SAD) Patients

	Control Subjects (n = 18)	SAD Patients (n = 12)
Age range (years)	19–44	23–44
	mean $\pm$ SD	mean $\pm$ SD
Age (years)	27.0 $\pm$ 5.8	30.3 $\pm$ 6.4
Body mass index (kg/m <sup>2</sup> ), BMI	23.4 $\pm$ 2.5	25.0 $\pm$ 4.1
Spielberger State Anxiety Score, STAI <sup>a</sup>	32.4 $\pm$ 4.5	46.8 $\pm$ 7.9
Spielberger State Anxiety Score, STAI <sup>b</sup>	32.3 $\pm$ 5.0	43.8 $\pm$ 7.8
Spielberger Trait Anxiety Score, STAI <sup>a</sup>	34.3 $\pm$ 6.6	51.3 $\pm$ 9.2
Spielberger Trait Anxiety Score, STAI <sup>b</sup>	33.3 $\pm$ 6.8	48.5 $\pm$ 9.4
Activity Injected, ID (MBq)	421.3 $\pm$ 15.7	398.7 $\pm$ 35.2
Radiochemical Purity, RCP (%)	97.8 $\pm$ 1.4	97.9 $\pm$ 1.1
Specific Activity (GBq/ $\mu$ mol)	29.7 $\pm$ 21.7	23.4 $\pm$ 13.9
Weight of WAY 100634 ( $\mu$ g)	7.2 $\pm$ 3.6	5.1 $\pm$ 2.9
Weight of unlabeled WAY100635 ( $\mu$ g)	10.7 $\pm$ 10.1	11.2 $\pm$ 10.3

<sup>a,b</sup> Spielberger State and Trait Anxiety Score, STAI (max = 80); <sup>a</sup>screening visit, <sup>b</sup>PET day.

scan). Both the patients and the healthy volunteers gave written informed consent after the procedures had been fully explained at the screening visit. The study was approved by the Ethics Committee of the Medical University of Vienna and the General Hospital of Vienna. All subjects received reimbursement for participation. No significant differences of demographic and radiochemical variables were found between the groups (unpaired *t* test, *p* > .05, corrected).

### PET Scanning Protocol and Preprocessing

Positron emission tomography scans were performed on a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, Wisconsin) at the Department of Nuclear Medicine, Medical University of Vienna, Austria. The heads of subjects were positioned in the scanner parallel to the orbitomeatal line using a laser beam system to ensure the covering of the cerebellum in the field of view (FOV). Head movements were minimized with a polyurethane molded cushion and straps about the forehead and chin, and subjects were instructed not to move or speak during the measurement. A 5-minute transmission scan was performed in two-dimensional mode for correction of tissue attenuation using a retractable <sup>68</sup>Ge ring source. Dynamic PET scans were acquired in three-dimensional mode. Measurements started simultaneously with intravenous bolus injection of [carbonyl-<sup>11</sup>C]WAY-100635 in phosphate-buffered saline using an average dose of 5.37  $\pm$  .86 MBq per kilogram body weight. A series of 30 successive time frames (15  $\times$  1 minute, 15  $\times$  5 minutes) were collected, resulting in a total acquisition time of 90 minutes. The emission data were scatter and attenuation corrected using the transmission data. Thirty-five contiguous slices (matrix 128  $\times$  128) with a slice thickness of 4.25 mm were reconstructed using an iterative filtered back-projection algorithm (FORE-ITER). The spatial resolution of the final reconstructed volume was 4.36 mm full-width at half maximum (FWHM) at the center of the FOV. No realignment for head movement or partial volume correction was applied. The 30 frames of dynamic PET imaging were summed (PET<sub>ADD</sub>) for MRI-PET co-registration.

### Radiochemistry

[Carbonyl-<sup>11</sup>C]WAY-100635 was prepared at the Cyclotron Unit of the PET center according to methods described previously (Matarrese *et al.* 2002; McCarron *et al.* 1996), with slight modifications, in a fully automated PET synthesizer (GE Healthcare, Uppsala, Sweden). Details of radiochemical variables are shown in Table 1. Injected activity, specific activity, radiochemical purity, weight of WAY-100634 (precursor), and weight of unlabeled WAY-100635 were used as covariates in the multivariate analysis of variance (ANOVA). The specific activity is defined as activity (GBq) per amount of substance (WAY 100635,  $\mu$ mol) determined by high-performance liquid chromatography (HPLC). The values were corrected for the time of tracer administration.

### Magnetic Resonance Imaging

High-resolution T1-weighted structural magnetic resonance (MR) images (magnetization-prepared rapid gradient-echo [MPRAGE] sequence, 256  $\times$  256 matrix, .78  $\times$  .86 mm voxel size, slice thickness 1.56 mm, 128 slices) were acquired in each subject using a 3 Tesla Medspec whole-body MR scanner (Bruker BioSpin, Ettlingen, Germany).

