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ORIGINAL ARTICLE

The serotonin-1A receptor distribution in healthy men and women measured by PET and [*carbonyl*-^{11}C]WAY-100635

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Abstract
Purpose The higher prevalence rates of depression and anxiety disorders in women compared to men have been associated with sexual dimorphisms in the serotonergic system. The present positron emission tomography (PET) study investigated the influence of sex on the major inhibitory serotonergic receptor subtype, the serotonin-1A (5-HT$_{1A}$) receptor.
Methods Sixteen healthy women and 16 healthy men were measured using PET and the highly specific radioligand [*carbonyl*-^{11}C]WAY-100635. Effects of age or gonadal hormones were excluded by restricting the inclusion criteria to young adults and by controlling for menstrual cycle phase. The 5-HT$_{1A}$ receptor BP$_{ND}$ was quantified using (1) the 'gold standard' manual delineation approach with ten regions of interest (ROIs) and (2) a newly developed delineation method using a PET template normalized to the Montreal Neurologic Institute space with 45 ROIs based on automated anatomical labeling.
Results The 5-HT$_{1A}$ receptor BP$_{ND}$ was found equally distributed in men and women applying both the manual delineation method and the automated delineation approach. Women had lower mean BP$_{ND}$ values in every region investigated, with a borderline significant sex difference in the hypothalamus (p=0.012, uncorrected). There was a high intersubject variability of the 5-HT$_{1A}$ receptor BP$_{ND}$ within both sexes compared to the small mean differences between men and women.
Conclusions To conclude, when measured in the follicular phase, women do not differ from men in the 5-HT$_{1A}$ receptor binding. To explain the higher prevalence of affective disorders in women, further studies are needed to evaluate the relationship between hormonal status and the 5-HT$_{1A}$ receptor expression.

Keywords Positron emission tomography · 5-HT$_{1A}$ receptor · Serotonin · Sex · [Carbonyl-^{11}C]WAY-100635

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Introduction

Twice as many women compared to men suffer from major depression and anxiety disorders, which affect nearly one-fifth of the Western population and cause an immense personal, social and economic burden (see, e.g. [1]). The striking sex difference in prevalence rates appears to be independent of country and culture and cannot be entirely

explained by psychosocial factors, social support or coping style [2]. Rather, it has been demonstrated that women and men differ significantly in brain structure and function (for reviews, see [3, 4]), suggesting a higher biological susceptibility to depression in females. Interestingly, a growing body of evidence suggests that the serotonergic system, which is known to be altered in affective disorders, may be sexually dimorphic. In the brain of female rodents, a higher tryptophan content and utilization rate [5], a higher serotonin synthesis and serotonin turnover [6] and overall higher serotonin levels [7] have been demonstrated. Several human studies reported a greater responsiveness to serotonergic challenges in female participants [8]. Acute tryptophan depletion, used as an experimental model for depression, was shown to affect females to a significantly larger extent than males [9]. And also, in vivo assessment of serotonergic structure and function with positron emission tomography (PET) revealed sex differences in the serotonin neurotransmission. Major methodological advancements and the development of selective radioligands allow now for a precise quantification and localisation of serotonergic receptors and the serotonin transporter [10]. The few studies that have been conducted in human subjects, however, report findings that are in part contrasting the results obtained in rodents. Using PET and the radioligand α-[^{11}C]methyl-L-tryptophan, the serotonin synthesis rate in the brain of healthy male subjects was found to be higher than the synthesis rate in females [11]. A lower serotonin transporter binding in women was observed using the radioligand [^{11}C]MADAM [12]. A lower 5-HT2 receptor-binding capacity in women was also reported [13]. The reason for the discrepant results is still unclear.

Several lines of evidence indicate a sexual dimorphism of the serotonin-1A (5-HT$_{1A}$) receptor subtype, which is suspected to be substantially involved in the pathogenesis of depressive illness [14], anxiety disorders [15] and suicide [16]. The 5-HT$_{1A}$ receptor binding is of particular interest for psychiatry as it was shown to correlate with the treatment effect of SSRIs (selective serotonin reuptake inhibitors) as recently demonstrated by our group [17]. The 5-HT$_{1A}$ receptors serve both as somatodendritic autoreceptors on serotonergic neurons in the raphe nuclei of the brainstem and as postsynaptic heteroreceptors. The highest densities of the postsynaptic receptor are found in limbic areas (in particular in the hippocampus and the anterior cingulate cortex), while basal ganglia and the cerebellum exhibit very low densities [18]. Postsynaptically located 5-HT$_{1A}$ receptors influence a wide range of physiological and behavioural states by modulating cholinergic, dopaminergic, glutamatergic and GABAergic neurotransmitter release (for review, see [19]), while autoreceptor activation in the raphe nuclei reduces serotonergic cell firing and inhibits excitation and neural activation in targeted cortical areas [20].

