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Serotonin 5-HT_{1A} Receptor Binding Potential Declines with Age as Measured by [^{11}C]WAY-100635 and PET

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Positron emission tomography (PET) and [^{11}C]WAY-100635 were used to examine the effect of age on serotonin-1A (5-HT_{1A}) receptor binding potential (BP) in 19 healthy subjects. Regions of interest (ROI) were drawn on the co-registered magnetic resonance imaging (MRI) in orbitofrontal (OFC), dorsolateral prefrontal (DLPFC), anterior cingulate (ACC), lateral (LTC), and mediotemporal (MTC), parietal, occipital and cerebellar cortex, and the raphe nuclei. BP values were calculated using a simplified reference tissue method. In addition, a voxelwise analysis was performed using SPM99. Voxelwise analysis revealed a significant global decrease of 5-HT_{1A} BP with age (set level $<$ .001). ROI analysis revealed significant age-related 5-HT_{1A} BP decreases in DLPFC ($r = -0.56$), ACC ($r = -0.44$), OFC ($r = -0.42$), LTC ($r = -0.40$), parietal ($r = -0.65$), and occipital cortex ($r = -0.43$), but not in MTC or raphe nuclei. Overall, cortical 5-HT_{1A} BP declined by approximately 10% per decade, except for the MTC, where we did not find a significant age effect. Hence, careful age matching may be recommended for future studies using PET and [^{11}C]WAY-100635 to examine 5-HT_{1A} receptors.
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KEY WORDS: *5-HT_{1A} receptors; Age effects; [^{11}C]WAY-100635; Positron emission tomography*

The serotonin-1 (5-HT_1) family of receptors is characterized by seven transmembrane loops linked to G-proteins and modulate adenylate cyclase activity (Zifa and Fillion 1992). The 5-HT_{1A} receptors are distinguished from other 5-HT_1 family members by their unique genetic sequence and ligand-binding profile (Zifa and Fillion). Until recently, there were no specific antagonist ligands for this receptor; therefore, most studies relied on selective agonists such as 8-hydroxy-2-di-n-propylaminotetraline (8-OH-DPAT) (Fletcher et al. 1993). Agonist studies are limited by the fact that they bind only to 5-HT_{1A} receptors in the high-affinity state. Furthermore, 8-OH-DPAT has never been approved for human use. The discovery of selective and potent antagonists, such as WAY-100135 and WAY-100635, which both can be used in humans, has opened new possibilities for 5-HT_{1A} research and therapeutics (Fletcher et al. 1993).

A human autoradiographic study showed the highest density of 5-HT_{1A} receptors in the temporolimbic cortex, followed by brainstem raphe nuclei, frontal cortex, and other neocortical regions, with very low or undetectable levels in the cerebellum (Burnet et al. 1997).

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The brainstem receptors are somatodendritic autoreceptors, whereas the cortical receptors are mainly postsynaptic. The cortical 5-HT_{1A} receptors are localized on axon hillocks of pyramidal cells, especially in the layers II, III, and V of the cortex (Burnet et al. 1997). These receptors are involved in the inhibitory modulation of cortico–cortical association and cortico–striatal efferent fibers (Bowen et al. 1993). Cortical 5-HT_{1A} receptors exert inhibitory control over striatal glutamate release, and 5-HT_{1A} antagonists increase glutamate release in the striatum via cortico–striatal efferents (Dijk et al. 1995). In addition, 5-HT_{1A} agonists increase the outflow of dopamine in the prefrontal cortex without a similar change in striatal dopamine release (Wędzony et al. 1996).

Post-mortem studies show a decline in 5-HT_{1A} receptor number with age (Dillon et al. 1991; Lowther et al. 1997; Matsubara et al. 1991). However, these studies have either used 8-OH-DPAT, an agonist ligand, which measures 5-HT_{1A} receptors in the high affinity state, or spiperone, an antagonist, which lacks specificity. These limitations have recently been overcome by the discovery of WAY-100635, a selective high-affinity (Kd $<$ 1 nM) 5-HT_{1A} antagonist (Fletcher et al. 1993). WAY-100635 labels almost twice as many 5-HT_{1A} receptors as 8-OH-DPAT, because it labels both low- and high-affinity receptors (Fletcher et al. 1993). To adapt WAY-100635 for human studies, it has been labeled at the [*carbonyl*-^{11}C] position (Farde et al. 1997) and can be used for the quantitative analysis of binding to 5-HT_{1A} receptors in humans (Farde et al. 1998).