### Region of Interest Analysis

The structural MRI images were co-registered to the PET<sub>ADD</sub> images and resliced using Statistical Parametric Mapping version

2002 (SPM2; The Wellcome Department of Imaging Neuroscience, University College London; [www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) (Meyer *et al.* 1999). Regions of interest (ROIs) were defined a priori to enable comparison to other 5-HT<sub>1A</sub> receptor studies (Bonne *et al.* 2005; Neumeister *et al.* 2004; Tauscher *et al.* 2001a) and based on the functional MRI and PET literature in anxiety disorders (Adolphs 2002; Liotti *et al.* 2000; Pine *et al.* 2001; Schneider *et al.* 1999; Tillfors *et al.* 2001) in areas expressing high levels of the 5-HT<sub>1A</sub> receptor (Rabiner *et al.* 2002; Varnas *et al.* 2004). Five ROIs in the anterior cingulate cortex, medial orbitofrontal cortex, insula, amygdala, hippocampus, and one reference region in the cerebellum were drawn bilaterally on co-registered MR images using the anatomical criteria established by Bremner *et al.* (1998). Due to the absence of MRI criteria for raphe boundaries, the raphe region in the midbrain was directly traced onto the PET<sub>ADD</sub> image. To delineate the dorsal raphe nucleus, we identified the slices showing the interpeduncular cistern on the triplanar view of co-registered MR images and placed a fixed-size cubic volume of interest (VOI) (747mm<sup>3</sup>) over the area with highest signal on the corresponding PET<sub>ADD</sub> slices (Abrams *et al.* 2004).

### Tracer Kinetic Modeling of [carbonyl-<sup>11</sup>C]WAY-100635 Emission Data

For quantification of the 5-HT<sub>1A</sub> receptor BP, we used the kinetic modeling tool of the biomedical image quantification software PMOD 2.7 (PMOD Technologies Ltd., Zurich, Switzerland) (Mikolajczyk *et al.* 1998). The simplified reference tissue model (SRTM), based on a two-tissue compartmental model (Gunn *et al.* 1998; Lammertsma and Hume 1996), was applied using the cerebellum as a reference region because of its low 5-HT<sub>1A</sub> receptor density (Burnet *et al.* 1997; Hall *et al.* 1997). Decay-corrected time activity curves (TACs) were obtained using the 30 frames of the dynamic PET data and the three-dimensional ROIs. We calculated the regional BP and the regional relative delivery of the radioligand normalized to the cerebellum (R1). Right and left ROIs were combined (except for the raphe region) to improve signal-to-noise ratio. The SRTM is sensitive to group differences in the radioligand kinetics of the reference region. We therefore examined the cerebellar TACs and found no significant group difference (two-tailed *t* test,  $p = .163$ ) between areas under the curve between cerebellar TACs of SAD patients and healthy control subjects.

### Statistical Analysis

Statistical analyses were performed using SPSS 12.0.1 (SPSS Inc., Chicago, Illinois). The Kolmogorov-Smirnov test ( $p > .05$ ) for normal distribution and the Levene's test for equality of variance ( $p > .05$ ) were used prior to parametric statistical analyses. All parametric tests were two-tailed. Significance was set at  $p < .05$ . Pearson product moment correlation coefficients were calculated between regional BP values and demographic (age, body mass index) and radiochemical variables. To exclude any possible bias by summation effects, we included all radiochemical variables (see Table 1) as covariates in the multivariate ANOVA. Age-dependent reduction of 5-HT<sub>1A</sub> BP is a matter of debate (Rabiner *et al.* 2002; Tauscher *et al.* 2001b) and there may be age-dependent regional effects on perfusion and tracer delivery, so age was controlled for. A multivariate ANOVA with all regional BP values as dependent variables; group (patients versus control subjects) as a fixed factor; and age, injected activity, specific activity, weight of WAY-100634, and radiochemical purity as covariates was performed. To exclude the influence

on regional BP of tracer delivery differences between groups, we performed an additional multivariate ANOVA with all regional R1 values as dependent variables, group as a fixed factor, and age and the four radiochemical variables as covariates. When significant group-by-region differences for BP values were obtained with ANOVA, post hoc *t* tests (unpaired, two-tailed, alpha level  $p < .05$ ) in eight ROIs were calculated. Significance levels were corrected for the multiple comparisons involved in employing eight ROIs. The Bonferroni adjusted *p* value was  $p < .00625$ . A Pearson product moment correlation and partial correlations controlling for age and radiochemical variables between anxiety scores (STAI) and regional 5-HT<sub>1A</sub> BP were performed (two-tailed,  $p < .05$ ).

