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# Age and sex effects on 5-HT$_4$ receptors in the human brain: a [$^{11}$C]SB207145 PET study

Karine Madsen[1,2], Mette T Haahr[1,2], Lisbeth Marner[1,2], Sune H Keller[3], William F Baaré[2,4], Claus Svarer[1,2], Steen G Hasselbalch[1,2,5] and Gitte M Knudsen[1,2]

[1]*Neurobiology Research Unit, Department of Neurology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark;* [2]*Center for Integrated Molecular Brain Imaging, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark;* [3]*PET and Cyclotron Unit, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark;* [4]*Danish Research Center for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Copenhagen, Denmark;* [5]*The Memory Clinic, Department of Neurology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark*

**Experimental studies indicate that the 5-HT$_4$ receptor activation influence cognitive function, affective symptoms, and the development of Alzheimer's disease (AD). The prevalence of AD increases with aging, and women have a higher predisposition to both AD and affective disorders than men. This study aimed to investigate sex and age effects on 5-HT$_4$ receptor-binding potentials in striatum, the limbic system, and neocortex. Positron-emission tomographic scans were conducted using the radioligand [$^{11}$C]SB207145 in a cohort of 30 healthy subjects (mean age 44 years; range 20 to 86 years; 14 men and 16 women). The output parameter, $BP_{ND}$, was modeled using the simplified reference tissue model, and partial volume correction was performed with the Muller–Gartner method. A decline with age of 1% per decade was found only in striatum. Women had a 13% lower 5-HT$_4$ receptor binding in the limbic system. The lower limbic 5-HT$_4$ receptor binding in women supports a role for 5-HT$_4$ receptors in the sex-specific differences in emotional control and might contribute to the higher prevalence of affective diseases and AD in women. The relatively stable 5-HT$_4$ receptor binding with aging contrasts others in subtypes of receptors, which generally decrease with aging.**
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## Introduction

Serotonergic neurotransmission is involved in the modulation of sleep, mood, aggression, neuroticism, sexual activity, and impulsivity, which may differ between genders and change with aging. In addition, depression (reviewed by Meyer, 2007) and anxiety (reviewed by Nutt, 2005) have been linked to serotonergic disturbances. The lifetime prevalence for both mood and anxiety disorders is nearly 14% in the western population, and the prevalence is twice as high in women compared with men (Alonso *et al*, 2004). Alzheimer's disease (AD) has also been linked to serotonergic disturbances (Salmon, 2007), and women have higher prevalence of the disease that causes severe personal, social, and economic burdens to societies worldwide.

Lowering the serotonin level by acute tryptophan depletion has larger memory-impairing effects in women (Sambeth *et al*, 2007), but *in vivo* positron-emission tomographic (PET) studies of sex differences of markers of the serotonin system have shown diverging results: lower 5-HT$_{2A}$ receptor binding in women was initially reported (Biver *et al*, 1996), but was not confirmed in larger samples (Adams *et al*, 2004; Frokjaer *et al*, 2009). Higher 5-HT$_{1A}$ receptor binding has been described in women in some (Costes *et al*, 2005; Jovanovic *et al*, 2008) but not all studies (Cidis Meltzer *et al*, 2001; Stein *et al*, 2008). Cerebral serotonin-transporter binding has not been consistently shown to depend on sex (Jovanovic *et al*, 2008; Kalbitzer *et al*, 2009; Meyer *et al*, 2004).

PET studies have primarily shown a decline or unchanged levels of serotonergic markers with normal aging: the 5-HT$_{2A}$ receptors decline most

Correspondence: Professor GM Knudsen, Neurobiology Research Unit N9201, The Neuroscience Centre, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, Copenhagen O DK-2100, Denmark.
E-mail: gmk@nru.dk
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pronouncedly, with 17% per decade without partial volume (PV) correction (Sheline *et al*, 2002) and 6% per decade with PV correction (Adams *et al*, 2004). A decline with age is described for the 5-HT$_{1A}$ receptors in both genders (Moller *et al*, 2007; Tauscher *et al*, 2001), in women only (Costes *et al*, 2005) and men only (Cidis Meltzer *et al*, 2001; Rabiner *et al*, 2002). Studies involving the serotonin transporter have described localized decreases with age in varying regions (Kalbitzer *et al*, 2009; Meyer *et al*, 2001; Reimold *et al*, 2008).