With regard to sex differences, the presynaptic function of the 5-HT$_{1A}$ receptor was proposed to be decreased [21] or increased in female rodents [22]. Animal studies suggested area specific sexual dimorphisms with higher 5-HT$_{1A}$ receptor binding in females in some regions (e.g. the anterior cingulate cortex) while lower in other regions (e.g. the hippocampus) [23, 24]. A higher 5-HT$_{1A}$ receptor density in women was reported post mortem in the dorsal raphe nucleus [25] and in the prefrontal cortex [26]. Several other human post-mortem studies, however, found no gender differences in binding sites [27-29]. The few PET studies investigating sex differences in the 5-HT$_{1A}$ receptor in humans in vivo resulted in controversial findings. Using the radioligand [*carbonyl*-^{11}C]WAY-100635, either a higher 5-HT$_{1A}$ receptor-binding potential (BP$_{ND}$, "BP non displaceable" according to the nomenclature established in the consensus paper by Innis et al. [30]) in female subjects was found [12, 31] or only age-related effects without an overall influence of sex [32].

Given the controversial results, the aim of the present study was to prove the hypothesis of sex differences in the 5-HT$_{1A}$ receptor-binding potential in 32 healthy volunteers (16 male and 16 female subjects) matched to age and socio-economic status. To account for the presumable influence of gonadal hormones on the receptor binding [33], all female subjects were measured within the follicular phase of the menstrual cycle. To control for a possible effect of age [32, 34], the age range of the subjects was limited to 20–35 years. Furthermore, a newly developed method for the anatomical delineation of the 5-HT$_{1A}$ receptor maps was introduced using a tracer-specific template, a coregistered region of interest (ROI) template and automated anatomical labelling (AAL) [35].

Materials and methods

Subjects

Thirty-six healthy subjects (18 females and 18 males) participated in the PET study approved by the Ethics Committee at the Medical University of Vienna. All subjects gave written informed consent at the screening visit and were recruited from the community via advertisements. Female and male subjects were matched for age and socio-economic status. The criteria for participation were age of 20 to 35 years and physical health as assessed by a general physical examination including neurological status, electrocardiogram and a routine laboratory screening. The narrow age range was chosen to minimize possible age effects on the 5-HT$_{1A}$ receptor-binding potential [34]. Exclusion criteria comprised any chronic medication or hormonal treatment including hormonal contraception

within 6 months prior to the study, drug abuse, pregnancy, irregular menstrual cycles, abnormalities in the physical examination or any Axis I, DSM IV, psychiatric disorder as assessed by the MINI International Neuropsychiatric Interview obtained by an experienced psychiatrist [36]. Female participants were tested for pregnancy at the screening visit and before each PET measurement using an human choriogonadotropin urine test (ACON Laboratories, Inc., San Diego, CA, USA).

Four subjects were excluded from the final analysis either because of outliers in radiochemical variables (one female, one male) or because the time activity curves of the cerebellar regions exceeded the mean cerebellar binding by more than two standard deviations (one female, one male) [37]. The final statistical analysis included 16 female (age 24.1±2.6 years, mean ± SD) and 16 male subjects (age 26.2±4.2 years, mean ± SD). To control for menstrual cycle phase at PET measurement, blood samples were collected in the morning prior to the first examination, prior to PET scans and on the day of the final examination from all female participants. Plasma levels of estrogen, progesterone, testosterone, follicle stimulating hormone and luteinizing hormone (LH) were quantified by the Clinical Institute for Medical and Chemical Laboratory Diagnostics at the Medical University of Vienna (for details of standards and references, see http://www.kimcl.at). All female subjects were measured within the follicular phase, i.e. within the first 3–10 days of the menstrual cycle. The female subjects were only measured when their hormonal plasmal levels lied within the follicular reference range of 0.5–1.0 ng/ml for progesterone, 22–215 pg/ml for 17β-estradiol and 2.4–12.6 mU/ml for LH.

PET image acquisition

Subjects were measured using an ADVANCE full-ring PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) at the Department of Nuclear Medicine, Medical University of Vienna. For quantification of the 5-HT_{1A} receptor binding, the radioligand [*carbonyl*-^{11}C]WAY-100635 was chosen, a highly selective and specific 5-HT_{1A} receptor antagonist [38]. The tracer was prepared in a fully automated PET synthesizer (GE Healthcare, Uppsala, Sweden) at the Cyclotron Unit of the PET centre at the Medical University of Vienna as recently described by our group [39]. For image acquisition, the head of the subject was positioned 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 by polyurethane moulded cushions and straps around forehead and chin. A transmission scan (5 min) was performed in two-dimensional mode for correction of tissue attenuation using a retractable ^{68}Ge ring source. The three-dimensional image acquisition started simultaneously with the intravenous bolus injection of the radioligand [*carbonyl*-^{11}C]WAY-100635, soluted in phosphate-buffered saline (pH 7.4). The mean injected activity of the radioligand was 5.65±0.8 (mean ± SD) MBq/kg body weight, with a mean specific radioactivity at the time of injection of 153±117 GBq/μmol, and a radiochemical purity of 97.5±1.3%. Dynamic scans were collected in three-dimensional mode and comprised a series of 30 successive time frames (15×1 min, 15×5 min) resulting in a total acquisition time of 90 min. Data were reconstructed in a 128×128×35 matrix, with a slice thickness of 4.25 mm using an iterative filtered back-projection algorithm (FORE-ITER). The spatial resolution of the scanner was 4.36 mm full-width at half-maximum at the centre of the FOV.

