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Summary

This PhD-thesis deals with in vivo imaging of the serotonergic transmitter system, more specifically the serotonin 4 (5-HT_4) receptor. Animal studies indicate that the 5-HT_4 receptor plays a role in cognitive function and affective diseases. Moreover, 5-HT_4 receptor activation is presumed to modulate Ach release and reduce the accumulation of cerebral β-amyloid (Aβ) in 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. Therefore, the 5-HT_4 receptor is interesting in relation to both gender, aging and AD.

With the radioligand [^{11}C]SB145207, it is now possible to measure the in vivo cerebral 5-HT_4 receptors in humans. However, the chemical amount (mass dose) allowed for injection, without risking pharmacological effects or bias the measurement, has been questioned as the radioligand binds with relatively high affinity to the 5-HT_4 receptor.

The main aims of the studies in this thesis were to:

- Estimate the upper mass dose limit and affinity of the radiotracer [^{11}C]SB145207, by conducting test-retest studies in healthy subjects with varying mass doses.
- In a cohort of healthy subjects investigate sex and age effects on regional 5-HT_4 receptor binding in striatum, limbic system and neocortex.
- Study the 5-HT_4 receptor binding in AD and its relation to Aβ by comparing AD patients to elderly healthy subjects.

[^{11}C]SB207145 PET scans were conducted with a GE Advance scanner. Brain regions of interest were automatically delineated on co-registered individual 3T MRIs. Partial volume correction was done with the Muller-Gartner method. The output parameter for 5-HT_4 receptor binding, the non-displaceable binding potential (BP_{ND}), was modeled using the simplified reference tissue model. The AD patients and eight of the elderly healthy subjects were also investigated with [^{11}C]PIB scans in a high-resolution brain-dedicated PET scanner to measure the accumulation of cerebral Aβ.

We found that in spite of its relatively high receptor affinity (mean in vivo K_D=2.8 nM (range 1.0-4.8)), [^{11}C]SB207145 does not require to be produced at extraordinarily high specific radioactivity and the upper mass dose limit was estimated to 4.5 μg ± 1.6 (receptor occupancy <5%).

Women were found to have 13% lower 5-HT_4 receptor binding in the limbic system compared to men, and the largest difference was found in amygdala, which is highly involved in affective functions. The observation supports a role for 5-HT_4 receptors in the sex specific differences of emotional control and cognitive function, and the lower limbic 5-HT_4 receptor binding may contribute to the higher prevalence of affective diseases and AD in women.

A decline with age of 1% per decade was only found in the striatum. Thus, cerebral 5-HT_4 receptor binding seems to be relatively stable with aging, which speaks against a direct involvement of 5-HT_4 receptors in the cognitive decline in normal aging.

No difference in 5-HT_4 receptor binding was found between 11 AD patients and 12 healthy elderly subjects. However, when groups were defined by the accumulation of cerebral Aβ, high levels of

Aβ accumulation was associated with high 5-HT_4 receptor binding. This finding could be interpreted as attempting to counteract Aβ accumulation and improve cognitive function.
Future investigations 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.

Dansk Resumé

Flere dyreeksperimentelle studier tyder på at serotonin 4 receptor subtypen (5-HT_4 receptoren) er af betydning for kognitiv funktion og ved affektive sygdomme. Stimulation af 5-HT_4 receptorerne menes desuden at modulere acetylkolinfrigivelse og reducere akkumuleringen af cerebral β-amyloid (Aβ) ved Alzheimers sygdom (AS). Prævalensen af AS øges med alderen, og kvinder har højere risiko end mænd for at udvikle AS og affektive sygdomme. Således er 5-HT_4 receptorerne interessante både i forhold til køn, aldring og AS. Det er nu blevet muligt at kvantificere hjernens 5-HT_4 receptorer in vivo med PET-radioliganden [^{11}C]SB207145.

De væsentligste mål i denne afhandling er:

- Udfra test-retest studier hos mennesker med varierende massedosis indgivet, at bestemme radioligandens affinitet samt øvre grænseværdi for massedosis af [^{11}C]SB207145.
- I en kohorte af raske personer at undersøge effekten af køn og alder på den regionale 5-HT_4 receptorbinding i striatum, det limbiske system og neokortex.
- At undersøge 5-HT_4 receptorbindingen ved AS og sammenhængen med Aβ ved at sammenligne patienter med diagnosen AS med raske jævnaldrende.

[^{11}C]SB207145 PET-skanningerne blev optaget med en GE Advance skanner. Hjerneregioner blev automatisk indtegnet på co-registrerede individuelle 3T MR billeder. Muller-Gartner metoden blev anvendt til korrektion for partial volume effekt. 5-HT_4 receptorbindingen (det ikke-displacerbare bindingspotentiale BP_{ND}) blev modelleret med " the simplified reference tissue model". Patienterne med AS samt 8 raske ældre blev yderligere undersøgt med [^{11}C]PIB PET-skanninger for at måle akkumuleringen af Aβ i hjernen.

På trods af relativ høj receptoraffinitet (middel in vivo K_D=2,8 nM (spænd 1,0-4,8)) er det rutinemæssige niveau af specifik aktivitet ved produktion af [^{11}C]SB207145 tilstrækkeligt, og den øvre grænse for massedosis blev estimeret til 4,5 μg ± 1,6 (receptor okkupans <5%).

Kvinder havde 13% lavere 5-HT_4 receptorbinding i det limbiske system i forhold til mænd. Den største forskel fandtes i amygdala, som er særligt involveret i affektive funktioner. Observationen støtter hypotesen om involvering af 5-HT_4 receptorerne i de kønsspecifikke forskelle i emotionel kontrol og kognitiv funktioner, og lavere 5-HT_4 receptorbinding i det limbiske system kan være medvirkende til den højere prævalens af affektive sygdomme og AS hos kvinder.

Et fald med alderen i 5-HT_4 receptorbindingen på 1% pr. dekade blev kun fundet i striatum og 5-HT_4 receptorniveauet synes at være relativt stabilt med alderen i modsætning til andre receptor-typer. Dette taler imod en direkte involvering af 5-HT_4 receptorerne i det fald, der ses i den kognitive funktion ved normal aldring.

Der var ingen forskel i 5-HT_4 receptorbinding mellem 11 patienter med AS og 12 raske ældre kontrolpersoner. Derimod var en stigning i 5-HT_4 receptorbinding associeret med høj Aβ akkumulation. Dette kan fortolkes som et forsøg på at modvirke Aβ-ophobningen samt at forbedre den kognitive funktion ved AS.

Det konkluderes at [^{11}C]SB207145 er en glimrende radioligand til fremtidige in vivo studier af 5-HT_4 receptorerne og disse studier kan medvirke til udvikling af nye behandlingsparadigmer ved affektive og neurodegenerative sygdomme. Fremtidige studier bør fokusere på sammenhængen mellem 5-HT_4 receptorbinding og såvel affektive symptomer som kognitive funktioner ved neuropsykiatriske sygdomme.

List of Papers

1: Karine Madsen, Lisbeth Marner, Mette Haahr, Nic Gillings and Gitte M. Knudsen. Tracer-dose Limits and in Vivo 5-HT_4 Receptor Affinity in Human Brain PET Studies with [^{11}C]SB207145 *(Submitted to Nuclear Medicine and Biology)*

2: Karine Madsen, Mette Haahr, Lisbeth Marner, Sune H. Keller, William Baaré, Claus Svarer, Steen G. Hasselbalch and Gitte M. Knudsen. Age and Sex Effects on 5-HT_4 Receptors in the Human Brain – A [^{11}C]SB207145 PET Study *(Journal of Cerebral Blood Flow and Metabolism,* advance online publication 2 March 2011; doi: 10.1038/jcbfm.2011.11*)*

3: Karine Madsen, Wolf-Julian Neumann, Klaus Holst, Lisbeth Marner, Szabolcs Lehel, Gitte Moos Knudsen and Steen Gregers Hasselbalch. Cerebral Serotonin-4 Receptors and β-amyloid are positively correlated in Early Alzheimer's Disease *(Submitted to Journal of Alzheimer's Disease)*

Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5-HT_4	serotonin 4 receptor
[^{11}C]PIB	[*N*-Methyl-^{11}C]$_2$-(4'-methylaminophenyl)-6-hydroxybenzothiazole
PET	positron emission tomography
Aβ	amyloid beta
Ach	acetylcholine
AchEI	acetylcholine esterase inhibitor
AD	Alzheimer's disease
ANCOVA	analysis of covariance
APP	amyloid precursor protein
ASYMAD	asymptomatic risk for Alzheimer's disease
BP	binding potential
BP_{Baseline}	non-occupied binding potential
BP_{ND}	binding potential relative to the non displaceable binding
B_{max}	receptor concentration
BMI	body mass index
BP_{Occupied}	underestimated binding potential by the mass dose
CSF	cerebrospinal fluid
D	mass dose
f_P	plasma free fraction
GABA	gamma-amino-butyric acid
I	image of voxels
ID_{50}	mass dose of ligand that saturates 50% of receptors
K_{D}	dissociation constant
HPLC	high pressure liquid chromatography
HRRT	high resolution research tomographs
PSF	point spread function
O	receptor occupancy
MCI	mild cognitive impairment
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association
PV	partial volume
sAPPα	soluble amyloid precursor protein
SCL-90-R	symptom check list revised
SERT	serotonin transporter
SPECT	single photon emission computed tomography
SPM	statistical parametric mapping
SRTM	simplified reference tissue model
SSRI	selective serotonin reuptake inhibitors
SUVR	standardized uptake value ratio
TACs	time activity curves
X	spatial distribution of voxels

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is believed to be involved in several normal brain functions, including mood, sleep, emotions, sexual activity, hunger and memory, all functions that may change with aging and differ between genders. Changes with aging and gender differences have been described within the serotonin system and disturbances have been shown in several neuropsychiatric disorders such as affective disorders, Alzheimer's disease (AD), schizophrenia, eating disorders and drug dependence.

The development of the new PET tracer [^{11}C]SB207145 has made it possible to quantify the serotonin 4 receptor (5-HT$_4$) in vivo in humans, and studies of the 5-HT$_4$ receptor with this tracer will be in focus in this thesis.

The background section presents principles in PET imaging and the partial volume effect which is particularly crucial in studies of atrophic brains. The serotonin system in general is described and sex differences and changes with aging of serotonergic markers in PET are summarized. The 5-HT$_4$ receptor is introduced separately. AD and the suggested involvement of 5-HT$_4$ receptors in the pathophysiology are presented.

The studies in this thesis include methodological issues regarding validation of the tracer (involving mass dose effects) and the partial volume correction. The more clinical studies investigate changes with aging, gender differences and 5-HT$_4$ involvement in the pathophysiology of AD. Results are discussed and related to other papers for a more general discussion and perspectives.

Background

Positron Emission Tomography

PET is an imaging technique that makes it possible to measure physiological, biochemical and pharmacological processes in the brain in vivo. PET imaging demands a chemical structure (precursor) designed to bind to the molecule of interest, and the precursor must be labeled with a positron emitting radioisotope (figure 1) with its decay being detected in the PET scanner (figure 2 and 3). A cyclotron that beams accelerated protons into a target with 11 MeV is needed for the production of radioisotopes. The most frequently used radioisotopes are [^{11}C] and [^{18}F], although they have short physical half lives which complicates the production ($T_{½}$ 20.3min and $T_{½}$ 109.8 min repectively). Labeling of the precursor is also complex, and radiochemists struggle to label as high a fraction of the precursor molecules as possible, so that enough radioactivity can be given for the PET scan (300-600 MBq) without giving high chemical amounts of the precursor which can cause pharmacological effects or bias in the measurements. The term *specific activity* is used to describe the relationship between radioactivity and chemical amount, SI unit [GBq/µmol]. The measurement of the target will be underestimated if a large fraction of target sites (for example a specific receptor) is occupied by the precursor, and reliable quantitative measurements require negligible occupancy of the target, the limit is arbitrary set and often referred to as <5% to 10% (Innis et al., 2007).

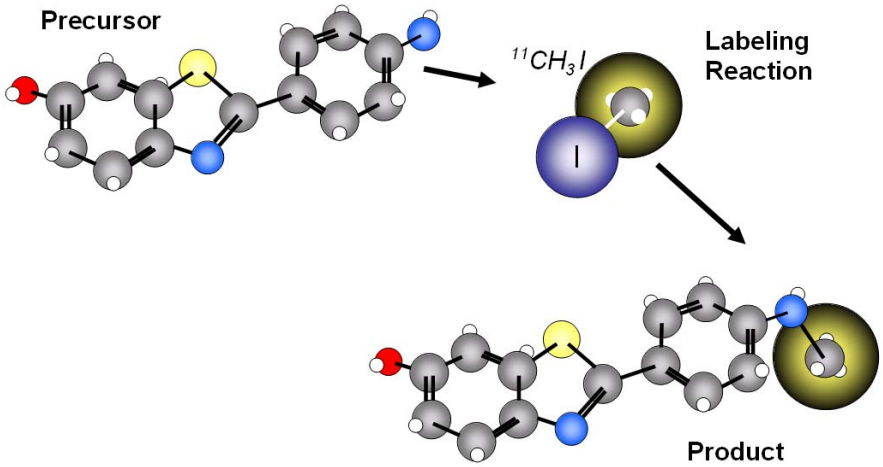

Figure 1 Labeling of the non-radioctive precursor PIB with [^{11}C] to produce the radioligand [^{11}C]PIB. (Drawing kindly provided by Brian Lopresti, PhD)

The PET scanner consists of rings of detectors surrounding the subject studied (figure 3). The two photons from the positron emission decay (figure 2) are detected by two detectors in the PET scanner and registered as an event when the time delay between registrations is less than 10ns, and the event is assigned to the line of response joining the two detectors. The original distribution of events can be reconstructed into a 3D image using either iterative algorithms or filtered back-projection algorithm.

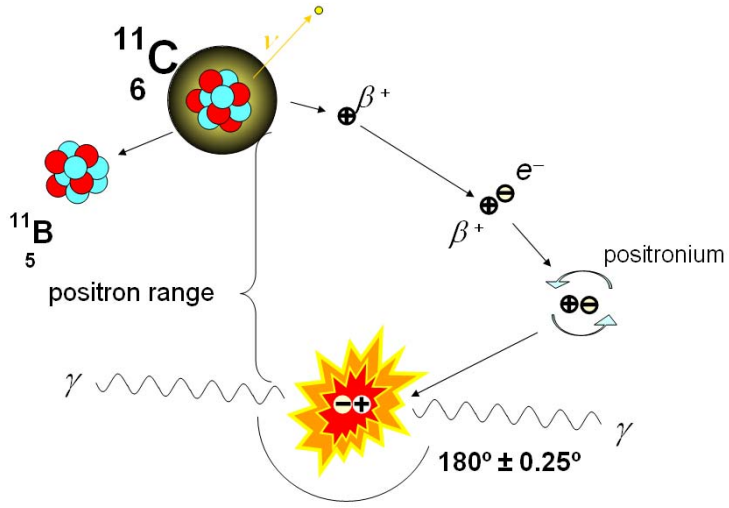

Figure 2 The decay of the radioisotope [^{11}C] by a positron emission (β^+) decay: The extra proton is converted to a neutron and a positron and a neutrino are emitted from the nucleus. The positron annihilate on contact with an electron producing two photons in opposite directions with an energy of 511 KeV. (Drawing kindly provided by Brian Lopresti, PhD)

The basic principle of PET imaging is that after intravenous injection the tracer distributes into the body, a fraction crosses the blood-brain barrier of which some tracer molecules binds to the target site (specific binding), some binds to other sites (unspecific binding) and some are free. Several requirements for a tracer are of importance (none of the existing tracers are perfect):

- Be able to cross the blood-brain barrier (sufficiently high lipophilicity)
- A favourable ratio between specific and non-specific binding (lipophilicity must not be too high)
- Binds to the target site with high selectivity and affinity (in nM range), but not too high affinity as binding then will be irreversible during the scan
- Have relatively fast kinetics, so that equilibrium between association and disassociation to target sites is reached within the time window of a scan
- Tolerable radioactivity dosages (ideally below 5 mSv)
- A reference region with negligible number of target sites is preferred for clinical practice, thus representing the free and non-specific tracer only
- High enough specific radioactivity must be attainable so that <5% to 10% of target sites are occupied.
- Metabolites should not cross the blood-brain barrier and bind to other target sites.

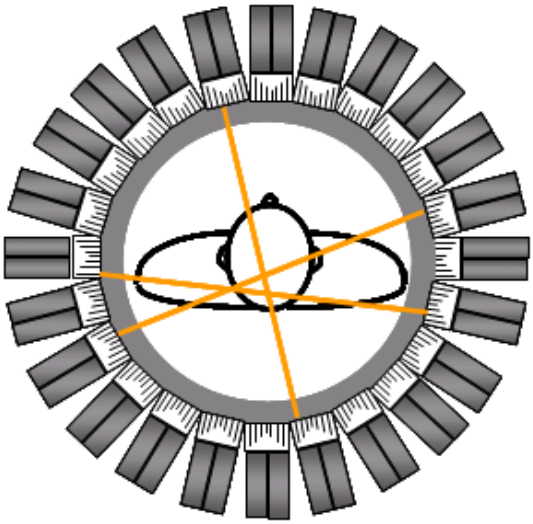

Figure 3 Detectors register the emitting photons from the subject in in the PET scanner (Drawing kindly provided by Brian Lopresti, PhD)

The detected events during the scan reflect the distribution of the tracer. Recording of events over time can either be detected in one time frame (static scan), usually some time after injection when equilibrium-like conditions of tracer distribution is reached, or in series of frames (dynamic scan) thereby creating a 4D image of the tracer distribution.

Anatomical regions are hard to define in a PET image, therefore regions are often defined on co-registered magnetic resonance images (MRI) (figure 4). The number of events in specific regions can be measured, and from 4D images regional time activity curves (TACs) or concentration curves can be extracted (figure 5). MRI scans can be segmented into grey matter, white matter and cerebrospinal fluid (CSF), and data can be extracted exclusively from the area that the target site is located in, for example serotonin receptors in grey matter. Quantitative measures of receptor concentration are obtained from the tracer data by taking the ratio between the concentrations in the regions of interest to a reference tissue concentration, ideally arterial blood. The ratio is either measured under equilibrium conditions or modeled from TACs. The ratio is proportional to the ratio between target density (e.g. receptor concentration, B_{max}) and the dissociation coefficient (K_D), often referred to as the binding potential (*BP*) (Innis et al., 2007). The simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996) is often used on TACs and the outcome parameter, BP_{ND}, is the *BP* relative to the non-displaceable binding of the tracer (including both non-specific binding and the free tracer) represented by a reference region in the brain with negligible concentration of target sites (like e.g. cerebellum for many tracers), so that arterial blood sampling is not necessary.

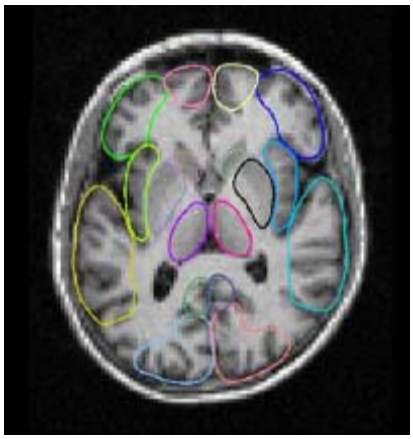

Figure 4 Automatic delineated regions of interest at the slice of an MRI (Svarer et al., 2005)

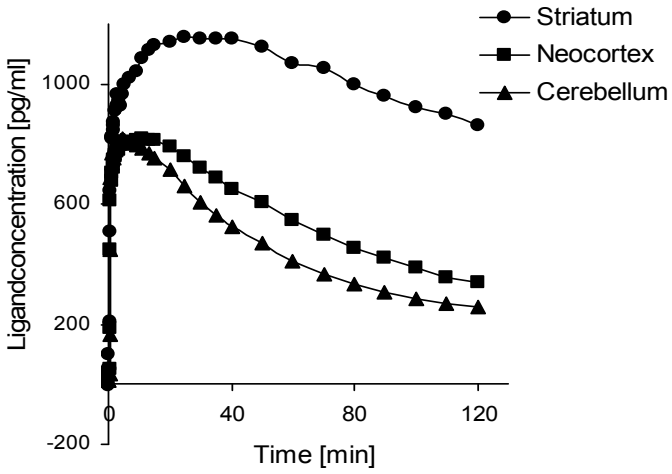

Figure 5 The time concentration curves for grey matter of three regions of interest in a two-hour dynamic PET scan with the tracer [^{11}C]SB207145

Partial volume correction

Due to movement artifacts, statistical noise and filtering, PET images have a limited resolution. Most scanners have a spatial resolution of 6-8mm full width at half maximum (FWHM), but the newer Siemens high resolution research tomographs (HRRT) have resolutions down to 2 mm

FWHM (Olesen et al., 2009). Activity in regions of interest will appear blurred and contaminated by the level of activity in the adjacent area, a phenomena known as the partial volume (PV) effect. The effect is more severe in regions with a large surface-area bordering low activity areas compared to more spherical shaped regions or regions surrounded by tissue with similar activity. Thus, for a target site localized in the brain grey matter (for example serotonin receptors) the spill-out is large from the cortical grey matter to the CSF and white matter (the grey matter thickness is only 2-3 mm) thereby underestimating the density of target sites. The effect is less in e.g. amygdala and putamen. The PV effect is especially prone to bias studies of age effects or when comparing groups with different brain morphology or degrees of atrophy. Increasing atrophy with aging (Raz et al., 2005) or for example in Alzheimer's disease increases the impact of the PV effect: the sulci widen, thereby increasing the spill-out of counts to the CSF in cortical regions, and further there is loss of grey matter. An example of this are studies of the 5-HT_{2A} receptor where a decrease with aging of 17% per decade was found in one study without PV correction(Sheline et al., 2002), while another study with PV correction showed a decrease of only 6% per decade (Adams et al., 2004).

The structural gender differences of brain structure may give rise to differences in PV effect and thus bias PET studies of gender differences. It has been documented that even though men have greater brain volumes than women, men also have greater volume of sulci (Gur et al., 1999), smaller grey/white matter ratios (Peters et al., 1998, Goldstein et al., 2001, Allen et al., 2003, Haier et al., 2005) and regional thinner cortical grey matter (Nopoulos et al., 2000, Allen et al., 2003, Carne et al., 2006, Sowell et al., 2007), which all may contribute to an increased PV effect in men.

