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Parametric and Regional Maps of Free Serotonin $5HT_{1A}$ Receptor Sites in Human Brain as Function of Age in Healthy Humans

Mette Møller*[,1,2], Steen Jakobsen[2] and Albert Gjedde[1,2]

[1]Center of Functionally Integrative Neuroscience, Aarhus University, Aarhus, Denmark; [2]PET-Centre, Aarhus University Hospital, Aarhus, Denmark

Serotonin $5HT_{1A}$-binding sites can be detected in living human brain with the positron-emitting antagonist [^{11}C]WAY-100635. Previous measurements of the availability of [^{11}C]WAY-binding sites in normal aging are equivocal, in part because of the greatly variable binding of this ligand. To test the null hypothesis that the binding potential (*p*B) of $5HT_{1A}$ sites remains constant with age; 19 healthy volunteers aged 23–73 years (8 women, 11 men) underwent positron emission tomography. To determine *p*Bs, we applied a novel tissue reference method of analysis, Estimation of Reversible Ligand Binding and Receptor Density (ERLiBliRD) (Gjedde, 2003; Rosa-Neto *et al*, 2004), which extrapolates measures of specific binding to an estimated steady-state. We compared these estimates in the two age groups with results obtained with the conventional Logan Plot and Simplified Reference Tissue Method (SRTM) applied to both regions of interest-based as parametric analyses. The regional distribution of specific binding of free sites [^{11}C]WAY-100635 was similar to that reported in previous studies, with the highest *p*Bs in limbic structures and the raphé nuclei. Although the results of the three methods differed, *p*Bs in the elderly subjects consistently were lower than those of young subjects. Thus, the correlation between *p*B and age applied to regions-of-interest revealed significant decline of *p*B at the rate of 3 or 4% per decade, and a 10% decline of the global mean $5HT_{1A}$ receptor-*p*B in elderly relative to young subjects. The results demonstrate that the number of available $5HT_{1A}$-binding sites declines with age.
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INTRODUCTION

Serotonin $5HT_{1A}$ receptors play a dual role in the modulation of serotonin neurotransmission, acting as presynaptic autoreceptors on serotonergic neurons, and as postsynaptic receptors throughout the cerebral cortex. *In vitro* autoradiography reveals presynaptic serotonin $5HT_{1A}$ receptors on the soma of serotonergic neurons in the raphé nuclei, where they regulate the firing of these neurons (Arango *et al*, 2001; Hall *et al*, 1997). Most binding sites are located postsynaptically on the axon hillock or soma of cortical pyramidal neurons (Amargos-Bosch *et al*, 2004; Azmitia *et al*, 1996). The density of cortical binding is heterogeneous with the highest binding in limbic structures such as hippocampus and cingulate gyrus, and the lowest binding in occipital lobe, thalamus, and basal ganglia.

Free serotonin $5HT_{1A}$-binding sites have been mapped in PET studies of the living human brain with the positron-emitting antagonist [^{11}C]WAY-100635 ([^{11}C]WAY) (Pike *et al*, 1995; Wilson *et al*, 1998). Subtle abnormalities in the availability of [^{11}C]WAY-binding sites were reported in clinical PET studies of personality differences (Borg *et al*, 2003), depression (Drevets *et al*, 1999; Bhagwagar *et al*, 2004; Sargent *et al*, 2000), schizophrenia (Tauscher *et al*, 2002), juvenile myoclon epilepsia (JME) (Meschaks *et al*, 2005), bulimia nervosa (Tiihonen *et al*, 2004), ALS (Turner *et al*, 2005), and Parkinson's disease (PD) (Doder *et al*, 2003). Together, these studies show that [^{11}C]WAY binding, as an index of serotonin $5HT_{1A}$ receptor availability, is correlated with scores of personality, increased in specific cortical regions of patients with schizophrenia and bulimia nervosa, globally decreased in patients with depression and ALS, and focally decreased in patients with JME and in the raphé nucleus of PD patients. Thus, PET images of the [^{11}C]WAY-binding potential (*p*B) in brain demonstrate disease-specific patterns of disturbed serotonergic neurotransmission.

