A Positron Emission Tomography Study of 5-Hydroxytryptamine-1A Receptors in Alzheimer Disease

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Objective: *The important role of serotonin-1A (5-hydroxytryptamine-1A [5-HT_{1A}]) receptors in cognition, behavior, and drug response is increasingly being recognized. Postmortem studies suggest decreased 5-HT_{1A} receptors in patients with Alzheimer disease (AD), but this has not been confirmed in vivo. Our primary objective was to assess the extent of 5-HT_{1A} receptor losses in mild to moderate AD.* **Methods:** *The authors examined 5-HT_{1A} receptors in 10 patients with mild to moderate AD and 10 healthy volunteers with the same sex and similar age using positron emission tomography imaging with the selective 5-HT_{1A} receptor radioligand, [^{11}C]WAY-100635. Regions of interest (ROIs) were manually drawn on coregistered magnetic resonance images for the frontal, lateral temporal, medial temporal (MTC), parietal, and cerebellar cortices. Using the simplified reference tissue model, 5-HT_{1A} binding potentials (BPs) were calculated relative to the cerebellum.* **Results:** *After adjusting for partial volume effects, ROI analysis showed a significant group effect (AD versus comparison group) on BP. Analysis of between-subjects factors showed significantly decreased 5-HT_{1A} BP in the right MTC, but not in the other ROIs.* **Conclusion:** *Given the strategic role of these receptors, loss of right medial temporal 5-HT_{1A} receptors might play an important role in AD symptomatology.* (Am J Geriatr Psychiatry 2007; 15:888–898)

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Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by amyloid plaques and neurofibrillary tangles as well as deficits in neurotransmitter function. Although cholinergic[1,2] and glutamatergic[3,4] deficits have been well documented and these systems have also shown efficacy as targets for pharmacotherapy,[5–7] other neurotransmitters are also implicated in this disorder and may be rational targets.[8]

Serotonin has been proposed to play a role in both the cognitive and behavioral symptoms of AD.[9] The 5-HT_{1A} receptor may be of particular importance as it is located presynaptically in the raphe nuclei, where it controls serotonin release, and postsynaptically in the limbic system and neocortex with the highest density in the hippocampus.[10,11] The 5-HT_{1A} receptor is thought to be involved with cognition,[12,13] psychosis,[14,15] anxiety,[16,17] depression,[16,17] and aggression.[18] In addition to putative direct effects, the 5-HT_{1A} receptor may also be indirectly implicated in cognition and the behavioral and psychological symptoms of dementia (BPSD) through influences on cholinergic[19,20] and glutamatergic neurotransmission.[19]

Postmortem ligand-binding studies[18,21–27] have suggested the possible involvement of the 5-HT_{1A} receptor in AD pathology, reporting decreases of 14%–53% in the frontal, temporal (including hippocampus and amygdala), and parietal cortices. Of these studies, however, the majority used ^{3}H-serotonin,[23,24,26,27] a ligand that is not specific for the 5-HT_{1A} receptor subtype. Studies that used the 5-HT_{1A} selective agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)[18,21,22,25] have varied in their results, with one study finding a 48% decrease in the frontal cortex and no significant decrease (30%) in the temporal cortex,[21] and others finding no significant differences between AD patients and controls.[18,22,25] Though specific for the 5-HT_{1A} receptor subtype, 8-OH-DPAT is an agonist, and will only label receptors in their high-affinity state.[28] In addition, because these studies were conducted postmortem, patients were likely in the final stages of AD. Therefore, earlier losses of 5-HT_{1A} receptors in AD remain unexplored.

WAY-100635 is a selective 5-HT_{1A} antagonist that labels both low- and high-affinity receptors.[29] WAY-100635 can be labeled at the [carbonyl-^{11}C] position to measure 5-HT_{1A} receptor binding in humans using positron emission tomography (PET). This study used [^{11}C]WAY-100635 PET imaging to examine 5-HT_{1A} binding potential in the mild to moderate stages of AD. Based on the results of human postmortem studies, our hypothesis was that 5-HT_{1A} binding potential would be reduced in patients with mild to moderate AD compared to a healthy elderly comparison group, particularly in the frontal, temporal, and parietal cortices of patients with mild to moderate AD.

