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Nigrostriatal Upregulation of 5-HT_{2A} Receptors Correlates with Motor Dysfunction in Progressive Supranuclear Palsy

Maria Stamelou, MD,[1,2] Andreas Matusch, MD,[1,2] David Elmenhorst, MD,[1] René Hurlemann, MD, PhD,[3] Karla M. Eggert, MD,[2] Karl Zilles, MD,[1,4] Wolfgang H. Oertel, MD,[2] Günter U. Höglinger, MD,[2]* and Andreas Bauer, MD[1,5]*

[1]*Institute of Neuroscience and Biophysics–Medicine (INB-3), Research Center Jülich, Jülich, Germany*
[2]*Department of Neurology, Philipps University, Marburg, Germany*
[3]*Department of Psychiatry, University of Bonn, Bonn, Germany*
[4]*C. & O. Vogt Institute for Brain Research, University of Düsseldorf, Düsseldorf, Germany*
[5]*Department of Neurology, University of Düsseldorf, Düsseldorf, Germany*

Abstract: A dysfunction of multiple neurotransmitter systems is assumed as a neurochemical basis of the akinetic-rigid syndrome of progressive supranuclear palsy (PSP). In vitro studies have produced conflicting results on the serotoninergic system in PSP. We, therefore, studied the binding potential of the serotonin 2A (5-HT_{2A}) receptor ligand [^{18}F]altanserin in 8 patients with clinically probable PSP and 13 healthy controls using positron emission tomography. We found an up-regulation of 5-HT_{2A} receptors in the substantia nigra and, to a lower degree, in the striatum, while neocortical 5- HT_{2A} receptor densities showed no changes upon partial-volume correction. Nigral and striatal receptor changes were significantly correlated with patients' scores of motor dysfunction (UPDRS III, PSP-rating scale) pointing to a functional relevance of the described findings. © 2009 Movement Disorder Society

Key words: PSP; Steele-Richardson-Olszewski syndrome; serotonin; 5-HT; 5-HT_{2A} receptor; PET

Symptoms of progressive supranuclear palsy (PSP) appear to originate from dysfunctions of multiple neurotransmitter systems.[1,2] So far, the majority of research on PSP,[3] however, was focused on the nigrostriatal dopaminergic system. Therefore, studies on nondopaminergic neurotransmitter systems are necessary.

Studies probing pre- and postsynaptic markers of the serotoninergic system in postmortem brains have shown conflicting results. Hornykiewitz and Shannak[4] found normal serotonin (5-HT) levels in PSP brains. In contrast, Kovacs et al.[5] have shown a significant increase in serotoninergic neurons in the raphe nuclei using immunohistochemistry. Inversely, Chinaglia et al.[6] suggested a decrease in serotoninergic transporters in the cortex and caudate nucleus using autoradiography. Using the same technique, Landwehrmeyer and Palacios[7] found no alterations.

With regard to postsynaptic 5-HT-receptors, Castro et al.[8] found an increase in [^{3}H]sumatriptan binding sites, a 5-$HT_{1B/1D}$-receptor ligand, most markedly in the substantia nigra. In contrast, Pascual et al.[9] found a decrease of 5-HT_1-receptors in the substantia nigra and globus pallidus. Maloteaux et al. reported a decreased specific binding of [^{3}H]ketanserin, labelling 5-HT_{2A} receptors (5-HT_{2A}R), in the temporal cortex but no significant alterations in the frontal cortex and in the striatum.

Long lasting effects of medication, specific circumstances of death and technical issues of postmortem

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Dr. Günter U Höglinger, Department of Neurology, Philipps University, Rudolf-Bultmann Str. 8, Marburg 35039, Germany or Dr Andreas Bauer, Institute of Neuroscience and Biophysics–Medicine (*INB-3*), Research Center Jülich, Jülich 52425, Germany. E-mail: guenter.hoeglinger@med.uni-marburg.de or an.bauer@fz-juelich.de

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TABLE 1. *Clinical and experimental data*