Algorithms to correct for the PV effect have been developed, all based on the segmentation of the co-registered brain MRI. Spill-over from brain tissue to the CSF can be corrected for with an algorithm developed by Meltzer et al. (Meltzer et al., 1990). Another algorithm correct for spill-out from grey matter to CSF and also for both spill-in from and spill-out to white matter (Muller-Gartner et al., 1992):

The point spread function (PSF) describes the image response to a point source, corresponding to the blurred resolution in the image. The observed image response (I_{obs}) can then be described by the true un-blurred values in grey matter voxels (I_{GM}), white matter voxels (I_{WM}) and CSF voxels (I_{CSF}).

$$I_{\text{obs}} = I_{\text{GM}} \otimes PSF + I_{\text{WM}} \otimes PSF + I_{\text{CSF}} \otimes PSF \qquad (1)$$

If only the target sites in the grey matter is of interest then

$$I_{\text{GM}} \otimes PSF = I_{\text{obs}} - I_{\text{WM}} \otimes PSF - I_{\text{CSF}} \otimes PSF \qquad (2)$$

If X is the spatial distribution of voxels in the image and the activity in white matter is assumed to be uniform ($\overline{I_{\text{WM}}}$) and zero in CSF then

$$I_{\text{GM}} \otimes PSF = I_{\text{obs}} - \overline{I_{\text{WM}}}(X_{\text{WM}} \otimes PSF) \qquad (3)$$

If the grey matter activity is considered to have little variation then

$$\widetilde{I}_{\text{GM}} = \frac{I_{\text{obs}} - \overline{I_{\text{WM}}}(X_{\text{WM}} \otimes PSF)}{X_{\text{GM}} \otimes PSF} \qquad (4)$$

The Muller-Gartner PV correction is theoretically more correct than the algorithm by Meltzer and co-workers, however, grey matter activity does variate and the method depend upon correct segmentation of the MRI and adequate co-registration between MRI and PET images. Another method only assumes uniform grey matter activity of voxels regionally (Rousset et al., 1998), however, only the Muller-Gartner method was available and evaluated in this thesis.

The Cerebral Serotonin System

5-HT is synthesized from tryptophan in neurons that have their soma in the raphe nuclei in the midbrain and project to all brain regions (figure 6). The 5-HT is released by vesicular transport from the presynaptic neuron to the synaptic cleft, and the signaling is mediated through postsynaptic receptor proteins (figure 7). There are 7 classes of 5-HT receptors numbered from 1-7, several classes have subtypes for which reason there are at least 14 types of receptor proteins (for instance 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors). All are primarily G_s protein-coupled receptors, except for the 5-HT_3 receptor. The serotonin transporter is located presynaptically and controls the 5-HT availability in the synaptic cleft by reuptaking released 5-HT.

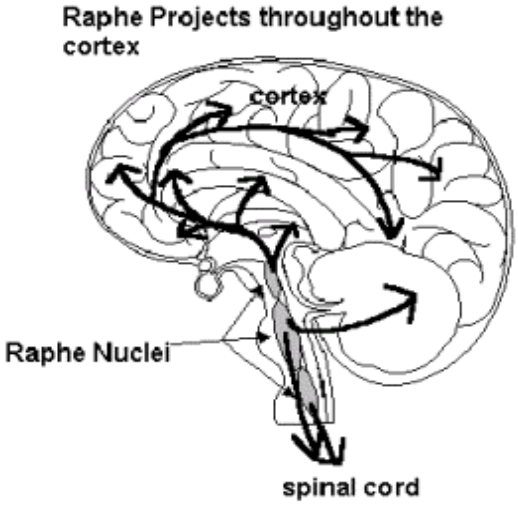

Figure 6 The projections throughout the brain of serotonin producing neurons that have their soma in the raphe nuclei in the midbrain (www.cellscience.com).

Serotonin can not be directly measured in vivo, and the association between the serotonin system and neuropsychiatric diseases, behavior and personality parameters has therefore been investigated with other serotonin markers including: genetic polymorphisms, post mortem receptor autoradiography, PET and CSF markers (5-HT and metabolites). Experimental studies may also include homogenate analysis and in vivo microdialysis. The interstitial serotonin concentration can be increased by treatment with Selective Serotonin Reuptake Inhibitors (SSRI) or decreased by ingestion of tryptophan-depleted mixture of amino acids and the effects of these manipulations has been investigated in several studies.

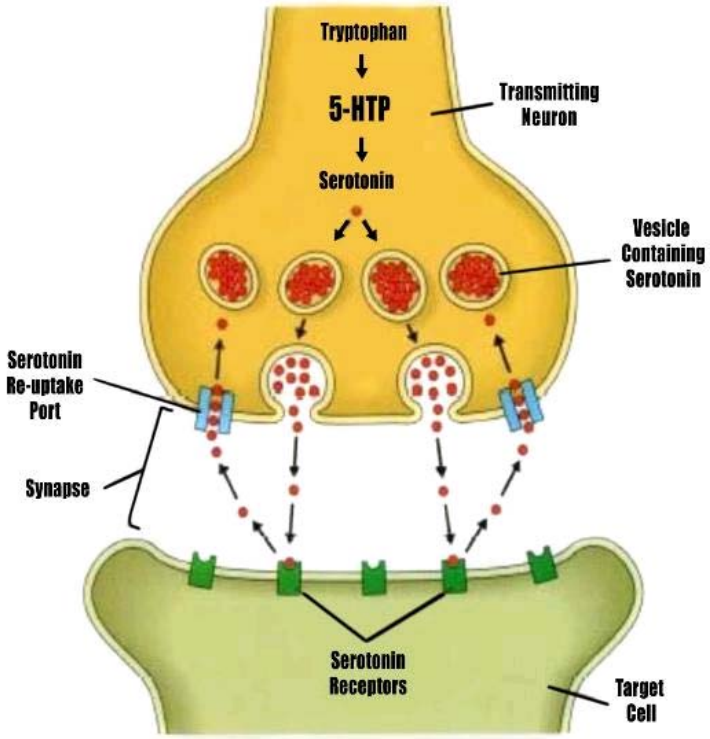

Figure 7 Illustrates the signaling from the presynaptic serotonergic neuron to the postsynaptic neuron mediated by serotonergic neurotransmission. Serotonin is synthesized from tryptophan released by vesicular transport. The serotonin transporter is located at the presynaptic neuron and mediates reuptake of serotonin from the synaptic cleft. The signal to the postsynaptic neuron is mediated by receptors.
(www.completehealthdallas.com)

Age and sex effects on serotonergic markers studied with PET

Depression (Meyer, 2007) and anxiety (Nutt, 2005) have been linked to serotonergic disturbances, and the prevalence for these is twice as high in women compared to men (Alonso et al., 2004). In contrast, men have a higher prevalence of schizophrenia which is also associated to changes in the serotonin system. AD has also been linked to serotonergic disturbances (Salmon, 2007), and the risk increases with aging and women have higher prevalence of the disease, suggesting a link between serotonergic dysfunction and neuropsychiatric disorders. which may be both age and gender specific.

Lowering the serotonin level by acute tryptophan depletion has larger memory-impairing effects in women (Sambeth et al., 2007), but in vivo PET imaging of sex differences in serotonin markers has shown diverging results (see details in table 1): 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 consistently been shown to depend on sex (Meyer et al., 2004, Jovanovic et al., 2008, Kalbitzer et al., 2009).

PET studies have primarily shown a decline or unchanged levels of serotonergic markers with normal aging: The 5-HT_{2A} receptor decline is most pronounced (Sheline et al., 2002, Adams et al., 2004). A decline with age is described for the 5-HT_{1A} receptors for both genders (Tauscher et al., 2001, Moller et al., 2007), in women only (Costes et al., 2005) and in 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 (Meyer et al., 2001, Reimold et al., 2008, Kalbitzer et al., 2009). Studies of other types of serotonin receptors have not been conducted.

Target Tracer	**Sample size (M/F)**	**Age range**	**Sex effect**	**Age effect**	**PV corr.**	**Reference**
SERT						
[^{11}C]DASB	8/9	22-51	ni	↓striatum	No	(Meyer et al., 2001)
	10/10	35±11	F=M	ns	No	(Meyer et al., 2004)
	11/8	44±10	F=M	↓thalamus	No	(Reimold et al., 2008)
	35/15	38±15	F<M caudate F>M midbrain	↓ thalamus	No	(Kalbitzer et al., 2009)
[^{11}C]MADAM	10/8	21-39	F<M global	ns	No	(Jovanovic et al., 2008)
5-HT$_{1A}$						
[^{18}F]MPPF	26/27	20-70	F>M, limbic, frontal	↓F only, global	No	(Costes et al., 2005)
[^{11}C]WAY-100635	10/11	21-80	F=M	↓M only, raphe, limbic and occipital cortex	Yes	(Cidis Meltzer et al., 2001)
	11/8	22-53	ni	↓neocortex	No	(Tauscher et al., 2001)
	61/0	24-53	ni	ns	No	(Rabiner et al., 2002)
	13/12	40±16	F>M global	ns	No	(Parsey et al., 2002)
	11/8	23-73	ni	↓global	No	(Moller et al., 2007)
	16/16	20-35	F=M	ni	No	(Stein et al., 2008)
	14/14	21-39	F>M global	ns	No	(Jovanovic et al., 2008)
5-HT$_{2A}$						
[^{18}F]setoperone	11/15	19-43	F=M	↓neocortex	No	(Lewis et al., 1999)
[^{18}F]altanserin	11/11	23-64	F<M global	ni	No	(Biver et al., 1996)
	6/16	21-69	ni	↓globally	No	(Sheline et al., 2002)
	30/22	21-79	F=M	↓cortex - occipital	Yes	(Adams et al., 2004)
	87/45	18-79	F=M global	↓globally	No	(Frokjaer et al., 2009)

Table 1 Effects of age and sex on serotonergic markers measured with PET in healthy subjects. ns = no significant effect, ni = not investigated, Age: range or mean ± SD, PV corr.= partial volume correction

The 5-HT$_4$ receptors

The regional distribution of the 5-HT$_4$ receptor has been described in humans from post mortem studies (Reynolds et al., 1995, Bonaventure et al., 2000, Varnas et al., 2003) showing a heterogenous distribution with high density in the striatum, intermediate in limbic regions and the temporal cortex and lower in other cortical regions. Cerebellum has only negligible number of 5-HT$_4$ receptors.

Stimulation of the 5-HT$_4$ receptor generally results in increased neuronal excitability, and the receptor is believed to act by modulating other neurotransmitter systems (Bockaert 2004). Experimental studies have suggested that the 5-HT$_4$ receptors modulate acetylcholine (Ach) release: Global 5-HT$_4$ stimulation increases Ach release in frontal cortex (Consolo et al., 1994), and local stimulation likewise increases Ach release in both hippocampus (Matsumoto et al., 2001) and frontal cortex (Yamaguchi et al., 1997). 5-HT$_4$ receptors are also modulating the dopaminergic system, and 5-HT$_4$ receptor agonism facilitates dopamine release and neuron impulse flow in nigral dopaminergic neurons (Lucas et al., 2001, Porras et al., 2002). Also GABAergic neurons in the prefrontal cortex have been shown to be influenced by the 5-HT$_4$ receptor (Cai et al., 2002).

5-HT release in the rat hippocampus and the firing rate of 5-HT neurons in the dorsal raphe nucleus are increased by acute 5-HT$_4$ agonism and decreased by antagonism (Ge and Barnes, 1996, Lucas and Debonnel, 2002). It has been discussed whether the 5-HT$_4$ receptor level could be an inverse marker of the (sub)chronic endogenous serotonin level: Animal studies have shown a down-regulation of 5-HT$_4$ receptors in response to chronic SSRI treatment and an upregulation in response to sub-chronic 5-HT depletion (Licht et al., 2009, Vidal et al., 2009), although no acute effect to SSRI treatment has been demonstrated in neither animals (Licht et al., 2009) nor humans (Marner et al., 2010b).

Experimental studies indicate that the 5-HT$_4$ receptor plays a role in memory acquisition and consolidation, and 5-HT$_4$ stimulation has mainly been found to improve memory performance (King et al., 2008). Further, 5-HT$_4$ stimulation also has an ameliorative effect on scopolamine induced cognitive deficits (Galeotti et al., 1998, Matsumoto et al., 2001), and has even a beneficial synergistic interaction with acetylcholine esterase inhibitors (AchEI) (Moser et al., 2002, Mohler et al., 2007, Cachard-Chastel et al., 2008). Animal studies have suggested that central 5-HT$_4$ receptor agonism gives a fast treatment response in depression (Lucas et al., 2007) where the cognitive function is impaired. Further, the 5-HT$_4$ receptor may be involved in reactions to stress (Compan et al., 2004), therefore stimulation of the receptor has been suggested in treatment for depression. Post mortem human studies have not described sex or age effects, but higher 5-HT$_4$ receptor density in frontal cortex and caudate nucleus has been found in depressed violent suicide victims (Rosel et al., 2004), which could be interpreted as a response to low levels of endogenous serotonin. Studies in neurodegenerative diseases have revealed normal 5-HT$_4$ receptor densities in Parkinson's disease but lower densities in putamen in Huntington's disease (Reynolds et al., 1995).

The 5-HT$_4$ receptor change in postmortem AD brains is discussed later in a separate section.

PET imaging of the 5-HT$_4$ receptor

The development of the PET tracer [^{11}C]SB207145 has made it possible to measure the 5-HT$_4$ receptor in vivo. The tracer was developed by GlaxoSmithKline Clinical Imaging Center in London who tested the tracer in pigs and humans (Gee et al., 2008), and they found the rank order of regional tracer concentration to be accordance with in vitro studies. The regional in vivo outcome measure for 5-HT$_4$ receptor binding for kinetic modeling with the simplified tissue model (SRTM) (Lammertsma and Hume, 1996) is the binding potential, BP_{ND}, that is relative to the non-displaceable binding of the tracer (including both non-specific binding and the free tracer) represented by the cerebellum. High correlations between regional binding potentials measured with SRTM and in vitro measurements (homogenate binding assays and autoradiography) have been shown in cortical and striatal regions of the Göttingen minipig, but not in hippocampus where PET studies with [^{11}C]SB207145 were underestimated, probably due to partial volume effects (Kornum et al., 2009). Evaluation of kinetic modeling in humans showed that SRTM with cerebellum as input yield low test-retest variability of BP_{ND} (6-10% in moderate to high-binding regions and 12%-14% in low-binding regions). In comparison to kinetic modeling using arterial input (which is considered the golden standard) in a 2-tissue compartment model, SRTM showed an underestimation of BP_{ND} in the high-binding striatal regions (20%-43%) and a slight overestimation in cortical regions (Marner et al., 2009). Although [^{11}C]SB207145 has high affinity to the 5-HT$_4$ receptor, it binds reversibly within the time window of a two hour dynamic PET-scan. It has a relatively slow metabolic rate and high free fraction in plasma (around 27%). We have previously made a population-based estimate of the upper limit of mass dose of 1.2 μg (occupancy < 5%), which requires a fairly high specific radioactivity, (Marner et al., 2010b). However, such estimate is biased by inter-individual differences in 5-HT$_4$ receptor binding, especially gender differences would bias the result: women were generally given higher doses than men and had lower BP_{ND}, therefore it was not possible to differentiate whether women had lower 5-HT$_4$ receptor levels or their lower BP_{ND} was caused by higher occupancy.

Another tracer for the 5-HT$_4$ receptor, the [^{123}I]SB207710, has been developed for single photon emission computed tomography (SPECT) and tested in rats (Pike et al., 2003), structural modification has been evaluated to design a high affinity PET tracer (Xu et al., 2010) but is not yet further evaluated.

Alzheimer's Disease

AD is a progressive neurodegenerative disease. It is the most frequent type of dementia and the prevalence doubles for every five years after the age of 65. Only a few percent of AD cases follow an autosomal dominant pattern, and the onset is often earlier compared to AD cases occurring sporadically. The primary symptoms in AD are cognitive impairment, behavioral changes and problems with practical skills. The cardinal symptom is loss of episodic memory, and usually loss of ability to learn and recall new material is the initial symptom. Behavioral changes may involve

depressive symptoms, apathy, aggression and others. Both cognitive and behavioral disturbances make daily life and practical skills an effort for both the patient and the caregivers, with an increased risk for institutionalization. (Waldemar and Burns, 2009)
I will here focus on the pathophysiology that begins developing decades before the criteria for clinical AD are fulfilled or even before subjects have cognitive complaints. Diagnosing AD and the biomarkers that may help diagnosing AD also at an early or even preclinical stage are discussed.

Neurodegeneration

Atrophy is increasing with normal aging, but even more severe atrophy is seen in AD including widening of sulci, enlargement of the third and the lateral ventricles and loss of both white and grey matter. Clusters of grey matter loss in MR imaging are described in AD patients in the mesial temporal lope, the posterior cingulated gyrus, precuneus, the temporoparietal cortex and the perisylvian neocortex (decreasing order of significance), figure 8 (Baron et al., 2001). Stereology studies have compared AD patients to age and sex matched healthy subjects (Pakkenberg et al., 2003) and shown decreased volume primarily in the limbic system but also in neocortex. Loss of neurons are consistently described in hippocampus in AD patients (in CA1, hilus and subiculum) (West et al., 2004) while cortical cell loss is controversial (Stark et al., 2005).

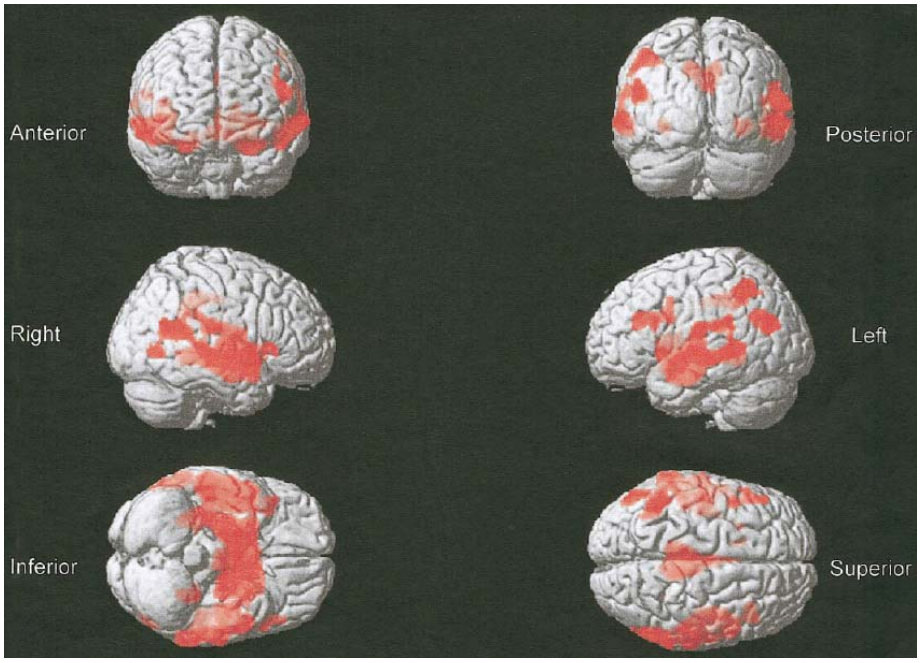

Figure 8
Clusters of voxels with grey matter density decrease in AD patients. (Baron et al., 2001)

Neuropathology

AD pathology is characterized by the deposition of intracellular neurofibrillary tangles and extracellular β-amyloid (Aβ). The accumulation of Aβ (figure 9) and tangles follow a regional pattern described first in post mortem studies (Braak and Braak, 1991). The extent of neurofibrillary tangles correlates with the severity of dementia in AD, while the Aβ plaques seem to start building up 10-20 years before symptoms of dementia occur (figure 10) (Jack et al., 2009), and the Aβ load is almost stabilized at the clinical stage of AD (Engler et al., 2006) and not associated to severity of

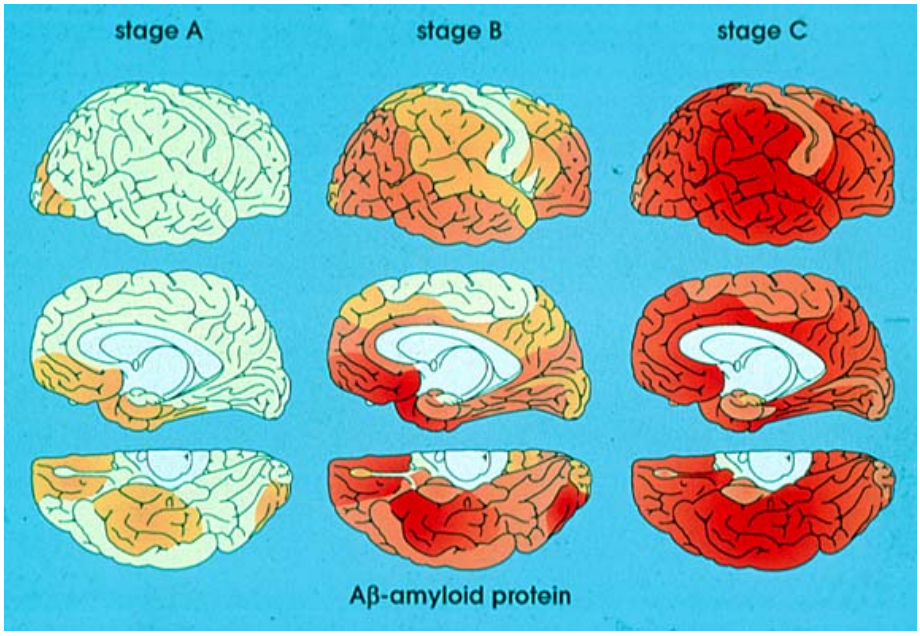

Figure 9
The pattern of Aβ accumulation (Braak and Braak, 1991)

dementia (Terry et al., 1991). The Aβ cascade model stating that increased production or decreased clearance of Aβ causes the disease was originally proposed in the 90s (Hardy and Higgins, 1992) and is currently the central hypothesis in AD pathogenesis: Aβ40 and Aβ42 peptides aggregate and form oligomers that subsequently form Aβ fibrils and result in deposition of Aβ plaques, this process trigger a cascade of deleterious changes, resulting in neuronal death and causing dementia. It has been discussed which component is the pathogenetic agent (Pimplikar, 2009), but the soluble oligomers of especially Aβ42 are now known to be highly neurotoxic although other factors not associated to Aβ also may be of relevance. Aβ40 or Aβ42 is a possible product of the proteolytic cleavage of the membrane-bound amyloid precursor protein (APP). APP has cleavage sites for the protein complexes called β-secretase, α-secretase and γ-secretase. Cleavage by the β-secretase and γ-secretase is responsible for formation of Aβ, while cleavage by the α-secretase leads to secretion of the non-amyloidogenic form of the soluble amyloid precursor protein (sAPPα) that may have neuroprotective effects (figure 11).

