Cortisol plasma levels in social anxiety disorder patients correlate with serotonin-1A receptor binding in limbic brain regions

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Abstract

Dysregulation of the hypothalamic-pituitary-adrenocortical axis with deficient glucocorticoid feedback and alterations in the serotonergic system have been identified as biological correlates of mood disorders. Close examination of the interaction between these systems may offer insights into the pathophysiology of anxiety disorders and depression to understand how stress and these disorders are related. In this study, we investigated the relationship between plasma levels of cortisol and the dominant inhibitory serotonergic receptor, serotonin-1A (5-HT$_{1A}$). Using positron emission tomography (PET) and the radioligand $[^{11}$C]WAY-100635, we quantified the 5-HT$_{1A}$ receptor binding. Data from 12 male patients with social phobia and 18 matched control subjects were analysed. Seven brain regions were investigated: the anterior and posterior cingulate cortices, hippocampus, amygdala, medial orbitofrontal and retrosplenial cortices, and dorsal raphe nucleus. Partial correlation analysis, controlled for age and radiochemical variables, was performed to demonstrate the association between cortisol plasma levels and 5-HT$_{1A}$ receptor binding. Cortisol plasma levels were significantly lower in patients with social phobia compared to healthy controls. Moreover, we found strong negative correlations between cortisol plasma levels and 5-HT$_{1A}$ binding in the amygdala ($r = -0.93$, $p = 0.0004$), hippocampus ($r = -0.80$, $p = 0.009$), and retrosplenial cortex ($r = -0.48$, $p = 0.04$) in patients with social phobia. Within the former two regions, these associations were significantly higher in patients than in healthy controls. This PET study confirms a negative association between plasma cortisol levels and the 5-HT$_{1A}$ receptor distribution consistent with studies in rodents and non-human primates. Dysregulation of the cortisol level might increase the vulnerability for mood disorders by altering limbic 5-HT$_{1A}$ receptors.

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Introduction

The role of the steroid hormone cortisol is still controversial as a mediator of short- or long-term effects of the stress reaction. Corticosteroids and serotonin have frequently been observed to interact functionally in the pathogenesis of depression and anxiety disorders (Farisse et al. 2000; Kasper, 1998; Kasper et al. 2005; Korte, 2001; Meijer & de Kloet, 1998; Millan, 2003; Porter et al. 2004). Acute stress increases both cortisol release and the activity of tryptophan hydroxylase raising the extracellular serotonin level. The therapeutic efficacy of either enhancement (by SSRIs) or attenuation (e.g. by tianeptine) of serotonin release is associated with the normalization of a dysfunctional hypothalamic–pituitary–adrenocortical (HPA) axis system (Bandelow et al. 2008; Lopez et al. 1994; Nickel et al. 2003).

As one of at least 25 different serotonin receptor subtypes, the serotonin-1A receptor (5-HT$_{1A}$) in particular is thought to play an important role in
the modulation of anxiety (Akimova et al. 2009; Spindelegger et al. 2009) and depression (Drevets et al. 2007; Savitz et al. 2009). Several studies have revealed reduced 5-HT \textsubscript{1A} receptor binding and dysfunctions of the HPA axis (Condren et al. 2002; Meijer & de Kloet, 1998; Nickel et al. 2003; Strohle & Holsboer, 2003; Young et al. 2004) in depressive (Bhagwagar et al. 2004, 2005; Drevets et al. 1999; Kasper et al. 2002; Pitchot et al. 2005; Sargent et al. 2000) and anxiety disorder (Condren et al. 2002; Lanzenberger et al. 2007; Neumeister et al. 2004; Young et al. 2004) patients. Receptor knockout models have demonstrated the important role of the 5-HT \textsubscript{1A} (Julius, 1998) and glucocorticoid receptors in regulating stress response. Studies in animals suggest a causal relationship between chronic stress-related glucocorticoid secretion and the 5-HT \textsubscript{1A} receptor down-regulation associated with affective disorders (Fernandes et al. 1997; Lopez et al. 1998).

Corticosteroids regulate 5-HT \textsubscript{1A} receptor expression by a transcriptional repression of the receptor promoter sequence via intranuclear mineralocorticoid and glucocorticoid receptors (Chalmers et al. 1993; Meijer, 2002; Meijer et al. 2000; Ou et al. 2001). This brain region-specific repression is cell type- and cell state-dependent as has been thoroughly demonstrated for the hippocampus (Lopez et al. 1998; Neumaier et al. 2000). The interaction between mineralocorticoid and glucocorticoid receptors rather than the absolute density of glucocorticoid binding sites, modulate the area-specific 5-HT \textsubscript{1A} receptor protein expression (Farisse et al. 2000; Meijer & de Kloet, 1998).

Encouraged by the persuasive animal study results mentioned above, several PET studies have examined the relationship between glucocorticoids and 5-HT \textsubscript{1A} receptor distribution in healthy and depressed human subjects in vivo. However, these studies have shown no significant correlations between 5-HT \textsubscript{1A} binding potential (BP) in different brain regions and cortisol plasma levels (Bhagwagar et al. 2003; Drevets et al. 1999; Montgomery et al. 2001).