However, the results of these studies must be weighed against the possible, but not yet proven effect of normal aging on receptor density. The evidence for an effect of normal aging on the availability of [^{11}C]WAY-binding sites is equivocal. Thus, normal aging showed significant decline

*Correspondence: Dr M Møller, PET-Centre, Aarhus University Hospital, 8000 Aarhus C, Denmark, Tel: +45 8949 4408, Fax: +45 8949 4400, E-mail: moller@pet.auh.dk
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of cortical [^{11}C]WAY binding in one study of rhesus monkeys (Tsukada *et al*, 2001) and three studies of humans (Tauscher *et al*, 2001; Cidis Meltzer *et al*, 2001; Bhagwagar *et al*, 2004), where as no correlation with age was reported in two other studies of humans (Parsey *et al*, 2002; Rabiner *et al*, 2002). In addition, the findings may reflect problems with kinetic analysis of binding due to the very rapid metabolism of the tracer in the circulation (Gunn *et al*, 1998; Osman *et al*, 1998) and the reported high inter-subject variability in [^{11}C]WAY binding, as demonstrated in a large cohort of healthy male subjects. The study found no correlation between *p*B and demographics, physiological or behavioral variables or levels of endogenous serotonin (Rabiner *et al*, 2002).

The present study tested the null hypothesis of no effect of normal aging on the results of non-invasive assays of [^{11}C]WAY binding in brain of healthy young and elderly normal subjects. To this end, we applied standard tissue reference methods to regions of interest (ROIs) and image voxels to obtain parametric estimates in the form of *p*B of [^{11}C]WAY. In addition, we compared the results to the results from the tissue reference method ERLiBiRD (Gjedde, 2003; Rosa-Neto *et al*, 2004). This method obtains estimates of extrapolation of specific uptakes ratios towards a predicted steady-state.

SUBJECTS AND METHODS

Recruitment

This study was approved by the Research Ethics Committee of Aarhus County. Nineteen healthy volunteers aged 23–73 years, divided in two groups with an average age in the elderly group of 12 subjects of 62.5 ± 6.8 years (five women and seven men), and an average age in the young group of seven subjects of 25.1 ± 2.0 years (three women, four men), gave written informed consent to the study. Exclusion criteria included cardiovascular disease and any history of neurological and psychiatric disease. All subjects were physically fit, free of prescribed medication, and did not meet the criteria for depression according to DSM IV.

Radiochemistry

[^{11}C-*carbonyl*]WAY was synthesized from the cyclotron-generated precursor according to the method of McCarron *et al* (1996). In brief, $^{11}CO_2$ was collected in a stainless steel cryotrap, warmed, and then flushed with nitrogen through a small coil of 1/16″ o.d. polypropylene tubing, which had been earlier flushed with a solution of cyclohexylmagnesium chloride (500 μl of 0.5 M in THF). The [^{11}C]-labeled Grignard adduct, [^{11}C]cyclohexanecarbonyl chloride, was eluted with a solution of thionyl chloride (10 μl in 400 μl THF) into a septum-sealed vial (2 ml) containing WAY-100634 (2 mg), 50 μl THF and triethylamine (60 μl) under a nitrogen atmosphere. The reaction proceeded in seven min at 85°C. The crude product was diluted with 500 μl of HPLC eluent, and purified by reversed phase HPLC on a Ultracarb 7 ODS 30 column (250 × 10 mm; Phenomenex Ltd.), eluting with sterile ethanol/70 mM NaH2PO4 (52:48) at 6 ml/min. The fraction containing [^{11}C-*carbonyl*]WAY-100635 (retention time ca. 10 min) was collected, evaporated to near-dryness at 90°C under vacuum, and then reformulated in sterile saline (10 ml) before passage through a sterile 0.22 μm filter into a sterile vial. Mean specific radioactivity was 42 GBq/μmol (SD 32).

PET and Data Analysis

Subjects were positioned in the ECAT EXACT HR47 tomograph (CTI/Siemens, Knoxville, TN, USA). After a 15 min attenuation scan, 60 min emission recordings in 3-D mode consisting of 22 frames were initiated after intravenous bolus injection of [^{11}C]WAY (150–430 MBq, mean 270, SD 111). High resolution T1-weighted MR scans were made with a 1.5 or 3 T magnet (GE Sigma Systems, Milwaukee, USA). The summed emission recordings were automatically co-registered to the individual MRI scans using published methods (Talairach and Tournoux, 1988; Collins *et al*, 1994; Iversen *et al*, 2006)). In brief, individual MR-images were co-registered to a common stereotaxic space (Montreal Neurological Institute) using a 12 parameter affine rigid body transformation; registrations were automatic in the young group, but manual in the elderly group because of inadequate registration of potentially atrophied brains. After calculation of the final PET-Talairach transformation matrix, dynamic emission recordings were resampled into the common coordinates and, using the program DISPLAY, templates of ROIs were drawn bilaterally on the average MR image: hippocampus, and the insular, cingulate and ventral medial prefrontal cortices. Owing to central and cortical atrophy in the elderly, each of the eight regions was applied to the individual resampled MR scan and adjusted to fit. The raphé nuclei were not visible on MR images and were outlined on the individual PET images in each subject. A ROI for the cerebellum (15 cm^3) was drawn manually on the MR anatomical template, excluding the vermis and carefully avoiding transverse sinuses and the inferior occipital lobe. Finally, time-radioactivity curves were extracted from the dynamic PET image based on the ROI templates of all 10 regions. We assumed that *p*Bs depend on both age and ROI. The linear relation between age, ROI and *p*B were assessed using linear regression for each of the binding potential estimates obtained with conventional methods Logan and SRTM and the method of ERLiBiRD.

