Research report

Gender-specific aging effects on the serotonin 1A receptor

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Abstract

The effects of age on serotonergic function have been hypothesized to underlie age-related changes in mood and behaviors such as sleep and eating. Of particular interest is the serotonin type-1A (5-HT1A) receptor, due to its putative role in mediating the therapeutic efficacy of antidepressant treatment. Using positron emission tomography (PET) and [11C–carbonyl] WAY100635, we assessed 5-HT1A receptor binding in 21 healthy subjects (10 men, 11 women) ranging in age from 21 to 80 years. Regional binding potential values were generated using a reference tissue model and corrected for partial volume effects. We observed an inverse relationship between age and binding of [11C–carbonyl] WAY100635 to the 5-HT1A receptor in men, but not women. This finding is in accord with observations reported in the postmortem literature. Gender-specific effects of age on central serotonergic function may relate to differences between men and women in behavior, mood, and susceptibility to neuropsychiatric disease across the adult lifespan. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

The central serotonin (5-HT) neurotransmitter system encompasses an extensive network of ascending projections from the brainstem raphe nuclei to cortical and limbic brain regions, as well as descending fibers to the spinal cord. Among its many putative functions are the regulation of mood and sleep cycles [36,47]. Conversely, serotonergic dysfunction has been implicated in depression, anxiety disorders, sleep disturbance, and eating disorders [23,26,44]. Several lines of evidence support substantial

aging changes in human serotonergic neurotransmission and receptor integrity. This has been demonstrated most convincingly for the well-studied postsynaptic 5-HT2A receptor. Age-related reductions in 5-HT2A receptor density and ligand affinity have been demonstrated in human frontal cortex by postmortem receptor pharmacology [3,9,16]. Also, marked decreases in 5-HT2A binding with advancing age have been found in vivo using positron emission tomography (PET) [7,22,34,52].

Relatively little is known about the influence of age on the 5-HT1A receptor, which exists both as a presynaptic autoreceptor in the dorsal raphe nucleus, and as a postsynaptic receptor in the hippocampus and cerebral cortex [42]. In some animal models [54] and human postmortem studies [1,27], aging reductions in 5-HT1A receptors have been noted. Arango et al. [1] reported an inverse relation-

ship between binding of [³H]8–OH–DPAT to prefrontal 5-HT1A receptors and age; however, other postmortem investigations failed to detect an association between age and density or affinity of 5-HT1A receptor binding in several cortical areas using the same probe [10,41].

Sex differences in the density or distribution of central 5-HT1A binding sites have been inconsistently observed. Arango and colleagues [1,2] noted significantly greater 5-HT1A receptor binding in frontal cortex specimens from women relative to men. Although most postmortem reports have not addressed the question of sex differences in 5-HT1A binding measures, Matsubara [28] demonstrated loss of receptor density with age in the frontal cortex of men, but not women. Also, Dillon et al. [11] noted that autoradiographic analysis of 5-HT1A receptor binding density revealed a greater age decrement in men than women in frontal cortex, hippocampus, and dorsal raphe nucleus. Indirect evidence of aging changes in 5-HT1A-mediated function is provided by the observation of blunting of 8-OH–DPAT-induced decreases in 5-HIAA in the frontal cortex and hippocampi of aged rats [45]. In humans, Gelfin et al. [15] noted that the body temperature reduction response to the 5-HT1A agonist ipsapirone was diminished in older subjects, with a significant inverse relationship observed with age but not sex.

Defining the influence of age and sex on the 5-HT1A receptor may have substantial implications for understanding the biological basis of mood and behavioral changes in the elderly. In this study, we used PET and the selective 5-HT1A antagonist [¹¹C–carbonyl] WAY100635 to characterize the interaction of age and gender effects on brain serotonergic function in vivo. A magnetic resonance (MR) imaging-based atrophy correction method was applied to the PET binding measures to correct for the dilutional effect of age-associated cerebral volume loss on quantitative PET measurements.