	Controls	PSP
N	13	8
Gender (female/male)	6/7	4/4
Age (years)	61.8 ± 7.0	67.1 ± 3.0
Disease duration	–	4.1 ± 1.4
PSP-RS (worst: 100)	–	43.6 ± 8.3
UPDRS II (worst: 52)	–	21.7 ± 5.0
UPDRS III (worst: 108)	–	24.4 ± 4.4
MMSE (best: 30)	–	27 ± 1.7
FAB (best: 18)	–	11.8 ± 2.5
MADRS (worst: 60)	–	8.1± 4.0
Injected dose (MBq)	229 ± 11	234 ± 13
Specific rdact. (GBq/μmol)	201 ± 146	175 ± 86
Plasma rdact. (KBq/mL)	1.28 ± 0.3	1.46 ± 0.51
Slope plasma rdact. (% /h)	4.0 ± 5.1	2.8 ± 5.1

PSP-RS, PSP rating scale; UPDRS, unified Parkinson's disease rating scale, part II (activities of daily living), part III (motor score); MMSE, mini mental status examination; FAB, frontal assessment battery; MADRS, montgomery-Åsberg depression rating scale; Rdact., radioactivity; average plasma radioactivity at equilibrium 120–180 min p.i.; slope between 120 and 180 min p.i.

preservation of human brain material are most likely to account for the reported discrepancies. In vivo studies using highly selective radioprobes and positron emission tomography (PET) represent a means to overcome these confounding factors. We, therefore, applied the selective 5-HT_{2A} receptor (5-HT_{2A}R) radioligand [^{18}F]altanserin in a cohort of PSP patients, naÿve of all medication affine to serotonergic targets, and an age- and gender-matched control cohort.

METHODS

Subjects

Patients qualified for participation, if they had 'clinically probable PSP'[3] to obtain the highest degree of diagnostic certainty possible in vivo, at disease stages II and III (of I–V, see Clinical Evaluation below and Table 1).

Of a pool of 40 controls all subjects within the age range between 51 and 75 years were selected to reflect the age- and gender-distribution of the PSP patients. Exclusion criteria for all participants were structural brain abnormalities, a history of drug abuse, other neurological or psychiatric conditions and any medication with affinity to 5-HT_{2A}R or interference with the serotoninergic system.

Written informed consent was obtained from all participants. The study protocol was approved by the ethics committees of the *Medical Faculties of the Universities of Bonn and Düsseldorf*, the *Federal Office for Radiation Protection* (BfS), and the *Federal Institute for Drugs and Medical Devices* (BfArM), Germany, and conducted in compliance with national legislation and the Declaration of Helsinki.

Clinical Evaluation

A single rater (MS) evaluated all patients using the PSP rating scale (PSP-RS),[10] the Unified Parkinson Disease Rating Scale (UPDRS) part III, the modified Hoehn & Yahr stage, UPDRS part II, the Frontal Assessment Battery (FAB),[11] and the Montgomery-Åsberg Depression Rating Scale (MADRS).[12]

Imaging

Cranial MRIs were obtained on a Siemens Avanto 1.5T using a 3D-MP-RAGE sequence.

Radiosynthesis of [^{18}F]altanserin, tracer application according to a bolus equilibrium schedule, venous blood sampling, metabolite correction of the plasma input function, PET image acquisition (120–180 min p.i. on an Siemens ECAT EXACT HR+) and image reconstruction were performed as published previously.[13]

Image Processing

Dynamic PET data were realigned, co-registered to MRI, parameterized and corrected for partial volume effects using PMOD *v.2.85* (PMOD, Zürich) and *SPM2 software* (Wellcome Trust Centre for Neuroimaging, London), as described previously.[13]

The binding potential BP_P was calculated from radioactivity concentrations averaged from 120 to 180 min p.i. in the cerebellum as reference region ($C_{Reference}$), the voxel or region of interest ($C_{Voxel/ROI}$), and plasma corrected for parent compound (C_{PPC}), according to the following equation:[14]

$$BP_P = (C_{Voxel}\text{-}C_{Reference})/C_{PPC}$$

Volume of Interest (VOI) Analysis

A set of VOIs was delineated by a blinded observer in the co-registered individual MRIs using PMOD. These individual VOI maps were applied to uncorrected and corrected BP_P maps. Statistical analyses, performed with *SPSS v.14.0* (SPSS Inc., Chicago, IL), included a univariate analysis of covariance (ANCOVA) to examine a putative influence of age and gender, heteroscedastic two-sample *t*-tests for between-group comparisons, bivariate Pearson's correlation of BP_P and clinical parameters.