Kinetic Analysis

5HT$_{1A}$ binding was quantified using ROI-based and voxel-wise parametric analysis (Table 1). The estimated quantity is the *p*B, which is a measure of the binding of serotonin to a class of receptors, relative to its concentration, and relative to the ratio between the affinity of serotonin and the radioligand. The class of receptors to which the measure applies is determined by the selectivity of the radioligand. In this case, the selectivity of the radioligand is strongly in favor of the serotonin 5HT$_{1a}$ receptors. The $pB = (B_{max} - B)/K_d$) is a measure of the number of unoccupied receptor sites relative to the affinity towards a ligand.

Parametric images of the *p*B were obtained in three different ways, including the tissue reference method of Logan (Logan, 2000), excluding the first 4 min of data, the multi-linear version of simplified tissue reference region

Table 1 Methods Applied to Imaging Data to Obtain Parametric and Regions-of-Interest (ROI) Maps of Binding Potentials

Methods of analysis	Linear regression	Nonlinear regression
Region-based (ROI)	Reference Logan Plot (Logan, 2000)	ERLiBiRD (Gjedde, 2003) & SRTM (Lammertsma and Hume, 1996)
Voxel-based	Logan & LSRTM (SRTM version of Zhou *et al*, 2003)	ERLiBiRD (Gjedde, 2003)

method (Zhou *et al*, 2003) and ERLiBiRD (Rosa-Neto *et al*, 2004), excluding the first minute of data.

Using cerebellum as reference region, average uptakes in ROIs were also analyzed by the Logan *et al*, SRTM (Gunn *et al*, 1998; Lammertsma and Hume, 1996) and ERliBiRD methods (Rosa-Neto *et al*, 2004). The ERLiBiRD method fits the following Equation

$$\rho^* = 1 + p_B\left(1 - e^{-\alpha\Theta}\right) + (R_1 - 1)e^{-\alpha\Theta}$$

to paired calculations of the ratio of areas-under-the-curves (AUC ratio $= \rho^*$) of voxels or regions of binding and reference, and the modified time variable Θ'' equal to the ratio

$$\Theta = \left[\frac{\int_o^T \int_o^u m^*_{ref}\, dt\, du}{\int_o^T m^*_{ref}\, dt}\right]$$

where m^*_{ref} is the time activity function of the reference region.

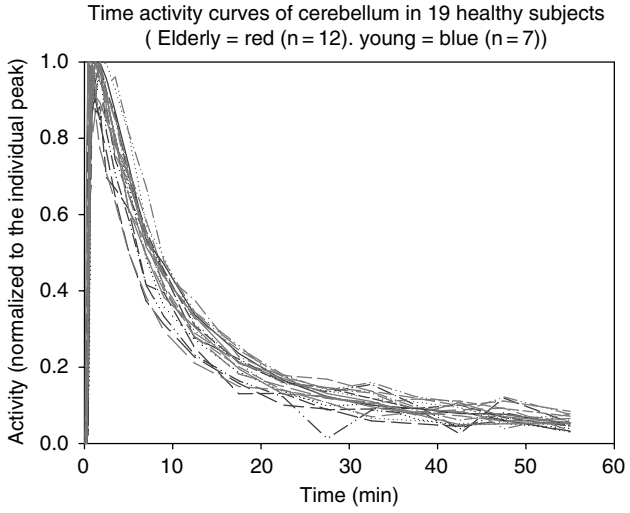

Figure 1 Time–activity curves recorded in cerebellum in the 19 healthy subjects of this study (red = elderly group, blue = young group). Abscissa: time (min). Ordinate: activity normalized to peak activity.

RESULTS

In both elderly and young subjects, the cerebellum time-activity curve, normalized to its peak value, had the same general form consistent with flow-limited clearance from the brain tissue (Figure 1). There was no difference between the patterns of washout from the cerebellum in the two groups of subjects. Figure 2 shows the average parametric image of [^{11}C]WAY binding in groups of young and elderly subjects, calculated by the methods of Logan *et al*, Zhou *et al* (multi-linear SRTM) and ERLiBiRD. The highest *p*Bs were determined in limbic cortical structures, frontal and temporal neocortex, and in the raphé nuclei, irrespective of the method used for calculating *p*B. *p*B images obtained with ERLiBiRD globally were 25% higher than by the two other methods.