METHODS

Subjects

A total of 10 normal subjects and 10 mild to moderate AD patients participated in this study. The AD participants were outpatients at the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre who met National Institute of Neurological and Communicative Disorders and Stroke–AD and Related Disorders Association criteria for probable AD,[30] *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for dementia of the Alzheimer type[31] and had mild to moderate AD based on a Global Deterioration Scale score[32] of $\leq$5 and a Mini-Mental State Exam (MMSE)[33] score $\geq$20. Subjects were assessed and excluded if they had an abnormal biochemical screening, non-AD dementia, significant vascular risk factors (Hachinski ischemic score $>$3)[34] or a brain magnetic resonance scan that revealed lesions not consistent with AD. Additional exclusion criteria were a history of significant medical illness or other conditions that diminish cognitive function, presence of depressive symptoms (Cornell Scale for depression in dementia[35] $\geq$8), the presence of any other premorbid or current psychiatric diagnosis (i.e., major mood, psychotic and substance abuse disorders) and the current or recent use of medications that affect serotonergic transmission. BPSD were measured with the Neuropsychiatric Inventory (NPI)[36] and cognition was characterized with the Mattis Dementia Rating Scale (DRS).[37]

The comparison group, recruited from the community, had normal cognition at screening based on an MMSE[33] score of $>$26 and a DRS[38] $>$130. Participants in the comparison group were recruited to

have an age and sex distribution comparable to the AD sample. Subsequent neuropsychological testing in the comparison group confirmed that learning and memory, language, visuospatial function, and executive function were within normal limits. Additional exclusion criteria were significant medical illness, presence of depressive symptoms (Geriatric Depression Scale score[39] ≥11), the presence of premorbid or current psychiatric diagnosis (i.e., major mood, psychotic and substance abuse disorders) and the current or recent use of medications that affect serotonergic transmission.

All healthy elderly participants and AD patients, or their legal substitute decision makers if applicable, provided written informed consent. The Research Ethics Boards of Sunnybrook Health Sciences Centre and that of the Centre for Addiction and Mental Health approved the study.

[^{11}C]WAY-100635 PET Scanning Procedures

A modified version of the McCarron method,[40] as used previously,[15,41] was used to obtain >98% radiochemically pure [*carbonyl*-^{11}C]WAY-100635 with high specific activity 2100 ± 500 mCi/μmol. A bolus injection of 9.5 ± 1.0 mCi of [^{11}C]WAY-100635 with a specific activity at injection of 1,062 ± 458 mCi/μmol was given in the right antecubital vein.

A GEMS-2048-15B PET scanner was used to obtain PET images of the subjects over a period of 90 minutes. The data were collected in 15 one-minute frames, then 15 five-minute frames. The images were attenuation-corrected using a ^{68}Ge transmission scan and reconstructed using filtered back projection (Hanning filter, 5-mm full-width at half maximum) yielding 15 axial slices, each 6.5-mm thick.

5-HT$_{1A}$ receptor binding was analyzed by two methods: predefined regions of interest (ROIs) and a voxel-wise analysis. For the ROI method, subjects underwent an magnetic resonance imaging (MRI) scan on a GE Signa 1.5T scanner acquired using a volumetric three-dimensional sequence covering whole brain. For each subject, 124 contiguous, 1.3-mm thick slices were obtained using a T1-weighted sequence, 192 phase-encoding steps, with a repetition time/echo time (TR/TE) of 35/5 msec and a flip angle of 35°, number of excitations (NEX) = 1, in an imaging time of 10.5 minutes. MRI scans were coregistered to the appropriate PET images using RView coregistration software.[42] ROIs were drawn manually on the coregistered MRI by a trained research assistant and reviewed by two of us (DH, KLL) using the Talairach and Tournoux atlas[43] as a guide. The frontal cortices, medial temporal cortices (MTC) and lateral temporal cortices (LTC), parietal cortices, and the cerebellar cortex (reference region) were delineated using previously defined guidelines.[15,44] These ROIs were selected based on the postmortem findings in AD.[18,21–27]

Decay-corrected time activity curves (TACs) were generated for the first 60 minutes of the data acquisition period for each subject. Following Lammertsma's noninvasive Simplified Reference Tissue Model (SRTM)[45] and using the cerebellar cortex (largely devoid of 5-HT$_{1A}$ receptors) as an area of reference, BP2 values were calculated in order to estimate the number of free 5-HT$_{1A}$ receptors in each ROI. The binding potential of 5-HT$_{1A}$ receptors obtained using Lammerstma's noninvasive SRTM[45] (BP2 values) have been shown to correlate with the invasive kinetically derived BP1 values obtained using arterial sampling, with a Pearson's correlation coefficient of >0.95.[46] However, because of the difficulties associated with arterial sampling of [^{11}C] WAY-100635, the use of an intracerebral reference region for the generation of BP2 values has improved reliability, as shown by test-retest analysis performed by Gunn et al.[46] The BP2 value acquired using the SRTM provides an estimate of binding potential shown to be proportionally related to the product of free (unbound) receptor density (B_{max}) and the affinity of the ligand for the receptor ($1/K_d$), but does not allow the two parameters to be determined separately. In this study, regional BP was estimated from BP2 values generated using the SRTM.