Statistical Parametric Mapping

Individual parametric maps of BP_P and gray matter segments were spatially normalized to the *Montreal Neurological Institute* 152 T1 template. Partial volume corrected BP_P maps were smoothed with a $3 \times 3 \times 3$ mm^3 Gaussian kernel, uncorrected BP_P maps and gray matter maps with a $10 \times 10 \times 10$ mm^3 kernel.

Voxel-wise group comparisons were performed with SPM2 by a two-sample *t*-test (without threshold masking; without sphericity correction; with the brain extracted from the T1 template, cut-off 0.1, as explicit mask). The level of significance was thresholded at $P < 0.001$ uncorrected.

RESULTS

Fifty-three patients were screened for eligibility. Forty-three patients did not meet the inclusion-criteria, ten thereof because of medication affecting the serotoninergic system. Two subjects refused to participate. Thus, in total 8 patients were included and compared to 13 controls. Basic clinical and experimental data of the participating subjects are given in Table 1.

Voxel-based morphometry demonstrated significant atrophy of cortical gray matter in the mesio-frontal, parietal, insular and fronto-opercular cortex (Fig. 1A). This pattern is in perfect agreement with published data.[15] Thus, for a valid assessment of 5-HT_{2A}R-binding unbiased by atrophy effects, partial volume correction of PET data was mandatory. Partial volume-corrected BP_P maps are presented in Figure 1B. Age and gender as covariates had no influence on regional BP_P.

Statistical parametric mapping (SPM) identified voxels, in which partial volume-corrected BP_P values differed significantly between patients and controls at a threshold of $P < 0.001$ (Fig. 1C). Voxels with reduced BP_P were observed in parts of the anterior cingulate, orbito-frontal and insular cortex. BP_P was increased in individual voxels in the right striatum (focus at the following stereotaxic Montreal neurological Institute (MNI)-coordinates given in mm: $x = 28$, $y = 12$, $z = -2$) and bilaterally in the substantia nigra (left: $x = -8$, $y = -12$, $z = -4$; right: $x = 8$, $y = -14$, $z = -4$).

The VOI-based analysis of partial volume-corrected BP_P data did not demonstrate significant differences in any of the cortical VOIs (e.g. entire neocortex: $BP_{P\text{-}PSP} = 2.4 \pm 0.8$, $BP_{P\text{-}Control} = 2.5 \pm 0.7$), whereas this method showed a trend for increased BP_P in the striatum ($BP_{P\text{-}PSP} = 1.3 \pm 0.8$, $BP_{P\text{-}Control} = 0.8 \pm 0.3$, $P = 0.061$; Supporting Information Table S1).

The VOI-analysis of the substantia nigra had to be performed without partial volume correction, because—consistent with its histological architecture—the volume fraction assigned to gray matter (22% in controls) was too small to allow reliable count statistics. In the substantia nigra, there was clearly measurable radioligand binding in PSP-patients, whereas it was hardly distinguishable from background in controls ($BP_{P\text{-}PSP} = 0.45 \pm 0.35$, $BP_{P\text{-}Control} = 0.02 \pm 0.36$, $P = 0.009$; Fig. 1D). The nigral signal mapped to the following MNI–coordinates left: $x = -7$, $y = -18$, $z = -12$; right: $x = 9$, $y = -19$, $z = -12$.

Although there was a broad overlap in the nigral BP_P in PSP patients and controls, the values in seven controls were equal to or lower than the lowest value measured in patients, the values in 2 patients were higher than the highest value measured in controls, and only one patient had a value lower than the mean value of the control group (Fig. 1E). This suggests a clear shift to higher values in the entire group of patients.

Nigral BP_P in PSP patients was significantly correlated with the clinical PSP-RS score ($r = 0.74$, $P < 0.05$; Fig. 1E) and the UPDRS-III ($r = 0.87$, $P <$