A strong evidence of the Aβ cascade hypothesis is that familiar AD cases have mutations in APP or presenilin genes, presinilin 1 and 2 are part of the γ-secretase complex. Further, Down's syndrome is associated with development of AD and the APP gene is located on chromosome 21.

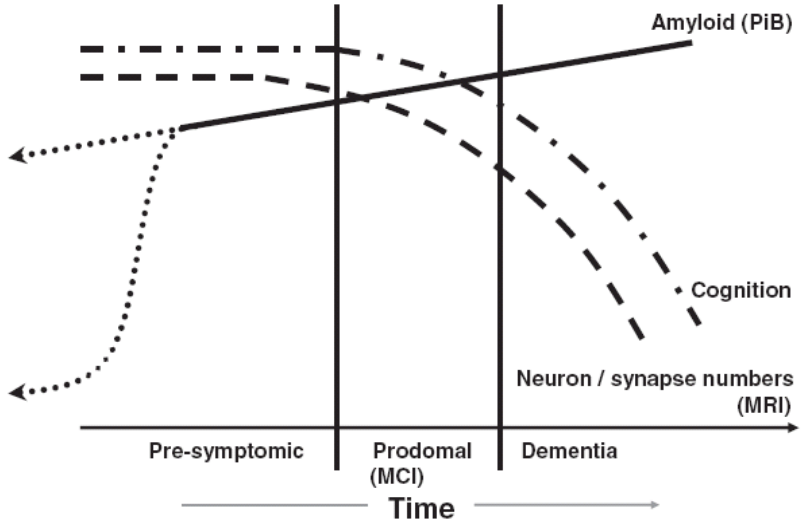

Figure 10 Aβ starts accumulating decades before symptoms of dementia occur. Therefore biomarkers for Aβ accumulation may help diagnosing AD even at preclinical stages (Jack et al., 2009).

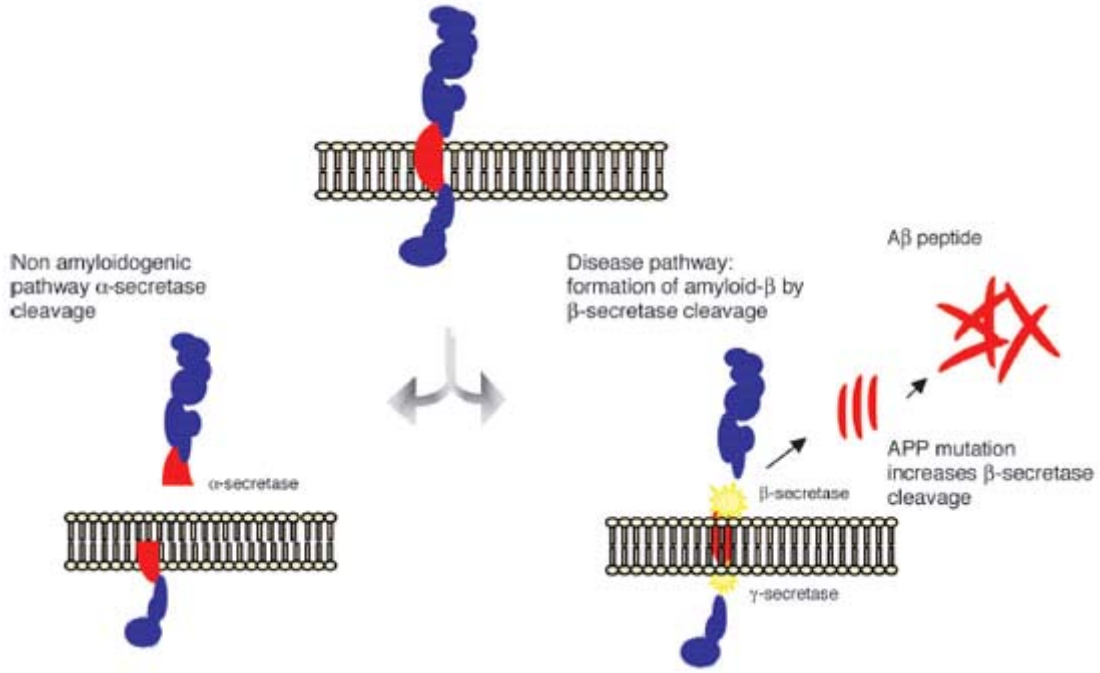

Figure 11 The membrane-bound APP and. At the left the non-amyloidogenic pathway with cleavage by α-secretase leading to the neuroprotective protein sAPPα. At the right the amyloidogenic pathological pathway by β-secretase and γ-secretase and the formation of Aβ (www.demneuropsy.com.br)

Neurotransmitter deficits

In AD several neurotransmitter systems seem to decline more rapidly than in normal aging (Nagren et al., 2010). The cholinergic axons projecting from the basal forebrain to the cortex (figure 12) are severely impaired already early in AD, and AChEI are the current standard treatment of AD despite their modest effects. PET imaging has consistently found reduced levels of tracers for markers of the cholinergic system in early AD including the nicotinic receptors, vesicular transporters and acetylcholine esterase activity but not muscarinic receptors (Herholz, 2008). The deficits in cholinergic neurotransmission seem to be related to attentional deficits rather than memory function (Bohnen et al., 2005).

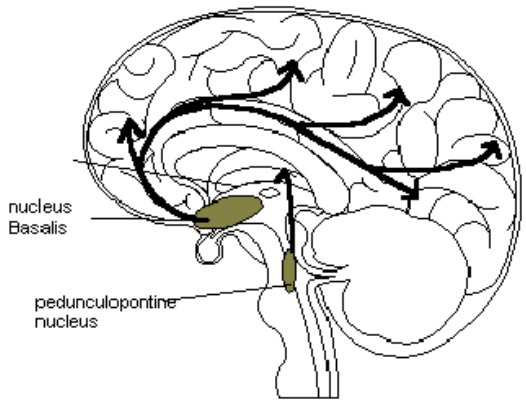

Figure 12 Cholinergic neurons located in the basal forebrain project to cortex and hippocampus. Cholinergic neurons located in the pedunculopontine lateral dorsal tegmentum in the brainstem projects primarily to thalamus.
(www.cellscience.com/CCA.htm).

The serotonin (5-HT) system is also affected in the course of AD and there are marked losses of presynaptic raphe neurons (Aletrino et al., 1992). Human post mortem studies (Meltzer et al., 1998) and in vivo PET studies (Nagren et al., 2010) have shown down regulations of the 5-HT transporter, the 5-HT_{2A} and 5-HT_{1A} receptor (See overview in table 2). The impaired serotonin transmitter system may account for emotional and behavioral symptoms that are common in AD, however, only very few studies have been able to associate alterations in the serotonergic system in AD patients with their clinical symptoms: The 5-HT_{1A} receptor binding correlates to cognitive impairment in a negative (Kepe et al., 2006) as well as a positive way (Lai et al., 2002) and a compensatory upregulation has been suggested to take place at the preclinical stage (Truchot et al., 2008). Depressive symptoms are found associated with decrease in the 5-HT_{2A} receptor binding (Hasselbalch et al., 2008) and the serotonin transporter (Chen et al., 1996, Ouchi et al., 2009).

Target Tracer	**Sample size (HC/MCI/AD)**	**Change in MCI/AD**	**PV corr.**	**Reference**
SERT				
[^{11}C]DASB	10/0/15	↓striatum	No	(Ouchi et al., 2009)
	10/0/12	↓mesial temporal, prefrontal and occipital cortex	Yes	(Marner et al., 2010a)
5-HT_{1A}				
[^{18}F]MPPF	5/6/8	MCI +AD: ↓hippocampus ↓raphe nuclei	No	(Kepe et al., 2006)
	21/11/10	AD: ↓Hippocampus MCI: ↑ hippocampus	Yes	(Truchot et al., 2007, Truchot et al., 2008)
[^{11}C]WAY-100635	10/0/10	↓medial temporal cortex	Yes	(Lanctot et al., 2007)
5-HT_{2A}				
[^{18}F]setoperone	37/0/9	↓neocortex	No	(Blin et al., 1993)
[^{18}F]altanserin	10/0/9	↓anterior cingulate, prefrontal and sensorimotor cortex	Yes	(Meltzer et al., 1999)
	17/16/0	↓global	Yes	(Hasselbalch et al., 2008)
	13/0/10	↓global	Yes	(Marner et al., 2010a)

Table 2 Change of serotonergic markers in AD and mild cognitive impairment (MCI) measured with PET, PV corr.= partial volume correction

Involvement of the 5-HT_4 receptor in Alzheimer's disease pathology

In addition to the evidence of involvement of the 5-HT_4 receptor in cognitive function and Ach release (discussed previously), the 5-HT_4 receptor is also suggested to be connected to the

accumulation of Aβ. Stimulation of the 5-HT_4 receptor has been found to activate a non-amyloidiogenic pathway of the APP cleavage with secretion of sAPPα (Robert et al., 2001, Lezoualc'h and Robert, 2003, Cachard-Chastel et al., 2007). Further, 5-HT_4 agonism in cell cultures from Tg2576 transgenic mice has been shown to inhibit the extracellular Aβ concentration in a concentration-dependent manner and also increase neuronal survival (Cho and Hu, 2007).
A human postmortem study of 10 AD patients showed decreased 5-HT_4 receptor expression in hippocampus and frontal cortex area 11, but not in temporal cortex area 22 and frontal cortex area 4 (Reynolds et al., 1995). However, a later study in a larger sample revealed no changes in 5-HT_4 receptor affinity and density in AD in frontal and temporal cortex (Lai et al., 2003) indicating that the 5-HT_4 receptor may not have a direct role in the pathogenesis of the disease.

Diagnosing Alzheimer's disease and biomarkers

Diagnosing AD has been based on a two step process of dementia recognition followed by the specific disease. The criteria have been defined by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). The NINCDS-ADRDA criteria for **probable AD** require both memory disorder and impairment of another cognitive domain, and the symptoms are required to interfere with activities of daily living (ADL). The onset must be insidious, and other diseases accounting for dementia must be excluded. The definite diagnosis of AD is only made after a histopathological confirmation, and the criteria for probable AD have limited sensitivity and specificity (Varma et al., 1999, Waldemar et al., 2007).
Because there is a long asymptomatic stage between the earliest pathogenic events of AD and the first cognitive impairment and again a stage before criteria for dementia are fulfilled, a higher diagnostic accuracy at the preclinical stages is critical, both for the ability to earlier treatment of the disease and correct classification of groups in clinical trials. The term **mild cognitive impairment (MCI)** is used for subjects with cognitive complaints and with cognitive impairment on formal testing, but who are not demented and ADL functions are unaffected. These subjects have an increased risk for developing AD with an estimated convertion rate of 10-15% per year, but not all MCI patients progress to clinical AD (Petersen et al., 1999). To improve the definition of the subset of MCI patients at high risk for AD, a subtype termed **amnestic MCI** has been used when memory is the only affected cognitive domaine (Petersen et al., 2001).
In 2005 a new set of criteria for AD diagnosing was discussed by an expert panel (Dubois et al., 2007) suggesting that the term **probable AD** is used when both a biomarker is positive and insidious hippocampal memory impairment is present (that is impaired delayed recall without cued improvement). These criteria allow for an earlier diagnose of AD (by including a subset of the patients that fulfill the criteria for amnestic MCI) and with a higher accuracy than the NINCDS-ADRDA criteria, by enrolling only cognitive deficits that are specific for AD and include the use of new biomarkers for AD. The discovery of biomarkers improves the possibilities of the clinician to recognize AD from other types of dementia, and may improve the selection of study subjects in

clinical research and the monitoring of treatment effects. Important biomarkers helping for correct and earlier diagnose include: CSF markers (Hansson et al., 2006), the APOE-ε4 allele, hippocampal volume measured with MRI (Colliot et al., 2008), regional cerebral blood flow measured with [^{99m}Tc]HMPAO SPECT (Waldemar et al., 1994, Talbot et al., 1995), hypometabolism measured with [^{18}F]FDG PET (Mosconi, 2005, Mosconi et al., 2008) and Aβ accumulation measured with PET. A range of PET tracers for Aβ and tangles have been developed over the last decade. The best accuracy has so far been shown with [^{11}C]PIB (Nordberg, 2008).

The term **asymptomatic risk for AD (ASYMAD)** or **preclinical AD** has been suggested for healthy subjects with a positive biomarker of AD. Around 20% of elderly healthy subjects in their sixties actually have abnormal [^{11}C]PIB binding (Rowe et al., 2010), corresponding to the delayed prevalence of 25% of dementia at age 85 (Ferri et al., 2005), similar prevalence of healthy subjects with abnormal [^{11}C]PIB binding has consistently been found in other studies (Nordberg, 2008, Villemagne et al., 2008). Further, abnormal [^{11}C]PIB binding in healthy elderly subjects has been shown to associate with progression to AD (Morris et al., 2009) and the level of [^{11}C]PIB binding is correlated to episodic memory impairment (Pike et al., 2007). The prevalence of abnormal [^{11}C]PIB binding in healthy elderly subjects increases with aging (Rowe et al., 2010).

Aims and Hypothesis

Study A (data not included in the papers of this thesis):

Evaluate the most appropriate size of the PSF for the Muller-Gartner PV correction by comparing scans with [^{11}C]SB207145 at the GE-Advance PET scanner with the high resolution Siemens HRRT PET scanner in four healthy subjects.

Study 1 (paper 1):

Estimate the upper mass dose limit and affinity of [^{11}C]SB145207 by doing test-retest studies in seven healthy subjects with varying mass doses. A higher mass dose limit than the previously population-based estimate of 1.2 μg (occupancy < 5%) was hypothesized.
Estimate the in vivo concentration of free tracer in tissue required to saturate 50% of receptors, the dissociation constant K_D. A higher K_D than the previous population-based of 0.59nM in humans and the in vitro value of 0.39nM in the Göttingen minipig was hypothesized.

Study 2 (paper 2):

Investigate sex and age effects on regional 5-HT$_4$ receptor binding in striatum, limbic system and neocortex, in a cohort of 30 healthy subjects. A decrease with aging and lower binding in women compared to men was hypothesized.

Study 3 (paper 3):

Study the 5-HT$_4$ receptor binding in AD compared to healthy subjects and evaluate the 5-HT$_4$ receptor binding in relation to Aβ. Lower 5-HT$_4$ receptor binding in AD was hypothesized.

Methods

Subjects

Healthy subjects were recruited by public advertisements or extracted from the civil registration system in Denmark. All subjects received a full explanation of the study and gave a written informed consent for participation according to the declaration of Helsinki II. The studies were approved by The Copenhagen Region Ethics Committee. In total 32 healthy subject and 11 AD patients were included in this thesis. Many of the healthy subjects participated in more than one study (figure 13). All subjects were scanned in the period from 2006 to 2009.

Exclusion criteria (except for the AD patients in paper 3) were:

- Significant medical history
- Drug or alcohol abuse
- Neurological or psychiatric disorders (including antidepressant treatment)
- Mentally deficiency (ensured with DART45 which is a Danish version of the National Adult Reading Test (Nelson and O'Connell, 1978), cut-off score 11)
- Contraindications to MRI or PET
- Significant head injury

All healthy subjects had a normal neurological examination and unremarkable brain MRI scans. Absence of psychiatric symptoms was ensured using the Symptom Check List Revised (SCL-90-R) (Derogatis and LR, 1994) on the day of the PET scan.

Study A:

4 healthy subjects

- 2 men, 2 women
- Mean age 54, range 53-56 years

Study 1 (paper 1):

7 healthy subjects

- 5 men, 2 women,
- Mean age 40 years, range 20-57

Study 2 (paper 2):

30 healthy subjects

- 14 men, 16 women
- Mean age 44 years, range 20-86

Study 3 (paper 3):

12 healthy subjects

- 6 men, 6 women
- Mean age 67 years, range 55-86

11 AD patients

- 6 men, 5 women
- Mean age 71 years, range 55-85
- Mean MMSE 24, range 19-27
- Newly diagnosed with AD according to the NINCDS-ADRDA criteria (McKhann et al., 1984)
- AchEI naive (except in one subject treated for four weeks prior to the PET scans)

Exclusion criteria for AD patients were

- Significant medical history
- Drug or alcohol abuse
- Contraindications to MRI or PET
- Significant head injury
- Neurological or psychiatric disorders not related to the AD diagnosis

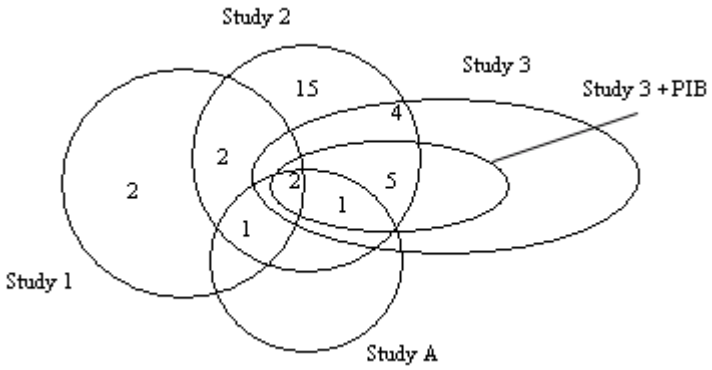

Figure 13 Overview the studies the 32 healthy subjects were included in.

Brain Imaging

The following section briefly describes and discusses the methods for image acquisition and data analysis that are used in the studies of this thesis. The methods are also described in more detail in the papers.

MRI (all studies)

Magnetic resonance imaging was conducted on a Siemens Magnetom Trio 3T MRI scanner (matrix 256x256; 1x1x1mm voxels). The T1 weighted MRIs were segmented into grey matter, white matter and CSF using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, UK). Segmented images were used for PV correction and made it possible to extract PET data from grey matter voxels only.

A set of regions were automatically delineated on each subject's MRI in a user-independent fashion using the Pvelab software package (Svarer et al., 2005).

PET Imaging of 5-HT$_4$ Receptors (all studies)

5-HT$_4$ receptor binding was measured with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA) starting with a bolus injection of [^{11}C]SB207145 given over 20 seconds. The scan was based on a 120 minute dynamic scan consisting of 38 time frames, increasing progressively in duration from 5 seconds to 10 minutes. After reconstruction, frames were aligned using AIR 5.2.5 (Woods et al., 1992) to correct for movements during the scan. The [^{11}C]SB207145 PET scans were automatically co-registered to the MRI with the AIR algorithm (Woods et al., 1992) using only the mean of the first 20 min of the PET scan corresponding to a flow-weighted image. The quality of each co-registration was evaluated by visual inspection in three planes. Regional time activity curves (TACs) were constructed from grey matter voxels both with and without the Muller-Gartner PV correction. Kinetic modeling of BP_{ND} was performed with the simplified reference tissue model (SRTM) using cerebellum as reference region. The kinetic modeling was performed using the PMOD software version 2.9, build 2 (PMOD Technologies). Different brain regions were included in the studies.

Immediately before tracer injection, venous blood samples were drawn and used to measure the plasma free fraction, f_P, with equilibrium dialysis (Kornum et al., 2009). Venous blood samples were also drawn 32 min and 55 min after injection, and the fraction of parent [^{11}C]SB207145 and its radiolabeled metabolites in plasma were measured with HPLC (Gillings, 2009). Blood analyses were analysed to control for bias in f_P or metabolism rate.

Test-retest studies of 5-HT$_4$ receptor binding with different PET scanners (study A)

In addition to the GE-Advance PET scan, four subjects were also scanned in the high resolution Siemens HRRT PET scanner with an identical set-up. The PV effect is directly related to the spatial resolution of the scan and is therefore less pronounced in the HRRT scanner which has a resolution down to 2 mm (Olesen et al., 2009) compared to the GE-Advance scanner's average resolution of 6 mm. Further, HRRT images were reconstructed with 3D-OSEM-PSF (Sureau et al., 2008) which reduces the partial volume effect and improves resolution by including the PSF in the reconstruction (Varrone et al., 2009). The BP_{ND} from the five regions were compared using the HRRT scan as a gold standard.

Test-retest studies of 5-HT$_4$ receptor binding with varying mass dose (study 1)

Test-retest studies were conducted in seven subjects in random order: one scan was performed with a high specific radioactivity (SA) and low mass dose (D_{low}) of unlabeled ligand, and one scan with low SA and high mass dose (D_{high}). In three subjects the mass dose difference was > 10 μg, in two subjects 3-5 μg and in two subjects 1-2 μg.

PET Imaging of Aβ (study 3)

All AD patients and eight elderly healthy subjects additionally underwent a [^{11}C]PIB PET scan to measure the Aβ burden. Subjects were given a bolus injection with a mean activity of 565 MBq (range 301-601 MBq) [^{11}C]PIB given over 20 seconds. The scan was performed with a high resolution Siemens HRRT PET scanner. A static scan at 40-70 minutes after injection was acquired. To minimize movement during the scan, the head was fixated using a moldable vacuum pillow. The images were reconstructed with 3D-OSEM-PSF (Sureau et al., 2008). The [^{11}C]PIB PET scan was co-registered to the MRI and the quality of each co-registration was evaluated by visual inspection in three planes.

The regional PET standardized uptake value (SUV) was measured by summing the data acquired from regional grey matter voxels. Data was normalized to cerebellum SUV, resulting in a region to cerebellar ratio (SUVR) (Lopresti et al., 2005, Pike et al., 2007). The time window 40-70 min post injection provides stability and an effective contrast between healthy subjects and AD patients (McNamee et al., 2009). The Aβ burden was expressed as a volume-weighted mean of SUVR of the following cortical regions that have high [^{11}C]PIB binding: posterior cingulate gyrus / precuneus, lateral prefrontal cortex (dorsolateral and ventrolateral part), parietal cortex and the lateral temporal cortex. A SUVR cut-of value at 1.5 to 1.6 has been generated in larger samples to categorize subjects as PIB-positive or PIB-negative (Pike et al., 2007, Jack et al., 2008) and a cut-off value of 1.6 was used in the study.