MR image acquisition

High-resolution T1-weighted images were acquired from all subjects using a 3-tesla whole-body MEDSPEC S300 MR-scanner (Bruker BioSpin, Ettlingen, Germany) and a magnetization-prepared rapid gradient-echo sequence (128 slices, 256×256 matrix, slice thickness 1.56 mm, voxel size 0.78×0.86 mm). The structural MR images were coregistered to summed PET images (PET_{ADD}) for definition of ROIs.

Data analysis

For quantification of the 5-HT_{1A} receptor BP_{ND}, we applied the Simplified Reference Tissue Model (SRTM) [40–42] as implemented in PMOD 2.9 (PMOD Technologies Ltd., Zurich, Switzerland, http://www.pmod.com) [43] using the cerebellum as reference region. Furthermore, in a second approach, the 5-HT_{1A} receptor BP_{ND} was quantified by applying the non-invasive Logan Plot method [44]. Cerebellar time activity curves were normalized to its peak to identify and exclude subjects with high specific binding in the reference region [37]. Two complementary approaches for the delineation of ROIs were applied, a manual delineation method with 10 ROIs (including the cerebellum as reference region) and an ROI-template-based, automated method with 45 ROIs (including the cerebellum as reference region), which was used to exclude possible bias caused by manual delineation.

Manual delineation of ROIs

For the manual delineation method, the individual MR images were coregistered to individual summed PET images (PET_{ADD}) of 30 dynamic time frames using Statistical Parametric Mapping software (SPM2, http://www.fil.ion.ucl.ac.uk/spm/) [45, 46]. Nine regions of

interest known for their high 5-HT_{1A} receptor density [18] were delineated on the coregistered MR images according to the standardised anatomical criteria established by Bremner et al. [47] and described by our group [15, 17]. Delineation was done by one investigator (C.S.) who was blind to gender of the subjects. The regions of interest (given in Fig. 3) included the anterior and posterior cingulate cortices, insula, hippocampus, hypothalamus, amygdala, the medial orbitofrontal cortex, the retrosplenial cortex and the cerebellum as region of reference [48]. The raphe nuclei were defined on the PET_{ADD} image by fixing a circular volume of interest (0.08 cm^3) over the highest binding signal in the dorsal midbrain area.

Automated delineation of regions of interest

For the automated delineation method, we used an ROI-template normalized to the 5-HT_{1A} distribution map in the stereotactic space of the MNI/ICBM brain (Montreal Neurologic Institute/International Consortium for Brain Mapping) and PMOD 2.9 [45]. Individual dynamic PET data were normalized to this standardised 5-HT_{1A} distribution map that corresponded to the ROI template. All 45 ROIs of this approach were based on the anatomical AAL atlas implemented in the SPM2 software [35]. The ROIs are given in Table 1. Figures 1 and 2 show representative ROIs overlayed on the 5-HT_{1A} receptor-binding potential map. Time activity curves of the 45 regions were used for quantification in PMOD 2.9.

Statistical analysis

Statistical analyses of the regional mean 5-HT_{1A} receptor BP_{ND} were done using the software SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). The threshold of significance was set at $p<0.05$, all tests were two-tailed. To control for normal distribution and equality of co-variance, the Kolmogorov–Smirnov test and the Levene's test were performed, respectively. Independent-samples t tests were used to test for sex differences in age, radiochemical variables and the normalized regional tracer delivery (R_1). Two-tailed Pearson product-moment correlation coefficients were calculated to test for a possible effect of age or radiochemical variables on the 5-HT_{1A} receptor BP_{ND} (i.e. injected activity, radiochemical purity, weight of WAY-100634, weight of unlabelled WAY-100635 and specific activity of the radioligand). Variables without influence on the 5-HT_{1A} receptor-binding potential were dropped from further analysis. To evaluate a mean effect of sex on the 5-HT_{1A} receptor BP_{ND}, a two-way analysis of variance (ANOVA) was conducted using sex as between-subject factor, region as within-subject factor, subjects as random factor and the interaction term sex by region. For an additional, exploratory analysis, independent-sample t tests, with sex as independent variable, were conducted in each region of interest. Bonferroni adjustment for multiple testing was used to correct for type I error. Both delineation methods were statistically analysed in the described way.

Results

The regional 5-HT_{1A} receptor BP_{ND} values separated for men and women are given in Fig. 3 for the manual delineation method and in Table 1 for the ROI-template-based approach. The regional distribution of the 5-HT_{1A} receptor BP_{ND} was in accordance with published in vivo and post-mortem studies showing the highest 5-HT_{1A} receptor expression in limbic areas as in the hippocampus and the anterior cingulate cortex [48]. Female and male participants did not differ significantly by age, radiochemical variables, regional tracer delivery (R_1) or binding in the reference region ($p>0.05$). There was no effect of age or radiochemical variables on the 5-HT_{1A} receptor BP_{ND} (Pearson correlation, $p>0.05$).