Recently, positron emission tomography (PET) and [^{11}C]WAY-100635 were used to show decreased 5-HT_{1A} receptor binding potential (BP [Mintun et al. 1984]) in patients with major depression (Drevets et al. 1999; Sargent et al. 2000) and in clozapine-treated schizophrenic patients (Bantick et al. 2000), as compared to healthy control groups.

Because of the noted decrease in 5-HT_{1A} receptor number with age in post-mortem studies, our hypothesis was that 5-HT_{1A} receptor BP as measured with [^{11}C]WAY-100635 and PET would decline with age in healthy subjects.

MATERIALS AND METHODS

Subjects

Nineteen healthy subjects (8 female/11 male; mean age 34 years; range: 22–53) were examined. Exclusion criteria were: (1) any Axis-I psychiatric diagnosis confirmed by the Structured Clinical Interview for DSM-IV, nonpatient edition (SCID-I/NP [First et al. 1997]); (2) presence of serious medical or neurological illness or of significant head injury; (3) a history of alcohol or substance dependence; (4) treatment with psychotropic medications within the last 3 months before the study; or (5) pregnancy. All subjects gave their written consent after the procedure had been fully explained. The study had been approved by the Human Subjects Review Committee of the University of Toronto.

To establish test–retest reliability, six of the 19 subjects were studied on a second occasion 1 to 2 weeks after the first scan.

[^{11}C]WAY 100635 PET Scanning Protocol

Radiolabeling. The selective 5-HT_{1A} receptor antagonist *carbonyl*-[^{11}C]-N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide ([^{11}C]WAY-100635) was synthesized by modifications of the McCarron method (McCarron et al. 1996) using a short *Teflon* tube loosely packed with polypropylene wool as a substitute for the narrow polypropylene tubing originally used (Houle et al. 2000). This procedure yielded syntheses with high purity and average specific activity of 41 GBq/μ mole at time of injection.

Data Acquisition. PET images were obtained with a GEMS PC2048-15B camera (General Electric Medical Systems, Milwaukee, WI, USA) in 15 1-minute frames followed by another 15 5-minute frames after bolus injection of 9.56 mCi [^{11}C]WAY-100635. The images were corrected for attenuation with a ^{68}Ge transmission scan and reconstructed by filtered back projection (Hanning filter, 5 mm full width at half maximum) and 15 6.5 mm-thick axial slices were obtained.

Image Analysis. For the quantification of 5-HT_{1A} receptor binding in human brain, two approaches were used: region of interest (ROI) and voxelwise analysis.

Each subject had a magnetic resonance imaging (MRI) scan (GE signa 1.5 T scanner; spin echo sequence T_1- and PD-weighted image; x,y,z voxel dimensions: 0.78, 0.78, and 3 mm, respectively). MRI scans were co-registered to each PET image by using RView8/mpr software (Studholme et al. 1997). For ROI analysis, nine distinct brain regions were delineated on the co-registered MRI using landmarks previously defined (Bremner et al. 1998). Anatomical ROI were drawn in dorsolateral prefrontal (DLPFC) and orbitofrontal (OFC), anterior cingulate (ACC), medial temporal (MTC), and lateral temporal (LTC), parietal and occipital cortex, and the cerebellum as a reference region. In addition, a raphe nuclei ROI was delineated in the following way: midbrain sections were identified on the co-registered MRI as those consecutive slices, where the interpeduncular cistern was clearly visible. A fixed-size circular ROI was then placed on a dorsal midbrain area with high tissue radioactivity in the corresponding two slices of a PET summation image yielding a constant volume of interest (VOI) of 0.6 cm^3 for the raphe nuclei.

Decay-corrected time activity curves (TAC) were ob-

tained for each ROI using the first 60 minutes of the data acquisition period. Additionally, for the six subjects scanned twice, TAC of 90 minutes data acquisition periods were generated. Regional binding potential (BP) values were calculated to estimate the 5-HT_{1A} receptor number in each ROI (Mintun et al. 1984). To obtain BP values, the cerebellum was used as the input function of a simplified reference tissue method, because the cerebellum is relatively devoid of 5-HT_{1A} receptors (Burnet et al. 1997) and this method proved to be superior to kinetic modeling using arterial data (Gunn et al. 1998).

For the voxelwise analysis, parametric 5-HT_{1A} receptor BP images were generated using the simplified reference tissue model (Gunn et al. 1997). In a next step, parametric images were spatially normalized within the standard Montreal Neurologic Institute (MNI) brain space using Statistical Parametric Mapping version 99 (SPM99) (Friston et al. 1995) and a ligand-specific template (Meyer et al. 1999).