Several PET studies also investigated 5-HT \textsubscript{1A} BP in patients with major depressive disorder, leading to inconsistent results (e.g. Hirvonen et al. 2008; Parsey et al. 2006). Recently, we showed that patients with social anxiety disorder (SAD) have reduced 5-HT \textsubscript{1A} binding in a number of limbic and paralimbic regions, compared to healthy control (HC) subjects (Lanzenberger et al. 2007). As a corollary to this finding, our objective was to further investigate the relationship between regional 5-HT \textsubscript{1A} receptor binding and the cortisol plasma levels in a group of 12 male patients suffering from anxiety disorders, and in 18 age-matched HCs. Two main hypotheses suggested by previous animal studies were tested:

1. Cortisol plasma levels will correlate negatively with 5-HT \textsubscript{1A} receptors in patients suffering from SAD and HCs.
2. The correlation will be more pronounced in brain regions expressing high levels of both mineralocorticoid and glucocorticoid receptors, which modulate 5-HT \textsubscript{1A} receptor protein levels by transcriptional suppression.

Several studies also indicate cortisol levels to be altered in patients suffering from anxiety disorders (Condren et al. 2002; Kallen et al. 2008; Roelofs et al. 2009; van West et al. 2008). For this reason, we were also interested whether cortisol plasma levels might differ between patients with social phobia and HCs. Furthermore, since patients with social phobia differ in regional 5-HT \textsubscript{1A} binding (Lanzenberger et al. 2007), we speculated the correlation between 5-HT \textsubscript{1A} receptors and plasma cortisol levels to be different in patients suffering from anxiety disorders and HC subjects.

Methods and materials

Subjects

Fourteen male anxiety disorder patients with social phobia (Brunello et al. 2000; Kasper, 1998) and 20 age- and gender-matched HCs underwent PET scans. Only male subjects were studied to rule out the confounding effects of sex steroid hormones on 5-HT \textsubscript{1A} receptor expression (Bethea et al. 2002; Bouali et al. 2003; Parsey et al. 2002; Rubinow et al. 1998; van Honk et al. 2005; Zhang et al. 1999). Two patients and two controls were excluded from data analysis because of incomplete dynamic PET data (one SAD patient and one HC for technical reasons), missing MR data (one HC), and drug consumption prior to the scan (one SAD patient). The analysis group comprised 12 medication-free outpatients [mean age (± S.D.) 30.25 ± 6.41 yr, range 23–44 yr] with SAD and 18 HCs (27.00 ± 5.84 yr, range 19–44 yr). Further details about sample characteristics and psychometric assessments have been described previously (Lanzenberger et al. 2007) where PET data from these subjects has been published. At the screening visit all volunteers underwent a medical examination, including assessment of general physical and neurological status and medical history, routine laboratory tests in order to exclude subjects with relevant abnormalities, history of drug abuse and/or psychiatric comorbidities. Diagnoses according to DSM-IV-TR criteria were established via a routine
clinical interview, the MINI International Neuropsychiatric Interview (Sheehan et al. 1998) and the Spielberger State-Trait Anxiety Inventory (Spielberger & Vagg, 1984). All patients were naive to psychotropic drug treatment including benzodiazepines (except for one, who had been off SSRI medication for 3 months prior to the PET measurement) in order to exclude treatment effects (Bhagwagar et al. 2004; Rabiner et al. 2000, 2002, 2004; Riad et al. 2004). Participants gave written informed consent after the procedures had been fully explained at the screening visit and they received reimbursement for their participation. The study was approved by the Ethics Committee at the General Hospital and the University of Vienna.

Radiochemistry of [carbonyl-^11^C]WAY-100635

The radiochemical preparation of [carbonyl-^11^C]WAY-100635 was performed at the Cyclotron Unit of the PET centre, as described previously by Wadsak et al. (2007). In brief, we used a method introduced by McCarron et al. (1996) with slight modifications (Matarrese et al. 2002; Mien et al. 2005) applying a fully automated PET synthesizer (GE Healthcare, Sweden). The injected dose was 405.35 ± 32.16 MBq (mean ± S.D., n=34) in the study. Specific radioactivity at the time of injection was 25.99 ± 18.18 GBq/μmol. The injected weight of precursors WAY-100634 and unlabelled WAY-100635 was 6.40 ± 3.58 and 11.86 ± 11.07 μg, respectively. The radiochemical purity of WAY-100635 was 98 ± 1%.