2. Materials and methods

2.1. Study subjects

Twenty-one healthy subjects (10 men, 11 women) were recruited from the community through the use of advertisements. Subjects ranged in age from 21 to 80 years, with no significant difference in the age distribution between men and women (Table 1). Exclusion criteria included signifi-

Table 1
Subject characteristics[a]

	N	Mean age± S.D.	Education (years)	17-item Hamilton
Men	10	52.0±21.7	16.2±2.0	1.0±1.8
Women	11	51.9±22.9	15.7±1.9	0.6±1.0

[a] No significant differences between men and women in age, education and Hamilton scores were found (P>0.05).

cant medical illness, history of neurological or psychiatric disease, and first-degree relative with a psychiatric or neurodegenerative disorder. Individuals using psychotropic or other medications with known central nervous system effects, including beta-blockers, were not studied. Subjects were also instructed to refrain from use of non-prescription cold preparations for 1 week prior to the PET study. The screening evaluation included a psychiatric interview using the Structured Clinical Interview for DSM-IV. A 17-item Hamilton Depression Rating Scale [19] was administered to exclude depressive symptomatology (scores <7). Three elderly women were receiving estrogen replacement therapy. Two pre-menopausal women were taking oral contraceptive agents. Confirmation of negative pregnancy status in women of reproductive age was achieved through serum pregnancy testing within 48 h prior to PET scanning. Informed consent was obtained at study entry in compliance with local Institutional Review Board requirements.

2.2. Image acquisition

Radiosynthesis of [¹¹C–carbonyl] WAY100635 was performed using a modification of previously described methods [30,43]. Dynamic PET scanning was performed for 60 min immediately following a bolus intravenous injection of 8–15 mCi [¹¹C–carbonyl] WAY100635 (specific activity: 0.96–3.01 mCi/nmol). Emission data were collected in three-dimensional mode using an ECAT HR+ tomograph (CTI PET Systems, Knoxville, TN), which acquires images in 63 transaxial planes [2.4-mm thick; in-plane resolution=4.1 mm full-width at half-maximum (FWHM)] over a 15.2-cm field-of-view. A Neuro-insert (CTI PET Systems, Knoxville, TN) placed in the camera gantry was used to reduce random coincidences [50]. Head movement was minimized by the use of a thermoplastic mask and headholder system. A 10-min transmission scan was acquired using rotating rods of ⁶⁸Ge/⁶⁸Ga immediately prior to the administration of [¹¹C–carbonyl] WAY100635 for attenuation correction of emission data. PET data were also corrected for radioactive decay and scatter and reconstructed with a Hanning frequency cut-off of 0.5.

Dynamic arterial blood sampling data were available in a subset of subjects. In 8/10 male and 9/11 female subjects, a quantitative cerebral blood flow (CBF) study was also performed prior to the [¹¹C–carbonyl] WAY100635 study. Twelve mCi of [¹⁵O]water was injected as an intravenous bolus using an automatic injection system and dynamic emission scanning performed for 3 min with arterial blood sampling over 3.5 min. A delay interval of 20 min allowed for radioactive decay of [¹⁵O]water before injection of [¹¹C–carbonyl] WAY100635. In 8/11 female and 7/10 male subjects, dynamic arterial blood sampling was performed during the

full 60-min acquisition of the [^{11}C–carbonyl] WAY100635 PET study. In these subjects, plasma data were corrected for the presence of radiolabeled metabolites of [^{11}C–carbonyl] WAY100635 as determined by high-performance liquid chromatography analyses.

The PET data were registered to volumetric spoiled gradient recalled (SPGR) MR images for the dual purpose of guiding region-of-interest (ROI) placement and performing partial volume correction. MR images were acquired using a Signa 1.5 T scanner (GE Medical Systems, Milwaukee, WI) with a standard head coil. The SPGR sequence (TE=5, TR=25, flip angle=40°, NEX=1; section thickness=1.5 mm with no intersection gap) was acquired in the coronal plane. In one subject in whom an SPGR image was not obtained, a high-resolution spin-echo T1-weighted sequence (TE=18, TR=400, NEX=1, section thickness=3 mm/interleaved) was used for ROI placement and partial volume correction. We have previously demonstrated that these two sequences yield comparable results using the two-component algorithm for partial volume correction [32]. Fast spin-echo axial T2 and proton density weighted images were also performed prior to PET imaging in order to screen subjects for unanticipated neuropathology. PET–MR registration was accomplished using the method of Woods et al. [53] as further validated in our laboratory [51]. Pixels corresponding to scalp and calvarium were removed from the SPGR MR images [46] to facilitate registration with the PET images.