Statistical Analysis. Statistical analyses of the ROI data were performed using SPSS for Windows 10.0.0, SPSS Inc., 1999. The mean and standard deviation of BP values were calculated for each ROI. To test the hypothesis that 5-HT_{1A} receptor BP declines with age, one-tailed Pearson product-moment correlation coefficients were calculated, using a threshold of $p < .05$ for significance. Potential correlations between age and the measured activity in the cerebellar ROI, between age and volume of interest (VOI) size, and between each ROI BP value and its respective volume were examined using two-tailed Pearson product-moment correlation coefficients.

The test–retest agreement of the estimates for BP in each ROI was assessed by calculating the difference between scan 1 and scan 2. Furthermore, we calculated average measure intraclass correlation coefficients (ICC) for each ROI and the repeatability coefficient (RC), which were calculated over the six subjects scanned twice. The RC is equal to twice the standard deviation of the difference between the BP values of scan 1 and 2. Thus, it can be expected that 95% of the differences are less than the RC (Bland and Altman 1986). In addition, to facilitate comparisons across regions, the RC was calculated as a percentage of the mean BP (RM):

$$\text{RM\%} = \frac{2 \times \text{SD}(\text{BPscan1} - \text{BPscan2})}{\text{Mean}(\text{BPscan1} + \text{BPscan2})}$$

Voxel-by-voxel analysis was carried out using SPM99. To test the hypothesis that age influenced the measured 5-HT_{1A} receptor BP in any given voxel, analysis of covariance (ANCOVA) was applied using age as the covariate of interest with no global normalization of brain activity levels, no grand mean scaling, and a gray matter threshold of 80%. Results of the ANCOVA were displayed as parametric maps using a height threshold of $p = .001$ with no extent threshold and two contrasts to examine increases and decreases with age. Furthermore, SPM99 was used to test: (1) whether the assumed age-related 5-HT_{1A} receptor BP decline occurred globally or in distinct brain areas; and (2) if gender influenced 5-HT_{1A} receptor BP.

RESULTS

After injection of 9.56 mCi (± .77 [SD]) [^{11}C]WAY-100635, ROI analysis of 60 minutes TAC by means of a simplified reference tissue method (Gunn et al. 1998) yielded 5-HT_{1A} BP values (mean ± SD) ranging from 5.10 (± 0.69) in MTC to 2.10 (± 0.65) in the raphe nuclei (Table 1). The rank order for BP values was MTC > LTC > ACC > OFC > DLPFC > parietal cortex > occipital cortex > raphe nuclei. Pearson correlation coefficients revealed significant age-related 5-HT_{1A} BP decreases in DLPFC ($r = -0.56$; $p = .006$ [Figure 1]), ACC ($r = -0.44$; $p = .029$), OFC ($r = -0.42$; $p = .036$), LTC ($r = -0.40$; $p = .047$), occipital ($r = -0.43$; $p = .034$) and parietal cortex ($r = -0.65$; $p = .001$ [Figure 2]). No significant correlation between 5-HT_{1A} BP and age was found in MTC ($r = -0.27$; $p = .132$ [Figure 3]) and raphe nuclei ($r = -0.16$; $p = .263$).

Volume of interest (VOI) values ranged from 54.4 cm^3 in parietel cortex to 0.6 cm^3 for the raphe nuclei (Table 1). There was no correlation of age and VOI size in most ROI and in the cerebellum, with the exception of the occipital cortex ($r = 0.52$; $p = .026$) and OFC ($r = -0.66$; $p = .002$). However, VOI size and their respective BP values were not significantly correlated for any given ROI. Furthermore, there was no correlation of age and the measured activity in the reference region cerebellum ($r = -0.09$; $p = .720$).

Table 1. Mean Regional 5-HT_{1A} Receptor BP Values and Their Respective Volume of Interest (in cm^3) for 19 Healthy Subjects

	Binding potential		Volume of interest	
ROI	**Mean**	**SD**	**Mean**	**SD**
Dorsolateral prefrontal cortex	3.07	0.46	39.8	6.5
Orbitofrontal cortex	3.28	0.53	19.7	3.5
Anterior cingulate cortex	3.85	0.46	16.1	1.8
Medial temporal cortex	5.10	0.69	9.1	2.5
Lateral temporal cortex	4.01	0.52	27.2	4.9
Parietal cortex	2.96	0.55	54.4	8.4
Occipital cortex	2.14	0.31	11.0	2.5
Raphe nuclei	2.10	0.65	0.6*	

