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Increased 5-HT_{2A} Receptors in the Temporal Cortex of Parkinsonian Patients with Visual Hallucinations

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Abstract: Well-formed visual hallucinations (VH) are common in patients with Parkinson's disease (PD). The pathophysiology of VH in PD is unknown but may involve structures mediating visual processing such as the inferior temporal cortex. Serotonergic type 2A (5-HT_{2A}) receptors have been linked to many psychiatric disorders, including psychosis. We hypothesized that enhanced 5-HT_{2A} receptor levels may be involved in VH in PD. Autoradiographic binding using [^{3}H]-ketanserin and spiperone, to define 5-HT_{2A} receptors, was performed in 6 PD patients with VH, 6 PD patients without VH, and 5 healthy, age-matched controls. The cerebral regions studied included the orbitofrontal cortex, inferolateral temporal cortex, motor cortex, striatum, and substantia nigra. There was a significant (45.6%) increase in the levels of [^{3}H]-ketanserin binding in the inferolateral temporal cortex of PD patients with VH when compared with PD patients without VH (54.3 ± 5.2 fmol/mg vs. 37.3 ± 4.3 fmol/mg, $P = 0.039$). Additionally, there was a significant increase in the levels of 5-HT_{2A} receptors in the motor cortex of all PD patients taken as a group when compared with controls (57.8 ± 5.7 fmol/mg vs. 41.2 ± 2.6 fmol/mg, $P = 0.0297$). These results suggest that enhanced 5-HT_{2A}-mediated neurotransmission in the inferolateral temporal cortex, a critical structure in visual processing, might be associated with the development of VH in PD. Our results provide new insights into the pathophysiology of VH in PD and provide an anatomical basis to explain why compounds with 5-HT_{2A} antagonist activity are effective at alleviating this debilitating complication. © 2010 Movement Disorder Society

Key words: Parkinson's disease; serotonin; 5-HT_{2A} receptors; visual hallucinations; autoradiography

The cardinal motor manifestations of Parkinson's disease (PD), rest tremor, rigidity, and bradykinesia, are secondary to dopamine (DA) deficiency in the striatum.[1] However, a range of non-motor symptoms, including psychosis, are increasingly recognized in PD. Such symptoms are often difficult to manage and represent a major cause of morbidity. Psychotic manifestations can begin as vivid dreams and progress to well-formed visual hallucinations (VH).[2–4] VH affect up to 60% of patients with PD.[5,6]

VH in PD have often been linked to DA receptor stimulation by dopaminergic drugs. However, not all patients with PD develop VH on dopaminergic medication, and often symptoms will continue despite a dose reduction. In addition, there is no consistent correlation with dose or type of dopaminergic drug.[3,7,8] Thus, VH in PD are no longer perceived as a pure drug-induced effect and are more likely due to a neuropathological dysfunction. One such target may be serotonin (5-HT)-mediated neurotransmission.

Traditionally, 5-HT has been considered to have a role in mood.[9,10] 5-HT_2 agonist actions are implicated in psychotic symptoms associated with the use of hallucinogens.[11] The atypical antipsychotics clozapine and quetiapine are both currently used to treat VH in

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PD, because of their low propensity to worsen motor symptoms.[12] In addition to being DA D_2 receptor antagonists, they are also 5-$HT_{2A/2C}$ receptor antagonists.[13,14] This raises the hypothesis that their effects on VH may reflect a 5-HT rather than DA action.

To date, the neural mechanisms underlying VH and potential site of 5-HT_{2A}-mediated action in PD are unknown. However, the inferior temporal cortex appears to be involved in visual processing of complex features.[15] This region may thus be involved in mediating the typical well-formed and complex VH characteristic of PD. Indeed, pathological studies have reported increased Lewy bodies in the inferolateral temporal cortex in PD patients with VH.[16] Of interest, a basal ganglia–thalamo–cortical loop has been described in primates that may be involved in visual processing.[17] This loop includes the inferolateral temporal cortex, substantia nigra (SN) pars reticulata, striatum, and the medial portion of the ventral anterior nucleus of the thalamus. Thus, reduced activity in the medial SN pars reticulata, as a result of changes to the striatopallidal pathways in advanced treated PD, may lead to increased thalamocortical excitatory drive and VH,[17] in a similar way to the neural mechanism underlying the expression of levodopa-induced dyskinesia (LID) in PD.[18]

We hypothesized that 5-HT_{2A} receptors within the inferolateral temporal cortex are involved in mediating VH in PD. This autoradiographic binding study measured 5-HT_{2A} receptor levels in five brain regions of PD patients experiencing VH compared with PD patients without VH and age-matched controls using [^{3}H]-ketanserin, a 5-HT_{2A} receptor antagonist.[19]

PATIENTS AND METHODS

Tissue Collection

Human postmortem tissue sections were obtained from the United Kingdom Parkinson's Disease Society Tissue Bank. The University Health Network Institutional Review Board approved the handling of the postmortem tissue. Tissue was collected according to established protocols[20] and stored at −80°C. Sections from 6 patients with a clinicopathological diagnosis of idiopathic PD and VH, 6 PD patients without VH, and 5 healthy, age-matched controls were used (Table 1). The neuropathological diagnosis was based on international neuropathological consensus criteria for the diagnosis of definite PD (http://www.ICDNS.org). The clinical diagnosis of PD was made using standard criteria,[21] and clinical details were provided from medical records by the tissue bank staff. Sections were 12-μm thick, unfixed, mounted on SuperFrost Plus slides, cut on the coronal plane, and stored at −80°C. Sections from inferomedial frontal cortex (Brodmann area 11; BA_{11}), inferolateral temporal cortex (BA_{21}), primary motor cortex (BA_4), striatum, and SN (compacta and reticulata) were studied (Fig. 1). There were no significant differences in postmortem interval (14.7 ± 3.0 hours for PD with VH, 21.8 ± 3.9 hours for PD without VH, and 16.3 ± 4.5 hours for controls; $P = 0.28$, one-way ANOVA) or brain weight (1360 ± 66 g for PD with VH, 1319 ± 79 g for PD without VH, and 1349 ± 48 g for controls; $P = 0.89$, one-way ANOVA).