The development and validation of the new PET tracer [$^{11}$C]SB207145 has now made it possible to quantify 5-HT$_4$ receptor binding *in vivo* in humans (Marner *et al*, 2009). The 5-HT$_4$ receptor is a $G_s$ protein-coupled 5-HT receptor, and its stimulation results in increased neuronal excitability (Bockaert, 2004). Experimental studies have suggested several beneficial effects from central 5-HT$_4$ receptor agonism: better cognitive performance (King *et al*, 2008), fast treatment response in depression (Lucas *et al*, 2007), modulation of acetylcholine release (Matsumoto *et al*, 2001), and beneficial effects on the accumulation of $\beta$-amyloid have been shown (Cho and Hu, 2007). No sex or age effects on the 5-HT$_4$ receptor density have been described in the human postmortem studies (Bonaventure *et al*, 2000; Reynolds *et al*, 1995; Varnas *et al*, 2003). In an earlier PET study with [$^{11}$C]SB207145 in a smaller younger cohort, we found that 5-HT$_4$ receptor binding declined with age, and a *post hoc* analysis suggested that women have lower binding in the hippocampus than men (Marner *et al*, 2010). The aim of this study was to evaluate age and sex effects on 5-HT$_4$ receptor binding in a larger cohort of healthy subjects, also including older individuals. Three brain regions were included in the study: striatum, limbic system, and neocortex.

## Materials and methods

### Subjects

A total of 30 healthy subjects were included (mean age, 44 years; range, 20 to 86 years; 14 men). Subjects were recruited by public advertisements ($N=26$) or extracted from the civil registration system in Denmark ($N=4$). All subjects gave a written informed consent for participation. The study was approved by The Copenhagen Region Ethics Committee ((KF)01-274821 and (KF)01 2006-2, with amendments).

Exclusion criteria were significant medical history, drug or alcohol abuse, neurological or psychiatric disorders, mental disorder (ensured with DART45, which is a Danish version of the National Adult Reading Test (Nelson and O'Connell, 1978)), pregnancy, or head trauma. All subjects had a normal neurological examination and unremarkable brain magnetic resonance imaging (MRI) scans. Absence of psychiatric symptoms was ensured using the symptom check list revised (SCL-90-R; Derogatis, 1994) on the day of the PET scan. All subjects were scanned in the period from 2006 to 2009. A younger subset of the cohort ($N=14$) participated in two earlier studies wherein the quantification approach and test–retest variability were evaluated (Marner *et al*, 2009) and sensitivity to acute 5-HT release was measured (Marner *et al*, 2010).

### MRI and Volumes of Interest

MRI was conducted on a Siemens Magnetom Trio 3T MR scanner. High-resolution 3D T1-weighted (matrix $256 \times 256$; $1 \times 1 \times 1$ mm voxels) and 2D T2-weighted sequences were acquired. The T1-weighted MRIs were segmented into gray matter, white matter, and cerebrospinal fluid using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, UK). The T2-weighted images served for brain-masking purposes.

In all, 17 regions were automatically delineated on each subject's MRI in a user-independent manner with the Pvelab software package (Svarer *et al*, 2005; freely available on http://www.nru.dk/downloads):

- Striatal regions (high 5-HT$_4$ receptor binding): caudate nucleus and putamen.
- Limbic regions (intermediate 5-HT$_4$ receptor binding): hippocampus, amygdala, anterior cingulate gyrus, posterior cingulate gyrus, and thalamus.
- Neocortical regions (low 5-HT$_4$ receptor binding): orbitofrontal cortex, medial and inferior frontal gyri, superior frontal gyrus, insula, superior temporal gyrus, medial and inferior temporal gyri, sensory motor cortex, parietal cortex, and occipital cortex.
- A region with negligible concentration of 5-HT$_4$ receptors: cerebellum excluding vermis.

### PET Imaging and Quantification of Nondisplaceable 5-HT$_4$ Receptor Binding

PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA) operating in 3D acquisition mode, producing 35 image slices with an inter slice distance of 4.25 mm. The total axial field of view was 15.2 cm, with an approximate in-plane resolution of 6 mm. To minimize movement during the scan, a light headband fixation was used.