Decreased signal intensity in patients with AD may occur due to atrophy causing partial volume effects (PVEs). These partial volume effects may contribute to apparent differences in binding potentials. Atrophy correction was performed to determine the extent to which observed BP differences were related to PVEs. PVEs were estimated using the Muller-Gartner method,[47] as summarized in the following equation:

$$I_{GM} = (I_{OBS} - I_{WM}X_{WM}{*}h - I_{CSF} \times {}_{CSF}{*}h)/X_{GM}{*}h1$$

where $*$ = the convolution operator, h = the three-dimensional point spread function (PSF) of the PET

scanner, I_{GM} = the radioactivity concentration within the gray matter (GM), I_{OBS} = the observed PET image; I_{WM} = the PET value for white matter (WM; assumed to be homogeneous), I_{CSF} = the PET value for cerebrospinal fluid (CSF; assumed to be homogeneous), X_{WM} = the spatial distribution of WM voxels, X_{CSF} = the spatial distribution of CSF voxels, and X_{GM} = the spatial distribution of GM voxels. The implementation was developed by one of the authors (PMR) following the eight steps described by Bencherif et al.[48] Briefly, the voxels in the subject MR image were probabilistically classified as gray matter (GM), white matter (WM), or CSF using SPM5. The probabilistic images were binarized according to the maximum probability encountered for each voxel across the three datasets. A rigid body transform matrix was calculated using the normalized mutual information metric implemented under SPM2. Then the binarized GM, WM, and CSF were rota-translated with this transformation. Voxel sizes and binary values were kept. The reoriented GM, WM, and CSF images were convolved with the PSF of the PET scanner (*h). Then the convolved data were resliced and resampled to the space of the PET image using trilinear interpolation. This gives us the value in each voxel in the PET space of the term X_{WM}*h, X_{CSF}*h, and X_{GM}*h in the above equation. ROIs of pure WM and CSF are created to estimate the values of I_{WM} and I_{CSF}. The above equation was solved for each voxel. The 3-Da PSF of the PET scanner was estimated as having a Gaussian distribution with a full width at half maximum (FWHM) of 6 mm. This value was calculated as the average of images of point sources placed in different positions in the field of view of the scanner and it is in agreement with other works with similar scanners.[49,50] To avoid overcorrection during the extraction of the activity data points on the PVE corrected image, the manually delineated ROIs were restricted to the places where X_{GM}*h >0.21, as has been done before.[48]

Next, for descriptive purposes, the mean percent of gray matter in each ROI was calculated in PET space as the ratio of the number of voxels of GM in the ROI to the total number of voxels in the ROI and compared between AD patients and healthy elderly controls using multivariate analysis of variance (MANOVA). Classification of voxels in PET space was obtained from the MRI classifications following these steps: 1) the probabilistically classified image of GM, WM, and CSF from step 1 (above) was reoriented with the MRI-PET coregistration transform and resampled using trilinear interpolation to PET space, and 2) the probabilistic maps was binarized according to the maximum probability encountered for each voxel across the three datasets.

Statistical Analysis

SPSS for Windows (version 14.0) was used to conduct the statistical analyses of the ROI data. All tests were two-tailed and α levels of $p < 0.05$ was considered significant. First, relevant demographic and clinical characteristics were compared for the two groups using independent *t*-tests or χ^2 as appropriate to establish comparability of the groups for possible confounders. Next, to test our hypothesis, regional 5-HT_{1A} receptor BP values of patients and the comparison group were compared using MANOVA with BPs from the eight ROIs (from frontal, temporal, and parietal cortices) as dependent variables and diagnosis as the fixed (between subjects) variable. Post-hoc tests were used to determine the contribution of each ROI to the overall difference. Effect sizes were calculated for each ROI using Cohen's *d* where $d = (M1\text{-}M2)/\delta$ where M1 = mean BP for the comparison group, M2 = mean BP for the AD group and δ = pooled SD and interpreted as small ($d \leq 0.2$), medium ($d = 0.21–0.79$), and large ($d \geq 0.8$). Pearson's product-moment coefficients were calculated to measure correlations between 5-HT_{1A} receptor BP and the DRS.