Data Analysis and Statistics

Detailed information about the employed models is included in the papers. Generally, possible biases in body mass index (BMI), injected mass, tracer metabolization rate and f_P were tested for in the cohort included in the studies.

Study A

Data analysis with Muller-Gartner PV correction was done with PSF in the range from 4 mm to 12 mm. Regional BP_{ND} weas related to the corresponding individual BP_{ND} measured at the HRRT scanner without PV correction. The deviation from line of identity ± SD was calculated, and the PSF with least deviation was used in studies 2 and 3. Five regions of interest were included in this analysis: Striatum, hippocampus, amygdala, cingulate gyrus and the neocortex.

Study 1

Under the assumption that full occupancy of receptor sites would be approached at high mass dose, the receptor occupancy is (O) determined by the mass dose (D) and the mass dose of ligand that saturates 50% of receptors (ID_{50})

$$O = \frac{D}{D + ID_{50}} \qquad (5)$$

Under the further assumption that the underestimated *BP* by mass dose (BP_{Occupied}) is given by the non-occupied *BP* (BP_{Baseline}) and *O* by the equation

$$BP_{\text{Occupied}} = (1 - O)BP_{\text{Baseline}} \qquad (6)$$

then the ID_{50} can be estimated from the test-retest studies with varying mass doses, by individual occupancy plots of 19 regions: The individual ID_{50} can then be calculated by using the slope (α) of the regression line in the occupancy plot, where α<1 represents the negative effect of higher mass dose on BP_{ND}

$$\alpha = \frac{1 - O_{\text{High}}}{1 - O_{\text{Low}}} = \frac{1 - \dfrac{D_{\text{High}}}{D_{\text{High}} + ID_{50}}}{1 - \dfrac{D_{\text{Low}}}{D_{\text{Low}} + ID_{50}}} = \frac{D_{\text{Low}} + ID_{50}}{D_{\text{High}} + ID_{50}} \quad \Leftrightarrow \quad ID_{50} = \frac{\alpha D_{\text{High}} - D_{\text{Low}}}{1 - \alpha} \qquad (7)$$

The ID_{50} was calculated for each of the seven subjects. The corresponding upper mass dose limits, D_5 (<5% ocuupancy) and D_{10} (<10% occupancy), are then calculated by rearranging eq. 5. to

$$D = \frac{O(ID_{50})}{1 - O} \qquad (8)$$

The in vivo concentration of free ligand in tissue required to saturate 50% of receptors, the dissociation constant K_{D}, was estimated individually using Scatchard plots: The slope equals $-1/K_{\text{D}}$ when regional *BP* is plotted against the concentration of receptor-bound ligand in the tissue of the same region.

Study 2

A linear ANCOVA was employed to model the effect of age, sex and their interaction on 5-HT_4 receptor binding for each of the three volume-weighted brain regions of interest: striatum, limbic system and neocortex. Regional PV corrected 5-HT_4 receptor binding was the primary dependent variable. To evaluate the effect of PV correction, analyses were also done without PV corrected binding measures.

Study 3

To correct for atrophy in AD, only PV corrected data were used for analyses of BP_{ND} of 5-HT_4 receptor binding. An AD-region was constructed as a volume-weighted average of five bilateral regions of interest for AD including: parietal cortex, lateral prefrontal cortex (including dorsolateral and ventrolateral prefrontal cortex), lateral temporal cortex, posterior cingulate gyrus and hippocampus.

The difference between groups in 5-HT_4 receptor binding in the AD-region was investigated with a Welch t-test; this was done both regarding clinical status (AD patients vs. HC) and PIB binding (PIB-positive SUVR>1.6 vs. PIB-negative SUVR<1.6,). The association between PIB binding and

5-HT_4 receptor binding was further investigated with a linear regression analysis using [^{11}C]PIB binding as a quantitative variable. The dependent variable was the volume-weighted 5-HT_4 receptor binding. The clinical degree of dementia, measured with MMSE, was investigated for correlation to 5-HT_4 receptor binding in the AD-region in a linear regression analysis.

It has previously been demonstrated for the 5–HT_{2A} receptor that there is a strong correlation between regions of 5–HT_{2A} receptor binding, indicating that a general level, rather than the regional levels, explains the variance between subjects (Erritzoe et al., 2010). This is also in agreement with PET studies of Aβ levels where one cortical level is the general accepted measurement. Therefore, as a supplement to the traditional statistical analysis a structural equation model was applied to analyze the association between the latent 5–HT_4 and PIB levels (Bollen, 1989). In this model we perceive the regional variables as indirect observations of an underlying/latent global 5–HT_4 and PIB level, ($\eta_{5\text{–HT4}}$ and η_{PIB}, respectively). To allow estimation of the linear association of the two latent variables, we set up a joint model where the correlation between latent variables $\eta_{5\text{–HT4}}$ and η_{PIB} were estimated. Thus, this model suggests a regional intercorrelation of both PIB load between regions of interest and between 5–HT_4 receptor binding in regions relevant for AD.

Results and Discussion

This section gives and discusses the main results of each aim in the studies, details are further described and discussed in the papers.

Study A

Using the raw data from the GE-Advance scan lead to an underestimation of the BP_{ND} of the four subjects' five brain regions of mean 24%±14% (mean±SD) compared to the HRRT data. This was expected as the PV effect is directly related to the spatial resolution of the scanner and is therefore less pronounced in the HRRT. When applying a Muller-Gartner PV correction with a point spread function of 6 mm, a mean difference in BP_{ND} of 0%±19% was generated (figure 14). Thus, when using a PSF <6mm data are not corrected enough and using a PSF > 6mm data are overcorrected. The latter may be the case in previous studies where the observed decrease in AD patients compared to healthy volunteers may be underestimated, as an 8 mm PSF was used when applying a Muller-Gartner PV correction (Hasselbalch et al., 2008, Marner et al., 2010a).

There were, however, regional differences and most importantly PV corrected BP_{ND} in the neocortex was generally higher than the HRRT data. In contrast, limbic and striatal regions showed no general pattern of neither under- or overestimation.

A more detailed comparison between HRRT scanner and the lower-resolution HR system with [^{11}C]MADAM has been presented (Nilsson et al., 2010). In correspondence with our study, they found that BP_{ND} without PV correction was approximately 20% lower compared to the HRRT. They found that applying the Meltzer method to the HR data provided the best agreement with the HRRT, while the Muller–Gartner method tended to overestimate both neocortical and limbic regions, but not subcortical regions. However, PV correction with the Muller–Gartner method depends heavily on the MRI segmentation, and the MRIs were 1.5 T and thereby of a lower quality than in our study, which make direct comparison of the studies difficult.

Another study comparing scans from the GE-Advance scanner with the HRRT with [^{18}F] Altanserin has shown that filtering the HRRT image with a 6mm (FWHM) filter matches the images (Svarer et al., 2010), indicating that using a PSF of 6 mm in the PV correction is appropriate.

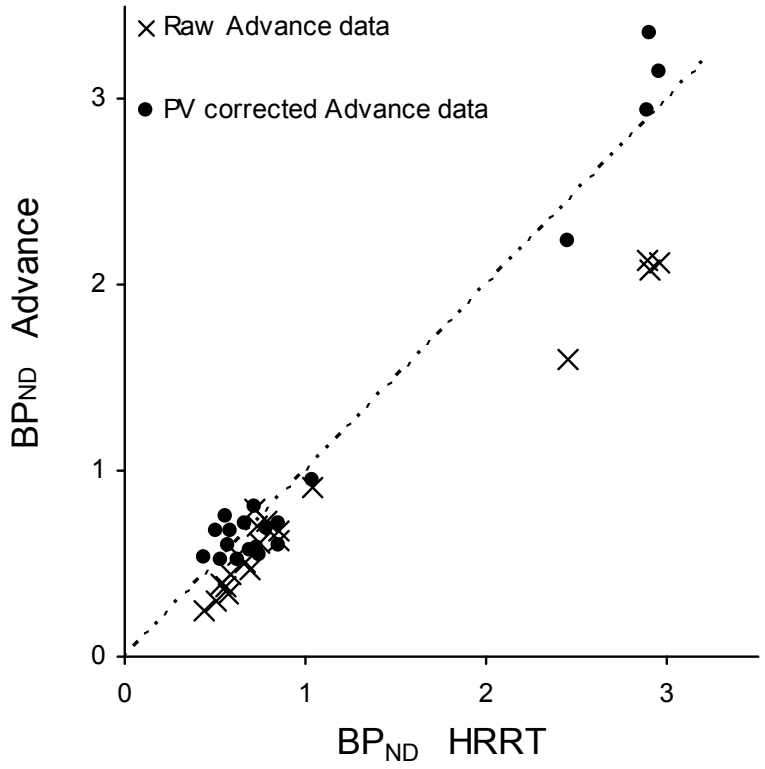

Figure 14 Plot illustrating the general underestimation of regional BP_{ND} measured at the GE-Advance scanner without PV correction compared to regional BP_{ND} measured at the HRRT scanner. After Muller-Gartner PV correction with a PSF = 6mm, the regional BP_{ND} are closer to the line of identity (dotted line).

Study 1 – Effect of mass dose

Increasing the mass dose reduced BP_{ND} in all test-retest studies (example in figure 15) whilst the relative cerebellar uptake was unchanged, thus supporting the use of cerebellum as a reference region in [^{11}C]SB207145 studies. Based on the individually measured mass dose effects (figure 16), an average ID_{50} of 85.4 μg ± 30.2 μg was found. Correspondingly (from eq. 8), if a receptor occupancy below 5% is intended an upper mass dose limit of 4.5 μg of [^{11}C]SB207145 per 70 kg body weight can be injected; if a receptor occupancy below 10% is accepted an upper mass dose limit of 9.5 μg for occupancies was found. The specific radioactivity required for this to be achieved is relatively easily obtained (Marner et al., 2009).

Inter-individual differences in blood-brain barrier uptake, systemic distribution volume, tracer metabolism, receptor affinity and plasma protein binding are all parameters that can influence the mass dose effects. Nevertheless, the interindividual variations in ID_{50} and mass dose limit determinations were relatively moderate. As hypothesized, the upper limit estimated from test-retest studies with high and low specific radioactivity was higher than the previous population-based estimate of the upper limit of mass dose (occupancy < 5%) of 1.2 μg (Marner et al., 2010b), which is biased by inter-individual variations in BP_{ND} due to confounders including sex and age effects.

The study provides a simple method for investigating the effects of mass dose in brain PET receptor studies. The method has not to our knowledge been presented previously, it does not require a true baseline scan, and only relatively low occupancies with a limited range are needed. Occupancy effects of mass dose should be assessed for all radiotracers, particularly agonists, in order to avoid pharmacological side effects and for the safety of patients and healthy volunteers. Bias from mass

dose should also be considered in studies with expected small group differences and in test-retest studies, where carryover effects of mass dose can be a problem, particularly if the affinity is high, and the time interval between scans is short (Ashworth et al., 2010). Mass dose effects are highly relevant in small rodents (Kung and Kung, 2005), but may also play a role in human PET studies where variations in bodyweight are expected, for example in studies of sex differences or diseases associated with altered bodyweight.

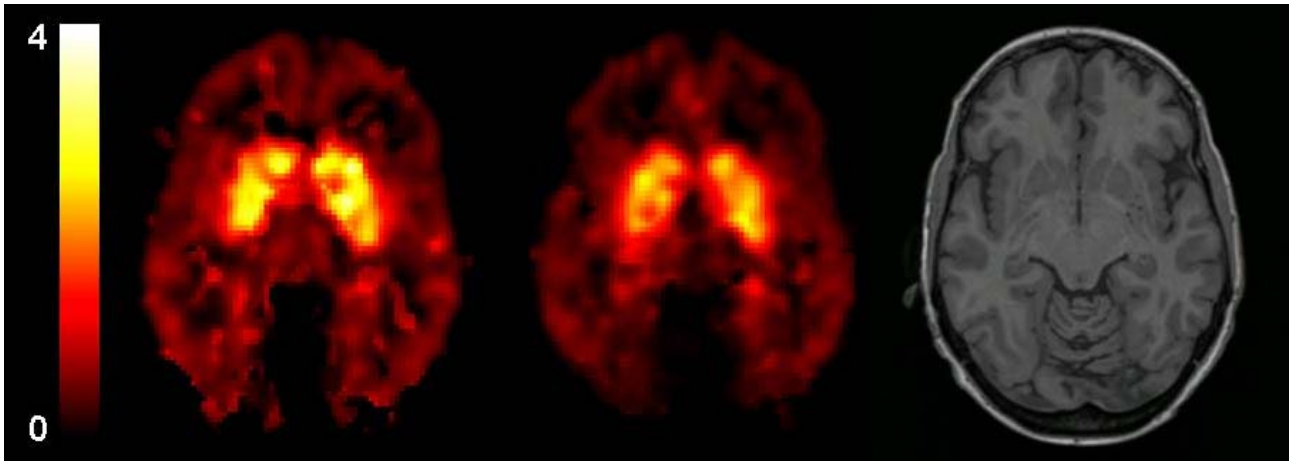

Figure 15 Illustration of the reduction of 15% in BP_{ND} by increasing the mass dose in one subject. Parametric image of BP_{ND} after injection of 4.9 µg ligand (left), 21.9 µg ligand (middle), and the corresponding MR image (right).

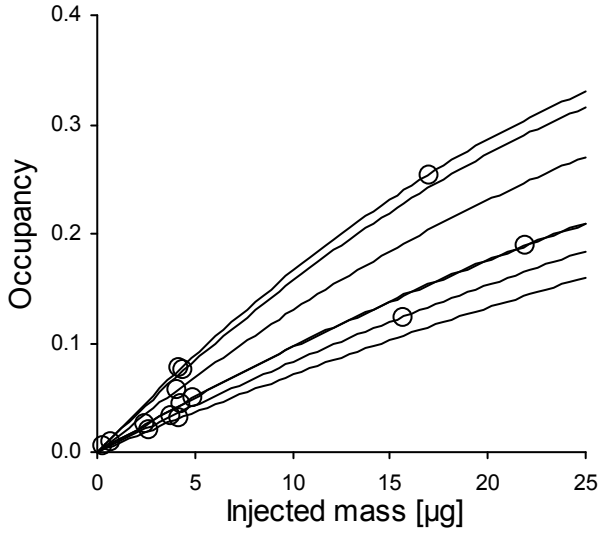

Figure 16 The lines describe (for each individual) the association between mass dose and occupancy defined by the ID_{50}. The scans are represented by two data points at the line for each individual. For comparison purposes, the mass doses are normalized to 70 kg bodyweight.

Study 1 – Affinity

K_D was estimated to an average value of 2.8 nM (range 1.0-4.8) in striatum and neocortex, despite the major effects on the slope of the Scatchard plot of small inaccuracies in the measurements of BP_{ND} (figure 17). No difference in the in vivo K_D in high versus low 5-HT_4 receptor density regions and the relatively low variation is in agreement with the general assumption that inter-individual variations in binding potential primarily reflect differences in the receptor density, whilst K_D,

determined by the structure of the receptor protein and the concentration of the neurotransmitter in question, is expected to be relatively constant between individuals and across brain regions. The estimated in vivo K_D is around sevenfold higher than the in vitro K_D found in the Göttingen minipig (Kornum et al., 2009) and also higher than the population-based estimate (Marner et al., 2010b) as hypothesized. An around tenfold higher K_D in vivo than in vitro is often found for tracers (Farde et al., 1995, Logan et al., 1997, Olsson et al., 2004) probably because in vivo only a fraction of the tracer is free.

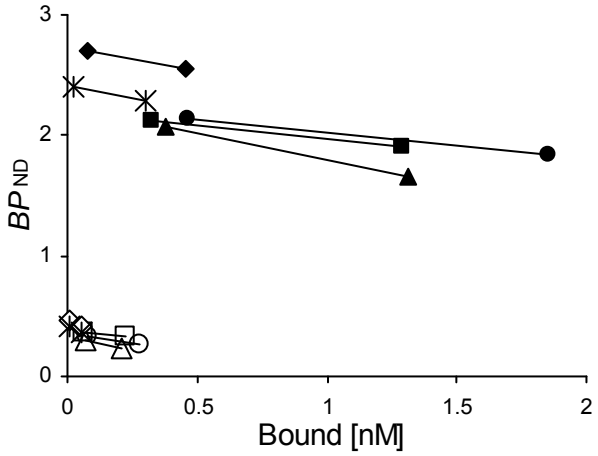

Figure 17 Scatchard plots of striatum (filled markers) and neocortex (open markers) from test-retest scans with varying mass dose in 5 subjects. The slope (α) equals -1 / K_D.

Study 2 – Effects of sex on 5-HT$_4$ receptor binding

Results of the linear ANCOVA are shown in table 3. Thirteen % lower 5-HT$_4$ receptor binding was found in women compared to men in the limbic system (figure 18) and similar findings were obtained without PV correction of data (11% difference). This 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 sub-regions showed that the difference was most pronounced with 19% in the amygdala (p=0.0056 without PV correction and p=0.012 with, uncorrected).

A borderline tendency of a 6% reduction in striatum of 5-HT$_4$ receptor binding in women compared to men was found both with and without PV correction of data. 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 for neither striatum nor neocortex. Post hoc analyses of neocortical sub-regions showed significant reductions of 9-13% 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). Thus, the hypothesis of lower 5-HT$_4$ receptor binding in women was confirmed for limbic regions, corroborating our previous finding in hippocampus in a smaller sample (Marner et al., 2010b).

The gender difference is highly interesting since the limbic system historically has been linked 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

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

Table 3 Linear ANCOVA analyses with regional 5-HT$_4$ receptor binding as dependent variable and age and sex as explanatory variables. Analyses are done for each region one by one, and sex differences are analyzed with men as reference.

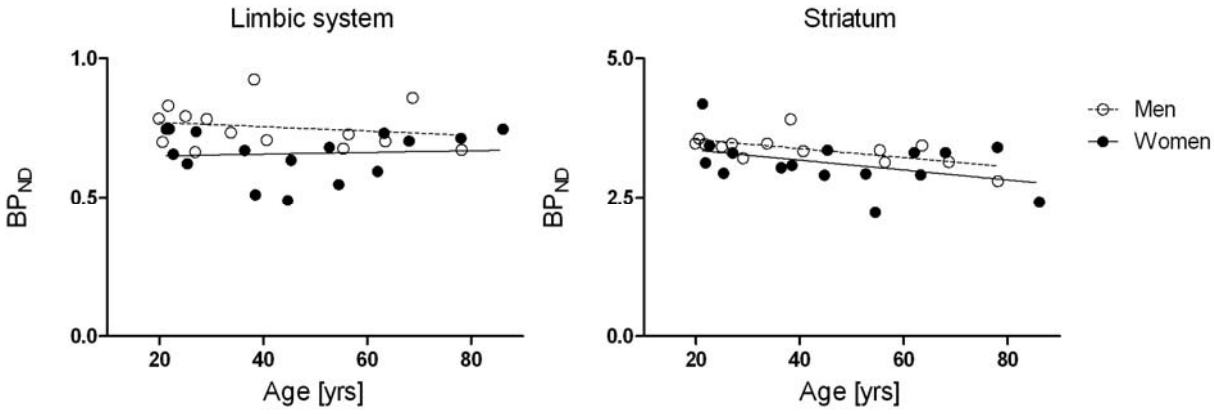

Figure 18 The association between regional PV corrected 5-HT$_4$ receptor binding for men and women separately; no interaction between sex and age was seen. The limbic 5-HT$_4$ receptor binding was by average 13% lower in women than in men. There was a decline with age in striatal 5-HT$_4$ receptor binding of 1% per decade.

emotions (Ehrlich et al., 2009) and was also found in the subregions of the neocortex that often are referred to as paralimbic: the orbitofrontal cortex, insula and superior temporal gyrus.

Thus, the observation support a role for 5-HT$_4$ receptors in the sex specific differences of emotional control, and the lower 5-HT$_4$ receptor binding may contribute to the observed higher prevalence of affective diseases and AD in women, since 5-HT$_4$ receptor binding agonism has a beneficial effects on depressive symptoms and AD pathology (Cho and Hu, 2007, Lucas et al., 2007), as discussed in the background section. The PV correction gave rise to an increased sex difference in 5-HT$_4$

receptor binding, as the PV effect caused a larger underestimation of BP_{ND} in men. This is expected due to the structural differences between genders that were summarized in the background section.
PET studies show as summarized in table 1, 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.

Study 2 – Effects of age on 5-HT_4 receptor binding

Results of the linear ANCOVA are shown in table 3. When correcting for the PV effect, and thereby for atrophy increasing with age, a decline of 5-HT_4 receptor binding with age was found in striatum only (figure 18) corresponding to a decline of 1% per decade; this finding was also significant after Bonferroni correction (p=0.04). Without correcting for the PV effect, a significant decline with age was found in all three regions corresponding to declines of 3-5% per decade, which is n agreement with a previous study (Marner et al., 2010b). Declines per decade are calculated as the change from 40 to 50 years.
When controlling for possible confounders an increase in cerebellar concentration of ligand was found with aging. This 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 non-displaceable binding with aging. All the same, and despite our hypothesis, 5-HT_4 receptor binding is relatively stable with aging compared to other in subtypes of receptors (overview of serotonin markers in table 1), of which primarily the 5-HT_{2A} receptors have been shown to decline with aging (figure 19). 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. One could speculate that the preserved level of the 5-HT_4 receptors is a response to decreased levels of interstitial 5-HT levels with aging or actually an attempt to maintain cognitive function.

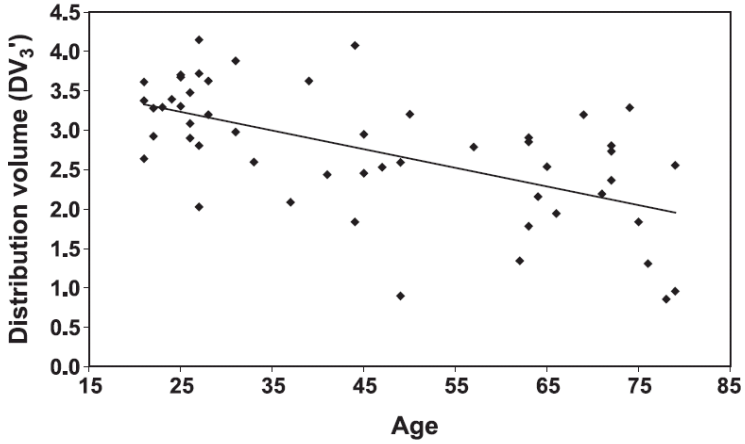

Fig. 4. The correlation between age and the averaged DV′3 for all cortical ROIs (reference region: cerebellum). The regression coefficient corresponds to an average decrease of 5-HT_{2A} receptors of about 6% per decade.