2.3. Image analysis

With the exception of the brainstem raphe, all ROIs were hand-drawn on the co-registered MR images by an unblinded single rater (CCM) and transferred to the dynamic PET data for regional sampling. ROIs were sampled on multiple consecutive MR images on which the structure was visualized, and right and left regions were combined to reduce noise. Regions sampled included areas known to contain moderate to high 5-HT1A receptor density and those implicated in depressive illness and responsivity to antidepressant therapy [29]: mesial temporal cortex (encompassing the amygdalo–hippocampal complex and adjacent portions of the parahippocampal gyrus), hippocampus alone, lateral orbitofrontal cortex [Brodmann area (BA) 47], pregenual anterior cingulate (BA 24/32), and subgenual cingulate (BA 25/32). In order to assess the regional specificity of our findings, an additional cortical region [occipital cortex (BA 18)] and two regions of relatively low 5-HT1A receptor density, the thalamus and basal ganglia (including both putamen and globus pallidus), were also sampled. Due to negligible 5-HT1A receptors in the cerebellum, this structure served as the reference region reflective of free and non-specific binding. The cerebellar hemispheres were sampled on three consecutive planes centered approximately at the level of the inferior portion of the fourth ventricle. (Care was taken not

to influence cerebellar measures with adjacent occipital lobe activity by sampling at least two sections below the inferior-most aspect of the occipital cortex). The dorsal raphe nucleus is a long, cylindrical structure, approximately 15 mm in length, located in the ventral pons/midbrain [38,49]. Since it does not have discernible borders on MR images, we used the MR to define z-axis landmarks from the upper pons to the midbrain on five consecutive sections (the inferior-most image was two planes below the interpeduncular cistern). The brainstem raphe region was then sampled directly on a late summed PET image (15–60 min post-injection interval) directly over the highest area of focal tracer uptake using a 8-mm-diameter circular ROI. Due to the limited spatial resolution of PET and the lack of clear imaging landmarks for the dorsal raphe nucleus, the sampled area likely reflects 5-HT1A binding in the dorsal as well as median raphe nucleus and surrounding brainstem structures such as the pontine reticular formation.

Time-activity data were generated from the dynamic image data for each ROI. The [^{15}O]water PET data were analyzed using a one-compartment model that accomodates for blood input function timing delays [20,39], where K_1 is used to estimate blood flow. This method generated kinetic parameter estimates of cerebral blood flow, which were then compared to [^{11}C–carbonyl] WAY100635 binding potential (BP) values. Regional BP values were determined using a three-parameter (simplified) reference tissue model [24], as implemented by Gunn et al. [17,18]. Since the cerebellum is virtually devoid of 5-HT1A receptors [42], this region was assigned as the reference region, in which radioactivity reflects the concentrations of free and non-specifically bound tracer. The reference tissue model utilizes the cerebellar time-activity data as an indirect input function to provide image-based measures of BP (with no arterial sampling). In those subjects in whom arterial blood sampling was performed during the [^{11}C–carbonyl] WAY100635 PET acquisition, a secondary analysis was performed using the Logan graphical method (applied over the 14–60 min post-injection interval) [25]. This was done to ensure that the reference tissue model generated binding measures comparable to an alternate method that utilized a true arterial input function.

2.4. Partial volume correction

A previously validated two-component MR-based atrophy correction algorithm [34,35] was applied to the regional BP values to correct for partial volume averaging between brain and cerebrospinal fluid (CSF). This was performed by first segmenting the MR images into brain and CSF voxels. This binary brain map was convolved with a Gaussian smoothing kernel approximating that of the final PET spatial resolution (FWHM=7.3 mm in the transverse and axial planes). ROI sampling of the convolved brain tissue image generated regional tissue correction factors with 1.0 representing pure brain and zero

corresponding to pure CSF. The regional BP values were then divided by the corresponding tissue correction factor to yield corrected BPs, as previously implemented [33,34].