Volumes of interest (VOIs) and the ratio of tracer delivery to each ROI relative to the cerebellum (R_1) were compared between AD patients and the comparison group using independent sample *t*-tests. VOIs were calculated by summing the product of the area of each ROI and its slice thickness (6.5 mm), for each slice in which the ROI appeared. Differential effects of blood flow, although not a particular problem with this tracer,[46] could, in the presence of atrophy, lead to changes in tracer binding between groups and be a possible confounder. The estimation of flow relative to the cerebellum within each subject group was calculated by taking the concentration of tracer (nCi/cc) from the TACs for each ROI for the first five frames and dividing it by the concentration within the cerebellum to correct for injected amounts across subjects. This ratio was computed for each

ROI and compared between patients and healthy elderly using unpaired *t*-tests. To compare cerebellar uptake between patients and the comparison group, an independent samples *t*-test was performed, with the area under the curve of the TAC for the cerebellum as the dependent variable. This was repeated with the area under the time-activity curve values adjusted by the activity at time of injection for each participant.

To find converging evidence for the ROI analysis, a whole-brain voxel-by-voxel comparison was also conducted using Statistical Parametric Mapping version 99 (SPM 99; Wellcome Department of Cognitive Neurology, London)[51] running under the MATLAB environment (version 6.1, MathWorks, Natick, MA). The SRTM was used to generate parametric 5-HT$_{1A}$ receptor BP images for voxelwise analysis using PMOD Medical Imaging Software (version 2.20). SPM 99 was used to generate statistical parametric maps of the difference between AD patients and healthy elderly for this voxelwise analysis. Parametric images were spatially normalized using the standard Montreal Neurologic Institute (MNI) template. An 8-mm Gaussian smoothing kernel was applied to the normalized images. To test whether the measured 5-HT$_{1A}$ receptor BP differed between patients and the comparison group in any given voxel, two-tailed *t*-tests were applied. SPM analysis provides both uncorrected and corrected p values where adjustment involves correction for multiple comparisons based on random field theory. Because data analysis was confirmatory rather than exploratory, uncorrected p values are reported. Results were displayed as statistical parametric maps using an uncorrected height threshold of $p < 0.001$. Two contrasts (AD 5-HT$_{1A}$ BP lower than that of the comparison group and vice versa) were applied to a search volume of 62,890 voxels, each with a size of $2 \times 2 \times 2$ mm.

RESULTS

Demographics

The demographic data are summarized in Table 1. Neither group showed significant depressive symptoms and there were no differences in age, sex, handedness, or years of education between AD patients and the comparison group. The AD group had been diagnosed an average of 4.8 (±4.2) years ago, they had Global Deterioration Scores between 3 (mild) and 5 (moderately severe) with a mode of 4 (moderate), and a mean Neuropsychiatric Inventory score of 6.8 (±2.6) indicating low levels of behavioral psychopathology. Concomitant psychotropic medications in the AD group included: cholinesterase inhibitors (N = 9) and vitamin E (N = 7). One AD patient had previously been treated with trazodone, which was discontinued 2 weeks before the PET scan date. None

TABLE 1. Demographic Characteristics of AD Patients and Controls

	AD Group	Comparison Group	Test Statistic	p Value
N	10	10		
Age[a]	75.7 (9.0)	72.8 (10.4)	0.67	0.51
Sex (N)[b]			0.00	1.00
Male	7	7		
Female	3	3		
Right handed (N)[b]	8	10	2.48	0.12
Years of education[a]	14.6 (2.6)	13.2 (3.4)	1.03	0.32
Mini-Mental State Exam[c]	24.6 (3.0)	28.8 (0.6)	4.35	0.002
Mattis Dementia Rating Scale (maximum 144)[c]	122.0 (16.3)	139.2 (2.4)	2.96	0.02
Depression				
Cornell scale for depression in dementia	3.3 (2.6)			
Geriatric Depression Scale		3.3 (3.2)		

Data are means (SD) unless otherwise indicated.
[a] *t*-tests, df = 18.
[b] χ^2 tests, df = 1.
[c] *t*-tests adjusted for unequal variances, df = 9.8 for MMSE, df = 7.24 for DRS.

of the comparison participants were taking psychotropic medications.