Results of the manual delineation method

Using the manual delineation method, the mean 5-HT_{1A} receptor BP_{ND} over all regions of interest was 3.38±0.07 (mean ± SE) for men and 3.11±0.07 (mean ± SE) for women. The two-way ANOVA did not confirm the hypothesis of a main effect for sex ($F_{1,30}=1.3$, $p=0.278$). However, a slightly lower mean 5-HT_{1A} receptor BP_{ND} was observed in all regions of interest in females (Fig. 3). Also, no significant interaction was found between sex and region. Post hoc independent-samples t tests done in nine manually drawn regions of interest (anterior and posterior cingulate cortex, amygdala, hippocampus, hypothalamus, insula, orbitofrontal cortex, retrosplenial cortex and raphe nuclei) did not reveal any significant sex differences except for a trend in the hypothalamus ($t_{30}=2.7$; $p=0.012$) that did not withstand the Bonferroni correction (adjusted significance level of $p<0.0056$). The results do not support the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} or in the 5-HT_{1A} receptor distribution.

Results of the automated delineation method

Using the automated delineation method, the mean 5-HT_{1A} receptor BP_{ND} over all regions of interest was 3.54±0.05 (mean ± SE) for men and 3.32±0.05 (mean ± SE) for women. The two-way ANOVA did not reveal any significant effect for sex ($F_{1,30}=0.8$, $p=0.378$), and there was no significant interaction between sex and region. Post hoc independent-samples t tests done in the 45 AAL regions of

Table 1 The regional serotonin-1A (5-HT_{1A}) receptor-binding potential receptor (BP_{ND}) in men and women using the automated delineation method (SD—standard deviation)

No. region of interest	5-HT_{1A} BP_{ND} males		5-HT_{1A} BP_{ND} females	
	Mean ± SD	Range	Mean ± SD	Range
Central region				
1 Precentral gyrus	2.63±0.43	1.88–3.27	2.51±0.54	1.70–3.70
2 Postcentral gyrus	2.94±0.54	1.94–3.81	2.74±0.55	1.79–3.84
3 Rolandic operculum	4.50±0.90	3.01–5.90	4.22±0.78	2.78–5.71
Frontal lobe				
4 Superior frontal gyrus	3.32±0.54	2.31–4.26	3.22±0.70	2.09–4.80
5 Middle frontal gyrus	3.51±0.60	2.54–4.51	3.41±0.74	2.34–5.31
6 Inferior frontal gyrus, opercular part	3.66±0.62	2.40–4.85	3.50±0.68	2.43–5.15
7 Inferior frontal gyrus, triangular part	3.25±0.52	2.25–4.11	3.17±0.64	2.10–4.51
8 Superior frontal gyrus, medial	3.56±0.60	2.31–4.59	3.48±0.75	2.30–5.18
9 Supplementary motor area	3.19±0.55	2.32–4.21	3.06±0.65	2.08–4.44
10 Paracentral lobule	3.09±0.63	1.93–4.17	3.00±0.58	2.02–4.06
11 Superior frontal gyrus, orbital part	3.76±0.64	2.73–4.79	3.63±0.75	2.49–5.34
12 Middle frontal gyrus, orbital part	3.69±0.71	2.62–4.78	3.48±0.73	2.48–5.18
13 Inferior frontal gyrus, orbital part	3.62±0.59	2.39–4.49	3.50±0.76	2.36–5.26
14 Gyrus rectus	4.70±0.81	3.15–5.85	4.54±1.06	2.95–7.03
15 Olfactory cortex	4.67±1.14	2.81–6.42	4.42±1.05	2.16–6.58
Temporal lobe				
16 Superior temporal gyrus	4.25±0.70	2.92–5.18	4.02±0.84	2.52–5.65
17 Heschl gyrus	4.22±0.76	3.06–5.43	4.00±0.83	2.67–5.77
18 Middle temporal gyrus	4.57±0.78	3.16–5.63	4.27±0.91	2.71–6.34
19 Inferior temporal gyrus	5.01±0.86	3.53–6.30	4.60±1.01	2.77–6.56
Parietal lobe				
20 Superior parietal gyrus	3.11±0.58	2.22–4.28	3.02±0.68	1.88–4.48
21 Inferior parietal, supramarginal/angular	3.47±0.57	2.48–4.45	3.41±0.70	2.29–4.96
22 Angular gyrus	3.70±0.63	2.75–4.97	3.57±0.74	2.29–5.13
23 Supramarginal gyrus	4.05±0.74	2.87–5.31	3.80±0.72	2.57–5.11
24 Precuneus	3.36±0.57	2.44–4.38	3.27±0.64	2.16–4.68
Occipital lobe				
25 Superior occipital gyrus	2.84±0.48	2.04–3.51	2.68±0.59	1.52–3.99
26 Middle occipital gyrus	3.57±0.64	2.57–4.40	3.37±0.71	2.03–4.99
27 Inferior occipital gyrus	3.67±0.61	2.64–4.52	3.42±0.66	2.22–4.71
28 Cuneus	2.77±0.43	2.06–3.41	2.62±0.60	1.44–3.88
29 Calcarine fissure	2.47±0.46	1.57–3.08	2.33±0.43	1.42–3.14
30 Lingual gyrus	3.17±0.49	2.26–3.87	2.98±0.57	1.77–4.05
31 Fusiform gyrus	4.98±0.87	3.41–6.24	4.62±0.95	3.07–6.59
Limbic lobe				
32 Temporal pole: sup. temporal gyrus	5.11±0.95	3.16–6.53	4.77±1.07	2.90–7.32
33 Temporal pole: middle temporal gyrus	5.27±0.90	3.33–6.60	4.91±1.18	2.78–7.43
34 Anterior cingulate/paracingulate gyri	4.02±0.75	2.50–5.06	3.72±0.90	2.43–5.75
35 Median cingulate/paracingulate gyri	3.33±0.60	2.32–4.33	3.18±0.75	2.12–4.84
36 Posterior cingulate gyrus	2.80±0.55	1.94–3.90	2.47±0.62	1.30–3.65
37 Hippocampus	4.51±1.35	2.34–6.78	4.18±1.02	1.98–5.51
38 Caput hippocampi	5.04±1.76	2.21–8.09	4.62±1.32	2.10–7.00
39 Parahippocampal gyrus	5.73±1.12	3.66–7.37	5.33±1.15	3.42–7.59
40 Insula	5.12±0.90	3.53–6.40	4.76±0.96	3.18–7.03
41 Amygdala	4.41±1.02	2.61–5.63	4.06±0.98	2.42–6.15
42 Subgenual cingulum	3.97±0.96	2.74–5.67	3.63±0.87	1.96–5.00
43 Superior frontal gyrus, medial orbital	4.19±0.77	2.84–5.43	4.03±0.81	2.73–5.90
Raphe nuclei (DRN) cerebellum as region of reference	1.98±0.98	1.06–5.17	1.65±0.71	0.59–3.36