2.5. Statistical analysis

Two-tailed, unpaired *t*-tests were used to test for differences in age and educational level between men and women subjects. Pearson correlations were applied to examine the relationship between age and BP (both uncorrected and corrected for atrophy) across all nine brain regions sampled. Pearson correlations were also used to examine the relationship between age and regional tissue correction factors in men and women. Lastly, atrophy-corrected regional K_1 CBF measurements and [^{11}C–carbonyl] WAY100635 BP values were compared. For all analyses, statistical significance was set at the $P < 0.05$ level due to the relatively small number of ROIs investigated.

3. Results

There were no differences in age or education level between men and women subjects (Table 1).

Overall, regional binding of [^{11}C–carbonyl] WAY100635 followed the expected rank order distribution of 5-HT1A receptors [42] (Fig. 1, Table 2). In all cortical areas and the brainstem, there was a significant inverse relationship between age and BP value in men, while no significant regional age-binding relationship was found among women subjects. The main study finding of a marked inverse relationship between age and [^{11}C–carbonyl] WAY100635 binding in men, but not women, was seen both before and after atrophy correction of the PET data (Table 2, Fig. 2). In men, corrected BP values showed statistically significant inverse correlations with age (ranging from $r = -0.82$ to -0.68) in cortical and the brainstem raphe regions. Although no significant relationship between age and binding was observed in women, a mild
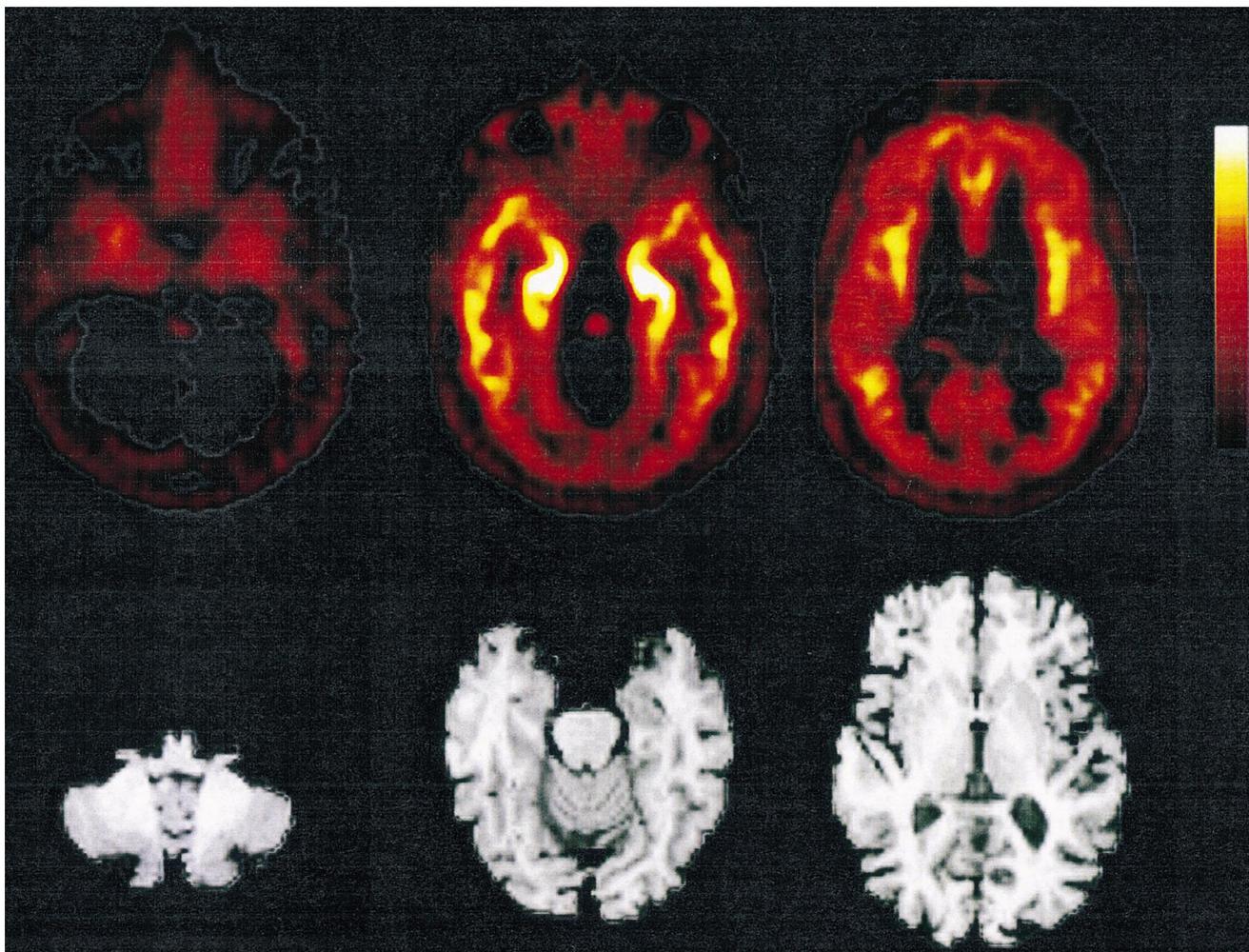