The mean magnitudes of *p*B by the ROI analysis in nine brain regions in the two groups are listed in Table 2. Also ROI-based analyses globally gave lower *p*B values with the Logan method than with the ERLiBiRD and SRTM methods. The correlations among the mean *p*B estimates of the several methods were consistently high (SRTM *vs* ERLi-BiRD: $r^2 = 0.90$, SRTM *vs* Logan: $r^2 = 0.73$) (Figure 3). Irrespective of method, the highest *p*Bs were seen in insula and hippocampus. Values of [^{11}C]WAY binding consistently were either high or low across the regions of all subjects, including the raphé nuclei.

A significant decrease in *p*B with age was demonstrated in the ROIs with postsynaptic-binding sites with a reduction in *p*B pr decade of 0.14 in SRTM ($p = 0.02$), 0.17 in ERLiBiRD ($p < 0.001$) and 0.13 in Logan ($p \ll 0.001$), when adjusting for the significant effect of region (data not shown), thus averaging a significant decrease of *p*B of 3–4% per decade. Figure 4 shows the correlation between *p*B and age in five regions-of-interest for the three methods of calculation. A decline in *p*B with age was demonstrated in all five regions measured by the three methods except in raphe calculated by the Logan method. A significant reduction was seen in hippocampus and insula using the methods of ERLiBiRD and Logan, the reduction in MPFC was only significant in Logan method. No method yielded a significant decrease in cingulate or the raphe region. SRTM showed a decline with age in all regions, but did not reach significance probably due to its lower precision compared to the Logan method. The ROI-based analysis also revealed a 10% decrease of the global mean magnitude of 5HT$_{1A}$ receptor magnitude of *p*B in elderly subjects relative to young healthy subjects. *T*-test for equal *p*B in young and old were significant in all three methods ($p = 0.02$ in SRTM, $p = 0.003$ in ERLiBiRD, $p = 0.004$ in Logan) (Table 2).

DISCUSSION

5HT$_{1A}$ receptors reside in all the cortical layers, but most densely so in the superficial layers (Burnet *et al*, 1995). Agonists of 5HT$_{1A}$ receptors evoke hyperpolarization of pyramidal neurons, unlike agonists of 5HT$_{2A}$ receptors, which cause depolarization (Amargos-Bosch *et al*, 2004; Aghajanian and Marek, 1997; Cruz *et al*, 2004). Therefore, 5HT$_{1A}$ receptors are believed to fundamentally modulate cortical excitation.

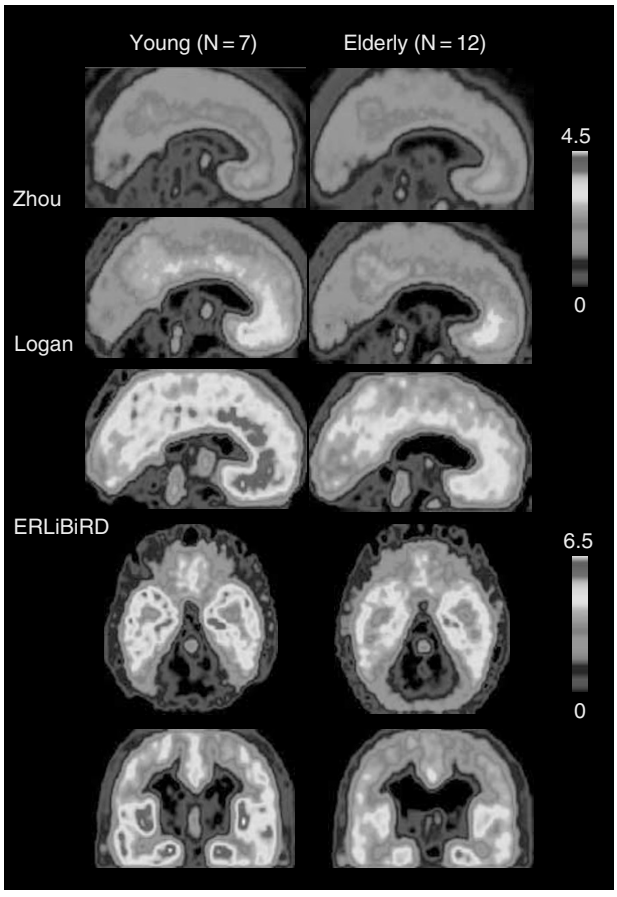

Figure 2 Parametric maps of *p*B of radioligand [^{11}C]WAY in selected sections of brain tissue determined by three different methods, multilinear simplified reference tissue regression method LSRTM (Zhou *et al*, 2003), non-linear regression method ERLIBiRD (Gjedde, 2003), and tissue reference version of Logan Plot method (Logan, 2000). Top three rows show sagittal views, second-to-last row transaxial view, and bottom row coronal view. Left column shows young adults, right column aged adults. Color bars shown to right of columns show color scale for LSRTM and Logan methods above and color scale for ERLiBiRD method below, respectively.