## Results

### Comparison Between SAD Patients and Healthy Control Subjects

The multivariate ANOVA revealed a significant reduction of 5-HT<sub>1A</sub> BP in SAD patients compared with healthy control subjects ( $p = .041$ ; Pillai's Trace). Including age and radiochemical variables as covariates in the multivariate ANOVA, we found a highly significant reduction in 5-HT<sub>1A</sub> binding in the amygdala ( $p = .003$ ;  $-21.4\%$ , uncorrected for multiple comparison, post hoc *t* test), in the anterior cingulate cortex ( $p = .004$ ;  $-23.8\%$ ), and in the insula ( $p = .003$ ;  $-28.0\%$ ), and a significant reduction in the raphe region ( $p = .030$ ;  $-36.4\%$ ) and the medial orbitofrontal cortex ( $p = .032$ ;  $-18.4$ ). Applying a Bonferroni correction for multiple comparisons, the amygdala region ( $p = .024$ ), the insula ( $p = .024$ ), and the anterior cingulate cortex ( $p = .032$ ) remained significant. Details are given in Table 2. Excluding age as a covariate in the multivariate ANOVA, we found a highly significant group difference in the anterior cingulate cortex ( $p = .003$ ), insula ( $p = .002$ ), and amygdala ( $p = .004$ ), and a significant difference in the raphe nuclei ( $p = .025$ ) and the medial orbitofrontal cortex ( $p = .028$ ), indicating a minor effect of age. To exclude possible bias introduced by ROI drawing, we additionally used a fixed size VOI in the amygdala and calculated a multivariate ANOVA, which further improved the significance level ( $p = .001$ ;  $-23.8\%$  group difference) of the post hoc *t* test. There was no relevant improvement applying a fixed size VOI in the hippocampal head ( $p = .234$ ;  $-9.7\%$  to  $p = .210$ ;  $-11.5\%$ ). Parametric 5-HT<sub>1A</sub> receptor binding potential maps superimposed on co-registered T1-weighted MR images are shown in Figure 1. Figure 2 shows scatter histograms of the 5-HT<sub>1A</sub> receptor BP values comparing patients with healthy control subjects.

An additional ANOVA with all regional R1 values as dependent variables and age as a covariate showed no significant group effect ( $p = .504$ ). Including the radiochemical variables additionally as covariates showed no significant effect ( $p = .361$ ). Both ANOVA results indicate no bias due to tracer delivery differences between groups. No significant correlations ( $p < .05$ , corrected) between 5-HT<sub>1A</sub> BP or R1 and demographic as well as radiochemical variables were found. There were no significant group differences between the SAD patients and the healthy control subjects concerning age, body mass index, and radiochemical variables.

To investigate the effect of comorbid agoraphobia on 5-HT<sub>1A</sub> BP, we performed calculations on subgroups. Excluding the six SAD patients with comorbid agoraphobia reduced the significance levels of the multivariate ANOVA only slightly ( $p = .075$ ,  $n = 24$ ; controlled for age and radiochemical variables). Exclud-

**Table 2.** Regional 5-HT<sub>1A</sub> Receptor Binding Potential Values in SAD Patients and Healthy Controls

Region of Interest	Binding Potential Values		Volume of Interest, VOI [cm <sup>3</sup> ] (n = 30)	Comparison of Means	
	Controls (n = 18)	SAD Subjects (n = 12)		Percentage Reduction <sup>a</sup>	p <sup>b</sup>
	Mean SD	Mean SD	Mean SD		
Hippocampus (head)	6.2 ± 1.9	5.6 ± 2.2	1.2 ± .2	−9.7%	.234
Hippocampus (fixed VOI) <sup>c</sup>	6.1 ± 1.9	5.4 ± 2.2	1.2 ± .1	−11.5%	.210
Insula	5.0 ± 1.0	3.6 ± 1.2	5.9 ± 1.3	−28.0%	.003 <sup>d</sup>
Anterior Cingulate Cortex	4.2 ± .8	3.2 ± 1.0	2.1 ± .6	−23.8%	.004 <sup>d</sup>
Amygdala	4.2 ± 1.1	3.3 ± 1.2	1.5 ± .3	−21.4%	.003 <sup>d</sup>
Amygdala (fixed VOI) <sup>c</sup>	4.2 ± 1.2	3.2 ± 1.2	1.3 ± .1	−23.8%	.001 <sup>d</sup>
Medial Orbitofrontal Cortex	3.8 ± .8	3.1 ± 1.1	9.3 ± 2.2	−18.4%	.032
Raphe Nuclei (fixed VOI) <sup>c</sup>	2.2 ± .7	1.4 ± .6	.7 ± .0	−36.4%	.030

<sup>a</sup>Calculation of mean BP reduction:  $(100 * BP_{patients} / BP_{controls}) - 100$ .

<sup>b</sup>Significance levels corrected for age and four radiochemical covariates as indicated in the statistical section (*post hoc* t tests, ANOVA).

<sup>c</sup>fixed VOI, fixed size for volume of interest.

<sup>d</sup>p values surviving the Bonferroni correction for multiple comparison implying eight ROIs (p < 0.00625; Bonferroni adjusted p value at 8 ROIs).

ing the six SAD patients without comorbid agoraphobia dramatically reduced the significance level of the multivariate ANOVA ( $p = .387$ ,  $n = 24$ ).

### Relationships Between Anxiety Scores (STAI) and Regional 5-HT<sub>1A</sub> Receptor Binding Potential

Spielberger State-Trait Anxiety Inventory scores obtained at the screening visit and on the day of the PET scan were significantly lower in the patient than the healthy control group ( $p < .001$ ). Details are given in Table 1. State and trait scores on both days correlated significantly ( $p < .001$ ). A Pearson correlation analysis revealed no significant correlation between state or trait anxiety scores and regional 5-HT<sub>1A</sub> BP in both groups. A partial correlation controlling for age and radiochemical variables also showed no significant relationship between 5-HT<sub>1A</sub> binding and anxiety scores. As shown in Figure 3, the values in the correlation plot are clustered into two groups, differentiating clearly between social anxiety disorder patients and healthy control subjects.