Fig. 1. Co-registered [^{11}C–carbonyl] WAY100635 PET (summed over the 15–60 min post-injection interval) and MR images in a healthy 74-year-old man. The PET images reflect the distribution of 5-HT1A receptors, including midbrain region of the raphe and hippocampus (top row, middle image). Lack of binding in the cerebellum, where there are negligible 5-HT1A receptors, is also demonstrated (top row, left image).

Table 2
Correlations between age and regional [^{11}C] WAY100635 BP values (corrected for partial volume effects)

| Region	Men			Women			Effect Size q
	Mean BP±S.D.	r	P value	Mean BP±S.D.	r	P value	
Subgenual cingulate	6.56±1.15	−0.81	0.005**	6.69±1.11	−0.11	0.75	1.017
Pregenual cingulate	6.09±1.29	−0.79	0.007**	6.42±1.19	+0.11	0.75	1.181
Lateral orbitofrontal	5.40±1.27	−0.82	0.004**	5.26±1.73	−0.08	0.81	1.0777
Brainstem raphe	4.87±1.58	−0.73	0.02*	3.92±1.45	−0.05	0.89	0.879
Mesial temporal	8.10±1.74	−0.68	0.03*	9.24±2.29	+0.04	0.91	0.869
Occipital	3.61±1.03	−0.70	0.03*	3.49±0.68	−0.44	0.18	0.395
Hippocampus	8.29±2.45	−0.69	0.03*	10.27±3.15	−0.06	0.86	0.788
Basal ganglia	0.89±0.40	−0.36	0.30	1.23±0.62	+0.40	0.23	0.801
Thalamus	0.61±0.46	−0.27	0.44	0.52±0.29	+0.39	0.23	0.689