ROI Analysis

The ROI-based analysis indicated a mean (±SD) decrease in 5-HT_{1A} receptor BP of 13.2 ± 10.8% across the eight cortical areas in A.D. patients versus the comparison group (Fig. 1A, Table 2). Effect sizes were of small magnitude in the frontal and right parietal cortices, medium in the left parietal cortex and large in the medial temporal and lateral temporal cortices. MANOVA revealed a significant effect of diagnosis on the BP values measured in the eight cortical ROIs (Wilks' $\lambda = 0.138$, $F_{8,11} = 8.623$, $p = 0.001$). Post hoc tests evaluating between-subject effects showed that 5-HT_{1A} BP values of AD patients were significantly lower in the right ($F_{1,18} = 15.7$, $p = 0.001$) and left MTC ($F_{1,18} = 6.6$, $p = 0.02$) and the right ($F_{1,18} = 5.6$, $p = 0.03$) and left LTC ($F_{1,18} = 10.4$, $p = 0.005$). There were no significant between-subject effects for BP values in any other ROIs.

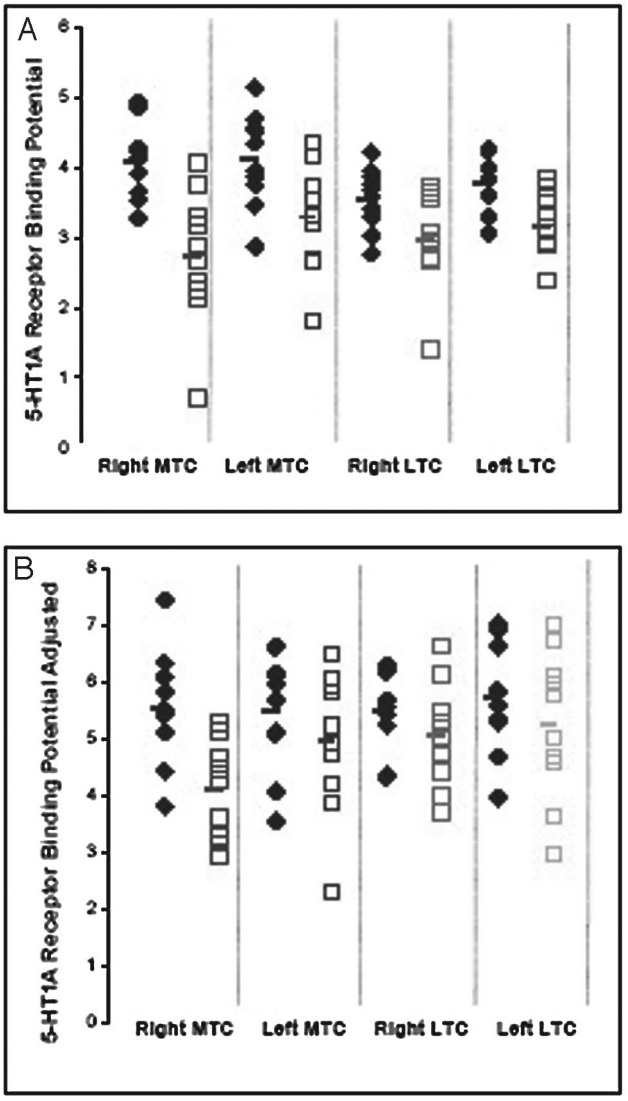
DRS scores were significantly correlated with 5-HT_{1A} BP values in the right ($r_{16} = 0.64$, $p = 0.004$) and left MTC ($r_{16} = 0.56$, $p = 0.015$) and the right ($r_{16} = 0.57$, $p = 0.014$) and left LTC ($r_{16} = 0.51$, $p = 0.029$).

Impact of Atrophy

BPs were adjusted for partial volume effects to determine the impact of atrophy. For the eight cortical ROIs, effect sizes decreased after adjustment for PVEs (Fig. 1B). MANOVA revealed a significant effect of diagnosis on the adjusted BP values (Wilks' $\lambda = 0.305$, $F_{8,11} = 3.136$, $p = 0.041$). Post-hoc tests evaluating between-subject effects showed that 5-HT_{1A} BP values of AD patients were significantly lower in the right MTC (Table 2, Fig. 2). Adjusted 5-HT_{1A} BP in the right MTC was correlated with DRS total score ($r_{16} = 0.56$, $p = 0.016$).

When comparing percent gray matter, MANOVA also revealed a significant effect of diagnosis (Wilks' $\lambda = 0.224$, $F_{8,11} = 4.773$, $p = 0.010$), with all ROIs except the right frontal ($p = 0.065$) and right parietal ($p = 0.093$) showing significant between subjects effects (Table 2).