The 5-HT_{1A} receptor BP_{ND} in 45 regions was quantified using the Simplified Reference Tissue Model and the cerebellum as region of reference. An independent-samples ANOVA revealed no significant sex differences in the mean 5-HT_{1A} receptor BP_{ND} between men and women ($p>0.05$), though men had a slightly higher 5-HT_{1A} receptor BP_{ND} in every region investigated. The range of the 5-HT_{1A} receptor BP_{ND} values in each region of interest demonstrates a high intersubject variability consistent to other PET studies investigating this receptor subtype [37]. The numbering of the ROIs corresponds to the PET templates shown in Figs. 1 and 2.

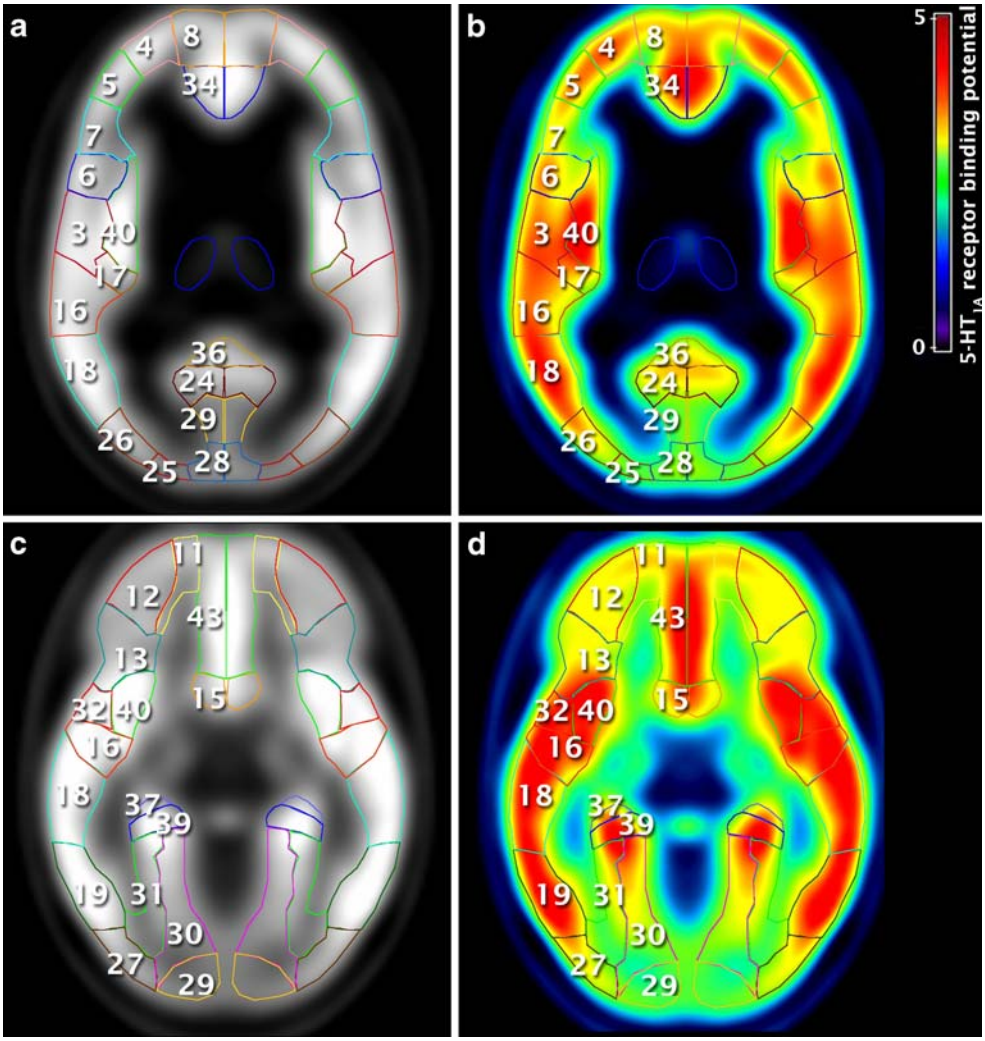

Fig. 1 PET template for automated delineation of ROIs. The 45 delineated ROIs are based on the AAL atlas implemented in the SPM2 software (toolbox) [35]. The figure shows representative ROIs on two axial slices (**a**/**b** vs. **c**/**d**) overlayed on a serotonin-1A receptor (5-HT_{1A}) receptor distribution map normalized to the MNI space either in black/white (**a**, **c**) or in colour (**b**, **d**). Low 5-HT_{1A} receptor BP_{ND} values are *dark blue* and high BP_{ND} values are *dark red* (see *colour bar*). The *numbering* of the regions corresponds to the regions of interest given in Table 1