* $P=0.05$.
** $P=0.01$.
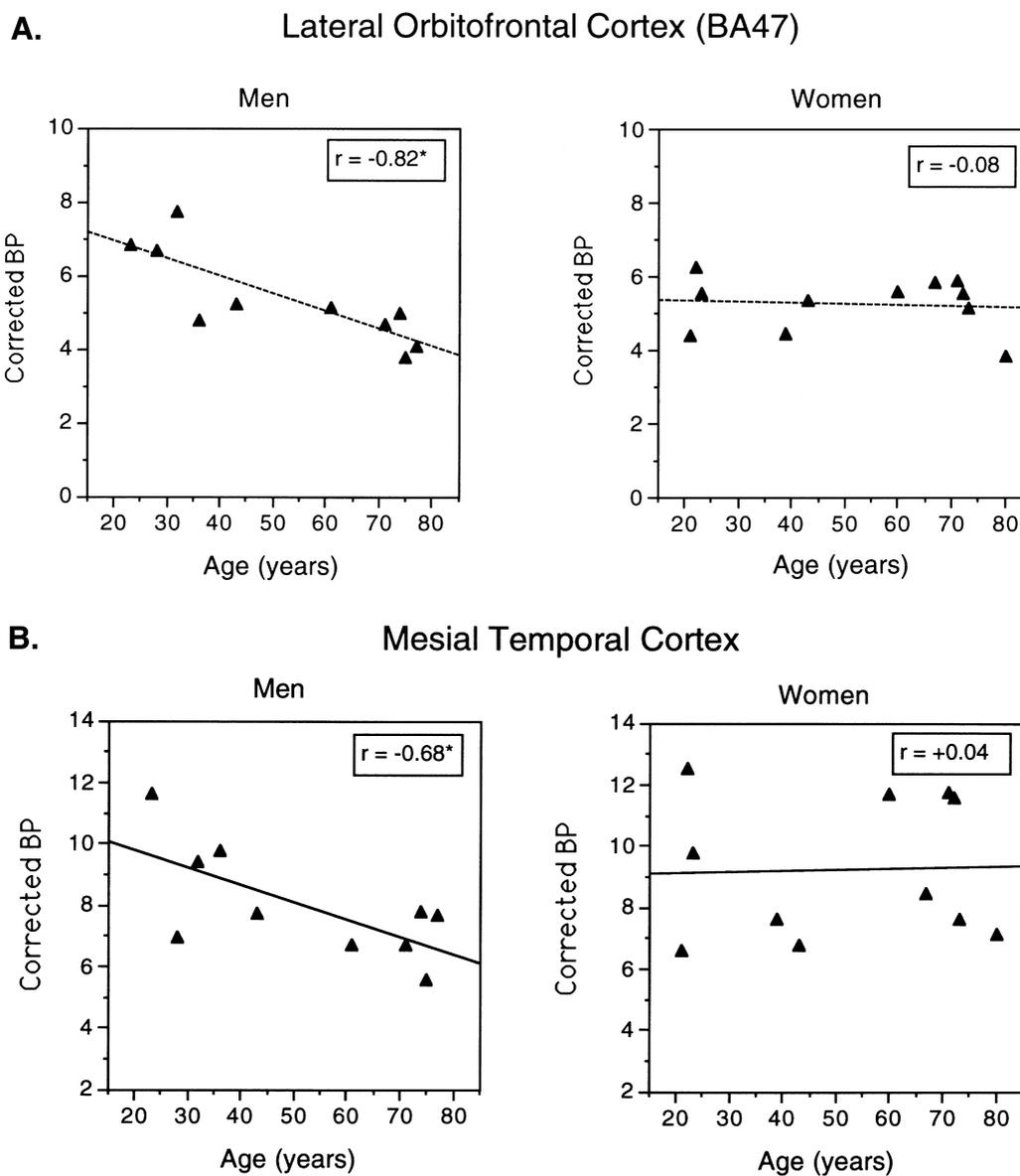
Fig. 2. A/B – Scatter plots of atrophy-corrected binding potential (BP) for [^{11}C–carbonyl] WAY100635 versus age in representative high 5-HT1A density brain regions: (A) lateral orbitofrontal cortex (BA47), (B) mesial temporal cortex. Note the strong aging decline in BP values in men (* $P<0.05$), and the lack of an age effect in women.

trend toward lower binding with age was observed in the occipital cortex ($r=-0.44$, $P=0.18$). There were no significant age effects on BP values in the receptor-poor control regions of the basal ganglia and thalamus. The magnitude of the MR-determined tissue correction factors correlated significantly with age in several brain regions for both men and women (Table 3), underscoring the need for partial volume correction. There was no significant difference in atrophy between gender groups.

There were no significant regional correlations between CBF and BP measurements either within the gender subgroups or across all subjects [$n=17$; mean correlation $(r)=0.06\pm0.17$]. The ratio of region-to-cerebellar CBF was well-correlated with R_I which represents the ratio of K_1 in the target region to that of the cerebellum [24], in both men ($r=0.92$) and women ($r=0.88$). Another important observation was the absence of a significant difference in R_I between the gender groups (men: mean $R_I=0.85\pm0.09$; women: mean $R_I=0.91\pm0.02$), and no consistent correlation between R_I and age across regions (mean $r=0.06\pm0.22$).