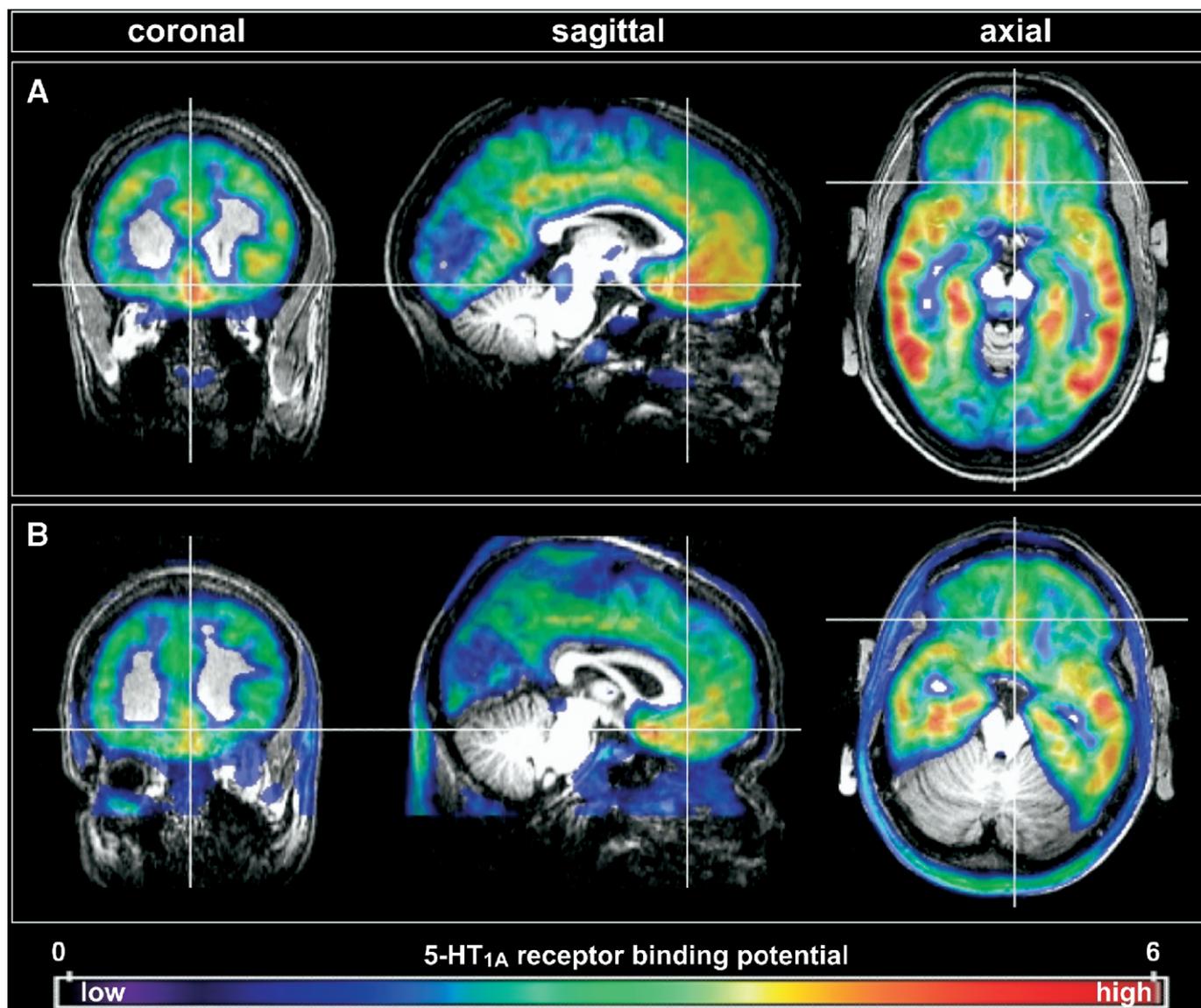
### Discussion

We have demonstrated a significantly lower 5-HT<sub>1A</sub> receptor binding potential in male patients suffering from SAD than in an age-matched healthy control group. Significant 5-HT<sub>1A</sub> BP reductions (corrected for multiple tests) were found in the amygdala (−21.4%), the anterior cingulate cortex (−23.8%), and insula (−28.0%), indicating alterations in limbic and paralimbic areas of SAD patients. The reduction was most prominent in the raphe nuclei (−36.4%) but did not survive correction for multiple testing in this area. Given the inclusion criteria of our subjects, we can exclude the possibility that the reduction in 5-HT<sub>1A</sub> BP was caused by exposure to psychotropic drugs or comorbid depression (Bailer *et al.* 2005; Drevets *et al.* 1999; Sargent *et al.* 2000; Sullivan *et al.* 2005). Our results are partly concordant with a recently published study in panic disorder patients (Neumeister *et al.* 2004). This group showed a reduction in 5-HT<sub>1A</sub> receptor binding of 27.6% in the anterior cingulate cortex, comparable with our result of 23.8%. In contrast to our study, they found no difference in the anterior insula and mesiotemporal cortex including both the hippocampus and amygdala. We differentiated the amygdala from the hippocampus region a priori because of comprehensive literature emphasizing the central role of the amygdala in neural processing of fear and anxiety. Investigating