The scan was based on a 120 minute dynamic acquisition starting with a bolus injection of mean 491 MBq (range, 206 to 611 MBq) [$^{11}$C]SB207145 given for more than 20 seconds. The mean mass dosage was 3.4 $\mu$g (range, 0.14 to 5.9 $\mu$g), the maximum upper dosage limit has been estimated to be 9.5 $\mu$g (occupancy $<10\%$; Madsen K *et al*, unpublished observations). The acquisition consisted of 38 time frames ($6 \times 5$, $10 \times 15$, $4 \times 30$, $5 \times 120$, $5 \times 300$, and $8 \times 600$ seconds). After acquisition, attenuation- and decay-corrected recordings were reconstructed by filtered back projection using a 6 mm Hann filter.

Frames were aligned using AIR 5.2.5 (Woods *et al*, 1992) to correct for movements during the scan. Before alignment, each frame was filtered with a 12 mm Gaussian filter, and the rigid transformation of each frame to a selected single frame with sufficient structural information (frame

**Table 1** Sex-specific values of demographic, tracer, and plasma data

| | *Men* | *Women* | *ANCOVA* | |
|---|---|---|---|---|
| | | | *Sex*, P-*value* | *Age*, P-*value* |
| *N* | 14 | 16 | | |
| Age (years) | 41 ± 20 | 47 ± 21 | 0.47 | |
| BMI (kg/m$^2$) | 27 ± 4 | 27 ± 7 | 0.70 | 0.53 |
| Injected mass per kg bodyweight (ng/kg) | 40 ± 22 | 49 ± 28 | 0.44 | 0.43 |
| Mean ligand concentration in cerebellum (fmol/ml) | 134 ± 76 | 202 ± 120 | 0.11 | 0.03 |
| $f_P$ (%), (*N*) | 26 ± 6 (10) | 27 ± 12 (13) | 0.74 | 0.94 |
| Parent compound in plasma, 32 minutes (%), (*N*) | 20 ± 8 (10) | 20 ± 6 (8) | 0.70 | 0.15 |
| Parent compound in plasma, 55 minutes (%), (*N*) | 13 ± 3 (12) | 13 ± 4 (9) | 0.97 | 0.81 |

ANCOVA, analysis of covariance; BMI, body mass index.
Values are mean ± s.d. Sex differences and age effects are tested on parameters one by one in a linear ANCOVA.

26: 15 to 20 minutes after injection) was estimated using the scaled least squares cost-function in AIR.

The [$^{11}$C]SB207145 was automatically co-registered to the MRI with the AIR algorithm using the mean of the first 20 minutes of the PET scan corresponding to a flow-weighted image. The quality of each co-registration was evaluated by visual inspection in three planes. Data were PV corrected using the Muller–Gartner method (Muller-Gartner *et al*, 1992), with a point spread function of 6 mm. The age and sex effects on 5-HT$_4$ receptor binding were assessed with PV-corrected data as the PV effect results in an underestimation of counts in high-count voxels because of spill-over to neighboring voxels due to insufficient resolution of the PET scanner. Amounts of spill-out and spill-in of brain regions depend on brain structure and are therefore influenced by the increased occurrence of brain atrophy with age (Raz *et al*, 2005) and the well-documented structural sex differences (Cosgrove *et al*, 2007).

Regional time activity curves were constructed both with and without PV correction, and kinetic modeling was performed with the simplified reference tissue model using cerebellum as the reference region, as validated previously (Marner *et al*, 2009). The regional *in vivo* outcome measure, the binding potential, $BP_{ND}$, is defined as:

$$BP_{ND} = f_{ND}\frac{B_{avail}}{K_D}$$

$f_{ND}$ being the nonprotein-bound fraction of nondisplaceable binding in the brain tissue, $B_{avail}$ the concentration of available receptors, and $K_D$ the dissociation constant. The kinetic modeling was performed using the PMOD software version 2.95, build 2 (PMOD Inc., Zürich, Switzerland). Volume-weighted means of $BP_{ND}$ were calculated for striatum, limbic system, and neocortex.

### Plasma Analysis: Metabolites and Free Fraction

Immediately before initiation of the scans, venous blood samples were drawn to measure the plasma-free fraction, $f_P$, with equilibrium dialysis ($N = 26$), as described previously (Kornum *et al*, 2009). Venous blood samples were drawn at 32 and 55 minutes after injection ($N = 21$ and $N = 24$, respectively), and the parent [$^{11}$C]SB207145 compound and its radiolabeled metabolites were measured in plasma with high-performance liquid chromatography.

### Statistics

To control for possible biases in age, body mass index, and plasma and tracer data, a linear analysis of covariance was used to test for sex differences and age effects on these variables (Table 1).