Figure 19 5-HT_{2A} receptors have shown pronounced decline with aging (Adams et al., 2004)

Study 3

When comparing groups defined by their clinical status, there was no difference in 5-HT_4 receptor binding between HC and AD patients (p-value=0.54). However, two HC were PIB-positive and therefore probably at a preclinical stage of AD, in correspondence with previous findings of abnormal [^{11}C]PIB binding in around 20% of elderly healthy individuals in their sixties (Rowe et al., 2010). Further, one AD patient was PIB-negative (figure 20). Though our patients were diagnosed according to NINCDS-ADRDA criteria, these criteria are clinical and show relatively low specificity (Varma et al., 1999). The importance of correct disease classification cannot be overemphasized in this context, and especially in studies of small samples misclassification may contribute to discrepant results in studies of AD. With the advent of specific biomarkers for AD such as [^{11}C]PIB, it is possible to increase both sensitivity and specificity of diagnosis and thus limit the needed sample size in future studies.

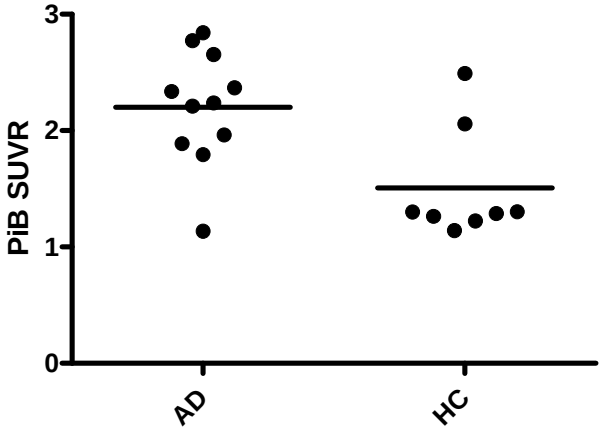

Figure 20 [^{11}C]PIB binding in AD patients and healthy controls. Two healthy controls were categorized as PIB-positive and one subject clinically diagnosed with AD was PIB-negative (cut-of value 1.6).

There was 13% higher 5-HT_4 receptor binding in the PIB-positive group compared to the PIB-negative (p-value=0.02). In addition, we found a positive correlation between PIB binding and 5-HT_4 receptor binding (p-value=0.03, estimate=0.09 ± 0.04, R^2=0.27) (figure 21). The structural equation model fitted and showed also a positive correlation between the latent variables $\eta_{5\text{–}HT4}$ and η_{PIB} was found (p-value=0.02, estimate=0.55, 95% confidence limits (0.23; 0.76)), thereby confirming the result of the linear regression analysis.

These results contrast findings of other serotonergic markers, which primarily are reported to be decreased in AD (Meltzer et al., 1998, Nagren et al., 2010). An increase is also against the hypothesis. There are several possible explanations based on experimental studies for our observation of an up-regulation of cerebral 5-HT_4 receptor binding in PIB-positive subjects: Firstly, it may be a counteractive response may be a selective response to compensate for the decreased levels of interstitial 5-HT levels found in AD (Ge and Barnes, 1996), because subchronic 5-HT depletion has been found to increase 5-HT_4 receptor binding in the dorsal hippocampus (Licht et al., 2009). Alternatively, an up-regulation of 5-HT_4 receptors may be an attempt to increase Ach

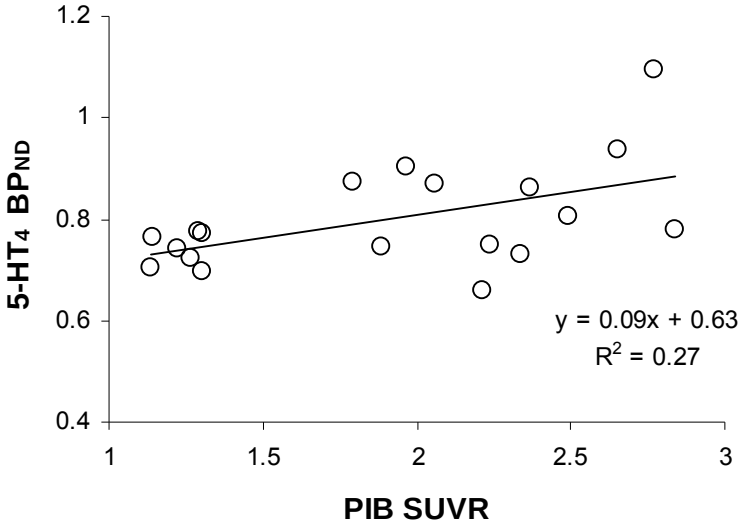

Figure 21 Correlation between amyloid load and the volume-weighted average 5-HT_4 receptor binding (representing parietal cortex, lateral prefrontal cortex, lateral temporal cortex, posterior cingulate gyrus and hippocampus). A statistically significant positive correlation was found (p =0.03).

release (Consolo et al., 1994, Matsumoto et al., 2001), improve cognitive function (King et al., 2008) or counteract Aβ accumulation (Cho and Hu, 2007). Future in vivo studies should clarify which of these mechanisms are relevant in humans. This study implies that at least the latter suggestion is plausible: A positive correlation between Aβ accumulation and 5-HT_4 receptor binding was found, and the between group differences were only found when including the two PIB-positive HC (possible *preclinical AD*) together with the AD group. This could imply that the up-regulation is initiated at a preclinical stage and follows the increase in Aβ accumulation, which is known to start decades before clinical symptoms occur in AD. Interestingly, a post hoc investigation of the 5 brain regions that were included in the volume-weighted outcome showed that the largest up-regulation in 5-HT_4 receptor binding (28%) was found in hippocampus (p=0.02) where no local increase in [^{11}C]PIB binding was seen. In line, no significant up-regulation was found in posterior cingulate gyrus (p=0.14) and lateral prefrontal cortex (p=0.23) where the highest local [^{11}C]PIB binding was found (figure 22). This could be interpreted as the up-regulation of 5-HT_4 receptors successfully counteracted Aβ accumulation *locally*, despite the *general positive* correlation between [^{11}C]PIB and [^{11}C]SB207145 binding. Although this explanation is rather speculative, it is, however, in line with the finding in cell cultures from Tg2576 transgenic mice where 5-HT_4 agonism inhibits the extracellular Aβ concentration (Cho and Hu, 2007).

There was a negative correlation between 5-HT_4 receptor binding and MMSE score in the patients diagnosed with AD (p=0.02, estimate=-0.03 ± 0.01, R^2 = 0.50) indicating that 5-HT_4 receptors are progressively up-regulated with increasing cognitive dysfunction, at least while dementia is still at a mild stage. This suggests that the up-regulation may not only be related to Aβ accumulation, as follow-up studies have shown limited further increase in Aβ accumulation in the course of AD (Engler et al., 2006, Jack et al., 2009). However, different regulatory patterns may emerge at a later stage of AD, and postmortem studies indicate that the 5-HT_4 receptor levels may be unchanged (Lai

et al., 2003) or even down-regulated in late stages of AD (Reynolds et al., 1995) and neither a correlation to cognitive or behavioral data was found (Lai et al., 2003) - maybe because compensatory mechanisms collapse when neurodegeneration becomes more severe.

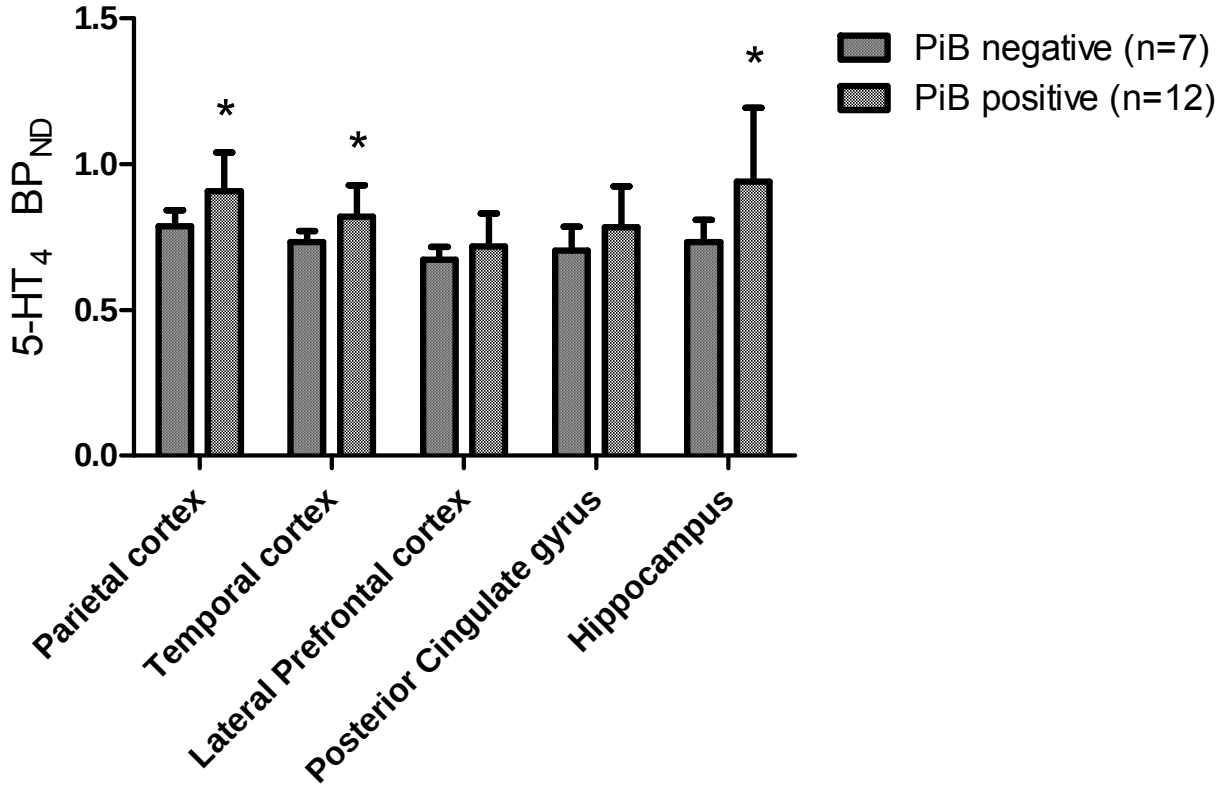

Figure 22 Regional 5-HT_4 receptor binding according to PIB-status. Statistically significant larger 5-HT_4 receptor binding was seen in PIB-positive individuals in parietal cortex (p=0.01), hippocampus (p=0.02) and temporal cortex (p=0.02), but not in posterior cingulate gyrus (p=0.14) and lateral prefrontal cortex (p=0.23). T-tests are not corrected for multiple comparisons.

Conclusion and Perspectives

This thesis investigates cerebral 5-HT_4 receptor in vivo binding with PET using [^{11}C]SB207145.

From tests-retest studies we determined the upper mass dose limit for 5% occupancy, and found that sufficient specific activity is relatively easily obtained. The method for evaluating mass dose effects has to our knowledge not been demonstrated before, and could be implemented in the validation of other tracers. The result differs from that in of a population-based estimate, where women were given higher doses than men, therefore it was not possible to differentiate whether lower 5-HT_4 receptor binding in women was accurate or caused by higher occupancies by the mass dose.

We showed that the Muller-Gartner PV correction lead to more accurate estimates of BP_{ND} in striatal and limbic regions, while cortical regions tend to be overcorrected with a PSF of 6mm. PV correction increases the noise in the data, but is necessary in studies comparing groups with structural differences such as gender differences and especially atrophy. For more accurate PV correction in cortical regions, future studies could focus on evaluating the Meltzer or Rousset PV correction methods using 3T MRI. However, development of the HRRT scanner has diminished the problem of PV effects and is recommended for studies involving differences in brain structure.

Gender differences in a cohort of 30 healthy subjects showed significantly lower 5-HT_4 receptor binding in women compared to men in the limbic system, especially in amygdala. The difference was significant both with and without PV correction and after Bonferroni correction for multiple comparisons. The lower 5-HT_4 receptor binding may contribute to the observed higher prevalence of affective diseases and AD in women, since experimental studies have indicated that 5-HT_4 receptor stimulation ameliorates depressive symptoms and AD pathology, but this remains to be confirmed. The gender difference in 5-HT_4 receptor binding may also contribute the known differences between genders in memory and learning.

PV correcting for cerebral atrophy had the anticipated effect on age related 5-HT_4 receptor binding: the decrease with aging was diminished. It was surprising that 5-HT_4 receptor binding is relatively stable with aging compared to other subtypes of receptors, and a decline of 1% per decade in 5-HT_4 receptor binding with age was found in striatum only. Experimental studies have suggested that 5-HT_4 receptors are involved in memory and learning, however, our study does not seem to support that 5-HT_4 receptors are directly associated with the cognitive decline in normal aging.

In study 3 we found that an up-regulation of 5-HT_4 receptor binding was associated to Aβ accumulation, but not to clinical status, and the study demonstrates the importance of correct classification of groups and the advance of [^{11}C]PIB as a biomarker. The up-regulation is highest in hippocampus where the PV correction is valid. Our data suggests that the cerebral 5-HT_4 receptor up-regulation starts at a preclinical stage and continues, at least as long as dementia is still at a mild stage. This contrasts most other receptor subtypes that seem to be down-regulated in AD, however, a compensatory up-regulation of the 5-HT_{1A} has also been suggested at the stage of MCI for then to decrease at the stage of dementia. Locally, the up-regulation of 5-HT_4 receptors may counteract Aβ

accumulation even though there is a positive correlation between the global Aβ accumulation and general 5-HT_4 receptor binding in regions involved in AD. The results suggest a preserved 5-HT_4 receptor pool for possible pharmacological treatment of AD patients with 5-HT4 receptor agonists. The 5-HT_4 receptor agonist PRX03140 has in 2005 been tested in a randomized placebo-controlled phase 1b study in 12 patients with mild to moderate AD. Side effects were not severe. An increase in reaction time was shown in cognitive testing, but it was not significant compared to the placebo group. A phase 2 study was terminated in 2009 because the pharmaceutical company went bankrupt. (www.alzforum.org/dis/tre/drc/detail.asp?id=113). However, such studies could indeed be of interest as the 5-HT_4 receptor is a possible target in treatment of both affective diseases and AD that cause personal, social and economical burden world wide. [^{11}C]SB207145 and [^{11}C]PIB PET scans could be important biomarkers in relation to possible future clinical trials evaluating 5-HT_4 receptor agonists.

Further studies of 5-HT_4 receptors with [^{11}C]SB207145 in PET in patients with AD and in relation to affective diseases are highly interesting: The level of 5-HT_4 receptors in relation to AD development is relevant and studies both at the stage of MCI and later stages of dementia in AD are of interest. Also the relation to cognitive function and neuropsychiatric symptoms in AD is highly relevant. Studies with [^{11}C]SB207145 in PET of patients suffering from affective diseases are relevant due to the antidepressant effect of 5-HT_4 agonism and the possible relation to the endogenous serotonin level demonstrated in experimental studies, alternatively studies of healthy subjects in relation to neuroticism or subjects predisposed to depression could be performed. Also the involvement of 5-HT_4 receptors in relation to cognitive function in healthy subjects should be investigated.

Finally, it is highly interesting to investigate whether the 5-HT_4 receptors measured with [^{11}C]SB207145 in PET is a marker of the endogenous level of cerebral serotonin in humans, as has been suggested in experimental studies. If so, this would make brain PET imaging with [^{11}C]SB207145 relevant in relation to many functions related to the cerebral serotonin system.

References

Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, Madsen K, Frokjaer V, Martiny L, Paulson OB, Knudsen GM (A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. Neuroimage 21:1105-1113.2004).

Aletrino MA, Vogels OJ, Van Domburg PH, Ten Donkelaar HJ (Cell loss in the nucleus raphes dorsalis in Alzheimer's disease. Neurobiol Aging 13:461-468.1992).

Allen JS, Damasio H, Grabowski TJ, Bruss J, Zhang W (Sexual dimorphism and asymmetries in the gray-white composition of the human cerebrum. Neuroimage 18:880-894.2003).

Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, de Girolamo G, Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lepine JP, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martinez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini P, Palacin C, Romera B, Taub N, Vollebergh WA (Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr Scand Suppl 21-27.2004).

Ashworth S, Rabiner EA, Gunn RN, Plisson C, Wilson AA, Comley RA, Lai RY, Gee AD, Laruelle M, Cunningham VJ (Evaluation of 11C-GSK189254 as a novel radioligand for the H3 receptor in humans using PET. J Nucl Med 51:1021-1029.2010).

Baron JC, Chetelat G, Desgranges B, Perchey G, Landeau B, de la Sayette V, Eustache F (In vivo mapping of gray matter loss with voxel-based morphometry in mild Alzheimer's disease. Neuroimage 14:298-309.2001).

Biver F, Lotstra F, Monclus M, Wikler D, Damhaut P, Mendlewicz J, Goldman S (Sex difference in 5HT2 receptor in the living human brain. Neurosci Lett 204:25-28.1996).

Blin J, Baron JC, Dubois B, Crouzel C, Fiorelli M, Attar-Levy D, Pillon B, Fournier D, Vidailhet M, Agid Y (Loss of brain 5-HT2 receptors in Alzheimer's disease. In vivo assessment with positron emission tomography and [18F]setoperone. Brain 116 (Pt 3):497-510.1993).

Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti B, Davis JG, Constantine G, Mathis CA, Moore RY, DeKosky ST (Cognitive correlates of alterations in acetylcholinesterase in Alzheimer's disease. Neurosci Lett 380:127-132.2005).

Bonaventure P, Hall H, Gommeren W, Cras P, Langlois X, Jurzak M, Leysen JE (Mapping of serotonin 5-HT(4) receptor mRNA and ligand binding sites in the post-mortem human brain. Synapse 36:35-46.2000).

Braak H, Braak E (Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259.1991).

Cachard-Chastel M, Devers S, Sicsic S, Langlois M, Lezoualc'h F, Gardier AM, Belzung C (Prucalopride and donepezil act synergistically to reverse scopolamine-induced memory deficit in C57Bl/6j mice. Behav Brain Res 187:455-461.2008).

Cachard-Chastel M, Lezoualc'h F, Dewachter I, Delomenie C, Croes S, Devijver H, Langlois M, Van Leuven F, Sicsic S, Gardier AM (5-HT4 receptor agonists increase sAPPalpha levels in the cortex and hippocampus of male C57BL/6j mice. Br J Pharmacol 150:883-892.2007).

Cai X, Flores-Hernandez J, Feng J, Yan Z (Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. J Physiol 540:743-759.2002).

Carne RP, Vogrin S, Litewka L, Cook MJ (Cerebral cortex: an MRI-based study of volume and variance with age and sex. J Clin Neurosci 13:60-72.2006).

Chen CP, Alder JT, Bowen DM, Esiri MM, McDonald B, Hope T, Jobst KA, Francis PT (Presynaptic serotonergic markers in community-acquired cases of Alzheimer's disease: correlations with depression and neuroleptic medication. J Neurochem 66:1592-1598.1996).

Cho S, Hu Y (Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. Exp Neurol 203:274-278.2007).

Cidis Meltzer C, Drevets WC, Price JC, Mathis CA, Lopresti B, Greer PJ, Villemagne VL, Holt D, Mason NS, Houck PR, Reynolds CF, 3rd, DeKosky ST (Gender-specific aging effects on the serotonin 1A receptor. Brain Res 895:9-17.2001).

Colliot O, Chetelat G, Chupin M, Desgranges B, Magnin B, Benali H, Dubois B, Garnero L, Eustache F, Lehericy S (Discrimination between Alzheimer disease, mild cognitive impairment, and normal aging by using automated segmentation of the hippocampus. Radiology 248:194-201.2008).

Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, Dumuis A, Brunner D, Bockaert J, Hen R (Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT4 receptor knock-out mice. J Neurosci 24:412-419.2004).

Consolo S, Arnaboldi S, Giorgi S, Russi G, Ladinsky H (5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. Neuroreport 5:1230-1232.1994).

Costes N, Merlet I, Ostrowsky K, Faillenot I, Lavenne F, Zimmer L, Ryvlin P, Le Bars D (A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. J Nucl Med 46:1980-1989.2005).

Derogatis, LR (Symptom Checklist-90-R. Administration, Scoring, and Procedures Manual, 3rd edition. National Computer Systems, Minneapolis, Minnesota.1994).

Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P (Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6:734-746.2007).

Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Luthi A (Amygdala inhibitory circuits and the control of fear memory. Neuron 62:757-771.2009).

Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, Wall A, Ringheim A, Langstrom B, Nordberg A (Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. Brain 129:2856-2866.2006).

Erritzoe D, Holst K, Frokjaer VG, Licht CL, Kalbitzer J, Nielsen FA, Svarer C, Madsen J, Knudsen G (A nonlinear relationship between cerebral serotonin transporter and 5-HT(2A) receptor binding: an in vivo molecular imaging study in humans. J Neurosci 30:3391-3397.2010).

Farde L, Hall H, Pauli S, Halldin C (Variability in D2-dopamine receptor density and affinity: a PET study with [11C]raclopride in man. Synapse 20:200-208.1995).

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M (Global prevalence of dementia: a Delphi consensus study. Lancet 366:2112-2117.2005).

Frokjaer VG, Erritzoe D, Madsen J, Paulson OB, Knudsen GM (Gender and the use of hormonal contraception in women are not associated with cerebral cortical 5-HT 2A receptor binding. Neuroscience 163:640-645.2009).

Galeotti N, Ghelardini C, Bartolini A (Role of 5-HT4 receptors in the mouse passive avoidance test. J Pharmacol Exp Ther 286:1115-1121.1998).

Ge J, Barnes NM (5-HT4 receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo. Br J Pharmacol 117:1475-1480.1996).