PET image acquisition

PET measurements were performed on a GE Advance PET scanner at the Department of Nuclear Medicine, Medical University of Vienna, Austria. Polyurethane moulded cushions and straps around the forehead and chin minimized head movements. A transmission moulded cushions and straps around the forehead and chin minimized head movements. A transmission scan of 5-min duration, using a retractable ⁴⁸Ge ring source, was performed in 2D mode for correction of tissue attenuation. Dynamic PET scans were acquired in 3D mode and measurements started simultaneously with an intravenous bolus injection of [carbonyl-^11^C]WAY-100635 in a phosphate-buffered saline. A series of 30 successive time-frames (15 × 1 min, 15 × 5 min) was collected resulting in a total acquisition time of 90 min. The emission data were scatter-corrected. Thirty-five contiguous slices (matrix 128 × 128) with a slice thickness of 4.25 mm were reconstructed using an iterative filtered back-projection algorithm. The final reconstructed volume had a spatial resolution of 4.36 mm full-width half-maximum (FWHM) at the centre of the field of view (FOV).

Hormone assay

Considering the diurnal rhythm and the influence of time awake on total cortisol plasma levels (Kudielka et al. 2005), the first blood sampling was performed at 08:51 hours ± 72.6 min (mean ± S.D.), approximately 90 min after awakening on the day of PET scans. A second sample was taken 21 ± 10.4 min later. Hormonal levels used for statistics are the mean values of both samples which were taken and measured independently. Assays were performed using the E170 module (Roche E170 Modular Analytical System®, USA) as published elsewhere in a multicentre study (Bieglmayer et al. 2004). Electrochemoluminiscence (ECLI A) was used for quantification of total cortisol plasma levels. The lower limit of sensitivity was 0.04 μg/dl and the inter-assay coefficient of variation (CV) was 6%.

Tracer kinetic modelling of [carbonyl-^11^C]WAY-100635 emission data

For quantification of the 5-HT₁₅ receptor BP₅₀ (Innis et al. 2007) the kinetic modelling tool of the biomedical image quantification software PMOD 2.7 was used (Mikołajczyk et al. 1998). The Simplified Reference Tissue Model (SRTM), based on a two-tissue compartmental model (Gunn et al. 1998; Lammerstma & Hum, 1996; Parsey et al. 2000), was applied using the cerebellar grey matter (GM) as the reference region, due to its low 5-HT₁₅ receptor density (Hall et al. 1997). Because of the current controversy concerning modelling (Hirvonen et al. 2007; Parsey et al. 2005), we further quantified 5-HT₁₅ BP₅₀ using cerebellar white matter. Decay-corrected time–activity curves (TACs) were obtained using the 30 frames of the dynamic PET data and the 3D regions of interest (ROIs). We calculated the regional BP₅₀ and the regional relative delivery of the radioligand normalized to the cerebellum (R₁, defined as KᵣROI/KᵣREF). Right and left ROIs (except for the raphe region and the medial orbitofrontal cortex) were combined to improve signal-to-noise ratio.

ROI analysis

The 30 frames of dynamic PET imaging were summed (PET integral image, PET₅₀₀₀₀₅₃ for MRI-PET co-registration. Structural magnetic resonance images (MPRAGE sequence, 256 × 256 matrix, 0.78 × 0.86 mm voxel size, slice thickness 1.56 mm, 128 slices) acquired
for each subject on a 3 T MR scanner (Bruker BioSpin, Germany) were co-registered to the PET ADD images using SPM5 (Meyer et al. 1999). Eight a priori-defined ROIs (six for post-synaptic, one for presynaptic 5-HT\textsubscript{1A} receptors and one control region without a relevant 5-HT\textsubscript{1A} receptor density) were drawn on these co-registered MR images (Fink et al. 2008; Stein et al. 2008) using a triplanar tracing technique (see Fig. 1) and PMOD 2.7 (http://www.pmod.com). The anterior (mean ± S.D., 2.1 ± 0.7 cm³) and posterior (1.4 ± 0.1 cm³) cingulate cortices, hippocampus head (1.2 ± 0.2 cm³), amygdala (1.5 ± 0.3 cm³), medial orbitofrontal cortex (9.0 ± 2.2 cm³), and one region of reference in the cerebellum (21.6 ± 3.0 cm³) were delineated using anatomical criteria adopted from Bremner et al. (1998). The cerebellum was delineated as four separated ROIs, two for each hemisphere, with a fixed volume of 4.5 cm³ as described by Parsey et al. (2005). The cerebellar ROIs were placed on PET signal intensities representing cerebellar GM (excluding vermis and venous sinus) and cerebellar white matter. Regarding the central role of the retrosplenial region and ventral posterior cingulate cortex in the processing of self-relevant emotional contents including episodic memory, we traced the retrosplenial region (0.6 ± 0.1 cm³) using criteria from Vogt et al. (2006). The raphe region (fixed ROI, 0.75 cm³), in the midbrain was directly traced on the PET ADD image according to standard procedures. Glucocorticoid levels have been shown to correlate with hippocampal volume (Buchanan et al. 2004; Lupien et al. 1998), therefore we additionally traced fixed ROIs for the hippocampus head (1.2 cm³) and amygdala (1.3 cm³).