*The raphe nuclei was a fixed size VOI

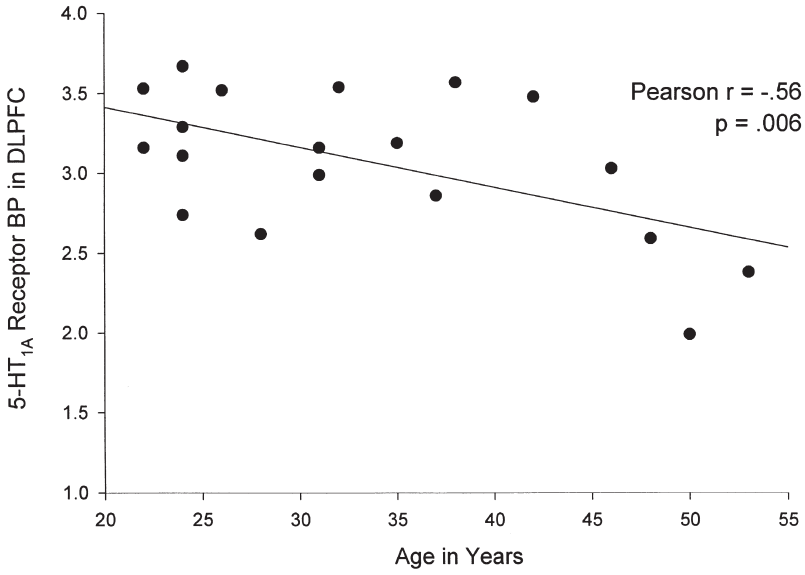

Figure 1. Scatterplot of the serotonin 5-HT$_{1A}$ receptor BP decline in the dorsolateral prefrontal cortex with age in 19 healthy subjects.

A test–retest study in six healthy subjects scanned twice revealed excellent reproducibility for BP values of each ROI except for the raphe nuclei (Table 2). Sixty minute TAC gave slightly better results for test–retest reproducibility than 90 minute TAC, as described by the mean error between scan 1 and 2 in percentage, the ICC for BP scan 1 and 2, the RC and the RM. With 60 minute TAC, the size of the mean error between scan 1 and scan 2 ranged from 2 to 7% in cortical ROI, and was 19% in the raphe nuclei. Average measure ICC confirmed a very low test–retest error between repeated 60 minute scans ranging for cortical regions from 0.93 to 0.99. Again, the raphe showed the poorest result with an ICC of 0.58.

Voxelwise analysis revealed a significant global decrease of 5-HT$_{1A}$ BP with age (set level $< .001$; 12 clusters). Four of these 12 clusters survived a correction for multiple comparisons over the entire volume (Table 3). Parametric mapping using SPM99 showed that the age-related decline in cortical 5-HT$_{1A}$ receptor BP was not limited to specific brain regions but occurred globally all over the cortex (Figure 4). The only exception was one cluster in the mediotemporal cortex of each hemisphere, which showed a significantly lesser decline in 5-HT$_{1A}$ receptor BP values with age (Figure 5).

A multivariate analysis of variance with the eight regional BP values as the dependent variables (DV), gender as a fixed factor and age as a covariate revealed no significant interaction between gender and 5-HT$_{1A}$ BP in any of the ROI. Testing for a differential influence of gender on the demonstrated age-dependent 5-HT$_{1A}$ receptor BP decline, the interaction term of "gender*age"

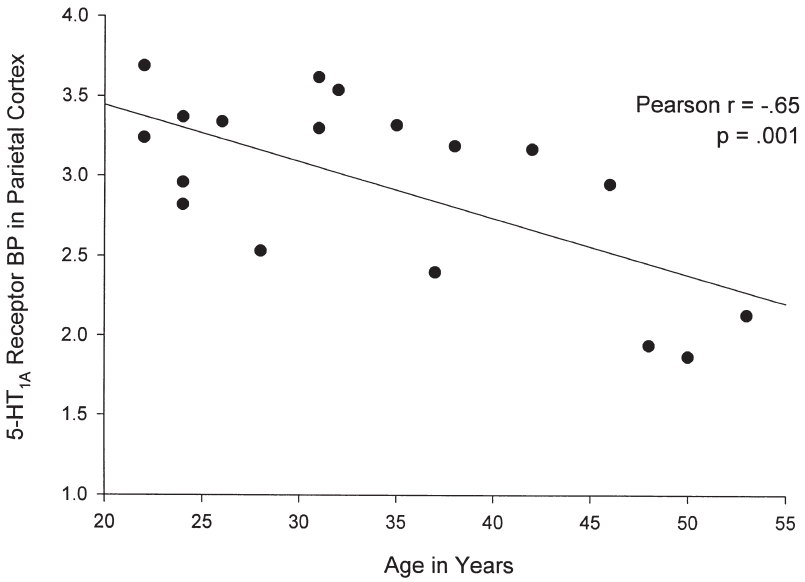

Figure 2. Scatterplot of the serotonin 5-HT$_{1A}$ receptor BP decline in the parietal cortex with age in 19 healthy subjects.