Gee AD, Martello L, Passchier J, Wishart M, Parker C, Matthews J, Comley R, Hopper R, Gunn R (Synthesis and Evaluation of [11C]SB207145 as the First *In Vivo* Serotonin 5-HT_4 Receptor Radioligand for PET Imaging in Man. Current Radiopharmaceu.2008).

Gillings N (A restricted access material for rapid analysis of [(11)C]-labeled radiopharmaceuticals and their metabolites in plasma. Nucl Med Biol 36:961-965.2009).

Goldstein JM, Seidman LJ, Horton NJ, Makris N, Kennedy DN, Caviness VS, Jr., Faraone SV, Tsuang MT (Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex 11:490-497.2001).

Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, Gur RE (Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. J Neurosci 19:4065-4072.1999).

Haier RJ, Jung RE, Yeo RA, Head K, Alkire MT (The neuroanatomy of general intelligence: sex matters. Neuroimage 25:320-327.2005).

Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 5:228-234.2006).

Hardy JA, Higgins GA (Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184-185.1992).

Hasselbalch SG, Madsen K, Svarer C, Pinborg LH, Holm S, Paulson OB, Waldemar G, Knudsen GM (Reduced 5-HT2A receptor binding in patients with mild cognitive impairment. Neurobiol Aging 29:1830-1838.2008).

Herholz K (Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. Eur J Nucl Med Mol Imaging 35 Suppl 1:S25-29.2008).

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE (Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 27:1533-1539.2007).

Jack CR, Jr., Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, Knopman DS, Boeve BF, Klunk WE, Mathis CA, Petersen RC (11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain 131:665-680.2008).

Jack CR, Jr., Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, Shiung MM, Gunter JL, Boeve BF, Kemp BJ, Weiner M, Petersen RC (Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. Brain 132:1355-1365.2009).

Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordstrom AL (Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. Neuroimage 39:1408-1419.2008).

Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FA, Hashemi SH, Baare WF, Madsen J, Hasselbalch SG, Kringelbach ML, Mortensen EL, Knudsen GM (The personality trait openness is related to cerebral 5-HTT levels. Neuroimage 45:280-285.2009).

Kepe V, Barrio JR, Huang SC, Ercoli L, Siddarth P, Shoghi-Jadid K, Cole GM, Satyamurthy N, Cummings JL, Small GW, Phelps ME (Serotonin 1A receptors in the living brain of Alzheimer's disease patients. Proc Natl Acad Sci U S A 103:702-707.2006).

King MV, Marsden CA, Fone KC (A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci 29:482-492.2008).

Kornum BR, Lind NM, Gillings N, Marner L, Andersen F, Knudsen GM (Evaluation of the novel 5-HT4 receptor PET ligand [11C]SB207145 in the Gottingen minipig. J Cereb Blood Flow Metab 29:186-196.2009).

Kung MP, Kung HF (Mass effect of injected dose in small rodent imaging by SPECT and PET. Nucl Med Biol 32:673-678.2005).

Lai MK, Tsang SW, Francis PT, Esiri MM, Hope T, Lai OF, Spence I, Chen CP ([3H]GR113808 binding to serotonin 5-HT(4) receptors in the postmortem neocortex of Alzheimer disease: a clinicopathological study. J Neural Transm 110:779-788.2003).

Lai MK, Tsang SW, Francis PT, Keene J, Hope T, Esiri MM, Spence I, Chen CP (Postmortem serotoninergic correlates of cognitive decline in Alzheimer's disease. Neuroreport 13:1175-1178.2002).

Lammertsma AA, Hume SP (Simplified reference tissue model for PET receptor studies. Neuroimage 4:153-158.1996).

Lanctot KL, Hussey DF, Herrmann N, Black SE, Rusjan PM, Wilson AA, Houle S, Kozloff N, Verhoeff NP, Kapur S (A positron emission tomography study of 5-hydroxytryptamine-1A receptors in Alzheimer disease. Am J Geriatr Psychiatry 15:888-898.2007).

Lewis R, Kapur S, Jones C, DaSilva J, Brown GM, Wilson AA, Houle S, Zipursky RB (Serotonin 5-HT2 receptors in schizophrenia: a PET study using [18F]setoperone in neuroleptic-naive patients and normal subjects. Am J Psychiatry 156:72-78.1999).

Lezoualc'h F, Robert SJ (The serotonin 5-HT4 receptor and the amyloid precursor protein processing. Exp Gerontol 38:159-166.2003).

Licht CL, Marcussen AB, Wegener G, Overstreet DH, Aznar S, Knudsen GM (The brain 5-HT4 receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. J Neurochem 109:1363-1374.2009).

Logan J, Volkow ND, Fowler JS, Wang GJ, Fischman MW, Foltin RW, Abumrad NN, Vitkun S, Gatley SJ, Pappas N, Hitzemann R, Shea CE (Concentration and occupancy of dopamine transporters in cocaine abusers with [11C]cocaine and PET. Synapse 27:347-356.1997).

Lopresti BJ, Klunk WE, Mathis CA, Hoge JA, Ziolko SK, Lu X, Meltzer CC, Schimmel K, Tsopelas ND, DeKosky ST, Price JC (Simplified quantification of Pittsburgh Compound B amyloid imaging PET studies: a comparative analysis. J Nucl Med 46:1959-1972.2005).

Lucas G, Debonnel G (5-HT4 receptors exert a frequency-related facilitatory control on dorsal raphe nucleus 5-HT neuronal activity. Eur J Neurosci 16:817-822.2002).

Lucas G, Di Matteo V, De Deurwaerdere P, Porras G, Martin-Ruiz R, Artigas F, Esposito E, Spampinato U (Neurochemical and electrophysiological evidence that 5-HT4 receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal, dopaminergic function. Eur J Neurosci 13:889-898.2001).

Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Pineyro G, Sadikot AF, Debonnel G (Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 55:712-725.2007).

Marner L, Frokjaer VG, Kalbitzer J, Lehel S, Madsen K, Baare WF, Knudsen GM, Hasselbalch SG (Loss of serotonin 2A receptors exceeds loss of serotonergic projections in early Alzheimer's disease: a combined [(11)C]DASB and [(18)F]altanserin-PET study. Neurobiol Aging.2010a).

Marner L, Gillings N, Comley RA, Baare WF, Rabiner EA, Wilson AA, Houle S, Hasselbalch SG, Svarer C, Gunn RN, Laruelle M, Knudsen GM (Kinetic modeling of 11C-SB207145 binding to 5-HT4 receptors in the human brain in vivo. J Nucl Med 50:900-908.2009).

Marner L, Gillings N, Madsen K, Erritzoe D, Baare WF, Svarer C, Hasselbalch SG, Knudsen GM (Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. Neuroimage 50:855-861.2010b).

Matsumoto M, Togashi H, Mori K, Ueno K, Ohashi S, Kojima T, Yoshioka M (Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. J Pharmacol Exp Ther 296:676-682.2001).

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939-944.1984).

McNamee RL, Yee SH, Price JC, Klunk WE, Rosario B, Weissfeld L, Ziolko S, Berginc M, Lopresti B, Dekosky S, Mathis CA (Consideration of optimal time window for Pittsburgh compound B PET summed uptake measurements. J Nucl Med 50:348-355.2009).

Meltzer CC, Leal JP, Mayberg HS, Wagner HN, Jr., Frost JJ (Correction of PET data for partial volume effects in human cerebral cortex by MR imaging. J Comput Assist Tomogr 14:561-570.1990).

Meltzer CC, Price JC, Mathis CA, Greer PJ, Cantwell MN, Houck PR, Mulsant BH, Ben-Eliezer D, Lopresti B, DeKosky ST, Reynolds CF, 3rd (PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. Am J Psychiatry 156:1871-1878.1999).

Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ, Reynolds CF, 3rd (Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. Neuropsychopharmacology 18:407-430.1998).

Meyer JH (Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. J Psychiatry Neurosci 32:86-102.2007).

Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, Goulding V, Kennedy J, Wilson AA (Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. Arch Gen Psychiatry 61:1271-1279.2004).

Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K, Houle S (Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. Am J Psychiatry 158:1843-1849.2001).

Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, Rutkowski J, Marantz Y, Dumuis A, Bockaert J, Gold PE, Ragozzino ME (VRX-03011, a novel 5-HT4 agonist, enhances memory and hippocampal acetylcholine efflux. Neuropharmacology 53:563-573.2007).

Moller M, Jakobsen S, Gjedde A (Parametric and regional maps of free serotonin 5HT1A receptor sites in human brain as function of age in healthy humans. Neuropsychopharmacology 32:1707-1714.2007).

Morris JC, Roe CM, Grant EA, Head D, Storandt M, Goate AM, Fagan AM, Holtzman DM, Mintun MA (Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 66:1469-1475.2009).

Mosconi L (Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging 32:486-510.2005).

Mosconi L, Tsui WH, Herholz K, Pupi A, Drzezga A, Lucignani G, Reiman EM, Holthoff V, Kalbe E, Sorbi S, Diehl-Schmid J, Perneczky R, Clerici F, Caselli R, Beuthien-Baumann B, Kurz A, Minoshima S, de Leon MJ (Multicenter standardized 18F-FDG PET diagnosis of mild

cognitive impairment, Alzheimer's disease, and other dementias. J Nucl Med 49:390-398.2008).
Moser PC, Bergis OE, Jegham S, Lochead A, Duconseille E, Terranova JP, Caille D, Berque-Bestel I, Lezoualc'h F, Fischmeister R, Dumuis A, Bockaert J, George P, Soubrie P, Scatton B (SL65.0155, a novel 5-hydroxytryptamine(4) receptor partial agonist with potent cognition-enhancing properties. J Pharmacol Exp Ther 302:731-741.2002).
Muller-Gartner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ (Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. J Cereb Blood Flow Metab 12:571-583.1992).
Nagren K, Halldin C, Rinne JO (Radiopharmaceuticals for positron emission tomography investigations of Alzheimer's disease. Eur J Nucl Med Mol Imaging 37:1575-1593.2010).
Nelson HE, O'Connell A (Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. Cortex 14:234-244.1978).
Nilsson MS, Tóth M, Cselény Z, Karlsson P, Halldin C, Farde L, Varrone A (2010) Quantification of serotonin transporter availability with [11C]MADAM - A comparison between the ECAT HRRT and HR systems. In: Neuroreceptor Mapping Congress Glasgow: Neuroimage.
Nopoulos P, Flaum M, O'Leary D, Andreasen NC (Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. Psychiatry Res 98:1-13.2000).
Nordberg A (Amyloid plaque imaging in vivo: current achievement and future prospects. Eur J Nucl Med Mol Imaging 35 Suppl 1:S46-50.2008).
Nutt DJ (Overview of diagnosis and drug treatments of anxiety disorders. CNS Spectr 10:49-56.2005).
Olesen OV, Sibomana M, Keller SH, Andersen F, Holm JJS, Svarer C, Højgaard L (Spatial Resolution of the HRRT PET Scanner Using 3D-OSEM PSF Reconstruction. In 2009 IEEE Nuclear Science Symposium Conference Record (MIC), IEEE.2009).
Olsson H, Halldin C, Farde L (Differentiation of extrastriatal dopamine D2 receptor density and affinity in the human brain using PET. Neuroimage 22:794-803.2004).
Ouchi Y, Yoshikawa E, Futatsubashi M, Yagi S, Ueki T, Nakamura K (Altered brain serotonin transporter and associated glucose metabolism in Alzheimer disease. J Nucl Med 50:1260-1266.2009).
Pakkenberg B, Pelvig D, Marner L, Bundgaard MJ, Gundersen HJ, Nyengaard JR, Regeur L (Aging and the human neocortex. Exp Gerontol 38:95-99.2003).
Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, Mann JJ (Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. Brain Res 954:173-182.2002).
Peters M, Jancke L, Staiger JF, Schlaug G, Huang Y, Steinmetz H (Unsolved problems in comparing brain sizes in Homo sapiens. Brain Cogn 37:254-285.1998).
Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L, Winblad B (Current concepts in mild cognitive impairment. Arch Neurol 58:1985-1992.2001).
Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 56:303-308.1999).
Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, Mathis CA, Klunk WE, Masters CL, Rowe CC (Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain 130:2837-2844.2007).

Pike VW, Halldin C, Nobuhara K, Hiltunen J, Mulligan RS, Swahn CG, Karlsson P, Olsson H, Hume SP, Hirani E, Whalley J, Pilowsky LS, Larsson S, Schnell PO, Ell PJ, Farde L (Radioiodinated SB 207710 as a radioligand in vivo: imaging of brain 5-HT4 receptors with SPET. Eur J Nucl Med Mol Imaging 30:1520-1528.2003).

Pimplikar SW (Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41:1261-1268.2009).

Porras G, Di Matteo V, De Deurwaerdere P, Esposito E, Spampinato U (Central serotonin4 receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: in vivo studies with morphine, amphetamine and cocaine. Neuropharmacology 43:1099-1109.2002).

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM (A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal male volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. Neuroimage 15:620-632.2002).

Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D, Acker JD (Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. Cereb Cortex 15:1676-1689.2005).

Reimold M, Batra A, Knobel A, Smolka MN, Zimmer A, Mann K, Solbach C, Reischl G, Schwarzler F, Grunder G, Machulla HJ, Bares R, Heinz A (Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [11C]DASB PET study. Mol Psychiatry 13:606-613, 557.2008).

Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM, Wong EH (5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. Br J Pharmacol 114:993-998.1995).

Robert SJ, Zugaza JL, Fischmeister R, Gardier AM, Lezoualc'h F (The human serotonin 5-HT4 receptor regulates secretion of non-amyloidogenic precursor protein. J Biol Chem 276:44881-44888.2001).

Rosel P, Arranz B, Urretavizcaya M, Oros M, San L, Navarro MA (Altered 5-HT2A and 5-HT4 postsynaptic receptors and their intracellular signalling systems IP3 and cAMP in brains from depressed violent suicide victims. Neuropsychobiology 49:189-195.2004).

Rousset OG, Ma Y, Evans AC (Correction for partial volume effects in PET: principle and validation. J Nucl Med 39:904-911.1998).

Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, Jones G, Fripp J, Tochon-Danguy H, Morandeau L, O'Keefe G, Price R, Raniga P, Robins P, Acosta O, Lenzo N, Szoeke C, Salvado O, Head R, Martins R, Masters CL, Ames D, Villemagne VL (Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 31:1275-1283.2010).

Salmon E (A review of the literature on neuroimaging of serotoninergic function in Alzheimer's disease and related disorders. J Neural Transm 114:1179-1185.2007).

Sambeth A, Blokland A, Harmer CJ, Kilkens TO, Nathan PJ, Porter RJ, Schmitt JA, Scholtissen B, Sobczak S, Young AH, Riedel WJ (Sex differences in the effect of acute tryptophan depletion on declarative episodic memory: a pooled analysis of nine studies. Neurosci Biobehav Rev 31:516-529.2007).

Sheline YI, Mintun MA, Moerlein SM, Snyder AZ (Greater loss of 5-HT(2A) receptors in midlife than in late life. Am J Psychiatry 159:430-435.2002).

Sowell ER, Peterson BS, Kan E, Woods RP, Yoshii J, Bansal R, Xu D, Zhu H, Thompson PM, Toga AW (Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. Cereb Cortex 17:1550-1560.2007).

Stark AK, Pelvig DP, Jorgensen AM, Andersen BB, Pakkenberg B (Measuring morphological and cellular changes in Alzheimer's dementia: a review emphasizing stereology. Curr Alzheimer Res 2:449-481.2005).

Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, Mien LK, Moser U, Dudczak R, Kletter K, Kasper S, Lanzenberger R (The serotonin-1A receptor distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-100635. Eur J Nucl Med Mol Imaging 35:2159-2168.2008).

Sureau FC, Reader AJ, Comtat C, Leroy C, Ribeiro MJ, Buvat I, Trebossen R (Impact of image-space resolution modeling for studies with the high-resolution research tomograph. J Nucl Med 49:1000-1008.2008).

Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM (MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 24:969-979.2005).

Svarer C, Marner L, Madsen K, Keller SH, Haahr MT, Siboma M, Knudsen GM (2010) Comparing HRRT and advance scanner data acquisition using a steady-state scan approach. In: Neuroreceptor Mapping Congress Glasgow: NeuroImage.

Talbot PR, Snowden JS, Lloyd JJ, Neary D, Testa HJ (The contribution of single photon emission tomography to the clinical differentiation of degenerative cortical brain disorders. J Neurol 242:579-586.1995).

Tauscher J, Verhoeff NP, Christensen BK, Hussey D, Meyer JH, Kecojevic A, Javanmard M, Kasper S, Kapur S (Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. Neuropsychopharmacology 24:522-530.2001).

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572-580.1991).

Truchot L, Costes N, Zimmer L, Laurent B, Le Bars D, Thomas-Anterion C, Mercier B, Hermier M, Vighetto A, Krolak-Salmon P (A distinct [18F]MPPF PET profile in amnestic mild cognitive impairment compared to mild Alzheimer's disease. Neuroimage 40:1251-1256.2008).

Truchot L, Costes SN, Zimmer L, Laurent B, Le Bars D, Thomas-Anterion C, Croisile B, Mercier B, Hermier M, Vighetto A, Krolak-Salmon P (Up-regulation of hippocampal serotonin metabolism in mild cognitive impairment. Neurology 69:1012-1017.2007).

Varma AR, Snowden JS, Lloyd JJ, Talbot PR, Mann DM, Neary D (Evaluation of the NINCDS-ADRDA criteria in the differentiation of Alzheimer's disease and frontotemporal dementia. J Neurol Neurosurg Psychiatry 66:184-188.1999).

Varnas K, Halldin C, Pike VW, Hall H (Distribution of 5-HT4 receptors in the postmortem human brain--an autoradiographic study using [125I]SB 207710. Eur Neuropsychopharmacol 13:228-234.2003).

Varrone A, Sjoholm N, Eriksson L, Gulyas B, Halldin C, Farde L (Advancement in PET quantification using 3D-OP-OSEM point spread function reconstruction with the HRRT. Eur J Nucl Med Mol Imaging 36:1639-1650.2009).

Vidal R, Valdizan EM, Mostany R, Pazos A, Castro E (Long-term treatment with fluoxetine induces desensitization of 5-HT4 receptor-dependent signalling and functionality in rat brain. J Neurochem 110:1120-1127.2009).

Villemagne VL, Fodero-Tavoletti MT, Pike KE, Cappai R, Masters CL, Rowe CC (The ART of loss: Abeta imaging in the evaluation of Alzheimer's disease and other dementias. Mol Neurobiol 38:1-15.2008).

Waldemar G, Bruhn P, Kristensen M, Johnsen A, Paulson OB, Lassen NA (Heterogeneity of neocortical cerebral blood flow deficits in dementia of the Alzheimer type: a [99mTc]-d,l-HMPAO SPECT study. J Neurol Neurosurg Psychiatry 57:285-295.1994).

Waldemar G, Burns A (eds.) (2009) Alzheimer's Disease. New York: Oxford University Press.

Waldemar G, Dubois B, Emre M, Georges J, McKeith IG, Rossor M, Scheltens P, Tariska P, Winblad B (Recommendations for the diagnosis and management of Alzheimer's disease and other disorders associated with dementia: EFNS guideline. Eur J Neurol 14:e1-26.2007).

West MJ, Kawas CH, Stewart WF, Rudow GL, Troncoso JC (Hippocampal neurons in pre-clinical Alzheimer's disease. Neurobiol Aging 25:1205-1212.2004).

Woods RP, Cherry SR, Mazziotta JC (Rapid automated algorithm for aligning and reslicing PET images. J Comput Assist Tomogr 16:620-633.1992).

Xu R, Hong J, Morse CL, Pike VW (Synthesis, structure--affinity relationships, and radiolabeling of selective high-affinity 5-HT4 receptor ligands as prospective imaging probes for positron emission tomography. J Med Chem 53:7035-7047.2010).

Yamaguchi T, Suzuki M, Yamamoto M (Facilitation of acetylcholine release in rat frontal cortex by indeloxazine hydrochloride: involvement of endogenous serotonin and 5-HT4 receptors. Naunyn Schmiedebergs Arch Pharmacol 356:712-720.1997).

Paper 1

Mass Dose Effects and In Vivo Affinity in Brain PET Receptor Studies

– A Study of Cerebral 5-HT_4 Receptor Binding with [^{11}C]SB207145

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Abstract

Attention to tracer dose principles is crucial in PET and deviations can induce serious errors. In this study we devise a method for determining receptor occupancy of the mass dose of the radioligand itself and the in vivo affinity. **Methods:** The approach was used for [^{11}C]SB207145, a new PET radioligand for imaging the cerebral 5-HT$_4$ receptors in humans. Test-retest PET studies with varying specific activity of [^{11}C]SB207145 were conducted in seven healthy subjects and the output parameter regional BP_{ND} was modeled. Individual occupancy plots were first computed to estimate the mass dose that saturates 50% of receptors (ID_{50}) and subsequently, the maximal mass dose that can be injected (arbitrarily set at an occupancy <5%) was calculated. Scatchard plots were computed to estimate the in vivo K_D. **Results:** Increasing the mass dose resulted in a decrease in BP_{ND}, whilst the relative cerebellar uptake was unchanged. The ID_{50} was 85.4 μg ± 30.2, and the upper mass dose limit was 4.5 μg ± 1.6 which does not require ultra-high specific activity. The estimated in vivo K_D was 2.8 nM (range 1.0-4.8) without any regional differences. **Conclusion:** The presented method for estimating the upper mass dose limit is suggested as part of validation of PET radioligands.

1. Introduction

Methods for the production of radioligands for positron emission tomography (PET) are continuously improving and over the years efforts have been made to improve the specific activity, such that injection of a chemical amount of a ligand (mass dose) of a few micrograms or less can be achieved. A low mass is in keeping with tracer principles and may also sometimes be needed to avoid pharmacological or potentially toxic effects. Reliable quantitative estimates of binding potentials (*BP*) require negligible ligand occupancy of the target receptors/transporters, often referred to as <5% to 10% [1]. Thus, investigation of mass dose effects should be considered a mandatory part of a thorough PET radioligand validation. We here present a method for estimating the mass dose of a ligand that saturates 50% of receptors (ID_{50}) from which the mass dose limits can be calculated. The method involves test-retest studies for the construction of individual occupancy plots (a modification of which is also termed the 'Lassen Plot' [2, 3]) but neither true "zero occupancy" scans nor very high occupancy scans are required.