interest did not reveal any significant sex differences (the hypothalamus, however, is not included in the AAL regions). Therefore, the results of the automated method do not support the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} or in the 5-HT_{1A} receptor distribution. A second analysis in the AAL regions applying the non-invasive Logan plot yielded similar results to the SRTM (results not shown).

Discussion

The main finding of this in vivo study is the lack of a sex-specific 5-HT_{1A} receptor binding. This in vivo investigation using PET and the highly specific radioligand [*carbonyl*-^{11}C] WAY-100635 did not confirm the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} suggested in some previous PET studies [12, 31]. Our results were obtained independently using two delineation methods for regions of interest and the non-invasive SRTM [41] and Logan Plot method [44]. The absence of sex effects in the 5-HT_{1A} receptor BP_{ND} is in line with several human post-mortem [27–29] and in vivo studies [32, 49]. Interestingly, the mean observed 5-HT_{1A} receptor BP_{ND} tended to be lower in females in all regions of interest (see Fig. 3), which is in contrast to three previous studies performed using the same radioligand in healthy subjects [12, 31, 50]. One of these studies reported a higher 5-HT_{1A} receptor BP_{ND} in females using a clearly broader age range compared to our study. The sex difference was found only when using arterial input function but not when using the non-invasive SRTM [31]. A second, subsequent study also found a higher 5-HT_{1A} receptor BP_{ND} in females using both an arterial input function and the SRTM for comparison [50]. Limits of this study were a small sample size (six females and eight males) and a broad age range in males (25–65 years), while the age range for females was quite narrow (47–52 years). Both groups did not control for the possible effects of gonadal steroids [51]. A third study, however, found a significantly higher 5-HT_{1A} receptor BP_{ND} using SRTM in females in a demographically comparable sample to ours [12]. Given the similar study design (control for hormonal status and young