To exclude potential bias from differences in GM volume, voxel-based morphometry (VBM) analysis was performed for the a priori-defined ROIs using the VBM toolbox 5.1 (http://dbm.neuro.uni-jena.de/vbm/). After normalizing individual MRI scans to

![Fig. 1. Parametric 5-HT\textsubscript{1A} receptor distribution superimposed on a high-resolution single-subject structural MR image from a representative healthy control subject. (a) Coronal, (b) sagittal, and (c) axial views and (d) 3D projection. Regions of interest used are indicated with white lines. Corresponding sections are indicated by a white cross. 5-HT\textsubscript{1A} receptor binding potential values are given in the colour scale. Low levels of 5-HT\textsubscript{1A} receptors are found in the primary sensory areas, high levels in the mesio-temporal region and limbic areas including the hippocampus (HIP) and amygdala (AMY), lateral temporal cortex, dorsolateral prefrontal area, orbitofrontal cortex (OFC) and subgenual anterior cingulate cortex (ACC). PCC, posterior cingulate cortex; RSC, retrosplenial cortex; DRN, dorsal raphe nucleus; CER, cerebellum (reference region).]
Montreal Neurological Institute (MNI) space, GM segments were extracted and smoothed with a Gaussian kernel of 8 mm FWHM. Group analysis was performed in SPM5 with correction for multiple comparisons (false discovery rate, \( p < 0.05 \)). Differences between HCs and patients were calculated by independent-sample \( t \) test. The influence of cortisol on GM volume was assessed by linear regression analysis for each group separately.

**Statistical analysis of ROI data**

SPSS version 12.0.1 (SPSS Inc., USA) was used for statistical analyses. Levene’s test for equality of variance and the Kolmogorov–Smirnov test for normal distribution were performed prior to statistical analyses. Significance levels were set at \( p < 0.05 \). The partial correlation analysis between cortisol and regional 5-HT \(_{1A}\) BP\(_{ND}\) was performed one-tailed according to the huge database on rodents and on non-human primates demonstrating a negative correlation. Age-dependent effects on regional perfusion, tracer delivery, BP\(_{ND}\) distribution (Rabiner et al. 2002; Tauscher et al. 2001) and hormonal plasma levels are possible, therefore the partial correlation was controlled for age and the radiochemical variables including injected activity, specific activity and the weight of WAY-100635. Considering the effects of cortisol on neurogenesis and brain structure (de Kloet et al. 2005; McEwen, 2000), we performed a partial correlation analysis between regional R1 values and cortisol plasma levels to exclude effects on regional BP\(_{ND}\) due to tracer delivery. Significance levels were corrected for multiple comparisons using Bonferroni–Holm adjusted thresholds. To compare correlation coefficients between patients and HCs, \( r \) values were normalized (\( r' \)) using Fisher’s \( r \)-to-\( z \) transformation and a \( Z \) statistic was calculated between regional correlation coefficients of the study populations (Howell, 2002).

**Statistical parametric mapping (SPM) analysis**

In an exploratory, rater-independent approach we verified the findings of the ROI analysis. For this whole-brain voxel-wise quantification we used the modelling tool pxmod of PMOD 2.7 (Mikolajczyk et al. 1998). The 5-HT\(_{1A}\) BP\(_{ND}\) of each brain voxel was calculated using SRTM with the anterior cingulate cortex as a 5-HT\(_{1A}\) receptor-rich region and the cerebellum as a reference region expressing very low 5-HT\(_{1A}\) receptor levels (Gunn et al. 1998; Parsey et al. 2000, 2005; Rabiner et al. 2002; Tauscher et al. 2002; Varnas et al. 2004).

The SPM software package (http://www.fil.ion.ucl.ac.uk/spm/) was used for (1) the generation of a ligand-specific, normalized 5-HT\(_{1A}\) BP\(_{ND}\) template (Andreasen et al. 1996) the spatial normalization of individual 5-HT\(_{1A}\) BP\(_{ND}\) maps to the standard MNI (Mnie-Filali et al. 2007) stereotactic brain space implemented in SPM5, and (3) a statistical group analysis of parametric data. The 5-HT\(_{1A}\) BP\(_{ND}\) template was created according to the method described by Meyer et al. (1999) and is described in detail elsewhere (Stein et al. 2008). The normalized parametric 5-HT\(_{1A}\) BP\(_{ND}\) maps of patients with social phobia (smoothed with a Gaussian filter of 8 mm FWHM) were included in the correlation analysis (linear regression) using cortisol levels as covariates. We did not apply grand mean scaling, global normalization or corrections for age or radiochemical variables in SPM. The results of the correlation are displayed as parametric maps using a threshold of \( p = 0.001 \) and an extent threshold of 100 voxels (see Fig. 3).