Moderate to high correlations between regional BP values obtained with the reference tissue model and the Logan graphical analysis were noted in the subset of subjects in whom arterial sampling had been performed ($r=0.86\pm0.06$). Further, high correlation coefficients for the linear regression across regions ($r=0.98\pm0.03$) supported the integrity of the Logan graphical analysis for description of these data. Interestingly, a positive correlation was found between age and cerebellar radioligand distribution volume (DV) generated by the Logan graphical analysis in the subjects in whom arterial blood samples were available (Fig. 3), consistent with a rise in the free and non-specific binding component with advancing age. This was true for both men ($r=0.49$, $P=0.27$.) and women ($r=0.88$, $P=0.003$), although significance in the relationship between age and cerebellar DV was attained only in the women.
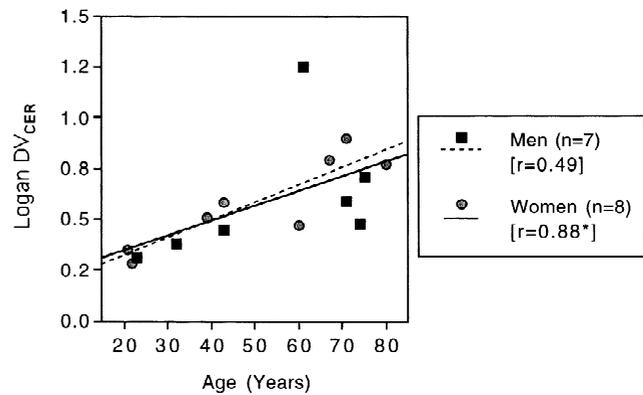

Fig. 3. Scatter plot showing the relationship between age and Logan cerebellar distribution volume (DV) for the subsets of men and women (* $P<0.05$) with arterial blood data.

4. Discussion

This in vivo human study demonstrated a differential influence of gender on the effect of age on the 5-HT1A receptor. These data are consistent with findings reported in the postmortem literature. Marcusson et al. [27] demonstrated a similar age–sex interaction in postmortem frontal cortex, and Dillon et al. [11] noted aging changes in males only in frontal cortex, hippocampus and dorsal raphe tissue specimens. Also, Arango et al. [2] reported significantly higher 5-HT1A receptor binding in multiple cortical layers of the ventrolateral prefrontal cortex of female compared to male brains in a sample spanning a large age range. Variability in the results of other postmortem studies examining aging and 5-HT1A receptor binding may result from limited power to probe the effects of gender, although some regional differences between men and women have been observed [10,41]. Further, lack of highly selective markers for the 5-HT1A receptor subtype limits the interpretation of several earlier studies [8,27,48]. In living human subjects, a diminished body temperature reduction response to the 5-HT1A agonist ipsapirone in older subjects has been demonstrated, with a significant inverse relationship with age, but no gender effect, observed [15]. However, these investigators also noted age–sex interactions in adrenocorticotrophic hormone (ACTH) and cortisol responses, which appear to decrease with age in men, but increase in aging women.

The biological basis of our finding of gender-specific aging alterations in 5-HT1A receptor binding is unclear. The influence of gonadal steroids on brain development has been associated with sex differences in brain organization, neuropsychological performance and learning/memory function (for review, see Ref. [31]); however, neuroendocrine effects on human brain aging have not been well-studied. Regional localization of estrogen (and progestin) receptors shows considerable overlap with that of 5-HT1A receptors, including limbic structures such as the hip-

Table 3
Correlations between age and regional MR-defined atrophy correction factors

Region	Men		Women	
	r	P value	r	P value
Subgenual cingulate	−0.80	0.006**	−0.68	0.03*
Pregenual cingulate	−0.80	0.006**	−0.69	0.02*
Lateral orbitofrontal	−0.69	0.03*	−0.52	0.10
Brainstem raphe	−0.46	0.18	−0.48	0.13
Mesial temporal	−0.66	0.04*	−0.74	0.009**
Occipital	−0.73	0.02*	−0.76	0.008**
Hippocampus	−0.81	0.005**	−0.70	0.02*
Basal ganglia	−0.33	0.35	−0.28	0.41
Thalamus	−0.77	0.009**	−0.49	0.12

* $P=0.05$.
** $P=0.01$.

pocampus and cingulate, and the midbrain raphe nuclei. Further, alterations in circulating estrogen levels have been shown to modulate 5-HT1A receptors and 5-HT1A mRNA expression [5,13,40]. Since loss of estrogen typically upregulates 5-HT1A receptor expression [13], lack of an aging reduction in 5-HT1A receptors in women may be, in part, due to post-menopausal reductions in circulating estrogen. Three of the six post-menopausal women subjects in our study had been exposed to exogenous estrogen, which may be one source of variability in the PET data. The potential influence of estrogen replacement strategies on the aging profile of 5-HT1A binding in women is an important focus of investigation for future larger studies. Indeed, there is preliminary in vivo evidence that hormone therapy can alter 5-HT2A receptor binding measures in humans [37].