[^{3}H]-Ketanserin Autoradiographic Binding

Sections were removed from the freezer and allowed to dry at room temperature overnight. The following day, sections were preincubated in 50 mM Tris buffer (pH 7.4) for 30 minutes at room temperature. Following the removal of the preincubation buffer, sections were incubated for 1 hour at room temperature in a 50 mM Tris buffer (pH 7.4) solution containing 2.5 nM [^{3}H]-ketanserin (specific activity: 67 Ci/mmol; PerkinElmer, Waltham, MA) to define total binding. As ketanserin has some potential to bind to alpha-1 adrenergic receptors (α_1) and to the vesicular monoaminergic transporter (VMAT),[19,22,23] 1 μM prazosin and 1 μM tetrabenazine (Tocris, Ellisville, MO) were added to the incubation buffer.[24,25] Nonspecific binding was defined by incubating sections with 10 μM spiperone (Tocris, Ellisville, MO), which has an affinity in the nanomolar range for the 5-HT_{2A} receptors.[26] Following incubation, sections were washed twice for 10 min in 4°C Tris buffer (pH 7.4). Sections were then dipped for 10 seconds in 4°C deionized water and allowed to dry overnight at room temperature.

Autoradiographic images for optical density analysis were obtained by apposing sections to [^{3}H]-sensitive films (Biomax MR; Sigma, St-Louis, MO) for 50 days at 4°C with [^{3}H]-microscale standards (GE Healthcare Life Sciences, Pittsburgh, PA). Images were also obtained using a MicroImager (BioSpace Lab, Cambridge, MA), as this allowed greater anatomical resolution.

Autoradiograms were analyzed using MCID 6.0 Elite Image analysis system software (InterFocus Imaging, Linton, UK). Densitometric analysis of each aforementioned brain region was performed, whereby a reference curve of radioactivity versus optical density was calculated from alpha-emitting [^{3}H]-microscale standards and used to quantify the intensity of signal as nCi

TABLE 1. *Clinical characteristics of study patients*

Patients	Sex	Age at onset (yr)	Age of death (yr)	Hoehn and Yahr	Motor fluctuations: wearing-off and/or dyskinesia	Phenomenology of VH	Mood	Cognitive impairment	PD drugs	5-HT binding drugs	Comorbidities
PD with VH											
PD1	M	54	78	4	Yes	VH and paranoid delusions	Possible depression, aggression	Yes	Levodopa/DCI, bromocriptine, amantadine, selegiline, cabergoline	Olanzapine, thioridazine	
PD2	M	67	79	4	Yes	VH of people; paranoid delusions	NR	Yes (MMSE 23/30)	Levodopa/DCI ropinirole		Hypertension
PD3	F	68	82	3	Yes	VH of insects	NR	Short-term memory problems	Levodopa/DCI, selegiline, entacapone, pergolide		IHD, hypothyroidism
PD4	M	73	82	3	Yes	VH and delusions	NR	Yes	Tolcapone, entacapone, levodopa/DCI pergolide, ropinirole, selegiline	Quetiapine	MI
PD5	M	67	73	4	Yes	VH	NR	Yes	Levodopa/DCI amantadine	Amitryptiline, haloperidol, levopromazine	Thyroid adenoma
PD6	M	78	86	3	Yes	VH of birds, animals, and spouse	NR	NR	Levodopa/DCI entacapone		Hypertension, Paget's disease
PD7	M	69	79	4	Yes	–	Alcoholism	NR	Levodopa/DCI, amantadine		Bladder and bowel cancer
PD without VH											
PD8	M	58	75	4	Yes	–	Impulse control disorder on ropinirole	Yes	Entacapone, levodopa/DCI, pergolide	Metoclopramide	Nocturnal epilepsy (phenytoin, phenobarbitone)
PD9	F	65	76	3	Yes	–	Depression	NR	Cabergoline, levodopa/DCI selegiline	Imipramine, prochlorperazine	Transient ischemic attack, atrial fibrillation
PD10	M	75	82	3	Yes	–	Depression	NR	Levodopa/DCI pramipexole		
PD11	M	77	80	3	No	–	NR	Possible	Levodopa/DCI		Hypertension
PD12	F	78	88	3	No	–	NR	NR	Levodopa/DCI, selegiline	Chlorpromazine, prochlorperazine	Migraine
Controls											
C1	M	–	76							Amitryptiline	IHD, trigeminal neuralgia
C2	M	–	88								IHD, hypertension
C3	M	–	58							Amitryptiline	Cervical ependymoma, IHD, neuralgia
C4	F		100								NR
C5	F	–	71								IHD

PD, idiopathic Parkinson's disease; VH, visual hallucination; DCI, decarboxylase inhibitor; NR, not reported; MMSE, mini-mental state examination; IHD, ischemic heart disease; MI, myocardial infarction.