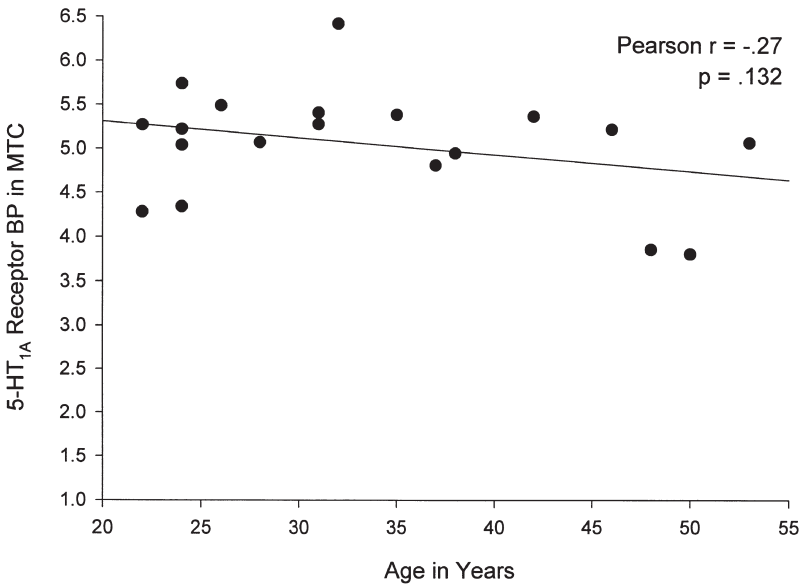

Figure 3. Scatterplot of the serotonin 5-HT_{1A} receptor BP in the MTC showing no significant decline with age in 19 healthy subjects.

did not predict the DV for six of the eight tested ROI. However, there was a significant interaction between "gender*age" and 5-HT_{1A} BP in the DLPFC ($F = 5.548$; $p = .015$) and in the parietal cortex ($F = 6.534$; $p = .008$), but none of these survived a correction for multiple comparisons. For the voxelwise analysis, we used SPM99 with "female" and "male" treated as two conditions, age as the covariate of interest and a corrected threshold level of $p < .05$ for statistical significance. There was no significant interaction between: (1) the covariate age and BP; and (2) the interaction term "age*gender" and BP for any given voxel. In summary, both voxelwise and ROI analysis did not reveal a significant influence of gender on 5-HT_{1A} receptor BP values, and no differential effect of gender on the demonstrated age-dependent 5-HT_{1A} receptor BP decline.

DISCUSSION

In vivo imaging of 5-HT_{1A} receptors in humans with PET and [^{11}C]WAY-100635 revealed a significant age-related decline of brain 5-HT_{1A} receptors, which occurred globally all over the cortex, with the exception of a mediotemporal area in both hemispheres. Both approaches—ROI and voxel-wise analysis—yielded similar results: 5-HT_{1A} receptor BP declined approximately by 10% per decade in our sample of healthy volunteers aged between 22 and 53 years.

Our findings, except for the apparent lack of age-dependent 5-HT_{1A} receptor BP decline in the MTC and in the raphe nuclei, are in correspondence with post-mortem data. Quantitative autoradiographic analysis of 5-HT_{1A} receptors with [^{3}H]8-OH-DPAT as a ligand

Table 2. Test-Retest Reproducibility in Six Healthy Subjects Described by the Mean Error in Absolute Percentage Between Scan 1 and Scan 2 for Each ROI, Their Respective 5-HT_{1A} Receptor Binding Potentials Intraclass Correlation Coefficients (ICC), Repeatability Coefficients (RC), and Their Associated Percentage of the Mean Parameter Value (RM)