FIGURE 1. **Serotonin-1A (5-hydroxytryptamine [5-HT_{1A}]) receptor binding potential values in the medial and lateral temporal cortices of 10 patients with Alzheimer disease (squares) and 10 healthy controls (triangles). Bars indicate group means before (A) and after (B) adjustment for partial volume effects. MTC: medial temporal cortex; LTC: lateral temporal cortex.**



SPM Analysis

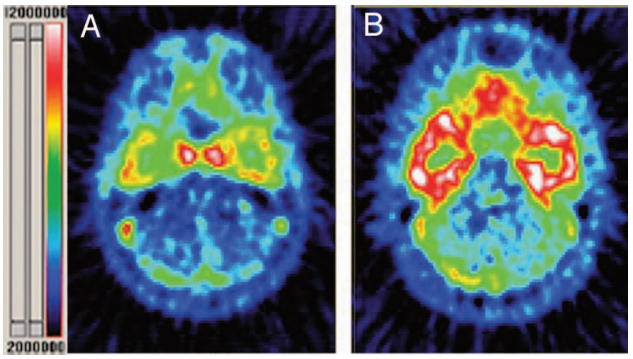
SPM analysis at the voxel level showed decreased WAY-100635 uptake in AD patients in the right MTC (parahippocampal gyrus, BA 35; MNI coordinates 28, −12, and −30 mm; $z = 3.61$; $p_{uncorrected} < 0.001$) and the left LTC (superior temporal gyrus BA 38;

TABLE 2. 5-HT1A Receptor Binding Potentials for the Eight Regions of Interest

	5-HT 1A Binding Potential						Percent Gray Matter	
Region	Comparison	AD	Percent Difference[a]	Effect Size[b]	p Value[c]	F Value	Comparison	AD
Frontal cortex								
Right uncorrected	2.4 (0.4)	2.3 (0.6)	3.7	.17	0.09	0.13	60.2 (7.4)	52.3 (10.3)
Right corrected	4.8 (0.8)	5.0 (1.5)	4.1	.2	0.72	0.13		
Left uncorrected	2.4 (0.4)	2.3 (0.5)	2.9	.16	0.75	0.11	58.1 (5.5)	50.9 (9.1)
Left corrected	4.7 (0.8)	4.9 (1.3)	5.1	.2	0.64	0.23		
Medial temporal cortex								
Right uncorrected	4.1 (0.5)	2.7 (1.0)	33.4	1.8	0.001	15.66	81.5 (5.2)	61.7 (16.2)
Right corrected	5.5 (1.0)	4.2 (0.9)	23.8	1.1	0.007	9.28		
Left uncorrected	4.1 (0.67)	3.3 (0.8)	19.9	1.1	0.03	6.56	80.6 (6.2)	62.5 (18.5)
Left corrected	5.5 (1.0)	5.0 (1.3)	9.9	0.5	0.31	1.11		
Lateral temporal cortex								
Right uncorrected	3.6 (0.4)	3.0 (0.7)	16.7	1.05	0.02	5.56	75.0 (6.5)	56.5 (20.2)
Right corrected	5.5 (0.7)	5.1 (0.9)	7.3	0.5	0.29	1.20		
Left uncorrected	3.8 (0.4)	3.2 (0.4)	15.8	1.44	0.005	10.43	76.8 (8.3)	57.4 (17.9)
Left corrected	5.7 (1.0)	5.3 (1.3)	8.0	0.4	0.39	0.78		
Parietal cortex								
Right uncorrected	2.2 (0.4)	2.2 (0.5)	1.2	0.07	0.89	0.02	57.1 (11.6)	48.2 (10.8)
Right corrected	4.5 (0.8)	5.0 (1.7)	11.8	0.4	0.38	0.82		
Left uncorrected	2.2 (0.4)	2.0 (0.5)	12.7	0.66	0.16	2.10	56.8 (12.5)	41.5 (7.1)
Left corrected	4.5 (0.9)	5.2 (1.7)	17.1	0.6	0.22	1.59		

Data are means (SD).
[a](Comparison BP – Patient BP) / Comparison BP × 100%
[b]Cohen's *d*.
[c]Between subject effects from corrected model using MANOVA, df=1,18.

FIGURE 2. [^{11}C]WAY-100635 PET scans from a representative AD patient (A) and a healthy elderly participant (B). Binding potential was significantly reduced in the right medial temporal cortex of AD patients compared with healthy elderly participants.