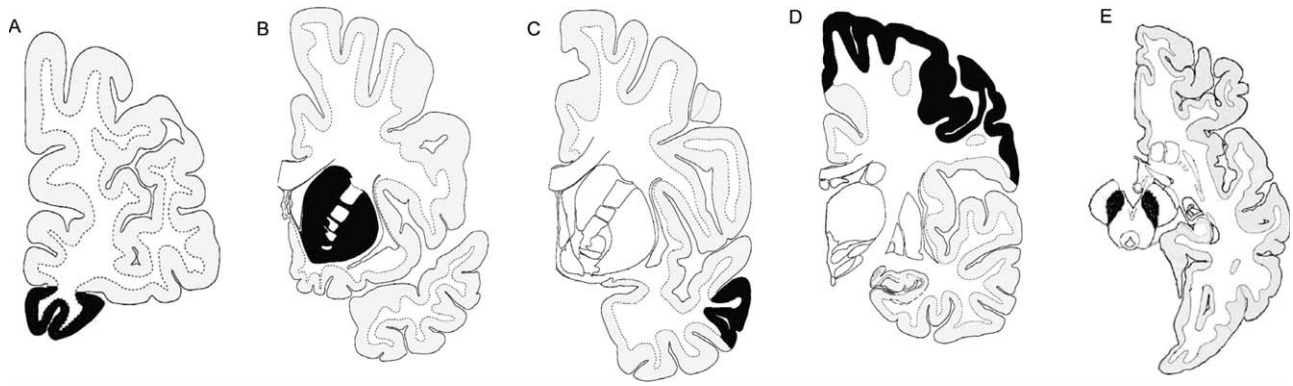

FIG. 1. Brain areas from which the sections included in the study were chosen are colored in black. From anterior to posterior, (**A**) orbitofrontal cortex (BA_{11}; AC: −48 mm); (**B**) striatum (AC: −10 mm); (**C**) inferolateral temporal cortex (BA_{21}; AC: +2.0 mm); (**D**) motor cortex (BA_4; AC: +17.2 mm); (**E**) SN (level of the mamillary bodies). All sections are coronal, except the SN, which is horizontal. AC, anterior commissure; BA, Brodmann area; SN, substantia nigra.

per mg of tissue. Background intensity was subtracted from each reading. For each brain area, three consecutive sections were processed to determine the total binding and one to evaluate the nonspecific binding. Both total and nonspecific binding were calculated in the same way, and nonspecific binding was subtracted from total binding to give specific binding. Intensity of signal was converted into fmol of receptor per mg of tissue. During the analytical process, the investigator was blinded to the clinical status of the patients. Illustrations were handled with the Adobe Photoshop 7.0.1 software (Adobe, San Jose, CA).

Statistical Analysis

Clinical characteristics were compared using appropriate parametric unpaired Student's *t* or ANOVA tests or nonparametric Mann-Whitney U or Kruskal-Wallis tests. One-way ANOVA followed by Tukey's post hoc test were used to compare specific binding between the three groups for each region of interest. Two-tailed unpaired Student's *t* test was performed to compare specific binding between the pooled parkinsonian patients and the controls. Significance was assigned when $P < 0.05$. Statistics were computed with the SPSS 13.0 for Windows (SPSS, Chicago, IL) and GraphPad Prism 5.02 (GraphPad Software, La Jolla, CA) softwares.

RESULTS

Patient Characteristics

Clinical features of patients are summarized in Table 1. The groups were matched for age (mean (±SD) age of death was 80 ± 4.5 years for PD with VH, 80 ± 4.7 years for PD without VH, and 78.6 ± 16.0 years for controls; $P = 0.96$, one-way ANOVA test). Each PD group contained only 1 female individual, whereas the control group contained 2 ($P = 0.61$, Kruskal-Wallis test). The two PD groups were matched for disease duration (12.2 ± 6.7 years for PD with VH, 9.8 ± 4.9 years for PD without VH; $P = 0.50$, unpaired Student's *t* test). No ratings for disease severity were performed prospectively in these patients; however, disease severity according to Hoehn and Yahr scale[27] was retrospectively scored using clinical information (3.5 ± 0.5 for PD patients with VH and 3.0 ± 0.375 for PD patients without VH, median ± semi-Q; $P = 0.65$, Mann-Whitney U test) (Table 1). All patients in the PD with VH group and 4 of the nonhallucinating group had levodopa-induced motor fluctuations. VH were recorded as present or absent according to clinical charts; no rating scale was used premortem to determine severity. Cognitive impairment, defined as either mini-mental state examination less than 26 or documented memory issues or dementia in the clinical records, was noted in 4 of the 6 PD patients with VH, 2 of 6 in the nonhallucinatory group, and none of the controls, although formal neuropsychological evaluations were not performed ($P = 0.082$, Kruskal-Wallis test). Three PD patients with VH were taking neuroleptics (D_2 antagonists) that also have 5-HT_{2A} binding potential (olanzapine, quetiapine, thioridazine, haloperidol, and levopromazine); 1 PD patient without VH was taking chlorpromazine, and 2 PD patients without VH were taking antiemetics with 5-HT_{2A} antagonist activity (metoclopramide and prochlorperazine) ($P = 0.93$, Mann-Whitney U test). Depression was reported in 1 PD patient without VH. One PD patient without VH, 1 PD patient with VH, and 2 control subjects were taking a tricyclic antidepressant ($P = 0.61$, Kruskal-Wallis test).

TABLE 2. *5-HT_{2A} receptor levels across the studied brain areas*

	[^{3}H]-Ketanserin binding (mean ± SEM)			
	Controls (fmol/mg)	All PD (fmol/mg)	No VH (fmol/mg)	VH (fmol/mg)
Orbitofrontal cortex (BA_{11})	46.2 ± 12.0	45.8 ± 4.1	42.1 ± 3.3	58.6 ± 6.2
Inferolateral temporal cortex (BA_{21})	42.7 ± 5.4	50.4 ± 4.1	37.3 ± 4.3	54.3 ± 5.2[a]
Motor cortex (BA_4)	41.2 ± 2.6	54.4 ± 5.1[b]	50.9 ± 9.4	57.8 ± 5.7
Striatum	33.8 ± 4.0	40.2 ± 3.4	35.3 ± 5.6	45.1 ± 3.4
Substantia nigra	14.2 ± 3.1	18.5 ± 3.3	17.0 ± 5.6	20.0 ± 4.4

[a]$P = 0.039$ between PD patients with VH and PD patients without VH.
[b]$P = 0.0297$ between PD patients and controls.