FIG. 1. 5-HT_{2A} receptor binding potential BP_P in n = 8 subjects with PSP and n = 13 controls. (**A**) *T* maps resulting from a comparison of gray matter segments in PSP patients and controls using statistical parametric mapping (SPM) highlight voxels with significantly reduced gray matter density in PSP. Significant gray matter atrophy of gray matter in PSP was particularly evident in the mesio-frontal, parietal, insular and fronto-opercular cortex, demonstrating the need for a partial volume-correction of the PET data. (**B**) Averaged partial volume-corrected parametric maps displaying 5-HT_{2A} binding potential BP_P in controls (CTRL) and PSP patients. (**C**) SPM *T* maps display individual voxels with significantly decreased and increased BP_P, respectively, in PSP patients compared to controls (thresholded at $P = 0.001$). Note that the anatomical regions containing the voxels of significant decrease are located in the anterior cingulate, in the orbito-frontal and in the insular cortex, while the voxels of significant increase were located in the substantia nigra and in the right striatum. (**D**) Comparison of averaged BP_P maps of the nigro-striatal region in coronal sections (upper panel) and of the midbrain in axial sections (lower panel) in controls (CTRL) and PSP patients. This presentation is without partial volume-correction, because—consistent with its histological architecture—the volume fraction assigned to gray matter was too small to allow reliable count statistics. Note increased specific binding bilaterally in the substantia nigra and striatum in PSP-patients. Note that partial volume-correction for gray matter atrophy would have increased the BP_P patients even further in patients. (**E**) Scatter plot demonstrating the correlation of the PSP-rating score in PSP patients with the 5-HT_{2A} binding potential BP_P in the substantia nigra. BP_P values were averaged from the left and right hemisphere. BP_P values of controls (CTRL) are shown on the left side of the graph. The horizontal bar shows the mean value of controls.

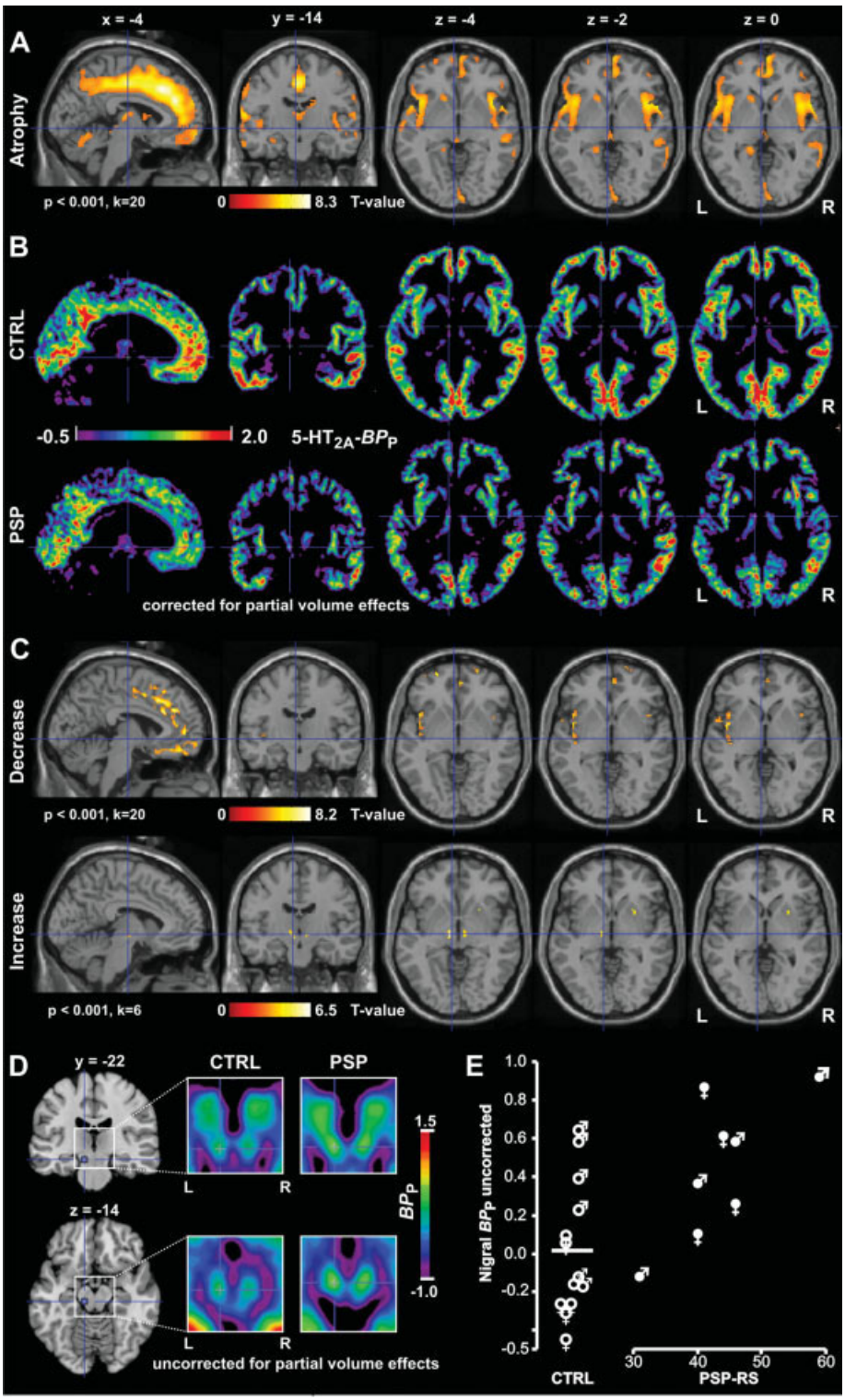
A
x = -4
y = -14
z = -4
z = -2
z = 0
Atrophy
p < 0.001, k=20
0
8.3 T-value
L
R
B
CTRL
-0.5
2.0
5-HT2A-BPP
PSP
corrected for partial volume effects
C
Decrease
p < 0.001, k=20
0
8.2 T-value
Increase
p < 0.001, k=6
0
6.5 T-value
D
y = -22
CTRL
PSP
z = -14
1.5
BPP
-1.0
uncorrected for partial volume effects
E
Nigral BPP uncorrected
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.5
CTRL
30
40
50
60
PSP-RS