**Results**

**Hormonal data**

Mean cortisol plasma levels were significantly lower (−20.4%; \( p = 0.016 \), unpaired \( t \) test, two-tailed) in patients with social phobia (15.32 ± 4.70 µg/dl, mean ± s.d.) than HC subjects (19.24 ± 4.14 µg/dl). There were no significant correlations between cortisol plasma levels and Spielberger state anxiety scores \((r = −0.160, p = 0.639, \text{age adjusted})\), but there were between cortisol plasma levels and Spielberger trait anxiety scores \((r = −0.618, p = 0.043, \text{age adjusted})\) in patients with social phobia. Conversely, in HC subjects there was neither a significant correlation between cortisol plasma level and Spielberger state nor trait anxiety scores \((r = 0.017, p > 0.05 \text{ and } r = −0.189, p > 0.05, \text{age adjusted, respectively})\).

**ROI analysis: relationship between regional 5-HT\(_{1A}\) BP\(_{ND}\) and hormone plasma levels**

In patients with social phobia the partial correlation analysis, controlled for age and radiochemical variables, revealed a highly significant negative correlation between cortisol plasma levels and regional 5-HT\(_{1A}\) BP\(_{ND}\) in the hippocampal head \((r = −0.80, p = 0.009)\), amygdala \((r = −0.93, p = 0.0004)\), retrosplenial cortex \((r = −0.85, p = 0.004)\) that survived Bonferroni–Holm correction. Details are shown in Table 1 and Fig. 2. The results improved slightly in the hippocampus \((r = −0.829, p = 0.0054)\), but not in the amygdala \((r = −0.902, p = 0.0011)\) when applying fixed ROIs, indicating no bias introduced by ROI drawing.
In the HC group, we found a significant correlation in the amygdala ($r = 0.49$, $p = 0.04$), the retrosplenial cortex ($r = 0.48$, $p = 0.04$), the anterior cingulate cortex ($r = 0.56$, $p = 0.02$) and the dorsal raphe nucleus ($r = 0.54$, $p = 0.02$), but significance levels did not survive Bonferroni–Holm correction. Comparing correlation coefficients between patients and HC subjects revealed significant group differences in the association within the amygdala ($z = 2.17$, $p = 0.0067$), hippocampus ($z = 2.05$, $p = 0.04$) and a trend for the retrosplenial cortex ($z = 1.7$, $p = 0.089$). Differences in the amygdala also survived correction for multiple comparisons.

There was no significant correlation between regional tracer delivery (R1) values and plasma cortisol levels in each ROI calculated for patients, HCs and pooled data ($p > 0.05$) indicating no bias due to tracer delivery effects.

When using cerebellar white matter as reference region for the calculation of 5-HT$_{1A}$ receptor binding, significant correlations were still found in the amygdala ($r = -0.71$, $p = 0.025$), hippocampus ($r = -0.65$, $p = 0.042$), retrosplenial cortex ($r = -0.8$, $p = 0.009$) and medial orbitofrontal cortex ($r = -0.64$, $p = 0.044$) for patients with social phobia. However, correlations did not survive correction for multiple comparisons. For HC subjects all correlations were revealed to be non-significant ($p > 0.05$).

**SPM analysis**

Results are shown in Fig. 3 using a threshold of $p < 0.001$ ($T = 3.72$, $n = 12$ SAD patients) and a cluster size minimum (extent threshold) of 100 voxels. The post-hoc SPM results were similar to those obtained with the ROI analysis. Significant clusters ($p < 0.001$) are superimposed on the high resolution T1-weighted MNI brain implemented in SPM5 (Fig. 3a, b, left) and the 5-HT$_{1A}$ receptor BP$_{ND}$ template (Fig. 3c) using a triplanar view. Corresponding areas are indicated by a blue cross. The SPM analysis confirms a significant relationship between cortisol plasma levels and the 5-HT$_{1A}$ BP$_{ND}$ in the hippocampus–amygdala region and the retrosplenial cortex, showing highly symmetrical patterns in both hemispheres. The SPM ‘glass brain’ (Fig. 3a, b, right) additionally revealed areas including the lateral parietal, lateral frontal and tempo-rabo-basal cortices that were not investigated in the ROI-based approach. Consistent with the ROI results, there was no significant cluster in the anterior cingulate cortex, but a small cluster in the posterior cingulate cortex, indicating an excellent concordance in both positive and negative findings in SPM and the ROI-based approach.

**VBM analysis**

VBM analysis showed no significant differences in GM volume between HC subjects and patients nor significant correlations between cortisol levels and GM volume. Even at uncorrected thresholds ($p < 0.001$), no main effects were found for any of the regions examined. However, using the lower threshold ($p < 0.001$) positive correlations between cortisol levels and GM volume were identified within the patient group for the superior medial frontal gyrus as well as other regions.