All PD patients were taking levodopa and other dopaminergic agents. Five PD patients were taking DA agonists, 3 in the VH group and 2 in the nonhallucinating group ($P = 0.66$, Mann-Whitney U test). Two of the PD patients without VH were taking a DA agonist with a 5-HT_{2A} effect (cabergoline and pergolide) ($P = 0.29$, Mann-Whitney U test). Potential 5-HT binding drugs taken at the time of death are listed in Table 1.

[^{3}H]-Ketanserin Binding in Human Postmortem Brains

[^{3}H]-Ketanserin binding levels across the different brain areas are reported in Table 2 and illustrated in Figure 2. In the control brains, the highest binding levels were found in the frontal cortex, followed by the temporal and the motor cortex. In these regions, specific binding represented ~80 to 90% of total. The lowest levels of [^{3}H]-ketanserin binding were encountered in the SN, in which the nonspecific binding was high (~50%).

In the inferolateral temporal cortex of PD patients with VH, [^{3}H]-ketanserin binding was increased by 45.6% compared with PD patients without VH (54.3 ± 5.2 fmol/mg vs. 37.3 ± 4.3 fmol/mg, respectively, $P = 0.044$, one-way ANOVA, $F = 3.93$; $P = 0.039$, Tukey's post hoc test) (Table 2, Fig. 3). There were no significant differences in [^{3}H]-ketanserin binding in

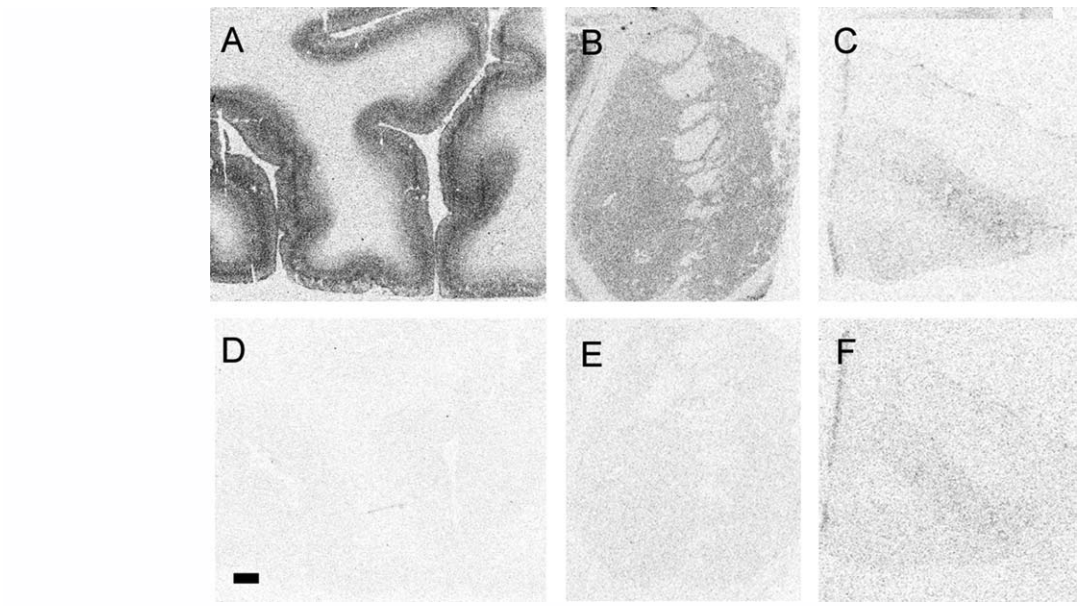

FIG. 2. Autoradiograms representative of [^{3}H]-ketanserin binding levels on the sections studied. The top row represents the total binding, whereas the bottom row is the nonspecific binding. As illustrated, nonspecific binding is relatively low in the temporal cortex (**D**) and the striatum (**E**). However, nonspecific binding was 50% in the SN (**F**). The total binding was higher in the temporal cortex (**A**) than in the striatum (**B**). The lowest levels (after subtraction of the nonspecific binding) were encountered in the SN (**C**). These pictures were taken following exposition in a MicroImager for 12 hours. Scale bar: 2 mm. SN, substantia nigra.