six regions of interest, we found the most significant group difference in the amygdala, suggesting an area-specific effect on serotonergic transmission in SAD. Given the high percentage (50.0%) of comorbid agoraphobia in the patient sample, our results could be thought to reflect a main effect of agoraphobia on reduced 5-HT<sub>1A</sub> BP. The subgroup analysis detailed in Results did not support the view that our results could reflect effects attributable solely to agoraphobia, although the small sample size might limit its validity.

### Modulation of Amygdala Activity by Mesiofrontal Areas

Given the central role of the amygdala in fear conditioning and processing (Adolphs *et al.* 2005; Morris *et al.* 2001), we expected and found significant differences in 5-HT<sub>1A</sub> binding of the amygdala region between SAD patients and control subjects. The amygdala is strongly modulated by mesiofrontal areas. The anterior cingulate cortex and neighboring prefrontal areas control the attention to threat-related stimuli and inhibit amygdala activity by top-down regulation (Bishop *et al.* 2004). In rats, destruction of ventral prefrontal cortex blocks recall of fear extinction, indicating the storage of long-term extinction memory in paralimbic areas and not in the amygdala (Milad and Quirk 2002). Studies in nonhuman primates have confirmed the central role of the anterior cingulate region (Kalin *et al.* 2005) and the orbitofrontal cortex (Kalin and Shelton 2003) in the regulation of amygdala activity. Increased amygdala activity is associated with diminished activation of the mesiofrontal cortex in anxiety disorders (Shin *et al.* 2005). The exaggerated amygdala responsiveness frequently shown in functional magnetic resonance imaging (fMRI) studies investigating SAD patients (Birbaumer *et al.* 1998; Lorberbaum *et al.* 2004; Schneider *et al.* 1999; Tillfors *et al.* 2002) might be associated with alterations of the serotonergic system in mesiofrontal areas. Several functional studies have reported increased or altered amygdala activation to facial stimuli in social phobia, confirming the specific association of faces with anxiety in this disorder. A recently published PET study of panic disorder patients demonstrated a lack of appropriate top-down regulation of the orbitofrontal region on amygdala activity (Kent *et al.* 2005). These results in limbic and paralimbic regions are consistent with our data, which demonstrate area-specific 5-HT<sub>1A</sub> alterations in anxiety circuitry including the amygdala, anterior cingulate, and orbitofrontal cortices.



**Figure 1.** Parametric 5-HT<sub>1A</sub> receptor binding potential (BP) maps superimposed on the co-registered T1-weighted MR images of a single healthy subject (A) compared with a representative patient suffering from social anxiety disorder (SAD) (B). White cross in the triplanar view (coronal, sagittal, axial) indicates the corresponding area in the mesial orbitofrontal cortex. The color table indicates the 5-HT<sub>1A</sub> receptor binding potential values. 5-HT<sub>1A</sub>, serotonin-1A; BP, binding potential; MR, magnetic resonance; SAD, social anxiety disorder.