Sex differences in the effect of age on the 5-HT1A receptor may be of etiologic importance to potential gender influences on susceptibility, course and treatment of mood disturbances in the elderly. Several investigators have suggested that loss of serotonergic reserve in aging may enhance the susceptibility of the elderly to depression, which is hypothesized to be a disorder of deficient serotonergic neurotransmission. This is consistent with epidemiologic evidence that the gender gap in prevalence of depressive disorders closes in later middle age, with women showing higher rates of depression than men predominantly in early to middle adulthood [4]. Interestingly, we noted a similar intersection point (at approximately 50–55 years of age) between the male and female regression lines for regional [^{11}C–carbonyl] WAY100635 BP values in our series and in the depression prevalence rates by Bebbington and colleagues (Fig. 2). This observation is consistent with our theory of a biologic basis to differing patterns of mood vulnerability in normal aging between men and women. Further, preclinical evidence for a key role of the 5-HT1A autoreceptor in the action and efficacy of antidepressant pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs) [6,21] suggests that sex differences in the effect of age on the 5HT1A receptor function may influence treatment response in depressive illness. Other potential behavioral correlates of an age–sex interaction in serotonergic function include age-related changes in sleep patterns. An age-dependent loss of slow-wave sleep has been observed in men but not women [14], which may relate to reduced serotonergic-mediated regulation of sleep in elderly men.

Advantages of the reference tissue model analysis of [^{11}C–carbonyl] WAY100635 PET data applied in this study include that this approach does not require arterial blood sampling to derive an input function in order to generate specific binding measures. This is of particular value in the elderly and in experimental protocols requiring multiple scanning sessions. Acceptable inter-subject variability using the reference tissue model as applied to [^{11}C–carbonyl] WAY100635 PET data has been demon-strated at Hammersmith Hospital [18] and in our laboratory [12]. Further, we found moderate to high correlations ($r=0.7–0.9$) between regional BP values obtained with the reference tissue model and the Logan graphical analysis [25] in the subset of subjects in whom arterial sampling had been performed (seven men, eight women). It is recognized that BP values obtained with the reference tissue model can be flow-sensitive [18]; however, our measures of R_I do not support a differential sensitivity between gender groups or a relationship to age.

Our observation of a positive relationship between age and cerebellar DV values suggests an elevated non-specific binding component for [^{11}C–carbonyl] WAY100635 in older individuals. Similar observations have been made using other serotonergic PET ligands [7,34]. In our study, the correlation between age and cerebellar DV reached statistical significance in women only. However, lack of significance in the men may be due to the small subset of subjects ($n=7$) in whom arterial sampling data was available. Notably, eliminating the outlier cerebellar DV value noted among the men in Fig. 3 produces a similarly high age correlation in men ($r=0.87$) as women ($r=0.88$). These findings underscore the need to account for non-specific binding in regional PET measurements, which is accomplished with use of the BP measurement, particularly in studies comparing subjects over a large age range.

5. Conclusion

Although sparse to date, knowledge of the interaction of age and sex on brain function can provide insight into alterations in human behavior with normal aging and shed light on treatment approaches for mood and cognitive dysfunction associated with late-life neuropsychiatric disease. In this PET study, an aging reduction in [^{11}C–carbonyl] WAY100635 binding in receptor-rich brain regions with age was observed in men but not women. Gender-specific effects of age on the 5-HT1A receptor may contribute to differences between men and women in behaviors, mood, and susceptibility to psychiatric disease across the adult lifespan. Future studies are needed to replicate this finding in a larger sample and to examine potential neuroendocrine influences and behavioral and therapeutic correlates of this finding.

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