As the primary investigation, a linear analysis of covariance was used to model the effect of age, sex, and their interaction on 5-HT$_4$ receptor binding for each of the three brain regions. Interaction between age and sex was excluded from the analysis if not significant. Regional PV-corrected 5-HT$_4$ receptor binding was the primary dependent variable. To evaluate the effect of PV correction, analyses were also performed without PV-corrected binding measures. Tests were two sided, and *P* values were considered significant when $< 0.05$. Parameter estimates and s.d. were given when appropriate.

## Results

The sex-specific values for demographic and tracer data are shown in Table 1. There was no significant effect of age and sex on demographic and tracer data, except for a significant increasing cerebellar mean concentration of unlabeled tracer with age ($P = 0.03$, estimate 2.1 fmol/mL years ± 0.9).

Regional 5-HT$_4$ receptor binding, with and without PV correction, and gray matter volumes are listed in Table 2. The regional distribution of the tracer is in concordance with previous studies of [$^{11}$C]SB207145 (Marner *et al*, 2010) showing the binding pattern: neocortex < limbic system < striatum. No interaction was found between age and sex effects on regional 5-HT$_4$ receptor binding, therefore, it was excluded from the analyses. Results of sex and age effects on regional 5-HT$_4$ receptor binding are shown in Table 3.

### Effects of Sex on 5-HT$_4$ Receptor Binding

In all, 13% lower 5-HT$_4$ receptor binding was found in women compared with men in the limbic system (see Figure 1A). The finding was similar without PV correction of data (11%), and the finding is also significant after Bonferroni correction for multiple comparisons ($P=0.014$ with PV correction and $P=0.048$ without). A *post hoc* analysis of limbic subregions showed that the difference was most pronounced, with 19% in the amygdala ($P=0.0056$ without PV correction and $P=0.012$ with; see Figure 2).

A borderline tendency of 6% reduction in striatum of 5-HT$_4$ receptor binding in women compared with men was found both with and without PV correction of data (see Figure 1B). For the neocortex, a significant reduction of 10% was found in PV-corrected data only. Thus, a similar pattern was found in striatum and neocortex, but after Bonferroni correction for multiple comparisons, there were no significant gender differences found neither for striatum nor for neocortex. *Post hoc* analyses of neocortical subregions showed significant reductions of 9% to 13% in women both with and without PV correction of data in orbitofrontal cortex, insula, and superior temporal gyrus ($P$ values range from 0.005 to 0.04, uncorrected).

**Table 2** Regional $BP_{ND}$ values with and without PV correction and the corresponding regional gray matter volumes

| | *Uncorrected* $BP_{ND}$ | *PV-corrected* $BP_{ND}$ | *Gray matter volume (ml)* |
|---|---|---|---|
| Neocortex | 0.36 ± 0.07 | 0.69 ± 0.08 | 350 ± 60 |
| Limbic system | 0.57 ± 0.09 | 0.70 ± 0.09 | 23 ± 3 |
| Striatum | 2.2 ± 0.3 | 3.2 ± 0.4 | 7 ± 1 |

PV, partial volume.
Values are mean ± s.d.

### Effects of Age on 5-HT$_4$ Receptor Binding

Without correcting for the PV effect, a significant decline with age was found in all three regions corresponding to declines of 3% to 5% per decade. However, when correcting for the PV effect, a decline in 5-HT$_4$ receptor binding with age was found in striatum only (see Figure 1B) corresponding to a decline of 1% per decade; this finding was significant after Bonferroni correction ($P=0.04$). Declines per decade are calculated as the change from 40 to 50 years.

## Discussion

### Sex Differences in 5-HT$_4$ Receptor Binding

We found that women had 13% lower limbic 5-HT$_4$ receptor binding than men (see Figure 1A). This is highly interesting because the limbic system has been linked historically to learning and memory, cognitive processing, and emotion. Further, *post hoc* analyses showed that the difference was most pronounced in the amygdala, which is highly involved in the control of emotions (Ehrlich *et al*, 2009), and was further found in the subregions of the neocortex that often are referred to as paralimbic: the orbitofrontal cortex, insula, and superior temporal gyrus, of which primarily the orbitofrontal cortex is involved in affective functions. Our observations support a role for 5-HT$_4$ receptors in the sex-specific differences of emotional control, and the lower 5-HT$_4$ receptor binding might contribute to the observed higher prevalence of affective diseases and AD in women, which persists even after controlling for the fact that women tend to live longer than men. An animal study has reported that 5-HT$_4$ receptor agonism exerts a fast antidepressive response, with modification of key markers of antidepressant action: desensitization of 5-HT$_{1A}$ autoreceptors, increased