PET studies of several classes of neuroreceptors, for example; dopamine D_1 (Wang *et al*, 1998) and D_2 (Farde *et al*, 1995; Antonini and Leenders, 1993), histamine H_1 (Yanai *et al*, 1992) and serotonin 5HT$_{2A}$ (Adams *et al*, 2004; Meltzer *et al*, 1998; Sheline *et al*, 2002) demonstrate age-dependent decline of the availability of receptors of the order of 10% pr decade. Transporters of dopamine (van Dyck *et al*, 2002) and serotonin (Yamamoto *et al*, 2002) also tend to decline with age. In contrast, ambiguous findings have made it difficult to confirm a similar decline of serotonin 5HT$_{1A}$ receptors. Thus, the results reported in the literature are inconclusive based on small sample sizes and biased by the lack of elderly participants, high intersubject variability in the range of 16–24% (Rabiner *et al*, 2002), and the fact that in no study did the researchers follow the same subjects with age. One study demonstrated an association with age in young and aged monkeys with decrease of binding to receptors in temporal and frontal cortices in the elderly monkeys, but less displacement of [^{11}C]WAY binding after injection of the agonist 8-OH-DPAT, suggesting impaired affinity in the aged monkeys (Tsukada *et al*, 2001). One study of humans subjects aged 22–53 years found a 10% decline per decade of *p*B in the whole brain, except in mesiotemporal cortex (Tauscher *et al*, 2001), and one other study of a wider age range of human subjects (21–80 years) found significant decrease in receptor-rich areas of the brain, but only in men. A more recent study on previously depressed subjects reported a decrease with age in 5HT$_{1A}$ receptor binding compared to controls in both raphé nuclei and postsynaptic regions (Bhagwagar *et al*, 2004). The decrease was more pronounced in the group of depressed subjects, but their ages were not specified. Two other studies found no association between age and *p*B: They included a large database of 61 males with a narrow age-span (24–53 years), and a smaller study of both genders (20–70 years) with higher *p*B in women than in men regardless of age (Parsey *et al*, 2002; Rabiner *et al*, 2002). Selected studies of WAY-100635 binding are listed in Table 3. In general, the present change is more modest than reported in the two previous studies showing a significant correlation with age, and reported in studies of the effects of diverse pathologies. The majority of studies listed in Table 3 used the 'gold standard' SRTM method but a few used the Logan plot. The magnitude of *p*B values determined in the present study are consistent with those reported in the literature. The definition of the *p*B as an index of unoccupied receptors makes a direct comparison with total receptor numbers somewhat tenuous, as reasons for decline may include changes of bound serotonin as well as changes of receptor affinity. Therefore, it is possible that the variability of past results is related to fluctuations of bound serotonin or receptor affinity in addition to receptor numbers.

The agonist 8-OH-[^{3}H]DPAT is most often used in postmortem studies of 5HT$_{1A}$ receptors. The radioligand binds to high-affinity receptors coupled to G-proteins only and hence reveals only half of the binding of the antagonist [^{3}H]WAY-100635. Direct comparison of receptor density *in vitro* and *in vivo* studies is not feasible with [^{11}C]WAY (Burnet *et al*, 1997) of the receptor density, although the distribution of the binding of [^{11}C]WAY *in vivo* studies generally fits with the postmortem findings (Hall *et al*, 1997).

As cerebellum is almost devoid of 5HT$_{1A}$ receptors (Hall *et al*, 1997), uptake of the tracer in this region is often used as a surrogate for an arterial input function in the absence of blood sampling. In some studies with [^{11}C]WAY (Rabiner *et al*, 2002; Doder *et al*, 2003; Turner *et al*, 2005), as many as 10% of the subjects were excluded because of abnormally high cerebellar time-activity curves potentially caused by specific binding in cerebellum, incorrect segmentation of cerebellum, or variable properties of the tracer. One of the known properties of [^{11}C]WAY is the very rapid metabolism in the circulation (Gunn *et al*, 1998; Osman *et al*, 1998), which may violate the underlying assumption of continuous exchange of brain tissue with circulation and thereby may induce biases to the results of previously applied kinetic models.