MNI coordinates −46, 8, and −16 mm; z = 3.32; $p_{uncorrected}$ <0.001) when compared with healthy elderly participants (both sets of coordinates indicate x, y, and z, respectively). Corrected p values were nonsignificant.

Delivery, Blood Flow, and Volumes of Interest

The variable R_1 in the SRTM is the ratio of tracer delivery of the tissue of interest relative to the reference tissue. Independent samples t tests revealed no significant differences in regional tracer delivery (R_1) between patients and the comparison group for the right medial temporal (t_{18} = 0.71, p = 0.48), left medial temporal (t_{18} = 0.30, p = 0.77), right lateral temporal (t_{18} = 1.37, p = 0.19) or left lateral temporal (t_{18} = 0.23, p = 0.82) ROIs. Lastly, the ratio of tracer uptake in the temporal and frontal cortices relative to cerebellum in the early frames was not significantly different between groups (right temporal/cerebellum, t_{18} = 1.70, p = 0.11; left temporal/cerebellum, t_{18} = 1.03, p = 0.51; right frontal/cerebellum, t_{18} = 1.03, p = 0.32; left frontal/cerebellum, t_{18} = 0.42, p = 0.68). Thus, results cannot likely be explained by differences in delivery or estimated blood flow.

Independent sample *t*-tests revealed no significant differences in volumes of interest between patients and healthy elderly for all ROIs (all p >0.05). Pearson correlation coefficients (r) did not reveal any significant correlation between regional BP values and

their respective volumes of interest (all $p > 0.05$). Furthermore, there was no significant difference in the area under the curve of the cerebellar TACs between groups ($t_{18} = 0.77$, $p = 0.45$), even when normalized to activity at time of injection ($t_{18} = 0.56$, $p = 0.59$).

CONCLUSIONS

We examined 5-HT_{1A} binding potential in the frontal, temporal and parietal cortices in patients with mild to moderate AD using [^{11}C]WAY-100635 PET imaging. By ROI-based analysis, we found that 5-HT_{1A} BP was reduced overall, with significant decreases of 20% to 33% in the medial and 16% to 17% in the lateral temporal cortices of mild to moderate AD patients compared to healthy elderly. When partial volume effects were considered, the magnitude of BP differences between groups was reduced. As a result, a significant decrease in 5-HT_{1A} BP was maintained only for the right medial temporal cortex. This finding was supported by voxelwise analysis using SPM99.

In this study, although volume of interest, blood flow effects, and tracer delivery did not appear to contribute to between-group differences, partial volume effects were present. Correcting for partial volume effects due to cerebral atrophy involves segmenting CSF, gray matter, and white matter and applying the composite tissue image to the PET image. Dilutional effects are consistent with previous literature.[52–54] Partial volume effects may be particularly prominent in AD because atrophy is associated with increased CSF, and gray matter losses may be greater than white matter losses in AD.[47] A previous longitudinal study in patients with mild AD indicated that the medial temporal cortex showed a faster and more extensive reduction of gray matter volume than of regional cerebral blood flow.[55] Similarly, we found that the AD group had significantly less gray matter than the comparison group in each ROI tested, particularly in the temporal cortices. As a result, the effect of atrophy was large compared to the difference due to Alzheimer disease in this mild to moderately impaired population. Both overall loss of 5-HT_{1A} receptors and loss of 5-HT_{1A} receptors per gram of remaining gray matter may have an impact on function.

Our results are consistent with previous postmortem studies using the 5-HT_{1A} agonist 8-OH-DPAT that demonstrated decreases in temporal 5-HT_{1A} binding of 20%[18] to 30%.[21] Postmortem studies using other ligands also found significant decreases in binding in the temporal cortex[24,26,27,56] and two areas of the medial temporal cortex high in 5-HT_{1A} receptors: the hippocampus[26,56] and the amygdala.[56] Although those studies did not consistently reach statistical significance, the present study used the antagonist [^{11}C]WAY-100635, considered right and left as well as medial and lateral temporal cortices separately, and sampled more broadly from the region of interest.