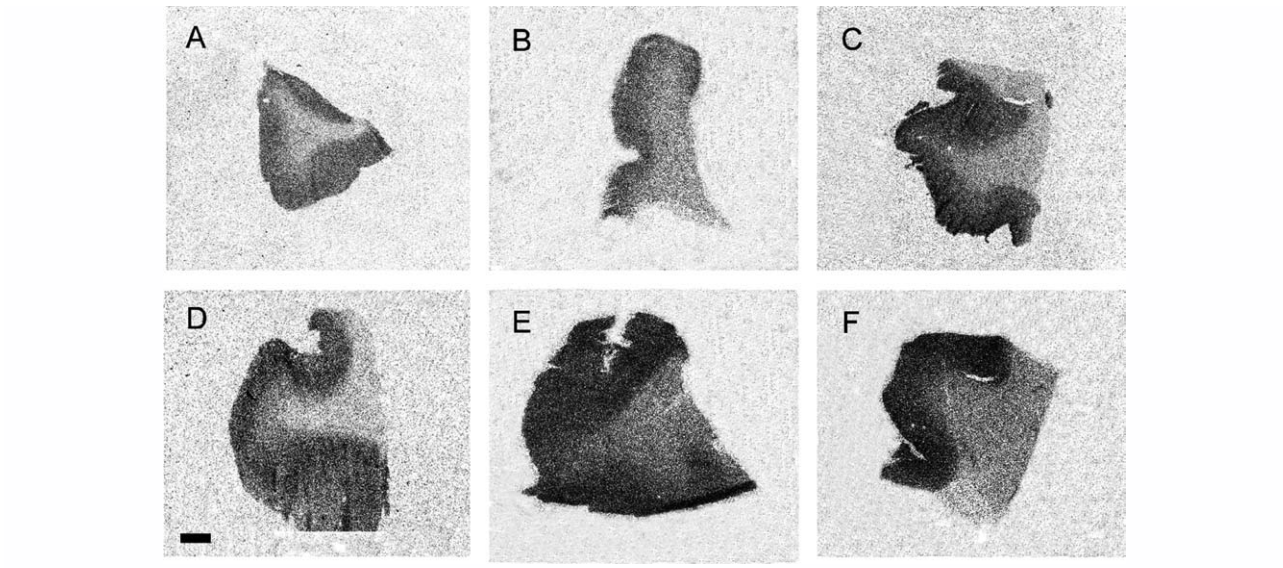

FIG. 3. Autoradiograms illustrating the different levels of [^{3}H]-ketanserin binding in the 3 patient groups studied. The upper row (A–C) represents the temporal cortex and the lower row (D–E) represents the motor cortex. As illustrated, in the temporal cortex, there was no difference in [^{3}H]-ketanserin binding density between the control patients (**A**) and PD patients without VH (**B**). However, [^{3}H]-ketanserin binding is darker, hence higher, in the temporal cortex of PD patients with VH (**C**). In the motor cortex, [^{3}H]-ketanserin binding levels are lower in the control group (**D**) when compared with either the PD patients without or with VH (**E** and **F**, respectively). These pictures were taken following exposition in a MicroImager for 12 hours. Scale bar: 2 mm.

the frontal cortex, motor cortex, striatum, and SN between any of the groups ($P = 0.149$ for the frontal cortex, 0.223 for the motor cortex, 0.139 for the striatum, and 0.637 for the SN, one-way ANOVA). These results are expanded in Table 2.

When all PD brains (with and without VH) were combined, there was a significant increase in [^{3}H]-ketanserin binding levels in the motor cortex when compared with the control group (54.4 ± 5.1 fmol/mg vs. 41.2 ± 2.6 fmol/mg, respectively; $P = 0.0297$, $t = 2.42$, Student's t test). No significant differences were found in the other brain regions when PD patients were pooled as a single group compared with control ($P = 0.631$ for the temporal cortex, 0.731 for the frontal cortex, 0.214 for the striatum, and 0.321 for the SN, Student's t test).

DISCUSSION

This study demonstrates that 5-HT$_{2A}$ receptor binding is increased in the inferior temporal cortex of PD patients with VH compared with PD patients without VH. This suggests that enhanced 5-HT$_{2A}$-mediated neurotransmission might underlie the pathophysiology of VH, and 5-HT$_{2A}$ antagonists may be useful in the treatment of VH in PD.

Technical Considerations

Ketanserin has some affinity for the α_1 adrenergic receptors and for the VMAT. Binding to these sites is unlikely to be a component of the binding observed as we included unlabeled prazosin and tetrabenazine to block these receptors. In addition, ketanserin binds to histaminergic-1 (H_1) receptors with a K_i of 10 nM.[19] However, as the concentration of ketanserin used in this study was low, i.e., 2.5 nM, it is unlikely that binding to the H_1 receptors was significant, as previously demonstrated in rat cerebral cortex.[22]

The levels of 5-HT$_{2A}$ receptors in control brains were comparable to prior studies in human brain tissue. Thus, [^{3}H]-ketanserin binding levels were higher in the cortex than in subcortical structures and higher in the striatum than in the SN.[28] The relative [^{3}H]-ketanserin binding densities across the different cortical areas (BA_4, BA_{11}, and BA_{21}) were also comparable to those obtained by Pazos et al.[28] The absolute levels obtained in the control brains (from 14.2 ± 3.1 to 46.2 ± 12.0 fmol/mg in the SN and frontal cortex, respectively) were in the same order as those obtained by Oquendo et al.,[24] although lower than those of Pazos et al.[28] Part of this discrepancy may due to the fact that Pazos et al. did not attempt to block the α_1 receptors and the VMAT.

Patient Characteristics

The number of patients included in our study, although relatively small (17, i.e., 6 PD patients with VH, 6 PD patients without VH, and 5 age-matched controls), is in accordance with prior postmortem studies published in the literature.[29] This number allows statistical analysis using a minimum number of samples. PD patients with and without VH were matched as far as possible for age, sex, and duration of disease. However, limitations of pathological studies with retrospective collection of clinical data may result in clinical variability of some disease parameters between the two groups. Thus, potential confounders that may affect 5-HT$_{2A}$ receptor binding include anxiety and depressive symptoms, use of 5-HT binding medications, and cognitive impairment.

The presence of significant anxiety and depression in PD may affect 5-HT$_{2A}$ receptor levels, as cortical 5-HT$_{2A}$ receptors are implicated in anxiety.[30] In general, 5-HT levels are lower in PD patients with depression, suggesting possible compensatory upregulation of postsynaptic cortical receptors. Indeed, postmortem studies have shown a lower density of neurons in the dorsal raphe nucleus in depressed versus nondepressed PD patients,[31] and 5-HT metabolite levels, in CSF, are reduced in depressed PD patients.[32] In our study, depression was reported in 1 subject in the PD with VH group and 2 in the non-VH group, with alcoholism and impulse control disorder in 2 others; however, it is possible that such symptoms were underreported, and the small size of our groups precluded subgroup analysis.