**Table 1. Correlation between cortisol plasma levels and regional 5-HT$_{1A}$ receptor binding**

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Healthy controls ($n = 18$)</th>
<th>Social phobia ($n = 12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ $p$</td>
<td>$r$ $p$</td>
</tr>
<tr>
<td>Amygdalaa</td>
<td>-0.49 0.04</td>
<td>-0.93* 0.0004</td>
</tr>
<tr>
<td>Hippocampus heada</td>
<td>-0.22 0.22</td>
<td>-0.8* 0.009</td>
</tr>
<tr>
<td>Retrosplenial cortex</td>
<td>-0.48 0.04</td>
<td>-0.85* 0.004</td>
</tr>
<tr>
<td>Medial orbitofrontal cortex</td>
<td>-0.37 0.094</td>
<td>-0.71 0.024</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>-0.56 0.02</td>
<td>-0.58 0.068</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>-0.36 0.1</td>
<td>-0.47 0.123</td>
</tr>
<tr>
<td>Dorsal raphe nucleus</td>
<td>-0.54 0.02</td>
<td>-0.27 0.258</td>
</tr>
</tbody>
</table>

* Regions in which correlations are significantly different between patients and healthy controls.

* $p$ values surviving the correction for multiple comparisons implying seven ROIs by means of Bonferroni–Holm.
Discussion

To our knowledge these data demonstrate, for the first time, a negative correlation between cortisol plasma levels and regional 5-HT\textsubscript{1A} receptor binding in humans, which confirms our central hypothesis.

In patients with social phobia, we have demonstrated a highly significant negative correlation between cortisol plasma levels and regional 5-HT\textsubscript{1A} receptor binding in the amygdala, the hippocampal head, and the retrosplenial cortex which was found both in ROI-based and independent voxel-wise approaches. For HC subjects, this association was generally weaker and group comparison between patients and controls showed significantly different correlations within the amygdala and hippocampus. However, the fact that correlations in HC subjects did not survive correction for multiple comparisons might be attributed to the small sample size. A negative correlation between cortisol plasma levels and regional 5-HT\textsubscript{1A} receptor binding in healthy humans might therefore not be ruled out and should be investigated in a greater sample in future studies.

The results in patients with social phobia are in agreement with studies in rodents, which report a region-specific down-regulation of hippocampal 5-HT\textsubscript{1A} receptor binding with a stress-related or treatment-induced change of the glucocorticoid plasma level (Andrews et al. 2004; Fernandes et al. 1997; Harvey et al. 2003; Judge et al. 2004; Lopez et al. 1998; Meijer & de Kloet, 1998).

We can exclude the conjecture that the area-specific results are simply based on higher signal-to-noise ratios of PET data in regions expressing high levels of 5-HT\textsubscript{1A} receptors (Rabiner et al. 2002; Varnas et al. 2004). The highest correlation coefficients and significance levels were found in the amygdala, which showed a medium level (3.89 \pm 1.16, mean \pm S.D.) of 5-HT\textsubscript{1A} receptor BP\textsubscript{ND} compared to the neighbouring hippocampus head (6.03 \pm 1.97), and a similar level to the anterior cingulate (3.87 \pm 1.00) and medial orbitofrontal (3.58 \pm 0.93) cortices. This confirms an area-specific relationship between cortisol and 5-HT\textsubscript{1A} receptor BP\textsubscript{ND} independent of absolute 5-HT\textsubscript{1A} BP\textsubscript{ND} levels in each ROI.

As shown in the SPM group analysis (Fig. 3), the central role of the retrosplenic region in processing self-relevant emotional contents such as episodic memory (Gilboa et al. 2004; Vogt et al. 2006) is mirrored by the highly significant correlation between as the bilateral temporal pole and medial temporal gyrus.
cortisol and 5-HT$_1$A BP$_{ND}$ in social phobia (see Figs 2c, 3b). We would like to emphasize the congruity between ROI-based results (Fig. 2a–c) and the SPM group analysis (see Fig. 3, SPM glass brain) as exemplified in the mesio-temporal hippocampus–amygdala region and the retrosplenial cortex.

In contrast to our results, several studies in healthy and depressed human subjects found no significant correlations between 5-HT$_1$A BP in different brain regions and cortisol plasma levels (Bhagwagar et al. 2003; Drevets et al. 1999; Montgomery et al. 2001). This discrepancy might partly be caused by the specific experimental conditions of the different studies. For example, Bhagwagar et al. (2003) and Montgomery et al. (2001) investigated 5-HT$_1$A Receptor binding after administration of hydrocortisone, which binds equally to mineralocorticoid and glucocorticoid receptors. As suggested by preclinical studies, the two receptor subtypes may have an opposing effect on 5-HT$_1$A expression (Chalmers et al. 1993; Mendelson & McEwen, 1991). Therefore, it is possible that equal activation of both receptor subtypes might cancel out the effects of each other.

By contrast, endogenous cortisol binds to the human mineralocorticoid $(K_i=0.13 \text{ nM})$ with a 100-fold higher affinity than glucocorticoid receptors $(K_i=15 \text{ nM})$ (Rupprecht et al. 1993). Therefore, the balance between mineralocorticoid and glucocorticoid receptor activation and the heterodimerization of both receptor subtypes regulates the cortisol-induced transcriptional repression of 5-HT$_1$A receptor expression (Korte, 2001; Liu et al. 1995; Meijer & de Kloet, 1998; Ou et al. 2001; Savory et al. 2001; Webster & Cidlowski, 1999). In addition, as corticotropin-releasing hormone (CRH) is an important regulator of the mineralocorticoid receptor expression, cortisol increases the
mineralocorticoid receptor level and shifts the balance between mineralocorticoid and glucocorticoid receptor activation (Gesing et al. 2001; Muller et al. 2003).