In contrast to some post-mortem studies that found significant decreases in 5-HT_{1A} binding in the frontal[21,23,24,26] and parietal[24] cortices, we found small decreases with no significant differences between AD patients and healthy elderly. Although those studies included AD patients in later stages of illness, our study sample excluded severe patients. This suggests that decreases in 5-HT_{1A} binding in the temporal cortices, particularly on the right, may precede losses in other areas. In support of this, previous literature implicates the temporal lobes as being specifically and severely affected in the early stages A.D.[57,58]

Our finding that 5-HT_{1A} receptor binding potential is decreased in the temporal cortices may be particularly germane in this population. In the temporal cortex, they are predominantly found postsynaptically in the hippocampus and amygdala.[10,11] In addition to being expressed on postsynaptic serotonergic neurons, the 5-HT_{1A} receptor is also found on cholinergic neurons in the septum and glutamatergic (pyramidal) neurons in the cortex and hippocampus.[11] In particular, 5-HT_{1A} receptors in the hippocampus, part of the medial temporal cortex, are involved in various facets of cognition.[12,59] In keeping with this, we found decreased right medial temporal 5- HT_{1A} binding potential was directly correlated to decreased cognition. Thus, decreased 5-HT_{1A} receptor dysfunction observed in this study may be implicated in the cognitive symptoms seen in early AD.

The 5-HT_{1A} receptor may also be involved in noncognitive behaviors.[14–17] In particular, a previous postmortem study, although finding no differences between AD patients and otherwise healthy elderly overall, found significant reductions in 5-HT_{1A} binding in the mid temporal cortices (BA21) of aggressive AD patients only.[18] In contrast to that study, we were able to demonstrate significant decreases in mild to moderate AD patients without aggression. This sug-

gests that right medial temporal cortex receptor loss precedes significant behavioral deterioration.

Our finding may have important treatment implications. The 5-HT$_{1A}$ receptors have been associated with the mechanism of action of atypical antipsychotic drugs,[60,61] anxiolytics,[17] and antidepressants,[17,62] all of which are frequently used in AD. Our results suggest that loss of 5-HT$_{1A}$ receptors may modify drug response to these medications. In addition, 5-HT$_{1A}$ antagonists have been proposed as a possible therapeutic approach to improve cognitive function in AD[13] by enhancing activation and signaling through the heterosynaptic neuronal circuits associated with cognitive processes[13] and by ameliorating AD-associated degradation of the cholinergic[63] and glutamatergic[64] systems. Our results suggest that 5-HT$_{1A}$ antagonists may not have the desired effect due to selective loss of 5-HT$_{1A}$ receptors in the medial temporal area.

Several points must be considered in interpreting these results. First, a larger sample size might detect more subtle differences in other regions of interest. A previous test-retest study indicated that we would be able to correctly identify a group difference of 20% to 79% with acceptable sensitivity,[41] which seems to have been borne out by our current study. However, to detect a between-group difference of 4% (e.g., frontal cortices) with a power ($1 - \beta$) of 80% and $\alpha = 0.05$, we would need a sample size of 250 per group. Thus, while we cannot rule out that such small differences in other regions exist, it is unknown whether differences of such a small magnitude would be clinically meaningful. Second, we did not perform quantitative measurement to determine absolute blood flow within each subject group, but instead used the SRTM with the cerebellum as the reference region. The SRTM provides an estimate proportional to the binding potential (B_{max}/K_d) but does not differentiate between density of binding sites (B_{max}) and affinity (K_d) of 5-HT$_{1A}$ receptors. Distinguishing B_{max} from K_d is not paramount because previous AD postmortem studies have shown a change in B_{max} with no alteration in K_d.[18,21–27,65] A second consequence of this model is that differences in BP may reflect differences in either the ROI or the reference region, or both. In this study, we confirmed that cerebellar TACs, even when adjusted for activity at time of injection, and cerebellar volumes did not differ between AD patients and the comparison group. Also, a general reference tissue error would be expected to lead to a systematic and consistent error across all the regions. In contrast, our results were selective for limited ROIs. Lastly, while participants were free of psychotropic medications known to influence the serotonergic system at the time of scanning, one AD patient had previously been taking trazodone. The results from this participant are similar to those of the other AD patients and removing this participant from the analysis did not change the results. Thus, although there is the possibility that prior use of this medication may impact 5-HT$_{1A}$ binding potential, this applied only to one AD patient and cannot, on its own, explain the group differences found in this study.

In summary, using [^{11}C]WAY-100635 PET imaging, we found decreased 5-HT$_{1A}$ BP in the right medial temporal cortices of patients with mild to moderate AD compared to healthy elderly participants comparable for age and sex. The 5-HT$_{1A}$ receptor mediates cognitive and noncognitive central functions highly relevant to AD and plays a critical role in regulating not only the serotonergic system, but also the cholinergic[19,20] and glutamatergic systems, all of which are affected by AD. Despite potential benefits of 5-HT$_{1A}$ receptor antagonism, medial temporal receptor loss may limit the efficacy of pharmacologic interventions targeting this receptor in those with AD.

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