Another potential confounder is the use of 5-HT binding medications. Indeed, antidepressants, which elevate the endogenous levels of 5-HT, are likely to downregulate 5-HT$_{2A}$ receptors, whereas antipsychotics and antiemetics, which antagonize these receptors, are likely to upregulate them. However, similar numbers of patients in both PD groups and controls had exposure to antidepressants, antipsychotics, or antiemetics. The fact that PD patients, both with and without VH, were taking these drugs possibly renders the two groups equally susceptible to such a variation in the 5-HT$_{2A}$ levels and makes it less likely that 5-HT$_{2A}$ levels of one group will be disproportionately affected when compared with the other group. However, further studies may be needed to clarify this potential confounder.

DA agonists may also affect 5-HT$_{2A}$ receptors, in particular cabergoline and pergolide, which possess 5-HT$_{2A}$ agonist action,[33] and could downregulate the levels of 5-HT$_{2A}$ receptors. As previously mentioned, there was no difference between the two PD groups in terms of individuals taking DA agonists. However, exact doses and duration of use were not available. In the PD with VH group, 1 patient received cabergoline and 2 received pergolide. Interestingly, as these drugs could potentially downregulate the levels of 5-HT$_{2A}$ receptors, they would have been likely to decrease 5-HT$_{2A}$ receptor levels in the PD with VH group to a greater extent than in the PD without VH group and might have decreased the extent of the difference we found.

The presence of cognitive impairment is also a potential confounding factor, as PD patients with VH are more at risk of developing subsequent dementia.[3] However, a recent postmortem study investigating 5-HT$_{2A}$ receptor binding, using [^{3}H]-ketanserin, reported no correlation with cognitive impairment in patients with vascular dementia.[34]

Abnormal 5-HT$_{2A}$-Mediated Neurotransmission in Parkinsonian Patients With VH

The 5-HT system is affected in PD. Hence, serotonergic neurons of the brainstem, which provide diffuse 5-HT innervation to the brain, undergo degeneration.[35,36] This results in a decrease in 5-HT levels in several brain areas.[37–39] In all of these brain regions, the decrease in 5-HT is not as pronounced as the DA decrease. Thus, simplistically, the increase in 5-HT$_{2A}$ receptor levels might represent a mechanism by which the brain attempts to compensate for the reduced ambient 5-HT levels.

In addition to the decrease in 5-HT, the decrease in DA could also contribute to increased 5-HT$_{2A}$ receptor levels. Indeed, in the 6-hydroxydopamine-lesioned rat, reduced DA levels secondary to the destruction of the nigrostriatal pathway led to an increase of 5-HT$_{2A}$ receptor levels in the striatum.[40,41] Thus, DA levels seem to exert some regulatory feedback on 5-HT$_{2A}$ receptor levels. This might be explained by the fact that DA can bind to a variety of 5-HT receptors, including 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_3$ receptors.[42–44] DA acts as a partial agonist at the 5-HT$_{2A}$ receptors.[44]

This interaction between DA and serotonergic receptors might be relevant to the pathogenesis of VH in PD. Indeed, in PD, it was shown that, following levodopa administration, the drug is taken up by the raphe neurons, metabolized into DA, and released into the striatum by serotonergic terminals, acting as a "false neurotransmitter."[45,46] The pulsatile administration of levodopa and the degree of DA depletion are linked to the development of dyskinesia.[47] A similar mechanism

could account for the development of DA-related VH, in which DA released from the 5-HT terminals would intermittently stimulate the 5-HT_{2A} receptors.

This hypothesis might also explain why VH tend to occur late in the disease process. Indeed, as the 5-HT system is not as affected as the DA system during the disease, it might take longer to deplete the amount of 5-HT below a critical threshold. Once this threshold is reached, intermittent nonphysiological stimulation of 5-HT_{2A} receptors by the levodopa-derived DA might lead to the appearance of VH.

Increased 5-HT_{2A} Receptor Levels in the Inferolateral Temporal Cortex of Parkinsonian Patients With VH

The temporal cortex is part of the ventral visual stream and plays a role in the recognition of objects and faces[48,49] as well as in auditory and visual integration.[50,51] As previously mentioned, degenerative changes occur in the temporal lobe of PD patients with VH, and Lewy bodies have been documented.[16] Another study demonstrated hippocampal atrophy in demented PD patients with VH.[52] Thus, the temporal lobe appears to be critically involved in visual processing, and in PD the integrity of this important visual processing area is affected. Under these conditions, an impaired 5-HT_{2A}-mediated neurotransmission might contribute to the genesis of VH. Indeed, we have also shown in a pilot positron emission tomography scan study that there is enhanced [^{18}F]-setoperone binding in the inferior temporal cortex in age- and sex-matched groups of PD patients with and without VH.[53]

However, altered 5-HT_{2A}-mediated neurotransmission might also occur beyond the inferior temporal cortex, as non-significant trends toward increases were encountered in both frontal cortex and striatum, when PD patients with VH were compared with PD patients without VH (P = 0.142 and 0.225, respectively, Tukey's post hoc test). Because these increases represent only trends, it is hard to fully appreciate their biological relevance; however, we speculate that they are linked to a broader disturbance in 5-HT_{2A} neurotransmission and could thus be associated with more severe VH.