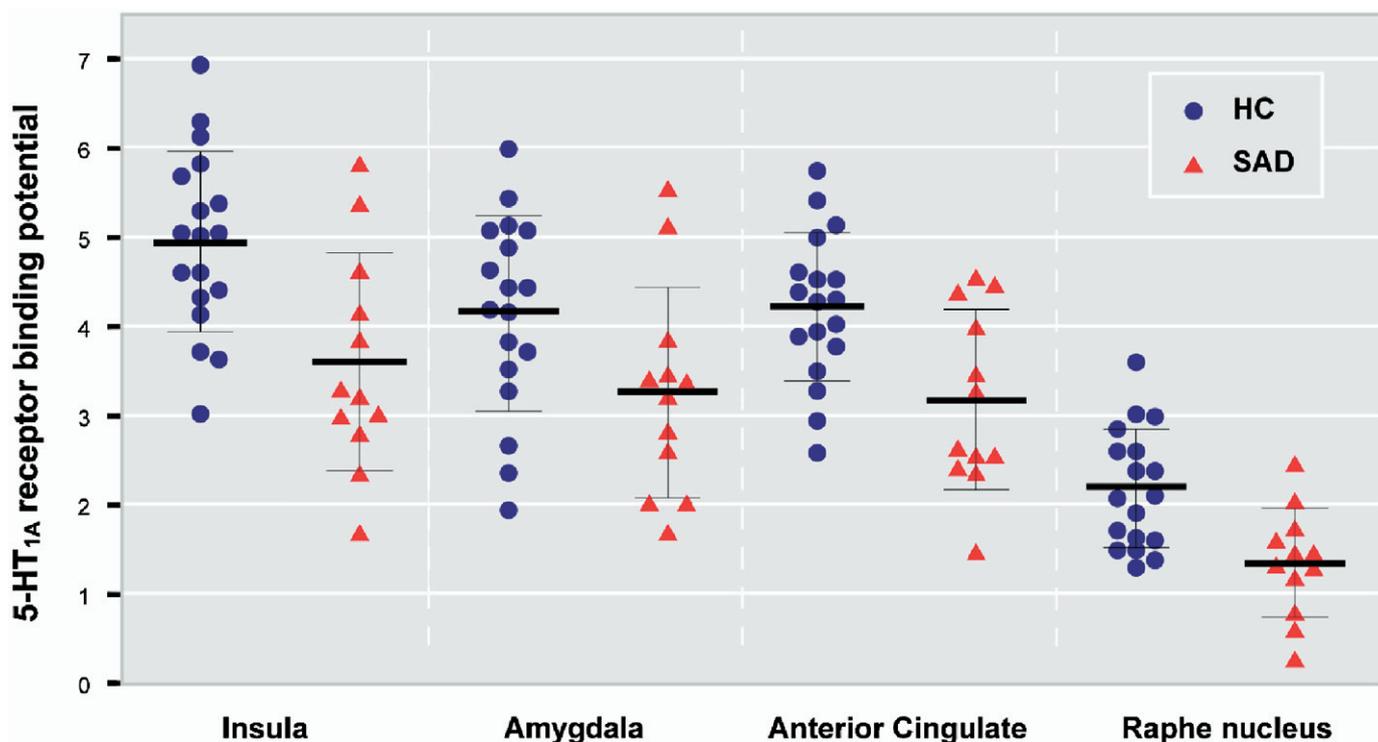
#### The Function of the Insula Region in the Anxiety Circuitry

In SAD patients, we found a significantly lower 5-HT<sub>1A</sub> receptor binding in the insula region, including the anterior and posterior parts, in the region of interest analysis. Neumeister *et al.* (2004), investigating the anterior insula, did not find lower 5-HT<sub>1A</sub> receptor binding in patients with panic disorder in this region. The same group reported no significant reduction in 5-HT<sub>1A</sub> binding in the anterior insula when investigating patients with posttraumatic stress disorder (Bonne *et al.* 2005). Given the important role of the polymodal insula region in the anxiety circuitry, we would expect changes of serotonergic transmission in this region. Patients suffering from social phobia had increased activity in the insula, amygdala, pons, and temporal pole, and decreased activity in the anterior cingulate and prefrontal cortex in an fMRI paradigm using anticipatory anxiety (Lorberbaum *et al.* 2004). Chua *et al.* (1999), investigating healthy subjects with fMRI, revealed an area-specific activation pattern restricted to

the insula, orbitofrontal region, anterior cingulate, and temporal cortices in anticipatory anxiety. Anxiety in healthy subjects was associated with specific activations in the ventral insula, orbitofrontal cortex, and anterior temporal region (Liotti *et al.* 2000). To summarize, functional data suggest the specific and strong involvement of the insula and mesiofrontal region in processing of anxiety consistent with our finding of reduced 5-HT<sub>1A</sub> receptor binding.

#### The 5-HT<sub>1A</sub> Receptor in the Hippocampus

In the hippocampus, we found no group difference in 5-HT<sub>1A</sub> receptor binding between patients and healthy control subjects. This is consistent with functional neuroimaging studies in healthy subjects (Benkelfat *et al.* 1995; Chua *et al.* 1999; Liotti *et al.* 2000), patients suffering from anxiety disorders (Bonne *et al.* 2005; Kent *et al.* 2005; Tillfors *et al.* 2001; Wright *et al.* 2003), and studies in nonhuman primates (Kalin *et al.* 2004, 2005). These studies have



**Figure 2.** Scatter histograms of the 5-HT<sub>1A</sub> receptor BP values for healthy control subjects (HC = ●, blue,  $n = 18$ ) and patients suffering from social anxiety disorder (SAD = ▲, red,  $n = 12$ ). Mean ( $\pm$  SD) bars indicate the significant differences between SAD and control group in the insula ( $p = .003^*$ ), amygdala ( $p = .003^*$ ), anterior cingulate cortex ( $p = .004$ ), and raphe nucleus ( $p = .030$ ). Asterisk (\*) indicates significant values ( $p < .05$ ) after adjustment for multiple comparison (Bonferroni correction) implying six ROIs. 5-HT<sub>1A</sub>, serotonin-1A; BP, binding potential; HC, healthy control subjects; SAD, social anxiety disorder; ROI, region of interest.

shown that the hippocampus is not a central part of anxiety circuitries. To exclude possible effects of hippocampal and amygdala size differences between the groups, we additionally calculated the binding potential using fixed volumes of interest (1.2 cm<sup>3</sup> for the hippocampus, 1.3 cm<sup>3</sup> for the amygdala). The significance levels were slightly higher in the amygdala ( $p = .003$  to  $p = .001$ ) and the hippocampus ( $p = .234$  to  $p = .210$ ), indicating that no bias was introduced in the manual tracing of ROIs on co-registered MR images. Given the long PET measurement time of 90 minutes, a relevant underestimation of BP by late binding equilibrium in receptor-rich regions such as the hippocampus or insula is unlikely (Parsey *et al.* 2000; Tauscher *et al.* 2002).