**Table 3** Linear ANCOVA analyses with regional 5-HT$_4$ receptor binding as a dependent variable, and age and sex as explanatory variables

| | *Uncorrected 5-HT$_4$ receptor binding* | | | *PV-corrected 5-HT$_4$ receptor binding* | | |
|---|---|---|---|---|---|---|
| | *Estimate ± s.e.* | P *value* | $R^2$ | *Estimate ± s.e.* | P *value* | $R^2$ |
| *Neocortex* | | | | | | |
| Age | −0.0020 ± 0.0005 | 0.0009 | | 0.00096 ± 0.0007 | 0.20 | |
| Sex | −0.028 ± 0.022 | 0.21 | 0.39 | −0.073 ± 0.029 | 0.017 | 0.21 |
| *Limbic system* | | | | | | |
| Age | −0.0021 ± 0.0006 | 0.001 | | −0.00016 ± 0.0008 | 0.84 | |
| Sex | −0.064 ± 0.025 | 0.016 | 0.42 | −0.095 ± 0.031 | 0.0048 | 0.27 |
| *Striatum* | | | | | | |
| Age | −0.0099 ± 0.0020 | < 0.0001 | | −0.0085 ± 0.0031 | 0.010 | |
| Sex | −0.13 ± 0.08 | 0.11 | 0.52 | −0.21 ± 0.12 | 0.10 | 0.31 |

ANCOVA, analysis of covariance; PV, partial volume.
PV corrected values are the primary outcomes. Analyses are performed for each region one by one, and sex differences are analyzed with men as reference.

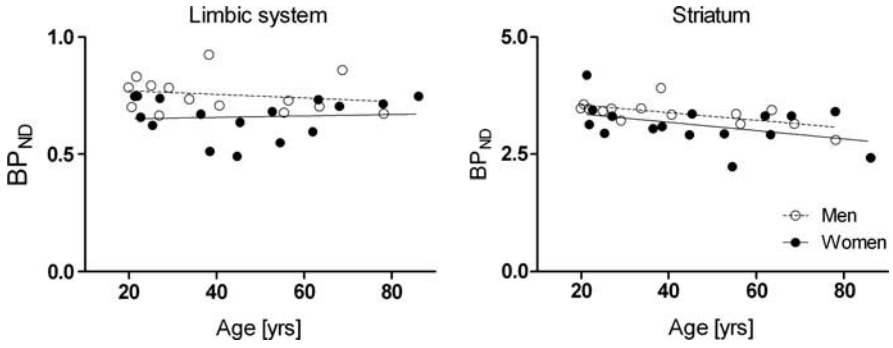


**Figure 1** The association between regional partial volume (PV)-corrected 5-HT$_4$ receptor binding for men and women separately. There is no interaction between sex and age. Mean 13% lower limbic 5-HT$_4$ receptor binding is found in women compared with men. There is a decline with age of 1% per decade in striatal 5-HT$_4$ receptor binding.

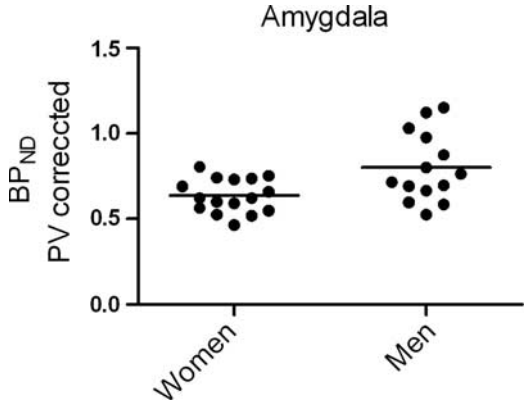


**Figure 2** A *post hoc* analysis of limbic subregions showed that the sex difference was most pronounced in the amygdala with 19% ($P = 0.012$).

tonus on hippocampal postsynaptic 5-HT$_{1A}$ receptors, and enhanced phosphorylation of the CREB protein and neurogenesis in the hippocampus (Lucas *et al*, 2007). Further, experimental studies have suggested 5-HT$_4$ receptor agonists to represent a valuable pharmacological target for the treatment of AD because they may provide both symptomatic relief of cognitive impairments as well as neuroprotection by reducing $\beta$-amyloid generation and toxicity (reviewed by Lezoualc'h, 2007). However, human postmortem studies revealed no changes in 5-HT$_4$ receptor affinity and density in AD in frontal and temporal cortex (Lai *et al*, 2003), whereas increased density of 5-HT$_4$ receptors in frontal cortex and caudate nucleus was found in violent suicide victims (Rosel *et al*, 2004).