Radioligands that may be particularly prone to exhibit pharmacological or occupancy effects include agonist radiotracers or compounds with a high plasma free fraction or slow metabolic rate (↑ brain uptake), a high transfer from the plasma to tissue (↑ K_1), or a high receptor affinity (low K_D). For example, high specific activity is needed with the high affinity D_2/D_3 receptor agonist [^{11}C](+)-PHNO because pharmacological effects are seen at mass doses above 3 μg [4], and the upper mass dose limit for human studies with [^{11}C](+)-PHNO has been estimated to be 0.5 μg (<10% occupancy) [5]. Likewise, the high affinity H_3 receptor antagonist [^{11}C]GSK189254 has an upper mass dose limit of around 0.2 μg (<5% occupancy) [6], and the upper mass dose limit of the high affinity D_2 receptor [^{11}C]FLB 457 is 0.5 μg [7]. The occupancy of the mass dose and the upper mass dose limit can be estimated from population-based studies [8] or from estimations of the oral dose of ligand that occupies 50% of available receptor sites (ED_{50}) [6]. Alternatively, the occupancy can be estimated by dividing the concentration of receptor-bound ligand in tissue with the density of receptors (B_{max}) in the same region, which can either be measured from Scatchard plots of test-retest studies [7] or from autoradiography studies.

[^{11}C]SB207145 is currently the only PET radioligand available for imaging the 5-HT_4 receptor in vivo in the central nervous system of humans, and since experimental studies have suggested the 5-HT_4 receptor to be involved in cognitive function [9], depression [10] and Alzheimer's disease [11, 12], in vivo investigations of the 5-HT_4 receptor in humans is highly interesting. We know that [^{11}C]SB207145 crosses the blood-brain barrier fairly well, and that it is a 5-HT_4 receptor antagonist with suitable kinetics for quantification of the 5-HT_4 receptor [13]. Although [^{11}C]SB207145 has high affinity to the 5-HT_4 receptor it binds reversibly within the time window of a two hour dynamic PET-scan. It has a relatively slow metabolic rate and high free fraction in plasma (around 27%). We have previously made a population-based estimate of the upper limit of mass dose of 1.2 μg (occupancy < 5%), which requires a fairly high specific activity [8]. Further, [^{11}C]SB207145 binding has been shown to be unaffected by acute increase of endogenous serotonin [8].

The aim of this study was to investigate the ligand mass dose effects on receptor occupancy, radioligand metabolism and free fraction in plasma. The mass dose required to saturate 50% of receptors (ID_{50}) and the upper limit for mass dose of [^{11}C]SB207145 for both maximum 5% (D_5) and 10% (D_{10}) occupancy are estimated from test-retest studies with varying mass dose, in a test-retest design. Finally, the in vivo concentration of free ligand in tissue required to saturate 50% of receptors (in vivo K_D) is calculated from the test-retest studies with larger differences in mass dose.

2. Methods

2.1 Subjects

Seven subjects were included in the study (five men and two women, mean age 40 years, range 20-57, mean body weight 81 kg, range 63-98). The subjects gave a written informed consent for participation, and 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, mentally deficiency (ensured with DART45 which is a Danish version of the National Adult Reading Test [14]), pregnancy or head trauma. All subjects had a normal neurological examination and unremarkable brain MRI scans. Absence of significant psychiatric symptoms was ensured using the Symptom Check List Revised (SCL-90-R) [15] on the day of the PET scans.

2.2 MRI and Regions of Interest

Magnetic resonance imaging (MRI) was conducted on a Siemens Magnetom Trio 3T MR scanner (matrix 256x256; 1x1x1mm voxels). The T1 weighted MRIs were segmented into grey matter, white matter and cerebrospinal fluid (CSF) using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, UK). The T2 weighted images served for brain masking purposes. Twenty regions were automatically delineated on each subject's MRI in a user-independent fashion with the Pvelab software package [16] (freely available on www.nru.dk/downloads), the mean grey matter volumes of the regions are given in parentheses:

- Striatal regions (high 5-HT_4 receptor binding): caudate nucleus (4.2 ml) and putamen (6.7 ml).
- Limbic regions (intermediate 5-HT_4 receptor binding): hippocampus (5.8 ml), entorhinal cortex (3.5 ml), amygdala (2.0 ml), anterior cingulate gyrus (6.6 ml), posterior cingulate gyrus (4.7 ml) and thalamus (8.5 ml).
- Neocortical regions (low 5-HT_4 receptor binding): orbitofrontal cortex (18.8 ml), medial and inferior frontal gyri (57.2 ml), superior frontal gyrus (43.4 ml), insula (14.9 ml), superior temporal gyrus (37.3 ml), medial and inferior temporal gyri (43.6 ml), sensory motor cortex (40.0 ml), parietal cortex (60.2 ml), occipital cortex (54.6 ml), dorsolateral prefrontal cortex (17.9 ml) and ventrolateral prefrontal cortex (12.0 ml).
- Reference region (negligible concentration of 5-HT_4 receptors): cerebellum, excluding vermis (79 ml)

2.3 PET scanning

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 542 MBq (range 366-604 MBq) [^{11}C]SB207145 given over 20 seconds. The acquisition consisted of 38 time frames, increasing progressively in duration from 5 seconds to 10 minutes.

After acquisition, attenuation and decay corrected recordings were reconstructed by filtered back projection using a 6mm Hann filter. Frames were aligned using AIR 5.2.5 [17] to correct for movements during the scan. Before alignment, each frame was filtered with a 12 mm Gaussian

filter, and the rigid transformation was estimated for each frame to a selected single frame with sufficient structural information (frame 26: 15-20 minutes. post injection). The [^{11}C]SB207145 PET scans were automatically co-registered to the MRI with the AIR algorithm [17] using only 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. Regional time activity curves (TACs) were constructed from grey matter voxels in VOIs, and kinetic modelling was performed with the simplified reference tissue model (SRTM) using cerebellum as reference region as previously validated [13]. The regional in vivo outcome measure for 5-HT_4 receptor density is the binding potential, BP_{ND}, relative to the non-displaceable binding of the radioligand (including both non-specific binding and the free radioligand) represented by the cerebellum. The kinetic modelling was performed using the PMOD software version 2.9, build 2 (PMOD Technologies).

Test-retest studies were conducted in all subjects in random order: one scan was performed with a high specific activity and low mass dose (D_{low}) of unlabelled ligand, and one scan with low specific activity and high mass dose (D_{high}) (see table 1). In three subjects the mass dose difference was > 10 μg, in two subjects 3-5 μg and in two subjects 1-2 μg. All mass doses were normalized to 70 kg body weight. Three of the test-retest studies were conducted with the high mass dose first and four with the low mass dose first. To avoid mass carry-over effects, the time interval between the first and the second scan was > 3 weeks, except in the two subjects with small mass dose differences (subjects #6 and #7) where studies were carried out the same day. No clinical side-effects were observed after [^{11}C]SB207145 injection.
Immediately before radiotracer injection, venous blood samples were drawn and used to measure the plasma free fraction, f_P, with equilibrium dialysis as described previously [18].
Venous blood samples were also drawn 55 minutes after injection, and fraction of parent [^{11}C]SB207145 and its radiolabelled metabolites in plasma were measured with HPLC, as previously described [19].

2.4 Data Analysis

At equilibrium conditions, the concentration of receptor bound ligand (B) is determined by the Michaelis-Menten equation

$$B = \frac{B_{\mathrm{Max}}\, F}{K_{\mathrm{D}} + F} \qquad (1)$$

Where B_{max} is the density of receptors, F is the concentration of free radioligand and K_D is the dissociation constant. At low dose mass of radioligand is $F \ll K_D$ and eq. 1 reduces to

$$\frac{B}{F} = \frac{B_{\mathrm{Max}}}{K_{\mathrm{D}}} = BP \qquad (2)$$

The outcome of SRTM is the non-displaceable binding potential, BP_{ND}, which is proportional (by the free tissue fraction, f_{ND}) to, but does not equal B_{max}/K_D.

Under the assumption that full occupancy of receptor sites would be approached at high mass dose, will the receptor occupancy (O) be determined by the mass dose (D) and ID_{50} of the ligand

$$O = \frac{D}{D + ID_{50}} \qquad (3)$$

Under the further assumption that the underestimated BP by mass dose ($BP_{Occupied}$) is given by the non-occupied BP ($BP_{Baseline}$) and O by the equation

$$BP_{\text{Occupied}} = (1 - O)BP_{\text{Baseline}} \qquad (4)$$

Then the ID_{50} can be estimated from the test-retest studies with varying mass doses, by individual occupancy plots: The 19 regional BP_{ND} of the scan using a high mass dose are plotted against the corresponding regional BP_{ND} of the scan using a low mass dose. The occupancy plots needs no true "zero occupancy" (baseline) scan, like in the 'Lassen Plot re-visited' [3], and the occupancy plots are here based on BP's rather than distribution volumes. The individual ID_{50} can then be calculated by using the slope (α) of the regression line in the occupancy plot, where $\alpha<1$ represents the negative effect of higher mass dose on BP_{ND}

$$\alpha = \frac{1 - O_{\text{High}}}{1 - O_{\text{Low}}} = \frac{1 - \dfrac{D_{\text{High}}}{D_{\text{High}} + ID_{50}}}{1 - \dfrac{D_{\text{Low}}}{D_{\text{Low}} + ID_{50}}} = \frac{D_{\text{Low}} + ID_{50}}{D_{\text{High}} + ID_{50}} \quad \Leftrightarrow \quad ID_{50} = \frac{\alpha D_{\text{High}} - D_{\text{Low}}}{1 - \alpha} \qquad (5)$$

The ID_{50} was calculated for each of the seven subjects. The corresponding upper mass dose limits, D_5 and D_{10}, are then calculated by rearranging eq. 3.

The fraction of parent compound in plasma 55 minutes post injection was compared between scans with high mass dose and low mass dose, using the Wilcoxon two-sample test (N=7), to measure the effect of mass dose on radioligand metabolic rate. The effect of mass dose on the free fraction of ligand in plasma (N=5) was analyzed similarly. In addition, we compared the concentration of free parent compound in plasma with the cerebellar concentration, and calculated the ratio between free and non-specific binding (N=9), under the assumption that 55 minutes post injection the free fraction in plasma equals that of cerebellum.

The in vivo concentration of free ligand in tissue required to saturate 50% of receptors, the dissociation constant K_{D}, was estimated individually using Scatchard plots: The slope equals $-1/K_{\text{D}}$ when regional BP is plotted against the concentration of receptor-bound ligand in the tissue of the same region. To minimize noise, the individual K_{D} was estimated only from volume weighted averages of the high-binding region striatum and the global neocortex, and only in subjects where the difference in mass dose in test-retest scans was above 3 μg (N=5). The concentration of receptor-bound ligand in a region was measured as the mean ligand concentration in the region 80-120 minutes post injection, subtracted by the mean cerebellar concentration (representing free and nonspecific bound ligand only). The time interval was chosen so that the cerebellar concentration was relatively stable (mean 15% ± 2% decrease during the interval 80-120 minutes post injection). An example of a time concentration curve is given in Figure 1a.

3. Results

Table 1 shows the individual mass doses and the estimated values of ID_{50}, D_{10}, and D_5. Occupancy plots were performed for each test-retest study (see example in Figure 1b). Increasing the mass dose reduced BP_{ND} in all test-retest studies (see slope<1 in Table 1), whilst the cerebellar area under the curve TAC (AUC) normalized to the mean injected activity (542 MBq) was unchanged (p=0.75, paired t-test). Based on the individually measured mass dose effects, an average ID_{50} of 85.4 μg ± 30.2 μg was found (eq. 5). Despite test-retest variability ID_{50} does not seem to vary more in subjects studied with low dose differences, and the association between mass dose and occupancy, described by eq. 3, is illustrated in figure 2. This corresponds to 5% receptor occupancy at a mass dose of 4.5 μg ± 1.6 μg and 10% receptor occupancy at 9.5 μg ± 3.3 μg.

The mean fraction of parent compound in plasma 55 minutes post injection was similar (p=0.70) in scans with low and with high mass dose; 10% ± 2% and 10% ± 3%, respectively. Similarly, f_P was similar (p=0.37) in scans with low and with high mass dose; 29% ± 7% and 25% ± 4%, respectively. Fifty-five minutes post injection, the concentration of free parent compound in plasma was mean 5 pM (range 0.1 – 17 pM) and the cerebellar concentration was mean 420 pM (range 11-1205 pM) 55 minutes post injection. Under the rigid assumption of equilibrium conditions at this time point, the free accounted for only 1.5% ± 0.6% of the non displaceable binding in cerebellum.

The individual graphically estimated average K_D was 3.3 nM (range 2.2-4.8) in the striatum and 2.4 nM (1.0-4.4) in neocortex (Figure 3). There was no significant difference in K_D between these regions (p=0.16, paired t-test).

4. Discussion

The study provides a new simple method for investigating the effects of mass dose in brain PET receptor studies. This is exemplified with the 5-HT_4 receptor antagonist [^{11}C]SB207145 for which the in vivo affinity also is investigated.

4.1 Mass effects

If a receptor occupancy below 5% is intended an upper mass dose limit of 4.5 μg of [^{11}C]SB207145 per 70 kg body weight can be injected; below 10% an upper mass dose limit of 9.5 μg for occupancies is required. Inter-individual differences in blood-brain barrier uptake, systemic distribution volume, radioligand metabolism, receptor affinity and plasma protein binding are all parameters that can influence the mass dose effects. Nevertheless, the interindividual variations in ID_{50} and mass dose limit determinations (see Figure 2 and Table 1) were relatively moderate.
The occupancy plots generally showed excellent correlations with R^2 between 0.97 and 1.00, and there did not seem to be any major regional differences in occupancy for [^{11}C]SB207145. The peak equilibrium occurs earlier in low density regions than in high density regions, which at least theoretically lead to a higher concentration of free and non-displacable ligand concentration in the low density regions [20]. This would in turn a non-linearity and intercept with y-axis>0 in the occupancy plot, which was not observed in this study.
We have previously made a population-based estimate of the upper limit of mass dose (occupancy < 5%) of 1.2 μg [8]. However, our study shows a larger range in mass dose, and test-retest studies with high and low specific activity are more reliable since a population-based study is influenced by inter-individual variations in BP_{ND} due to for example sex and age effects.
The presented method for estimating upper mass dose limits does not require a true baseline scan, and only relatively low occupancies with a limited range are needed. The method can also be used for estimating upper mass dose limits for radiotracers where no suitable reference region is available by using total distribution volumes instead of *BP*.
Occupancy effects of mass dose should be assessed for all radiotracers, particularly agonists, in order to avoid pharmacological side effects and for the safety of patients and healthy volunteers. Bias from mass dose should also be considered in studies with expected small group differences and in test-retest studies, where carryover effects of mass dose can be a problem, particularly if the affinity is high, and the time interval between scans is short [6]. Mass dose effects are highly relevant in small rodents [21], but may also play a role in human PET studies where variations in bodyweight are expected, for example in studies of sex differences or diseases associated with altered bodyweight. Furthermore, PET is increasingly used in CNS drug development for investigating the receptor occupancy of drugs [22, 23]. In studies of the dose-regimen of drugs, it could also be relevant to consider the blocking by the ligand itself, especially when only a small

range in receptor occupancy is beneficial, as is the case for the D_2 receptors in antipsychotic treatment [24].
The AUC for cerebellum normalized to injected activity was unaffected by the mass dose, inferring that [^{11}C]SB207145 does not bind specifically in cerebellum, thus supporting the use of cerebellum as a reference region.
We found up to a threefold difference between individuals in the fraction of unmetabolised ligand 55 minutes post injection. Metabolic rate can be affected by many factors; however, radiotracer metabolism was not systematically altered by the mass doses given in the present study, i.e. below 22 μg. Only small inter-individual variability was found in f_p, and the parameter was unaffected by the mass dose. The large range in concentration of free parent compound and cerebellar concentration 55 minutes post injection is primarily due to differences in injected mass doses. We estimated that the free parent compound accounted for only about 1.5% of the non displaceable binding with the remaining must be non-specifically bound. However, since equilibrium conditions do not apply 55 minutes post injection, we consider that the true free concentration probably constitutes a larger fraction. In any instance, based on our estimates cerebellum does not mainly respresent free compound.

4.2 Affinity

K_D was estimated to an average value of 2.8 nM (range 1.0-4.8) in striatum and neocortex, despite the major effects on the slope of the Scatchard plot of small inaccuracies in the measurements of BP_{ND}. We did not find any difference in the in vivo K_D in high versus low 5-HT_4 receptor density regions; this supports our assumption that free and non-displaceable ligand was similar in high- and low-binding brain regions at time 80-120 minutes post injection and the use of measuring bound at a time interval where the time concentration curves were relatively stable for both regions, rather than time of approximated peak equilibrium, which has been used previously [7]. The relatively low variation is in agreement with the general assumption that inter-individual variations in binding potential primarily reflect differences in the available receptor density (B_{avail}), whilst K_D, determined by the structure of the receptor protein and the concentration of the neurotransmitter in question, is expected to be relatively constant between individuals and across brain regions. The estimated in vivo K_D is around sevenfold higher than the in vitro K_D found in the minipig [18]. An around tenfold higher K_D in vivo than in vitro is often found for radioligands [7, 25, 26] probably because in vivo only a fraction of the radioligand is free and because of non-steady state conditions.

5. Conclusion

This study describes a method for estimating ID_{50} and the upper mass dose limit in validations of new radioligands using test-retest studies with different mass dose. The method can be applied with limited range in occupancy, when no true baseline scan is available, and despite the absence of a true reference region. Increased mass doses consistently reduced BP_{ND}, as measured with [^{11}C]SB207145 whilst the relative cerebellar uptake was unchanged, thus supporting the use of cerebellum as a reference region in [^{11}C]SB207145 studies. The mass dose required to saturate 50% of receptors (ID_{50}) was 85.4 μg ± 30.2, and the upper mass dose limit (occupancy <5%) was 4.5 μg ± 1.6. The specific activity required for this to be achieved is relatively easily obtained. Within the tested range, radiotracer metabolism and f_P were unaffected by mass dose. In vivo K_D was 2.8 nM (range 1.0-4.8) with no regional differences.

Disclosures

All authors report no actual or potential financial or personal conflicts of interest.

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References

[1] Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 2007;27:1533-9.

[2] Lassen NA, Bartenstein PA, Lammertsma AA, Prevett MC, Turton DR, Luthra SK, et al. Benzodiazepine receptor quantification in vivo in humans using [11C]flumazenil and PET: application of the steady-state principle. J Cereb Blood Flow Metab 1995;15:152-65.

[3] Cunningham VJ, Rabiner EA, Slifstein M, Laruelle M, and Gunn RN. Measuring drug occupancy in the absence of a reference region: the Lassen plot re-visited. J Cereb Blood Flow Metab 2010;30:46-50.

[4] Mizrahi R, Houle S, Vitcu I, Ng A, and Wilson AA. Side effects profile in humans of (11)C-(+)-PHNO, a dopamine D(2/3) agonist ligand for PET. J Nucl Med 2010;51:496-7.

[5] Rabiner EA and Laruelle M. Imaging the D3 receptor in humans in vivo using [11C](+)-PHNO positron emission tomography (PET). Int J Neuropsychopharmacol 2010;13:289-90.

[6] Ashworth S, Rabiner EA, Gunn RN, Plisson C, Wilson AA, Comley RA, et al. Evaluation of 11C-GSK189254 as a novel radioligand for the H3 receptor in humans using PET. J Nucl Med 2010;51:1021-9.

[7] Olsson H, Halldin C, and Farde L. Differentiation of extrastriatal dopamine D2 receptor density and affinity in the human brain using PET. Neuroimage 2004;22:794-803.

[8] Marner L, Gillings N, Madsen K, Erritzoe D, Baare WF, Svarer C, et al. Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. Neuroimage 2010;50:855-61.

[9] King MV, Marsden CA, and Fone KC. A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci 2008;29:482-92.

[10] Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, et al. Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 2007;55:712-25.

[11] Cho S and Hu Y. Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. Exp Neurol 2007;203:274-8.

[12] Cachard-Chastel M, Lezoualc'h F, Dewachter I, Delomenie C, Croes S, Devijver H, et al. 5-HT4 receptor agonists increase sAPPalpha levels in the cortex and hippocampus of male C57BL/6j mice. Br J Pharmacol 2007;150:883-92.

[13] Marner L, Gillings N, Comley RA, Baare WF, Rabiner EA, Wilson AA, et al. Kinetic modeling of 11C-SB207145 binding to 5-HT4 receptors in the human brain in vivo. J Nucl Med 2009;50:900-8.

[14] Nelson HE and O'Connell A. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. Cortex 1978;14:234-44.

[15] Derogatis and LR. Symptom Checklist-90-R. Administration, Scoring, and Procedures Manual, 3rd edition. National Computer Systems, Minneapolis, Minnesota 1994.

[16] Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 2005;24:969-79.

[17] Woods RP, Cherry SR, and Mazziotta JC. Rapid automated algorithm for aligning and reslicing PET images. J Comput Assist Tomogr 1992;16:620-33.

[18] Kornum BR, Lind NM, Gillings N, Marner L, Andersen F, and Knudsen GM. Evaluation of the novel 5-HT4 receptor PET ligand [11C]SB207145 in the Gottingen minipig. J Cereb Blood Flow Metab 2009;29:186-96.

[19] Gillings N. A restricted access material for rapid analysis of [(11)C]-labeled radiopharmaceuticals and their metabolites in plasma. Nucl Med Biol 2009;36:961-5.

[20] Olsson H and Farde L. Potentials and pitfalls using high affinity radioligands in PET and SPET determinations on regional drug induced D2 receptor occupancy--a simulation study based on experimental data. Neuroimage 2001;14:936-45.

[21] Kung MP and Kung HF. Mass effect of injected dose in small rodent imaging by SPECT and PET. Nucl Med Biol 2005;32:673-8.

[22] Cunningham VJ, Gunn RN, and Matthews JC. Quantification in positron emission tomography for research in pharmacology and drug development. Nucl Med Commun 2004;25:643-6.

[23] Lee CM and Farde L. Using positron emission tomography to facilitate CNS drug development. Trends Pharmacol Sci 2006;27:310-6.

[24] Howes OD, Egerton A, Allan V, McGuire P, Stokes P, and Kapur S. Mechanisms underlying psychosis and antipsychotic treatment response in schizophrenia: insights from PET and SPECT imaging. Curr Pharm Des 2009;15:2550-9.