Increased 5-HT_{2A} Receptor Levels in the Motor Cortex of Parkinsonian Patients

As discussed above with respect to the inferior temporal cortex, the finding of increased 5-HT_{2A} receptor levels in the motor cortex may represent a compensatory change due to degeneration of 5-HT innervation in PD. Interestingly, in the striatum, another key structure involved in motor processing,[54] there was a non-significant trend toward an increase in 5-HT_{2A} receptor levels when PD patients were compared with controls. This increase in the motor cortex, and perhaps striatum, might be related to the development of motor fluctuations, including dyskinesia. Indeed, 5-HT_{2A} receptors were shown to be involved in movement in the rat and the rabbit.[55,56] Pharmacological studies have also suggested that enhanced 5-HT_{2A}-mediated neurotransmission could be involved in the pathophysiology of dyskinesia. Hence, compounds with an antagonist action at the 5-HT_{2A} receptors were shown to reduce dyskinesia in parkinsonian monkeys[57] and humans.[58] However, further studies are needed to define more clearly the fate of 5-HT_{2A} receptors in LID.

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REFERENCES

1. Marsden CD. The mysterious motor function of the basal ganglia: the Robert Wartenberg Lecture. Neurology 1982;32:514–539.
2. Diederich NJ, Goetz CG, Raman R, Pappert EJ, Leurgans S, Piery V. Poor visual discrimination and visual hallucinations in Parkinson's disease. Clin Neuropharmacol 1998;21:289–295.
3. Fenelon G, Mahieux F, Huon R, Ziegler M. Hallucinations in Parkinson's disease: prevalence, phenomenology and risk factors. Brain 2000;123(Part 4):733–745.
4. Agid Y, Arnulf I, Bejjani P, et al. Parkinson's disease is a neuropsychiatric disorder. Adv Neurol 2003;91:365–370.
5. Diederich NJ, Goetz CG, Stebbins GT. Repeated visual hallucinations in Parkinson's disease as disturbed external/internal

perceptions: focused review and a new integrative model. Mov Disord 2005;20:130–140.
6. Williams DR, Lees AJ. Visual hallucinations in the diagnosis of idiopathic Parkinson's disease: a retrospective autopsy study. Lancet Neurol 2005;4:605–610.
7. Goetz CG, Pappert EJ, Blasucci LM, et al. Intravenous levodopa in hallucinating Parkinson's disease patients: high-dose challenge does not precipitate hallucinations. Neurology 1998;50:515–517.
8. Merims D, Shabtai H, Korczyn AD, Peretz C, Weizman N, Giladi N. Antiparkinsonian medication is not a risk factor for the development of hallucinations in Parkinson's disease. J Neural Transm 2004;111:1447–1453.
9. Jacobs BL, Fornal CA. Serotonin and behavior. A general hypothesis. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology the fourth generation of progress. New York: Raven Press; 1995. p461–469.
10. Naughton M, Mulrooney JB, Leonard BE. A review of the role of serotonin receptors in psychiatric disorders. Hum Psychopharmacol 2000;15:397–415.
11. Gresch PJ, Strickland LV, Sanders-Bush E. Lysergic acid diethylamide-induced Fos expression in rat brain: role of serotonin-2A receptors. Neuroscience 2002;114:707–713.
12. The Parkinson Study Group. Low-dose clozapine for the treatment of drug-induced psychosis in Parkinson's disease. The Parkinson Study Group. N Engl J Med 1999;340:757–763.
13. Meltzer HY. The importance of serotonin-dopamine interactions in the action of clozapine. Br J Psychiatry Suppl 1992;17:22–29.
14. Schotte A, Janssen PF, Gommeren W, et al. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology (Berl) 1996;124:57–73.
15. Grill-Spector K, Sayres R, Ress D. High-resolution imaging reveals highly selective nonface clusters in the fusiform face area. Nat Neurosci 2006;9:1177–1185.
16. Harding AJ, Broe GA, Halliday GM. Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. Brain 2002;125(Part 2):391–403.
17. Middleton FA, Strick PL. The temporal lobe is a target of output from the basal ganglia. Proc Natl Acad Sci USA 1996;93:8683–8687.
18. Bezard E, Brotchie JM, Gross CE. Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. Nat Rev Neurosci 2001;2:577–588.
19. Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J, Janssen PA. Receptor binding profile of R 41 468, a novel antagonist at 5-HT2 receptors. Life Sci 1981;28:1015–1022.
20. Vonsattel JP, Aizawa H, Ge P, et al. An improved approach to prepare human brains for research. J Neuropathol Exp Neurol 1995;54:42–56.
21. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. Neurology 2001;57:1497–1499.
22. Leysen JE, Niemegeers CJ, Van Nueten JM, Laduron PM. [^{3}H]Ketanserin (R 41 468), a selective 3H-ligand for serotonin2 receptor binding sites. Binding properties, brain distribution, and functional role. Mol Pharmacol 1982;21:301–314.
23. Hoyer D, Vos P, Closse A, Palacios JM, Engel G, Davies H. [^{3}H]ketanserin labels serotonin 5-HT2 and alpha 1-adrenergic receptors in human brain cortex. J Cardiovasc Pharmacol 1987;10(Suppl 3):S48–S50.
24. Oquendo MA, Russo SA, Underwood MD, et al. Higher postmortem prefrontal 5-HT2A receptor binding correlates with lifetime aggression in suicide. Biol Psychiatry 2006;59:235–243.
25. Darchen F, Scherman D, Laduron PM, Henry JP. Ketanserin binds to the monoamine transporter of chromaffin granules and of synaptic vesicles. Mol Pharmacol 1988;33:672–677.
26. Lyon RA, Davis KH, Titeler M. 3H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT2 receptors. Mol Pharmacol 1987;31:194–199.
27. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17:427–442.
28. Pazos A, Probst A, Palacios JM. Serotonin receptors in the human brain. IV. Autoradiographic mapping of serotonin-2 receptors. Neuroscience 1987;21:123–139.
29. Fox SH, Brotchie JM. 5-HT2C receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. Mov Disord 2000;15:1064–1069.
30. Weisstaub NV, Zhou M, Lira A, et al. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science 2006;313:536–540.
31. Paulus W, Jellinger K. The neuropathologic basis of different clinical subgroups of Parkinson's disease. J Neuropathol Exp Neurol 1991;50:743–755.
32. Mayeux R, Stern Y, Cote L, Williams JB. Altered serotonin metabolism in depressed patients with Parkinson's disease. Neurology 1984;34:642–646.
33. Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther 2002;303:791–804.
34. Elliott MS, Ballard CG, Kalaria RN, Perry R, Hortobagyi T, Francis PT. Increased binding to 5-HT1A and 5-HT2A receptors is associated with large vessel infarction and relative preservation of cognition. Brain 2009;132(Part 7):1858–1865.
35. Halliday GM, Blumbergs PC, Cotton RG, Blessing WW, Geffen LB. Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. Brain Res 1990;510:104–107.
36. Hillarp NA, Fuxe K, Dahlstrom A. Demonstration and mapping of central neurons containing dopamine, noradrenaline, and 5-hydroxytryptamine and their reactions to psychopharmaca. Pharmacol Rev 1966;18:727–741.
37. Fahn S, Libsch LR, Cutler RW. Monoamines in the human neostriatum: topographic distribution in normals and in Parkinson's disease and their role in akinesia, rigidity, chorea, and tremor. J Neurol Sci 1971;14:427–455.
38. D'Amato RJ, Zweig RM, Whitehouse PJ, et al. Aminergic systems in Alzheimer's disease and Parkinson's disease. Ann Neurol 1987;22:229–236.
39. Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y. Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. Brain Res 1983;275:321–328.
40. Radja F, Descarries L, Dewar KM, Reader TA. Serotonin 5-HT1 and 5-HT2 receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. Brain Res 1993;606:273–285.
41. Laprade N, Radja F, Reader TA, Soghomonian JJ. Dopamine receptor agonists regulate levels of the serotonin 5-HT2A receptor and its mRNA in a subpopulation of rat striatal neurons. J Neurosci 1996;16:3727–3736.
42. Woodward RM, Panicker MM, Miledi R. Actions of dopamine and dopaminergic drugs on cloned serotonin receptors expressed in Xenopus oocytes. Proc Natl Acad Sci USA 1992;89:4708–4712.
43. Oz M, Zhang L, Rotondo A, Sun H, Morales M. Direct activation by dopamine of recombinant human 5-HT1A receptors: comparison with human 5-HT2C and 5-HT3 receptors. Synapse 2003;50:303–313.
44. Bhattacharyya S, Raote I, Bhattacharya A, Miledi R, Panicker MM. Activation, internalization, and recycling of the serotonin 2A receptor by dopamine. Proc Natl Acad Sci USA 2006;103:15248–15253.
45. Carta M, Carlsson T, Kirik D, Bjorklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain 2007;130(Part 7):1819–1833.
46. Carta M, Carlsson T, Munoz A, Kirik D, Bjorklund A. Serotonin-dopamine interaction in the induction and maintenance of