We thoroughly examined potential confounders that could have influenced the observed sex difference in this limited sample size. No differences were found in the fraction of nonprotein-bound tracer molecules ($f_P$), metabolic rate, injected mass, or cerebellar concentration, indicating that bias from tracer availability did not explain the sex difference in limbic 5-HT$_4$ receptor binding. Even though the $BP_{ND}$ in limbic regions is moderate, the simplified reference tissue model yields low test–retest differences (Marner *et al*, 2009).

In this study, we were not able to examine whether differences in gonadal hormones and menstrual cycle phase had a role. However, no change in limbic 5-HT$_4$ receptor binding was found with age, and no interaction was found between age and sex in any region, indicating that menopause does not affect the 5-HT$_4$ receptor binding. Further, no difference in limbic 5-HT$_4$ receptor binding was found between premenopausal and postmenopausal women ($P = 0.64$, *t*-test with cutoff at 40 years).

The PV correction gave rise to an increased sex difference in 5-HT$_4$ receptor binding, because the PV effect caused a larger underestimation of $BP_{ND}$ in men. This is not surprising because it has been documented that men not only have greater brain volumes than women but also have greater volume of sulci, smaller gray/white matter ratios, and regional thinner cortical gray matter (Cosgrove *et al*, 2007), which all might contribute to an increased PV effect in men.

PET studies show, if anything, a pattern of higher levels of inhibitory receptors and lower levels of excitatory serotonergic receptors in women, and this is compatible with the finding in our study of lower 5-HT$_4$ receptor binding in women.

### Age Effects on 5-HT$_4$ Receptor Binding

PV corrected data showed an age-related decrease only in striatal 5-HT$_4$ receptor binding, 1% per decade. In agreement with our previous study (Marner *et al*, 2010), we found a decline of 3% to 5% per decade in $BP_{ND}$ with age without PV correction in all investigated regions. Increasing atrophy with aging (Raz *et al*, 2005) increases the impact of the PV effect: the sulci widen and there is loss of gray matter, leading to increasing spill-out of counts to the cerebrospinal fluid and white matter especially in cortical regions. Particularly for PET scanners with medium-to-low spatial resolution, age effects cannot be reliably estimated without considering the PV effect, even though PV correction depends heavily on the MRI segmentation,

co-registration, and size of point spread function, and the method introduces additional noise to the data.

We controlled for possible confounders that could have caused this outcome: body mass index, $f_P$, metabolic rate, $C_{FP}$, and injected mass were unaffected by age (see Table 1). However, the increasing cerebellar concentration of ligand could bias the measurement of $BP_{ND}$ and give an overestimation of the decrease with aging. Thus, the discrete striatal age-related decrease with aging may be caused by higher nondisplaceable binding with aging. Even though the simplified reference tissue model yields low test–retest differences in striatum, the model has been found to underestimate $BP_{ND}$ in the high-binding striatal regions (Marner *et al*, 2009). All the same, 5-HT$_4$ receptor binding is relatively stable with aging compared with other subtypes of receptors. This speaks against a direct involvement of 5-HT$_4$ receptors in the cognitive decline in normal aging, despite the beneficial effects of central 5-HT$_4$ receptor agonism on memory and learning found in experimental studies.

### Conclusion

In this study, we found a 13% lower 5-HT$_4$ receptor binding in the limbic system in women compared with men, with the largest difference of 19% being observed in amygdala. Whether the sex difference in 5-HT$_4$ receptors explains part of the observed difference in the prevalence of AD and affective disorders between men and women remains to be elucidated. We found a decrease with aging of 1% per decade in striatal 5-HT$_4$ receptor binding only, suggesting that this receptor subtype differs from the more pronounced age-related decline of other serotonergic markers. Future studies of the 5-HT$_4$ receptor *in vivo* should focus on associations between the 5-HT$_4$ receptor binding and affective symptoms as well as cognitive performance in neuropsychiatric disorders, and investigations might contribute to the development of new treatment paradigms in affective and neurodegenerative diseases.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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