	90 minute TAC				60 minute TAC			
ROI	**Mean error[a]**	**ICC[b]**	**RC[c]**	**RM[d]**	**Mean error**	**ICC**	**RC**	**RM**
Dorsolateral prefrontal cortex	9%	0.89	0.56	19%	7%	0.93	0.51	16%
Orbitofrontal cortex	4%	0.92	0.17	5%	2%	0.99	0.18	6%
Anterior cingulate	8%	0.85	0.71	19%	5%	0.95	0.49	12%
Medial temporal cortex	10%	0.93	0.73	14%	6%	0.97	0.73	14%
Lateral temporal cortex	6%	0.94	0.66	17%	6%	0.96	0.49	12%
Parietal cortex	9%	0.95	0.44	15%	5%	0.96	0.46	16%
Occipital cortex	2%	0.99	0.10	5%	6%	0.96	0.32	15%
Raphe nuclei	16%	0.66	0.56	31%	19%	0.58	0.48	27%

[a]Mean error = Absolute value ([BP_{ROI} scan 1 − BP_{ROI} scan 2]/mean [BP_{ROI} scan 1 + 2])
[b]ICC = Average measure intraclass correlation coefficient
[c]RC = 2 × SD of BP_{ROI} scan 1 minus BP_{ROI} scan 2
[d]RM = RC/(average of all BP_{ROI} [scan 1 + 2])

Table 3. Voxelwise Analysis with SPM99

Set-Level		Cluster-Level			Voxel-Level						
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{corrected}$	T	Z	$p_{uncorrected}$	x, y, z {mm}		
<.001	12	<.001	22683	<.001	0.005	7.63	4.96	<.001	48	28	10
					0.005	7.62	4.95	<.001	42	−48	−16
					0.012	6.99	4.74	<.001	62	−64	12
		<.001	8529	<.001	0.019	6.68	4.62	<.001	−52	−62	16
					0.029	6.41	4.51	<.001	−14	14	−22
					0.043	6.12	4.39	<.001	−68	−54	−14
		<.001	683	<.001	0.118	5.45	4.09	<.001	−26	−54	−14
		.001	581	.001	0.214	5.04	3.89	<.001	−40	50	−20
					0.497	4.37	3.53	<.001	−12	60	−10
					0.615	4.15	3.41	<.001	−30	62	−14

Age was entered as covariate of interest (n = 19), volume summary (p-values corrected for entire volume) for contrast −1 ("5-HT_{1A} receptor BP decreases with age").
Height threshold: T = 3.65, p = .001 (.877 corrected)
Extent threshold: k = 0 voxels, p = 1.000 (.877 corrected)
Expected voxels per cluster, [k] = 37.570
Expected number of cluster, [c] = 2.09
Degrees of freedom = [1.0, 17.0]
Smoothness FWHM = 19.2 21.2 18.9 {mm} = 9.6 10.6 9.5 {voxels}
Search volume: S = 749,272 mm3 = 93,659 voxels = 81.6 resels
Voxel size: 2.0, 2.0, 2.0 {mm} (1 resel = 962.71 voxels)

revealed age-related decreases in receptor density (B_{max}) without apparent changes in affinity (K_d) in several cortical and hippocampal regions (Dillon et al. 1991). As in our study, gender had no effect on 5-HT_{1A} receptors density. Another autoradiographic post-mortem examination with [^{3}H]8-OH-DPAT found a significant negative correlation between age and the number of 5-HT_{1A} receptors in the frontal cortex of suicide victims, but not in a control group (Lowther et al. 1997). In contrast, an earlier study had reported a significant negative correlation of age and 5-HT_{1A} B_{max} in frontal cortex of a control group but not in suicide victims (Matsubara et al. 1991). The interpretation of these post-mortem results is compromised by the fact that the agonist ligand 8-OH-DPAT measures 5-HT_{1A} receptors only in the high-affinity state.

Previous *in vivo* studies in healthy volunteers with PET and [^{11}C]WAY-100635 failed to show a significant decrease of 5-HT_{1A} receptors with age. In two studies, it may be attributed to a lack of power because of a relatively small sample size of six (Farde et al. 1998; Gunn et al. 1998) as opposed to 19 in our study. In a recent study reporting a widespread reduction of 5-HT_{1A} receptor binding with major depression, no correlation with age was found in 18 healthy controls (Sargent et al. 2000). The age range of the control group (27–56 years) was comparable to our sample (22–53 years). Unfortunately, the individual ages were not reported, but the distribution of age seemed slightly narrower in the Sargeant et al. (2000) study, as indicated by a standard deviation of 8.3, as compared to 10.2 in our study. Finally, another study reporting a reduced 5-HT_{1A} receptor binding potential in the raphe and MTC of 12 depressive patients in comparison to eight healthy controls, reported a trend toward an inverse correlation with age (r = −0.6) in the controls, which did not reach significance at the $p < .05$ level (Drevets et al. 1999). Again, the failure to show a significant relationship might be attributed to the small sample size (n = 8).