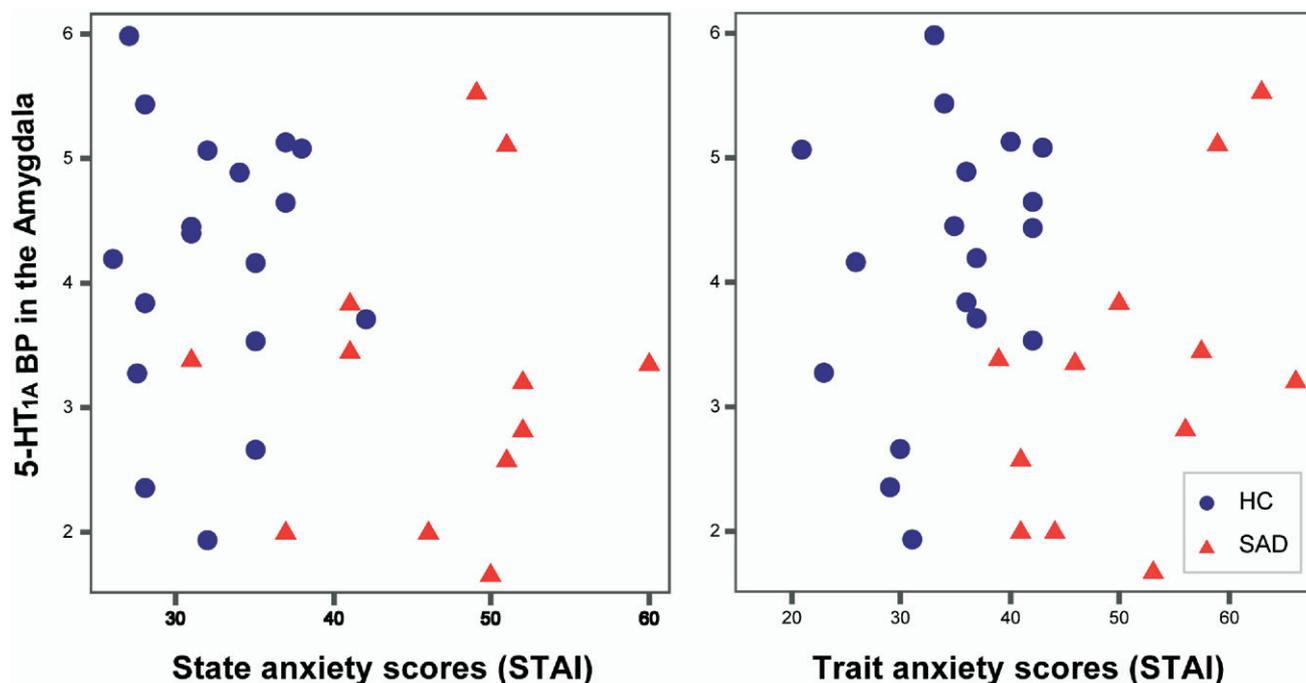
#### Anxiety Scores and Regional 5-HT<sub>1A</sub> Binding

Investigating 30 subjects, we found no significant relationship between either trait or state anxiety scores (STAI) and regional 5-HT<sub>1A</sub> BP in male SAD patients and healthy control subjects. These results are consistent with the comprehensive PET study published by Rabiner *et al.* (2002) performing the STAI in 66 healthy male subjects. In addition, Neumeister *et al.* (2004) compared patients suffering from panic disorder to healthy control subjects (67.7% female subjects) using the Panic Disorder Severity Scale (PDSS) and did not report a correlation between PDSS scores and regional 5-HT<sub>1A</sub> binding. Investigating patients with chronic fatigue syndrome and healthy control subjects, Cleare *et al.* (2005) also found no significant correlation between STAI scores and regional 5-HT<sub>1A</sub> receptor binding in 21 ROIs. We reported an inverse relationship between 5-HT<sub>1A</sub> receptor binding and anxiety scores in a group of 19 healthy subjects (42% female

subjects) using the Revised NEO Personality Inventory (Tauscher *et al.* 2001a). However, none of the correlations in the four ROIs delineated (prefrontal, parietal, occipital, and anterior cingulate cortices) survived a correction for multiple comparisons (Tauscher *et al.* 2001a). In addition, Sullivan *et al.* (2005) recently reported a positive correlation between 5-HT<sub>1A</sub> binding and anxiety scores in 28 patients with major depressive disorder (MDD) including comorbid anxiety disorders (43%) and found lower 5-HT<sub>1A</sub> BP in MDD patients with comorbid anxiety disorder than in those with MDD only. It is possible that our finding of no significant relationship between regional 5-HT<sub>1A</sub> BP and patients' anxiety scores arises because of insufficient statistical power, with a sample size of only 12. Given the high intersubject variability of 5-HT<sub>1A</sub> BP (Gunn *et al.* 1998; Rabiner *et al.* 2002) and the strong effects of steroid hormones on 5-HT<sub>1A</sub> receptor expression (Bethua *et al.* 2002; Parsey *et al.* 2002), the results in our homogeneous group of 18 male control subjects do not support a significant relationship between trait or state anxiety and 5-HT<sub>1A</sub> BP in the amygdala, hippocampus, insula, anterior cingulate, and orbitofrontal cortices in healthy male subjects.