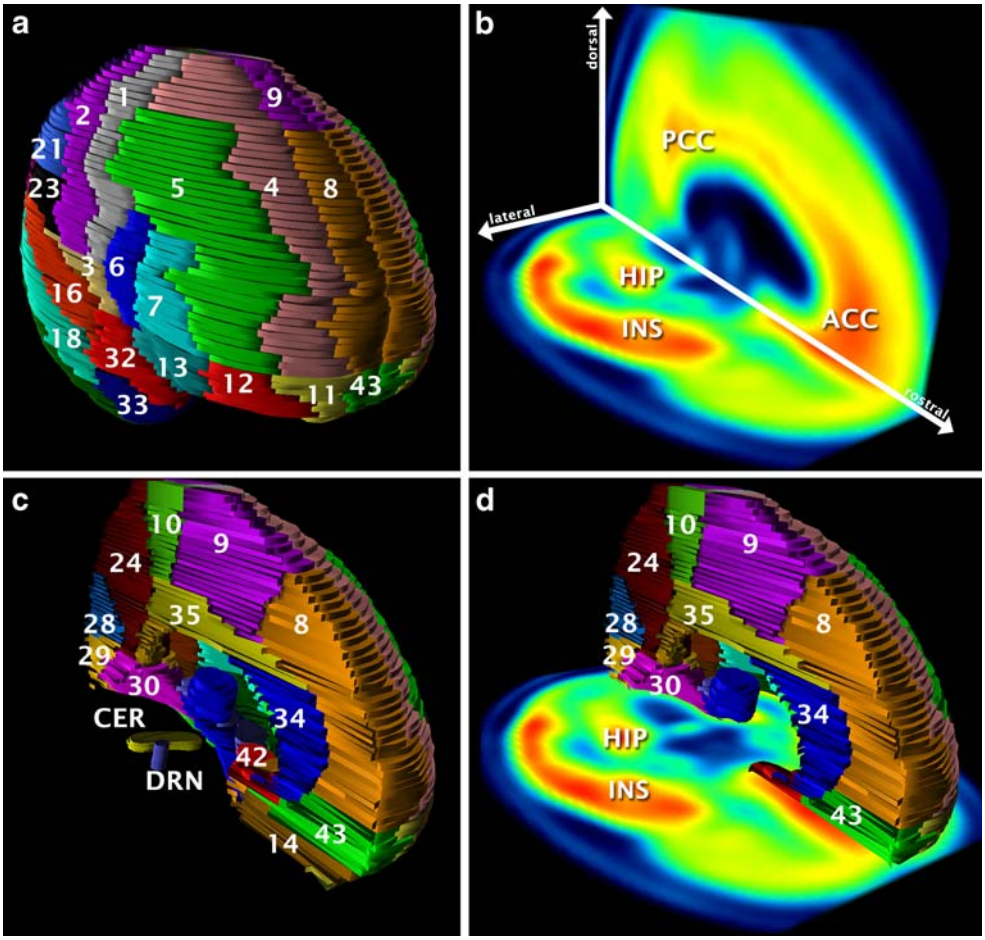

Fig. 2 A three-dimensional view on the PET template for automated delineation of ROIs. **a** Dorsolateral view on the ROI template. The *numbering* of the regions corresponds to the regions of interest given in Table 1. **b** Sagittal and an axial views of the serotonin-1A receptor (5-HT_{1A}) receptor distribution map. The *labelling* indicates the localisation of regions of interest used for the manual delineation method. *ACC* Anterior cingulate cortex, *PCC* posterior cingulate cortex, *HIP* hippocampus, *INS* insula. **c** Three-dimensional sagittal view on the ROI template. *CER* Cerebellum (region of interest), *DRN* dorsal raphe nuclei (used as an ROI for the quantification of the presynaptic 5-HT_{1A} receptor BP_{ND}). **d** Three-dimensional sagittal view on the ROI template projected on an axial slice of the 5-HT_{1A} receptor distribution map. The *numbering* of the regions corresponds to the regions of interest given in Table 1

age of the participants), it remains unclear why the suggested sex difference in the 5-HT_{1A} receptor binding was not replicated in the present investigation. Even more surprising, the mean 5-HT_{1A} receptor BP_{ND} in the present study was lower (though not significantly) in females compared to males in every region examined, which is the opposite of the findings of Jovanovic et al. [12].

Several issues and limitations need to be considered in the interpretation of these results. First, the disaccording results may reflect a small effect size of sex, which would

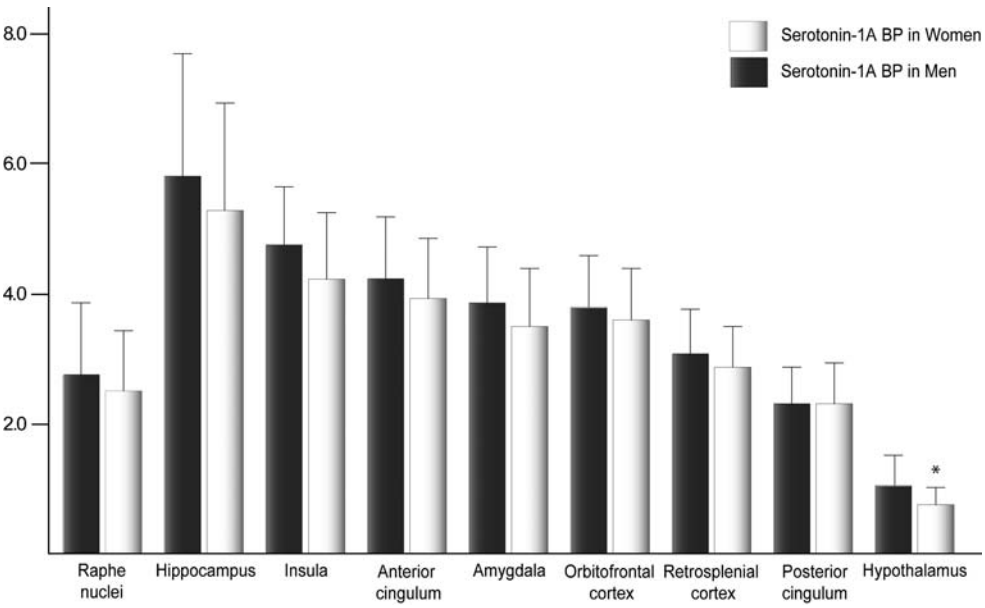

Fig. 3 The serotonin-1A receptor (5-HT_{1A}) binding potential (BP_{ND}) in men and women using a manual delineation approach in nine regions of interest and the cerebellum as region of reference (*black bars* men, *white bars* women). An independent-samples ANOVA revealed no significant sex differences in the mean 5-HT_{1A} receptor BP_{ND} between men and women ($p>0.05$). The *asterisk* (*) indicates a lower 5-HT_{1A} receptor BP_{ND} in women ($p=0.012$) in the hypothalamus that did not withstand the Bonferroni correction for multiple testing

conflict with the inherent limitations of PET studies. These include the particularly high intersubject variability compared to the broad overlapping range between the male and female 5-HT_{1A} receptor BP_{ND} [37] and the small sample size (because of ethical considerations and high costs of PET measurements). Second, given the significant correlations between the 5-HT_{1A} receptor BP_{ND} and personality traits as aggression [31] or anxiety [52], recruiting by advertisement and random inclusion of subjects in the lower or higher range in personality scales might bias the study sample. Methodological bias, however, is unlikely as the present results have been obtained using two independent approaches for definition of brain regions after a careful control for sex differences in binding of the reference region. In addition, two independent approaches for quantification have been applied, the SRTM and the Logan non-invasive approach that yielded comparable results in the ANOVA (data derived from the Logan approach not shown). By using an automated delineation, for the first time, a comprehensive distribution map of the 5-HT_{1A} receptor BP_{ND} in 45 regions of interest has been obtained in males and females.