**Hippocampus and cortisol**

The special relevance of the hippocampus is that this limbic region has both the highest mineralocorticoid and glucocorticoid receptor densities in the brain, and also the highest mineralocorticoid receptor levels outside the hypothalamus (de Kloet et al. 1998). This area-specific expression of high mineralocorticoid receptor densities, restricted to the hippocampus and a few smaller areas such as the hypothalamic nuclei (de Kloet et al. 1998; Morimoto et al. 1996; Otte et al. 2003), is an important mechanism in the region-specific regulation of 5-HT₁A receptors (Neumaier et al. 2000). As mentioned previously, the balance between mineralocorticoid and glucocorticoid receptor activation and the heterodimerization of both receptor subtypes regulates the cortisol-induced transcriptional repression of the hippocampal 5-HT₁A receptor expression (Korte, 2001; Liu et al. 1995; Meijer & de Kloet, 1998; Ou et al. 2001; Savory et al. 2001; Webster & Cidlowski, 1999). Pharmacological challenge studies using mineralocorticoid or glucocorticoid agonists in humans have further confirmed that the balance of mineralocorticoid and glucocorticoid receptors modulates the serotonin receptor subtype binding sites as shown in patients with major depression (Murphy et al. 1993; Young et al. 2003).

We found a highly significant correlation between hippocampal 5-HT₁A receptor B₉ ND and the cortisol plasma level in patients with social phobia (see Fig. 2a showing the ROI-based analysis, Fig. 3a showing the SPM analysis). However, in contrast to other limbic and paralimbic regions, there was no significant reduction of 5-HT₁A receptor B₉ ND or ROI size in patients with social phobia compared to HCs (Lanzenberger et al. 2007), which in turn indicates no significant reduction of hippocampal 5-HT₁A receptor B₉ ND by specific social stress-related change of cortisol levels in social phobia. This is consistent with a PET study on post-traumatic stress disorder reporting no change in 5-HT₁A receptor binding in patients (Bonne et al. 2005).

**Amygdala**

Several functional MRI (fMRI) and PET studies have shown hyper-responsiveness and hyperexcitability of the amygdala in stress-related affective disorders (Shin et al. 2005) and social phobia (Lorberbaum et al. 2004; Schneider et al. 1999; Tillfors et al. 2001). Efferent pathways from the amygdala and hippocampus regulate the expression of CRH in the hypothalamus (Schulkin et al. 1998), therefore the balance between CRH in the amygdala and hypothalamus regulates stress- and anxiety-associated cortisol secretion via positive and negative feedback loops. This balance might be altered in patients suffering from SAD given the specific hyperreactivity of the amygdala in this patient group. The area-specific and high correlation between peripheral cortisol level and the 5-HT₁A B₉ ND of the amygdala in social phobia is compatible with a strong direct or indirect feedback loop between the amygdala and the adrenal gland. In addition, elevated CRH levels in the amygdala increase the local glucocorticoid level that suppresses the local 5-HT₁A receptor expression (Linhorst, 2005; Meijer et al. 2000; van Gaalen et al. 2002). The activation of 5-HT₁A receptors inhibits GABA release in the amygdala (Koyama et al. 2002), therefore the reduced 5-HT₁A receptor levels in the amygdala found in our patients with social phobia might be linked to a reduced GABAergic inhibition in limbic areas (Amargos-Bosch et al. 2004) associated with elevated vulnerability to social stress.

**Serotonergic neurotransmission and cortisol**

Hormones of the HPA axis modulate serotonergic transmission on several neural levels (Millan, 2003; Pfennig et al. 2005). Studies in rodents have found a distinct repression of 5-HT₁A receptor transcription by glucocorticoids in the hippocampus but not in the raphe nuclei (de Kloet et al. 2000; Holmes et al. 1995; Le Corre et al. 1997; Neumaier et al. 2000). This would imply that cortisol reduces mainly postsynaptic 5-HT₁A receptor binding sites such as the hippocampus (Ogren et al. 2008), although high levels of glucocorticoid receptors have been found in the dorsal raphe nucleus (Morimoto et al. 1996). This is consistent with our results showing highly significant correlations between cortisol plasma levels and postsynaptic hippocampal 5-HT₁A receptors, but not presynaptic 5-HT₁A receptors in the raphe region. The area-specific up- and down-regulation of 5-HT₁A receptors by steroid hormone levels shift the relative influence of serotonergic transmission in different brain areas. This mechanism allows area-specific changes of inhibitory serotonergic effects in limbic areas, with unchanged serotonergic tone regulated by presynaptic 5-HT₁A receptors in the dorsal raphe nucleus (Amargos-Bosch et al. 2004). To conclude, the reciprocal effects of glucocorticoids on inhibitory 5-HT₁A receptor (down-regulation) and excitatory
5-HT₁A receptor (up-regulation) expression implicate an increase of excitatory serotonergic influence on glutaminergic and GABAergic neurons by cortisol (Farisse et al. 2000; Fernandes et al. 1997). The effect size of cortisol-dependent changes in excitatory serotonergic influence might be highest in the hippocampus, amygdala and retrosplenial cortex in SAD patients, given the area-specific differences in steroid receptor distribution.