One shortcoming of the present study might be that we studied healthy volunteers over a restricted age range of 22 to 53 years. Although it would have been possible to enroll slightly younger adults, we must point out that, for ethical reasons, we were not allowed to include volunteers younger than 18 years in this PET study comprising injection of a radioactive labeled compound. On the other hand, the inclusion of older subjects might have provided additional scientific insight. Further studies including younger and older subjects seem to be warranted, especially to address the question whether the reported age dependent 5-HT_{1A} receptor decline is a linear phenomenon over the whole life span or not.

Previously, 60 and 90 minute TAC were used for tracer kinetic modeling of [^{11}C]WAY-100635. We used 60 minute TAC for the calculation of the 5-HT_{1A} receptor binding potential with a simplified reference tissue model. In our own test–retest study, 60 minute TAC gave comparable if somewhat better repeatability measures as 90 minutes (Table 2). In a kinetic modeling study Gunn et al. (1998) reported a better (RC) for the MTC with 60 minutes; whereas, 90 minutes were superior for the insula and cingulate cortex, and the raphe nuclei. In comparison, all RC for each single ROI and their associated percentage of the mean parameter value (RM) were far better with both 60 and 90 minute

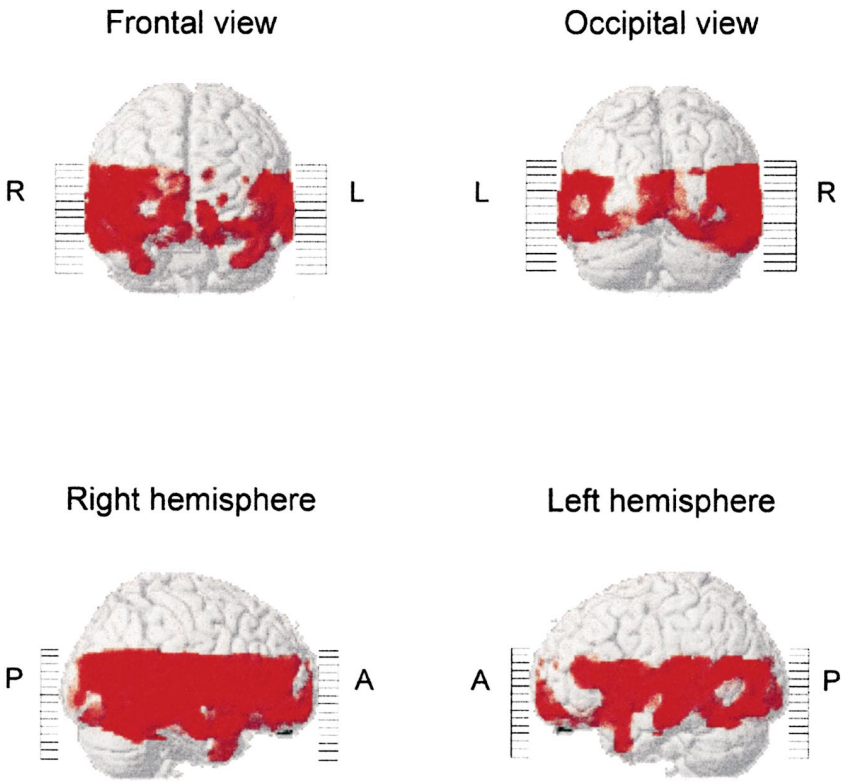

Figure 4. Parametric maps generated with SPM99 show a widespread and significant decrease of 5-HT_{1A} receptor BP with age (for a summary of statistics, see Table 3). Red colored voxels denote brain regions with a significant age-related decline in 5-HT_{1A} receptor BP. A grid shows the area of the brain which was actually scanned.

TAC in our study (Table 2). Because we intend to investigate 5-HT_{1A} receptors with PET and [^{11}C]WAY-100635 in psychotic patients in the future, the point can be made that scanning time should be kept as brief as possible to avoid possible behavioral problems and movement artifacts.