L-DOPA-induced dyskinesias. Prog Brain Res 2008;172:465–478.
47. Fabbrini G, Brotchie JM, Grandas F, Nomoto M, Goetz CG. Levodopa-induced dyskinesias. Mov Disord 2007;22:1379–1389; quiz 1523.
48. Goodale MA, Milner AD. Separate visual pathways for perception and action. Trends Neurosci 1992;15:20–25.
49. Halgren E, Dale AM, Sereno MI, Tootell RB, Marinkovic K, Rosen BR. Location of human face-selective cortex with respect to retinotopic areas. Hum Brain Mapp 1999;7:29–37.
50. Beauchamp MS, Argall BD, Bodurka J, Duyn JH, Martin A. Unraveling multisensory integration: patchy organization within human STS multisensory cortex. Nat Neurosci 2004;7:1190–1192.
51. Beauchamp MS, Lee KE, Argall BD, Martin A. Integration of auditory and visual information about objects in superior temporal sulcus. Neuron 2004;41:809–823.
52. Ibarretxe-Bilbao N, Ramirez-Ruiz B, Tolosa E, et al. Hippocampal head atrophy predominance in Parkinson's disease with hallucinations and with dementia. J Neurol 2008;255:1324–1331.
53. Ballanger B, Strafella AP, Zurowski M, et al. Investigating 5HT2A receptor binding in PD patients with visual hallucinations: a [^{18}F]setoperone PET study. Mov Disord 2008;23(Suppl 1):S75.
54. Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 1995;20:91–127.
55. Bishop C, Tessmer JL, Ullrich T, Rice KC, Walker PD. Serotonin 5-HT2A receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT2A/2C agonism. J Pharmacol Exp Ther 2004;310:687–694.
56. Dave KD, Fernando GS, Quinn JL, Harvey JA, Aloyo VJ. Serotonin 5-HT2A receptors in the CA1 field of the hippocampus mediate head movements in the rabbit. Psychopharmacology (Berl) 2004;176:287–295.
57. Iravani MM, Jackson MJ, Kuoppamaki M, Smith LA, Jenner P. 3,4-Methylenedioxymethamphetamine (ecstasy) inhibits dyskinesia expression and normalizes motor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. J Neurosci 2003; 23:9107–9115.
58. Durif F, Debilly B, Galitzky M, et al. Clozapine improves dyskinesias in Parkinson's disease: a double-blind, placebo-controlled study. Neurology 2004;62:381–388.