Richardson-Jones et al. (2010) recently demonstrated that reduced 5-HT₁A expression is associated with an increased autonomic response to an acute stressor. Our finding of no correlation between anxiety scores and regional 5-HT₁A BPND might therefore suggest that reduced 5-HT₁A binding increases the vulnerability in the pathogenesis of anxiety disorders and depression independent of current anxious feelings, which is in line with studies suggesting a role of 5-HT₁A receptors in the early development of anxiety-related circuitry (Gross et al. 2002). Regarding alterations in cortisol levels in SAD, the direction of changes remains a matter of debate since studies report both increases (Kallen et al. 2008; Roelofs et al. 2009; van West et al. 2008) and decreases (Furlan et al. 2001). Consistent with a recent study covering around 100 patients (de Rooij et al. 2009), we found reduced cortisol plasma levels in patients suffering from SAD as well as a negative correlation between trait anxiety scores and the cortisol plasma levels. In contrast, the reduced 5-HT₁A receptor binding seems to be a consistent finding across several anxiety disorders (Lanzenberger et al. 2007; Nash et al. 2008; Neumeister et al. 2004).

Still, alterations in any of these two variables (i.e. cortisol and 5-HT₁A binding) would not necessarily change the correlation per se, but only shift the regression line. The fact that we indeed found significantly stronger correlations in SAD patients indicates an increased dependency of 5-HT₁A receptors on cortisol levels within this patient group. However, this might be modulated by an independent regulatory factor which is altered in patients. In turn, this would lead to a separation of the two groups rather than a continuum from HCs to patients with SAD, and hence, explain our finding of a negative correlation despite reduced 5-HT₁A binding and cortisol levels. Specifically, our data might suggest an altered regulation of 5-HT₁A receptor expression through impaired corticosterone modulation of transcriptional repression (Meijer, 2002; Ou et al. 2001). This potentially results in a higher vulnerability of 5-HT₁A receptor expression to stress-induced cortisol changes in SAD patients.

**Limitations of the study results**

The data concerning mineralocorticoid and glucocorticoid receptor distributions discussed in this paper are based on post-mortem studies (de Kloet et al. 1998, 2005; Lopez et al. 1998). In-vivo imaging of human mineralocorticoid and glucocorticoid receptor distributions and comparisons with the 5-HT₁A receptor distribution (Lopez et al. 1998) are an important aim for the further investigation of steroid hormone-dependent regulation of brain functions and dysfunctions in affective and anxiety disorders, but suitable steroid PET ligands are still lacking (Wust et al. 2003).

Methodologically, the data analysis was limited to the calculation of BPND since no arterial blood samples were obtained during the PET scans. Considering that BPND depends on non-specific binding (Ichise et al. 2001) and recent concerns using the cerebellum as reference (Hirvonen et al. 2007; Parsey et al. 2005) our findings still need to be replicated using an arterial input function. However, 5-HT₁A BP values calculated with the SRTM are linearly related to those obtained from arterial blood samples (Gunn et al. 1998). Therefore we would expect a constant shift between BPND and BP; however, a correlation is rather insensitive to such constant changes. This is also true even if absolute BP values would differ between healthy subjects and patients (Parsey et al. 2008). Specifically, this would only shift the regression line up- or downwards, but would not change the association between 5-HT₁A binding and cortisol itself.

Using cerebellar white matter as reference region, correlations were still significant for the patient group but did not survive correction for multiple comparisons, which might again be related to limited statistical power. More importantly, considering that specific 5-HT₁A receptor binding is markedly higher for all ROIs than within the cerebellar cortex (Varnas et al. 2004), the use of cerebellar white matter would hardly contribute to improve the modelling procedure. In fact, this might even add noise due to unfavourable kinetic characteristics of white matter compared to GM.

**New therapeutic approaches**

Our results are consistent with the hypothesis of an area-specific modulation of serotonergic neurotransmission by steroid hormones in humans. Area-specific co-activator and co-repressor peptides influence the steroid receptor-modulated expression of serotonergic receptors (Hultman et al. 2005; McKenna et al. 1999; Meijer, 2002; Wang et al. 2004). This area-specific modulation of serotonergic neurotransmission
in limbic areas by steroid receptor modulation could provide opportunities for new pharmacological treatment options.

Conclusion

The PET results of our study demonstrate a highly significant negative correlation between the plasma cortisol level and 5-HT1A receptor BPND. This was most pronounced in brain areas involved in anxiety processing, including limbic areas. Thus, these data indicate an area-specific modulation of serotonergic neurotransmission by cortisol in the brain.

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Statement of Interest

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