Because we used the cerebellum for a simplified reference tissue model, the age-related decline of 5-HT_{1A}

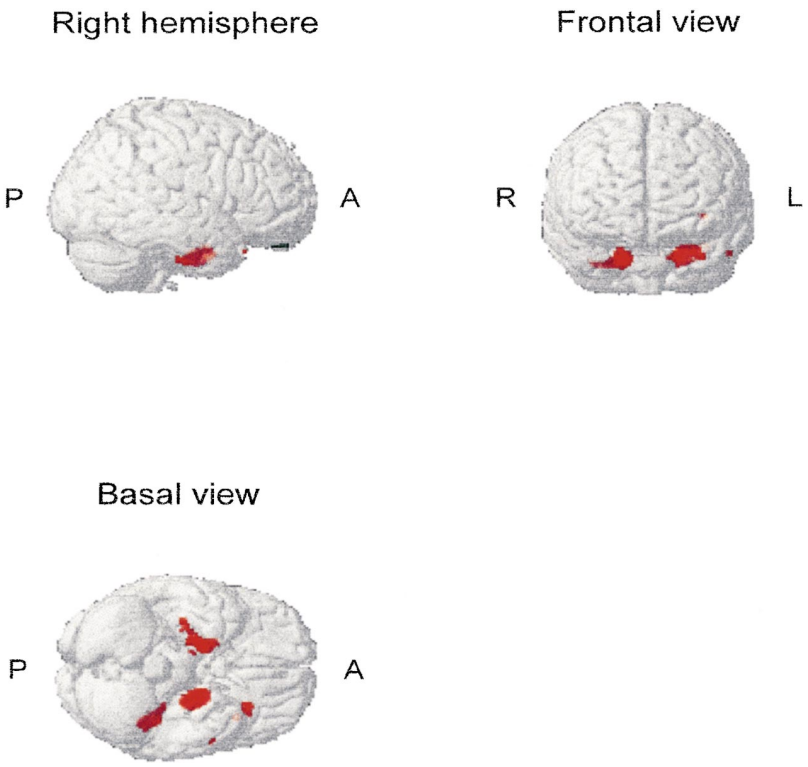

Figure 5. Parametric maps using proportional scaling to depict areas with significantly lesser than average age-related 5-HT_{1A} receptor BP decline. Note two clusters, occurring bilaterally one in each MTC, which survived correction for multiple comparisons (left MTC: $p_{corrected}$ = .003, cluster size: 285 voxels; right MTC: $p_{corrected}$ = .006, cluster size: 253 voxels).

receptor BP could theoretically be attributed to a higher [^{11}C]WAY-100635 uptake with age. However, in our sample, there was no correlation of cerebellar uptake as measured by the area under the cerebellar TAC and age.

In the age range of our sample; that is, between 22 and 53 years, a linear decline of gray matter cortical volume of approximately 6–7% per decade had been shown in healthy subjects (Zipursky et al. 1998; Pfefferbaum et al. 1994). To rule out cortical atrophy as a possible source of error, we examined a possible correlation of the actual VOI size and the measured BP. There was no correlation of VOI size and BP in any given VOI. Pearson correlation coefficients did not reveal any correlation between age and VOI size for the cerebellum, DLPFC, ACC, LTC, MTC, parietal cortex, and raphe nuclei. However, there was a positive correlation between age and VOI size in occipital cortex, and a negative correlation between age and the OFC VOI size. Because VOI size did not decrease with age, with the exception of the OFC, it is quite unlikely that the age-related decline of 5-HT_{1A} receptor BP was attributable to partial volume effects. Furthermore, in the occipital cortex, a positive correlation between age and VOI might have lead to an underestimation of the age-related decline of 5-HT_{1A} receptor BP.

On the other hand, partial volume effects might have contributed to a possible underestimation of 5-HT_{1A} receptor BP values in the raphe nuclei. With 0.6 cm^3, this was by far the smallest VOI and the mean BP value found in our study was considerably lower than those reported earlier by other groups (Farde et al. 1998; Gunn et al. 1998). Furthermore, it could not be delineated on the co-registered MRI, and showed the poorest test–retest reproducibility in our study (Table 2) and in another test–retest study (Gunn et al. 1998). These limitations must be taken into account when interpreting results of 5-HT_{1A} receptor binding potential in raphe nuclei with PET and [^{11}C]WAY-100635.

Great emphasis was laid on the accurate delineation of the ROI on the co-registered MRI. We used previously defined landmarks (Bremner et al. 1998) to delineate the ROI on the MRI and transferred the obtained template on a summation PET image where it was adjusted accordingly. The validity of our ROI approach is underlined by the fact that voxelwise analysis with SPM99 yielded similar results.

CONCLUSION

The main finding of this in vivo brain-imaging study in healthy volunteers was a significant decline of 5-HT_{1A} receptor density in the range of approximately 10% per decade, except for the medial temporal cortex and the raphe nuclei, where we did not find such a decline. Hence, the effect of age must be considered in future studies using PET and [^{11}C]WAY-100635 for the visualization of brain 5-HT_{1A} receptors. Based on our data, careful age matching must be advised for group comparisons of psychiatric patients and healthy controls to avoid this source of error.

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