FIG. 1

0.01). The same was true for the striatal BP_P (PSP-RS: $r = 0.82$, $P < 0.01$; UPDRS-III: $r = 0.79$, $P < 0.01$). All other obtained clinical scores were not correlated to the cerebral binding potential.

DISCUSSION

In the present study, we report an up-regulation of 5-HT_{2A}Rs in the substantia nigra and in the striatum, which is correlated with the severity of clinical motor dysfunction in PSP. These data provide therefore first *in vivo* evidence for a functional implication of serotonin receptors in the pathophysiology of PSP.

The presence of serotoninergic nerve terminals in the substantia nigra is well proven.[6] 5-HT_{2A}Rs have been detected in the substantia nigra in monkeys by autoradiography[16] and in humans by immunohistochemistry. They are particularly expressed on dopaminergic neurons.[17] The observed increase in [^{18}F]altanserin binding in the substantia nigra and the striatum of PSP patients most likely reflects an upregulation of postsynaptic 5-HT_{2A}Rs. As both parts of the substantia nigra cannot be discrimated by PET, an increase of 5-HT_{2A}R binding on dopaminergic neurons is likely to either modulate local nigral dopaminergic activity or modify upstream dopaminergic projections within the basal ganglia circuitry.

The reason for serotonin receptor upregulation is most likely a region-selective presynaptic serotoninergic denervation. Supporting the concept of an upregulation of postsynaptic 5-HT_{2A}Rs that occurs as a consequence of reduced presynaptic serotoninergic innervation, Chen et al.[18] reported in Parkinson's disease an increase of cortical 5-HT_{2A}Rs that coincides with a decrease in presynaptic serotoninergic nerve terminals.

On the other hand, the apparent correlation of 5-HT_{2A} receptor binding and the level of disability could result from complex indirect plastic changes, e.g. an increased 5-HT_{2A}R expression on pyramidal cells secondary to the loss of dopaminergic function.

Further, as suggested by the partial overlap of the nigral signal from patients and controls the aforementioned effects may be restricted to a subgroup of patients or advanced stages of disease.

It is, however, remarkable that in the present sample of PSP patients, there were relatively subtle reductions in neocortical 5-HT_{2A}Rs compared to the substantial neocortical atrophy. A potential explanation is that presynaptic and postsynaptic degeneration coincide in the neocortex so that receptor-upregulation on postsynaptic neurons—caused by presynaptic denervation—is counterbalanced by neocortical cell loss.

In conclusion, our finding of upregulated nigro-striatal postsynaptic 5-HT_{2A}Rs and a subtle reduction in cortical 5-HT_{2A}Rs, demonstrates a serotoninergic contribution to the pathophysiology of PSP. It suggests a presynaptic serotoninergic deficit in PSP, which should be verified in future studies by assessing the presynaptic serotoninergic innervation.

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