Another contributing factor for diverging results may be the sex-specific effect on age-dependent increase or decrease in the 5-HT_{1A} receptor binding. Several postmortem studies using the 5-HT_{1A} receptor agonist [^{3}H]8-OH-DPAT as radioligand did not report any significant sex differences in receptor binding or distribution, while an age-dependent decline of binding sites was found in men but not in women [27–29]. In women, the 5-HT_{1A} receptor binding in the occipital cortex even seemed to increase with age [27]. A similar trend was also observed in recent human PET studies using the radioligand [*carbonyl*-^{11}C]WAY-100635. Here, a significant inverse correlation of the 5-HT_{1A} receptor BP_{ND} with age was found in men [34, 49] but not in women [32]. Therefore, comparing the 5-HT_{1A} receptor expression in inhomogeneous groups of subjects with regard to age may yield deceptive results. The 5-HT_{1A} receptor BP_{ND} may be higher in females when comparing men and women in older age but lower in young adulthood. The results of the present study would be in line with the hypothesis since the female subjects included in the present study showing a trend towards a lower mean 5-HT_{1A} receptor BP_{ND} were particularly young (24.1±2.6 years, mean age ± SD).

Furthermore, the sex-dependent age effect on the 5-HT_{1A} receptor binding might be associated with lifetime changes in the production of gonadal steroids in men and women. An abundant amount of literature demonstrates the influence of steroid hormones on the 5-HT_{1A} receptor expression. Long-term administration of estrogen was demonstrated to increase the activity of the tryptophan hydroxylase [53] while down regulating the pre- and postsynaptic 5-HT_{1A} receptor binding [33, 54]. The effect was particularly pronounced in the raphe nuclei and in the hippocampus, i.e. in regions with a lower 5-HT_{1A} receptor-binding potential in female rodents [24]. A phase effect of the menstrual cycle on the 5-HT_{1A} receptor BP_{ND} was demonstrated in preclinical research [55] and indicated in a recent PET study, which, however, lacked statistical power [56]. A correlation of the 5-HT_{1A} receptor binding with progesterone plasma levels has been observed by our group [unpublished results]. Since we observed a slightly lower 5-HT_{1A} receptor BP_{ND} in women measured in the early follicular phase vs. in women measured in the late follicular phase, this might be indicative of a down regulation of the receptor by high progesterone levels at the end of the menstrual cycle, with a recovery of the 5-HT_{1A} receptor BP_{ND} later in the cycle. However, as long as the temporal relation of changes in the 5-HT_{1A} receptor BP_{ND} with regard to steroid hormone plasma levels is not known, these assumptions remain highly speculative.

A heightened serotonin neurotransmission caused by the long-term down regulation of somatodendritic 5-HT_{1A} receptors by higher estrogen and progesterone levels in females would be in line with animal studies that demonstrated a higher serotonin synthesis and turnover [6] and overall higher 5-HT levels [7] in the brain of female rodents. According to this hypothesis, a slightly lower overall 5-HT_{1A} receptor expression in females, as indicated in the present study, would lead to a reduced serotonergic inhibition on postsynaptic mainly glutamatergic neurons [19] and increased serotonin turnover [57]. This would be compatible with a greater serotonergic responsiveness in females. Indirectly, the higher prevalence rate of mood disorders in women that becomes apparent only with the onset of puberty while diminishing after the menopause might be an evidence for the effect of ovarian steroids on the serotonin neurotransmission [58]. Therefore, the restriction of female participants to women with regular menstrual cycle duration and the conductance of PET measurements in a restricted phase of the menstrual cycle appear as important prerequisites for revealing an either higher or lower 5-HT_{1A} receptor BP_{ND} in women compared to men.

Finally, it must be considered that the hypothesis of sex differences in the human serotonergic system may be simply false. Indeed, the present study is the fourth example of differing results with regard to sex effects on the serotonergic system. The 5-HT synthesis has been reported both lower in females [11, 59] and lower in males [60]. The 5-HTT binding potential was reported to be both higher in females [61] or higher in males [12]. In a large study sample of 88 subjects, Praschak-Rieder et al. found no sex differences in 5-HTT binding [62]. Another study with 52 healthy subjects did not reveal any sex differences in the 5-HT_{2A} receptor binding [63], which was suggested before

by Biver et al. [13]. Therefore, sex differences in the incidence of affective disorders might be not adequately explained by sex differences in serotonergic receptor or transporter densities.

In summary, our results do not confirm the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} in 16 healthy young women when compared to 16 healthy young men. This was demonstrated using two independent delineation methods (manual vs. normalized ROI-template-based) and two non-invasive quantification approaches (SRTM vs. Logan). The automated delineation method was shown to be reliable in comparison with the manual delineation with the additional benefit of a high number of regions of interest and a lower variability in regional volumes. A slightly, though not significantly, lower mean 5-HT_{1A} receptor BP_{ND} in female subjects was observed in all regions investigated, which is in contrast with some previous studies. Given the high intersubject variability of the 5-HT_{1A} receptor BP_{ND} within both sexes and the usually low sample size in PET studies, the inclusion criteria of the study sample might significantly influence results on sexual dimorphism. Therefore, sex differences in 5-HT_{1A} receptor binding remain a matter of debate.

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