The issue of use of the cerebellum as reference biased by binding of the tracer to receptors in different subdivisions of the cerebellum has been addressed in the literature in various ways, most of them based on the use of arterial

Table 2 Mean Magnitude of Binding Potentials (*p*B) in Nine Brain Regions in Elderly and Young Healthy Volunteers, Respectively

Method	1	2	3	4	5	6	7	8	9	Mean *p*B
Mean pB values in 9 regions in 7 young subjects										
SRTM	4.73	4.45	6.50	6.78	5.09	5.41	6.43	6.25	3.52	5.46[a]/5.70[b]
	±0.66	0.66	1.19	1.35	0.63	1.55	1.07	1.20	1.01	1.11/0.90
ERLiBiRD	4.46	4.07	5.76	5.53	4.58	4.65	5.90	5.65	3.06	4.85/5.08
SD	0.71	0.62	1.08	0.99	0.87	0.86	1.07	1.05	0.63	0.94/0.71
LOGAN	2.82	2.82	3.64	3.47	3.34	3.37	3.48	3.37	1.73	3.12/3.29
SD	0.50	0.52	0.78	0.74	0.77	0.51	0.60	0.57	0.37	0.59/0.30
Mean pB values in 9 regions in 12 elderly subjects										
SRTM	4.37	4.77	5.77	5.24	4.84	4.82	5.69	5.75	3.29	4.95/5.16
SD	0.98	1.41	2.63	1.21	1.13	1.58	1.49	1.23	0.64	0.8/0.54
ERLiBiRD	4.01	4.07	4.43	4.39	4.06	4.21	4.68	4.74	2.89	4.16/4.32
SD	1.01	1.13	1.36	1.21	0.92	1.53	1.21	0.97	0.80	0.55/0.28
LOGAN	2.58	2.72	3.02	3.06	2.99	2.76	2.94	3.08	1.87	2.78/2.89
SD	0.37	0.55	0.72	0.67	0.50	0.63	0.46	0.37	0.22	0.38/0.18

1. Left cingulate. 2. Right cingulate. 3. Left hippocampus (postsynaptic). 4. Right hippocampus. 5. Left medial prefrontal cortex. 6. Right medial prefrontal cortex. 7. Left insula. 8. Right insula. 9. Raphe nuclei. [a]All 9 regions. [b]Region 1–8.

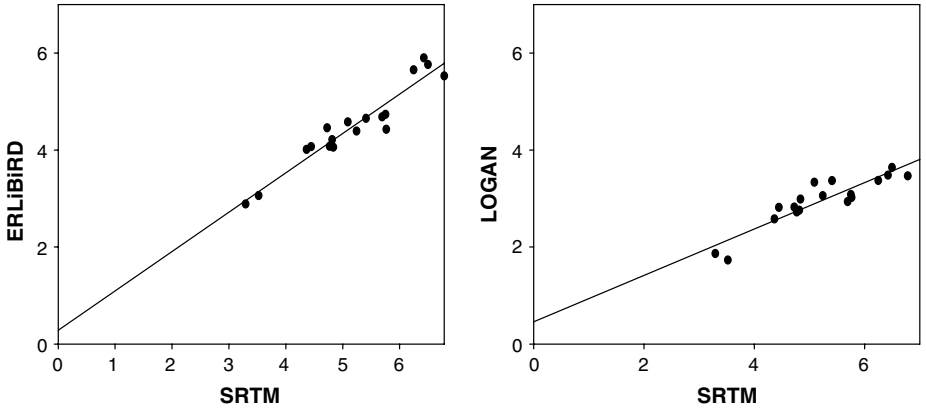

Figure 3 Correlation among methods used to obtain *p*B values in anatomically defined ROIs in 19 healthy subjects. Abscissae: values of *p*B obtained with method SRTM. Ordinates: Values of *p*B obtained with methods ERLiBiRD and Logan, left and right graph, respectively. Correlation coeeficients are $r^2 = 0.90$ and 0.73, left and right graph, respectively.

samples to determine binding in cerebellum on the basis of an input function. This approach is seriously limited because of the uncertainty of tracer and tracer metabolite concentrations in arterial blood. An alternative has been to examine *p*Bs in other parts of the brain with the use of cerebellar reference tissues segmented into gray and white matter. In the present study, the cerebellar reference was limited to white matter, as described recently (Hirvonen *et al*, 2007).