#### The Relationship Between Postsynaptic 5-HT<sub>1A</sub> Levels and Serotonergic Neurotransmission

The 5-HT<sub>1A</sub> receptor is the major inhibitory serotonergic receptor on glutaminergic and gamma-aminobutyric acid (GABA)ergic neurons in the frontal cortex (Amargos-Bosch *et al.* 2004; Puig *et al.* 2005; Santana *et al.* 2004). In addition, 5-HT<sub>1A</sub> receptors are involved in the control of serotonergic neurons in the dorsal raphe nuclei by the medial prefrontal cortex (Celada *et al.* 2001, 2002), suggesting an important feedback mechanism



**Figure 3.** Relationship between 5-HT<sub>1A</sub> receptor BP values in the amygdala and the Spielberger State-Trait Anxiety Inventory state (left) and trait (right) anxiety scores. Values are clustered into two groups, and grouping differentiates between social anxiety disorder (SAD) patients ( $\blacktriangle$ , red) and healthy control subjects ( $\bullet$ , blue). There was no significant correlation between STAI state or trait scores and 5-HT<sub>1A</sub> binding in patients (SAD) and healthy control subjects (HC). 5-HT<sub>1A</sub>, serotonin-1A; BP, binding potential; STAI, Spielberger State-Trait Anxiety Inventory; SAD, social anxiety disorder; HC, healthy control subjects.

between serotonergic firing in the raphe region and prefrontal activity. Therefore, the balance of inhibitory 5-HT<sub>1A</sub> and excitatory serotonin-2A (5-HT<sub>2A</sub>) receptors strongly influences the effects of serotonergic firing on glutaminergic and GABAergic neurons in the frontal cortex but also in the amygdala, hippocampus, and insula (Martin-Ruiz *et al.* 2001). Pharmacological studies in rodents have shown the anxiolytic effect of both 5-HT<sub>1A</sub> agonism and 5-HT<sub>2A</sub> antagonism (Delgado *et al.* 2005). Lower postsynaptic 5-HT<sub>1A</sub> receptor levels reduce the inhibitory influence of serotonergic neurons on glutaminergic and GABAergic neurons in the frontal cortex (Amargos-Bosch *et al.* 2004; Santana *et al.* 2004). Increased serotonergic phasic firing by reduced presynaptic 5-HT<sub>1A</sub> receptor levels in the raphe nuclei and increased serotonergic excitatory influence on glutaminergic and GABAergic neurons might shift the serotonergic influence on frontal neurons. Lower 5-HT<sub>1A</sub> receptor levels might be induced by a downregulation mechanism in the adult subject, e.g., by hormonal influence on receptor expression (Andrade *et al.* 2005; Bethea *et al.* 2002, 2005; Bouali *et al.* 2003; Fernandes *et al.* 1997; Meijer and de Kloet 1998) or congenital and genetic effects (Gross and Hen 2004; Gross *et al.* 2002). Downregulation of 5-HT<sub>1A</sub> receptors in the anterior cingulate cortex, insula, and amygdala suggests an area-specific regulation of 5-HT<sub>1A</sub> receptor expression in these areas involved in processing of anxiety-associated stimulation. Given the lower 5-HT<sub>1A</sub> BP in depression (Bhagwagar *et al.* 2004; Drevets *et al.* 1999; Sargent *et al.* 2000), reduced 5-HT<sub>1A</sub> receptor levels might increase the vulnerability for affective disorders, including both anxiety disorders and depression. Our data are not sufficient to differentiate between neuroplastic processes on 5-HT<sub>1A</sub> receptor expression in glutaminergic and GABAergic neurons and primarily reduced 5-HT<sub>1A</sub> receptor levels changing the serotonergic influence on frontal and limbic neurons. In addition, steroid hormones such as glucocorticoids regulate the 5-HT<sub>1A</sub> receptor

expression by transcriptional repression of the receptor promoter sequence (Meijer *et al.* 2000). Dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis have been frequently shown in anxiety disorders and depression. Therefore, reduced 5-HT<sub>1A</sub> receptor levels and changes in the serotonin system across different types of anxiety disorders and depression might be caused by HPA axis dysregulation.

### Conclusion

The main finding of this PET study is a significantly lower 5-HT<sub>1A</sub> receptor binding in patients suffering from social anxiety disorder compared with healthy control subjects, indicating an altered serotonergic neurotransmission in SAD and specifically implicating 5-HT<sub>1A</sub> receptors as a potential target of interest for novel therapeutics to treat this condition. These results further emphasize the central role of the amygdala, the mesiofrontal cortex, and the polymodal insula in the modulation of anxious states.

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