In the study, both parametric maps and ROI-based analysis were performed to estimate the binding of [^{11}C]WAY to 5HT$_{1A}$ receptors using linear and non-linear regression methods. As reported in previous studies, *p*B values calculated by non-linear methods were higher, but less precise than the values calculated by linear models. The ROI-based results showed an agreement between the gold standard SRTM, and the model-independent method ERLiBiRD with values ranging from 4 to 6. In the parametric maps the multi-linear SRTM (Zhou *et al*, 2003 version) gave low values of 2–3.5 in line with the Logan method results, whereas ERLiBiRD findings were about 25% higher.

The point of the study was to avoid the use of arterial sampling because of the uncertainty associated with the rapid tracer metabolism in arterial blood. Of the three methods, the SRTM conventionally is considered the gold standard because dependent and independent variables are fully separated. However, the method is sensitive to low cerebellar concentrations towards the end of the circulation time, and hence was supplemented with integral methods, which tend to lower statistical noise. Of these, the Logan method still uses the actual concentration of the tracer in ROI in the denominator of both axes and hence retains a residual sensitivity to noise in regions of low specific binding. Only the ERLiBiRD method is based exclusively on the use of integrals. Thus, the Logan method has a known bias in the direction of low *p*Bs, which we confirm in the

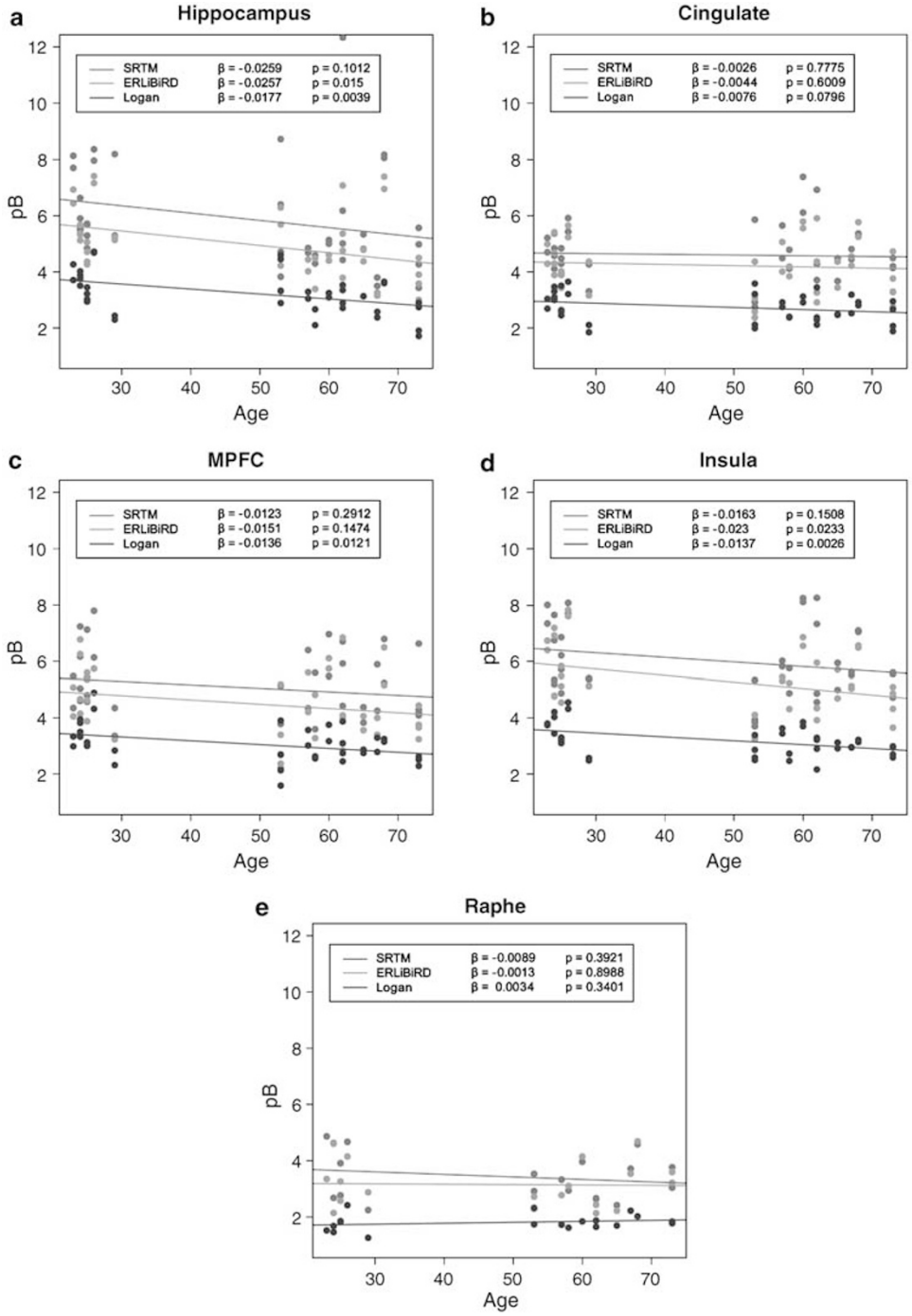

Figure 4 Correlation among measures of *pB* and age in five regios-of-interest; (a) Hippocampus, (b) Cingulate, (c) Medial prefrontal cortex, (d) Insula, and (e) Raphe nuclei. The slopes found in the three methods SRTM (red), ERLiBiRD (green), and Logan (blue) are listed in the panel including *p*-values. Abscissae: Age (years). Ordinates: *p*B values (ratio).

present study. For these reasons, we find that the ERLiBiRD has the best balance of precision and accuracy.

Limitations

A potential bias induced by differences in gender specific effects of age cannot be excluded and may magnify or dilute the effects of age in a group of both genders. Owing to the small sample size of this study, it was not possible to demonstrate a difference in *p*B between women and men. In the literature, only two studies (Parsey *et al*, 2002; Cidis Meltzer *et al*, 2001) addressed the issue of gender. In the first study, women had higher *p*Bs than men, but also higher volumes of distribution in cerebellum. In the second study, women had a non-significant trend towards higher *p*B than men, whereas an age-related decline in *p*B was seen only in men. Thus, the literature is limited and inconclusive on the subject of the influences of age and gender on the *p*B of WAY.

Table 3 Human Studies of 5HT$_{1A}$ Receptors with WAY-1000635

Author/journal	Sample size	Change in *p*B
Bhagwagar *et al* (2004) *Mol Psychiatry*	18 depressed patients/14 controls (males)	17% decrease in patients compared to controls
Cidis Meltzer *et al* (2001) *Brain Res*	21 controls (10 males, 11 females)	Decrease in *p*B with age (men only), % not listed
Doder *et al* (2003) *Neurology*	23 PD patients (16 males, 7 females)/8 controls (6 males, 2 females)	27% decrease in raphe of patients compared to controls
Parsey *et al* (2002) *Brain Res*	25 controls (12 females, 13 males)	No change in *p*B with age
Drevets *et al* (1999) *Biol Psychiatry*	12 depressed patients (7 females, 5 males)/8 control (4 females, 4 males)	42% decrease in raphe and 27% decrease in mesial temporal cortex (MTC) in patients compared to controls
Rabiner *et al* (2002) *Neuroimage*	61 male controls	No change in *p*B with age
Sargent *et al* (2000) *Arch Gen Psychiatry*	25 depressed patients/(22 males, 3 females) 18 controls (17 males, 1 female)	11–12% decrease in patients compared to controls
Tauscher *et al* (2002) *Arch Gen Psychiatry*	14 schizophrenic patients (6 females, 8 males)/14 controls (8 females, 6 males)	20% increase in *p*B in left MTC, 13 % in *p*B in right MTC in patients compared to controls
Tauscher *et al* (2001) *Neuropsychopharmacology*	19 controls (8 females, 11 males)	10% decrease per decade
Turner *et al* (2005) *Brain*	21 ALS patients (4 females, 17 males)/19 controls (5 females, 14 males)	21% decrease in patients compared to controls
Meschaks *et al* (2005) *Archives of Neurology*	11 patients with juvenile myoclonic epilepsy (6 females, 5 males)/11 controls (6 females, 5 males)	20–28% decrease in hippocampus, dorsolateral prefrontal cortex, and raphe in patients compared to controls
Tiihonen *et al* (2004) *Biological Psychiatry*	8 female bulimic patients/10 female controls	Increase in *p*B in patients compared to controls, % not listed

Varying degrees of atrophy were observed in the elderly brains, but we did not specifically correct for atrophy. This means that an overestimation of the decline in *p*B with age is possible. One study observed the same age-related decline with and without correction for atrophy (Cidis Meltzer *et al*, 2001). On the other hand, it is known that the currently available methods for correction may induce other biases due to overestimation of cortical thickness and masking of a true decline of the total number of receptors because the correction addresses concentrations of receptors in the tissue rather than their total numbers.

The findings confirm a significant correlation of the *p*B with age regardless of the chosen model, but the issue of whether the decrease reflects a reduced receptor affinity, decline in density because of loss of receptors due to neuronal loss or atrophy or caused by methodological biases awaits resolution in future studies.

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