[25] Farde L, Hall H, Pauli S, and Halldin C. Variability in D2-dopamine receptor density and affinity: a PET study with [11C]raclopride in man. Synapse 1995;20:200-8.

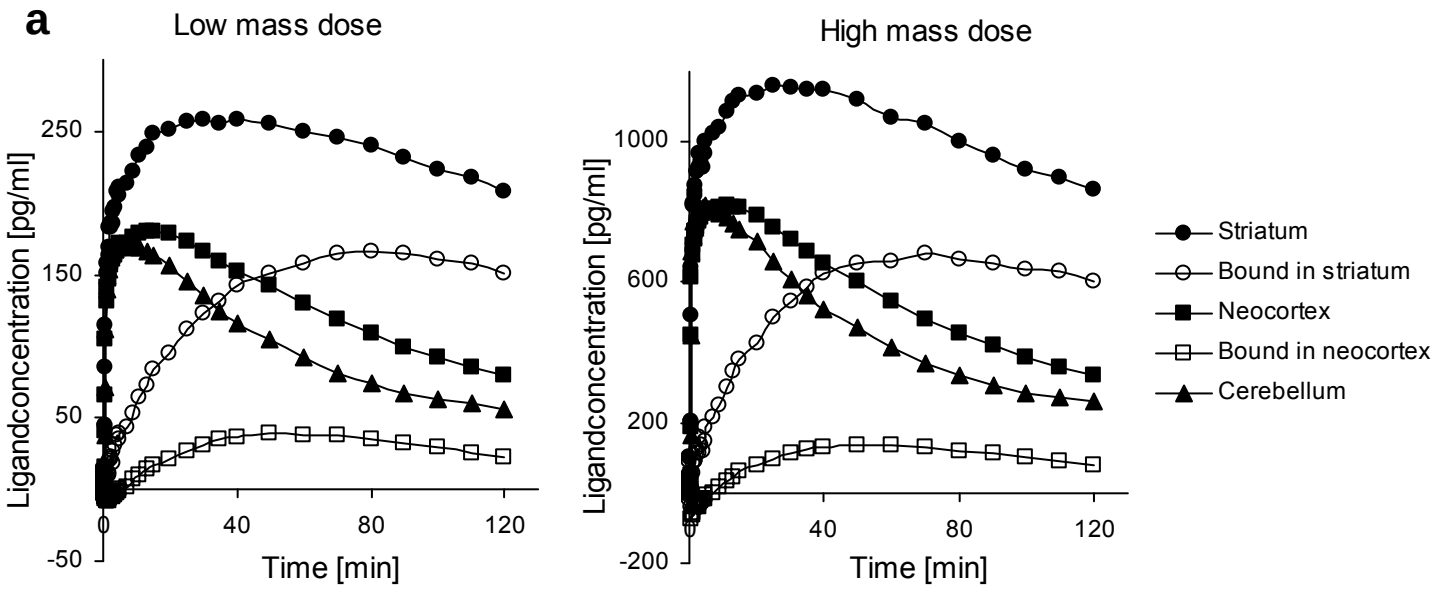
[26] Logan J, Volkow ND, Fowler JS, Wang GJ, Fischman MW, Foltin RW, et al. Concentration and occupancy of dopamine transporters in cocaine abusers with [11C]cocaine and PET. Synapse 1997;27:347-56.

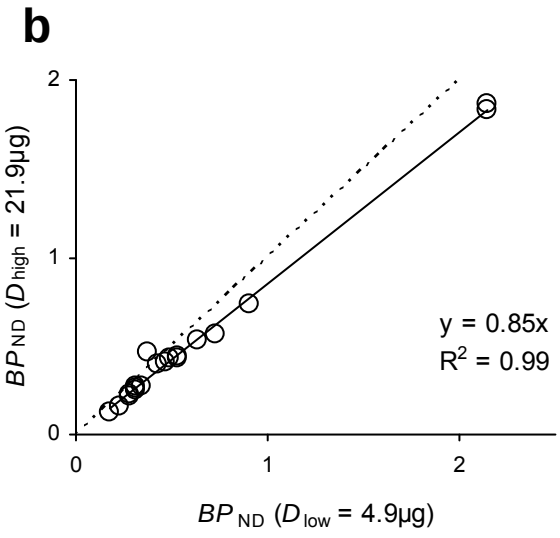
Tables and Figures

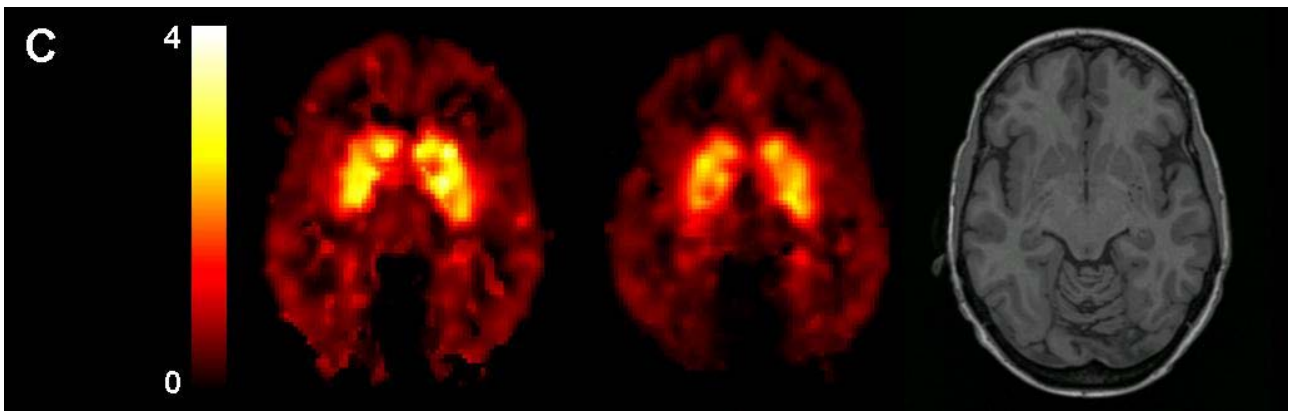
	Mass Dose [µg]		Occupancy plots		Mass dose limits [µg]		
	D_{Low}	D_{High}	α	R^2	ID_{50}	D_5	D_{10}
Subject 1	4.9	21.9	0.85	0.99	94.3	5.0	10.5
Subject 2	4.2	17.0	0.80	0.97	50.2	2.6	5.6
Subject 3	3.7	15.6	0.91	0.99	110.7	5.8	12.3
Subject 4	0.3	4.4	0.93	0.99	53.6	2.8	6.0
Subject 5	0.7	4.1	0.95	1.00	66.9	3.5	7.4
Subject 6	2.4	4.3	0.98	0.99	93.7	4.9	10.4
Subject 7	2.7	4.2	0.99	0.99	131.1	6.9	14.6
Mean ± SD	2.7 ± 1.7	10.2 ± 7.7	0.92 ± 0.07		85.8 ± 30.2	4.5 ± 1.6	9.5 ± 3.3

Table 1 Individual mass doses normalized to 70 kg bodyweight and the corresponding estimates of ID_{50}, D_{10}, and D_5 based on graphical analysis of occupancy plots (see example in Figure 1b). The slope α is the ratio between the high and low dose studies, R^2 designates the correlation coefficient. The mass doses giving 50% occupancy, ID_{50}, are listed together with the upper mass dose limits (occupancy <5% and <10%).

Fig 1 Examples from one volunteer (#1) **a:** Regional time concentration curves after intravenous administration of 4.9 µg ligand (left) and 21.9 µg [^{11}C]SB207145 (right) . The regional concentration of receptor-bound ligand is the regional concentration subtracted by the cerebellar concentration, which represents the non-specific and the free ligand. **b:** Occupancy plot from the test-retest study, for a graphical presentation of the mass effect on BP_{ND}. Each data point represents a single region (n=19), and the deviation from line of identity (dotted line) corresponds to the effect of higher mass on the BP_{ND}, i.e., in this example, occupancy is 14%. ID_{50} can be calculated from the slope of the regression line and the upper mass doses limits calculated by eq. 3. **c:** Parametric image of BP_{ND} after injection of 4.9 µg ligand (left), 21.9 µg ligand (middle), and the corresponding MR image (right).


a
Low mass dose
High mass dose
Ligandconcentration [pg/ml]
Time [min]
250
150
50
-50
1000
600
200
-200
0
40
80
120
Striatum
Bound in striatum
Neocortex
Bound in neocortex
Cerebellum


b
BP ND (D high = 21.9µg)
BP ND (D low = 4.9µg)
y = 0.85x
R2 = 0.99
0
1
2


C
4
0

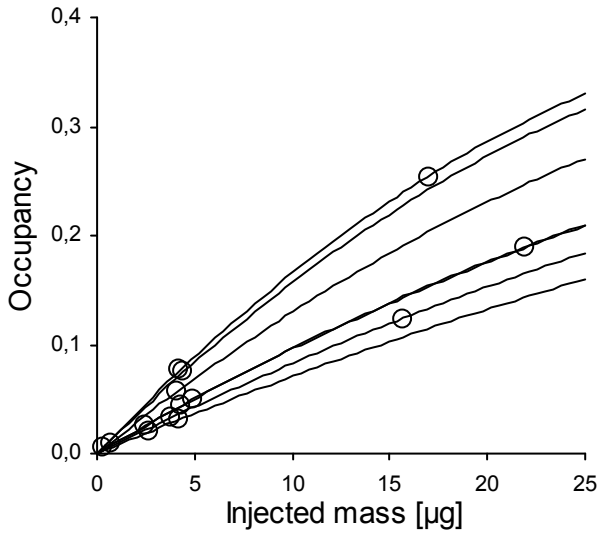


Fig 2 The lines describe for each individual the association between mass dose and occupancy defined by the ID_{50}. The scans are represented by two data points at the line for each individual. For comparison purposes, the mass doses are normalized to 70 kg bodyweight.

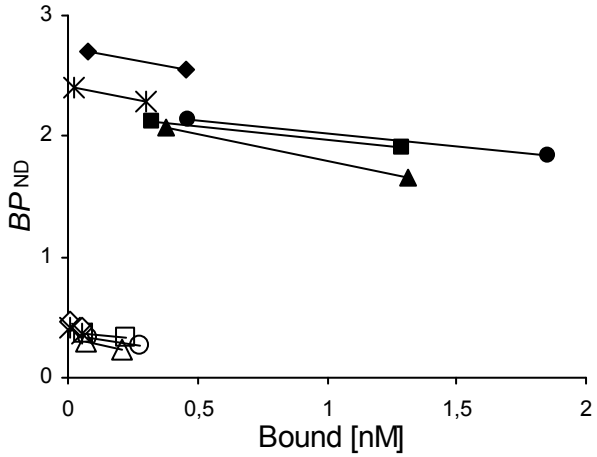


Fig 3 Scatchard plots of striatum (filled markers) and neocortex (open markers) from test-retest scans with varying mass dose in 5 subjects. The slope (α) equals -1 / K_D.

Paper 2

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Age and sex effects on 5-HT_4 receptors in the human brain: a [^{11}C]SB207145 PET study

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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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Keywords: aging; gender; imaging; partial volume; receptor; serotonin

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

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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 β-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 256×256; $1\times1\times1$ 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 μg (range, 0.14 to 5.9 μg), the maximum upper dosage limit has been estimated to be 9.5 μg (occupancy $<10\%$; Madsen K *et al*, unpublished observations). The acquisition consisted of 38 time frames (6×5, 10×15, 4×30, 5×120, 5×300, and 8×600 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.

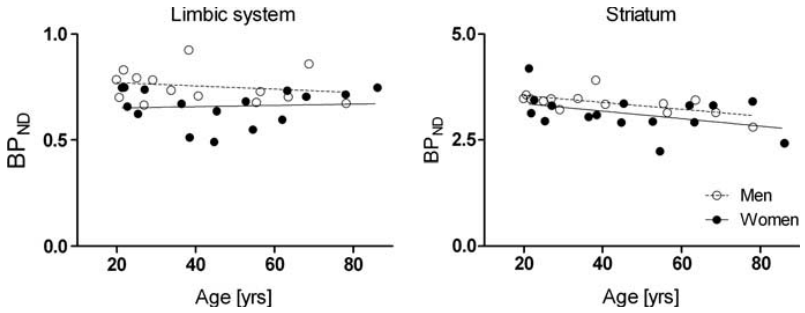

Figure 1 The association between regional partial volume (PV)-corrected 5-HT$_4$ receptor binding for men and women separately. There is no interaction between sex and age. Mean 13% lower limbic 5-HT$_4$ receptor binding is found in women compared with men. There is a decline with age of 1% per decade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

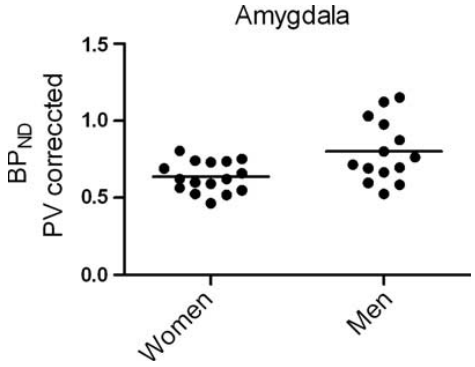

Figure 2 A *post hoc* analysis of limbic subregions showed that the sex difference was most pronounced in the amygdala with 19% ($P = 0.012$).

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 β-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.

References

Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, Madsen K, Frokjaer V, Martiny L, Paulson OB, Knudsen GM (2004) A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. *Neuroimage* 21:1105–13

Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, de Girolamo G, Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lepine JP, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martinez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini P, Palacin C, Romera B, Taub N, Vollebergh WA (2004) Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatr Scand Suppl* 420:21–7

Biver F, Lotstra F, Monclus M, Wikler D, Damhaut P, Mendlewicz J, Goldman S (1996) Sex difference in 5HT2 receptor in the living human brain. *Neurosci Lett* 204:25–8

Bockaert J, Claeysen S, Compan V, Dumuis A (2004) 5-HT4 receptors. *Curr Drug Targets CNS Neurol Disord* 31:39–51

Bonaventure P, Hall H, Gommeren W, Cras P, Langlois X, Jurzak M, Leysen JE (2000) Mapping of serotonin 5-HT(4) receptor mRNA and ligand binding sites in the post-mortem human brain. *Synapse* 36:35–46

Cho S, Hu Y (2007) Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp Neurol* 203:274–8

Cidis Meltzer C, Drevets WC, Price JC, Mathis CA, Lopresti B, Greer PJ, Villemagne VL, Holt D, Mason NS, Houck PR, Reynolds CF, III, DeKosky ST (2001) Gender-specific aging effects on the serotonin 1A receptor. *Brain Res* 895:9–17

Cosgrove KP, Mazure CM, Staley JK (2007) Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry* 62:847–55

Costes N, Merlet I, Ostrowsky K, Faillenot I, Lavenne F, Zimmer L, Ryvlin P, Le Bars D (2005) A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women overaging. *J Nucl Med* 46:1980–9

Derogatis LR (1994) *Symptom Checklist-90-R. Administration, Scoring, and Procedures Manual*, 3rd ed. Minneapolis, Minnesota: National Computer Systems

Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Luthi A (2009) Amygdala inhibitory circuits and the control of fear memory. *Neuron* 62:757–71

Frokjaer VG, Erritzoe D, Madsen J, Paulson OB, Knudsen GM (2009) Gender and the use of hormonal contraception in women are not associated with cerebral cortical 5-HT 2A receptor binding. *Neuroscience* 163:640–5

Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordstrom AL (2008) Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *Neuroimage* 39:1408–19

Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FA, Hashemi SH, Baare WF, Madsen J, Hasselbalch SG, Kringelbach ML, Mortensen EL, Knudsen GM (2009) The personality trait openness is related to cerebral 5-HTT levels. *Neuroimage* 45:280–5

King MV, Marsden CA, Fone KC (2008) A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. *Trends Pharmacol Sci* 29:482–92

Kornum BR, Lind NM, Gillings N, Marner L, Andersen F, Knudsen GM (2009) Evaluation of the novel 5-HT4

receptor PET ligand [11C]SB207145 in the Göttingen minipig. *J Cereb Blood Flow Metab* 29:186–96
Lai MK, Tsang SW, Francis PT, Esiri MM, Hope T, Lai OF, Spence I, Chen CP (2003) [3H]GR113808 binding to serotonin 5-HT(4) receptors in the postmortem neocortex of Alzheimer disease: a clinicopathological study. *J Neural Transm* 110:779–88
Lezoualc'h F (2007) 5-HT4 receptor and Alzheimer's disease: the amyloid connection. *Exp Neurol* 205:325–9
Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Pineyro G, Sadikot AF, Debonnel G (2007) Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* 55:712–25
Marner L, Gillings N, Comley RA, Baare WF, Rabiner EA, Wilson AA, Houle S, Hasselbalch SG, Svarer C, Gunn RN, Laruelle M, Knudsen GM (2009) Kinetic modeling of 11C-SB207145 binding to 5-HT4 receptors in the human brain *in vivo*. *J Nucl Med* 50:900–8
Marner L, Gillings N, Madsen K, Erritzoe D, Baare WF, Svarer C, Hasselbalch SG, Knudsen GM (2010) Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. *Neuroimage* 50:855–61
Matsumoto M, Togashi H, Mori K, Ueno K, Ohashi S, Kojima T, Yoshioka M (2001) Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. *J Pharmacol Exp Ther* 296:676–82
Meyer JH (2007) Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatr Neurosci* 32:86–102
Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, Goulding V, Kennedy J, Wilson AA (2004) Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 61:1271–9
Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K, Houle S (2001) Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. *Am J Psychiatry* 158:1843–9
Moller M, Jakobsen S, Gjedde A (2007) Parametric and regional maps of free serotonin 5HT1A receptor sites in human brain asfunction of age in healthy humans. *Neuropsychopharmacology* 32:1707–14
Muller-Gartner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ (1992) Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. *J Cereb Blood Flow Metab* 12:571–83
Nelson HE, O'Connell A (1978) Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. *Cortex* 14:234–44
Nutt DJ (2005) Overview of diagnosis and drug treatments of anxiety disorders. *CNS Spectr* 10:49–56
Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM (2002) A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normalmale-volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. *Neuroimage* 15:620–32
Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, Dahle C, Gerstorf D, Acker JD (2005) Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex* 15:1676–89
Reimold M, Batra A, Knobel A, Smolka MN, Zimmer A, Mann K, Solbach C, Reischl G, Schwarzler F, Grunder G, Machulla HJ, Bares R, Heinz A (2008) Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [11C]DASB PET study. *Mol Psychiatry* 13:606–13, 557
Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM, Wong EH (1995) 5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br J Pharmacol* 114: 993–8
Rosel P, Arranz B, Urretavizcaya M, Oros M, San L, Navarro MA (2004) Altered 5-HT2A and 5-HT4 postsynaptic receptors and their intracellular signalling systems IP3 and cAMP in brains from depressed violent suicide victims. *Neuropsychobiology* 49:189–95
Salmon E (2007) A review of the literature on neuroimaging of serotoninergic function in Alzheimer's disease and related disorders. *J Neural Transm* 114:1179–85
Sambeth A, Blokland A, Harmer CJ, Kilkens TO, Nathan PJ, Porter RJ, Schmitt JA, Scholtissen B, Sobczak S, Young AH, Riedel WJ (2007) Sex differences in the effect of acute tryptophan depletion on declarative episodic memory: a pooled analysis of nine studies. *Neurosci Biobehav Rev* 31:516–29
Sheline YI, Mintun MA, Moerlein SM, Snyder AZ (2002) Greater loss of 5-HT(2A) receptors in midlife than in late life. *Am J Psychiatry* 159:430–5
Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, Mien LK, Moser U, Dudczak R, Kletter K, Kasper S, Lanzenberger R (2008) The serotonin-1A receptor distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-100635. *Eur J Nucl Med Mol Imaging* 35:2159–68
Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM (2005) MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* 24:969–79
Tauscher J, Verhoeff NP, Christensen BK, Hussey D, Meyer JH, Kecojevic A, Javanmard M, Kasper S, Kapur S (2001) Serotonin 5-HT1A receptor binding potential declines with age asmeasured by [11C]WAY-100635 and PET. *Neuropsychopharmacology* 24:522–30
Varnas K, Halldin C, Pike VW, Hall H (2003) Distribution of 5-HT4 receptors in the postmortem human brain—an autoradiographic study using [125I]SB 207710. *Eur Neuropsychopharmacol* 13:228–34
Woods RP, Cherry SR, Mazziotta JC (1992) Rapid automated algorithm for aligning and reslicing PET images. *J Comput Assist Tomogr* 16:620–33

Paper 3

Cerebral Serotonin 4 Receptors and Amyloid-β in Early Alzheimer's Disease

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Short Running Title: The 5-HT_4 Receptor in Alzheimer's Disease

Abstract

The 5-HT_4 receptor may play a role in memory and learning and 5-HT_4 receptor activation has been suggested to modulate acetylcholine release and to reduce amyloid-β (Aβ) accumulation. The aim of this study was for the first time to investigate the in vivo cerebral 5-HT_4 receptor binding in early Alzheimer Disease (AD) patients in relation to cortical Aβ burden. Eleven newly diagnosed untreated AD patients (mean MMSE 24, range 19-27) and twelve age- and gender-matched healthy controls underwent a two-hour dynamic [^{11}C]SB207145 PET scan to measure the binding potential of the 5-HT_4 receptor. All AD patients and eight healthy controls additionally underwent a [^{11}C]PIB PET scan to measure the cortical Aβ burden.

When AD patients were defined on clinical criteria, no difference in cerebral 5-HT_4 receptor binding between AD patients and healthy controls was found ($p=0.54$). However, when individuals were reassigned to groups according to their amyloid status, the PIB-positive individuals had 13% higher 5-HT_4 receptor levels than PIB-negative individuals ($p=0.02$) and the importance of classification of groups is emphasized. The 5-HT_4 receptor binding was a positively correlated to Aβ burden ($p=0.03$) and negatively to MMSE score of the AD patients ($p=0.02$).

Our data suggests that cerebral 5-HT_4 receptor *upregulation* starts at a preclinical stage of and continues while dementia is still at a mild stage, which contrasts other receptor subtypes. We speculate that this may either be a compensatory effect of decreased levels of interstitial 5-HT, an attempt to improve cognitive function, increase acetylcholine release or to counteract Aβ accumulation.

Keywords

Positron-Emission Tomography, 5-HT4 Receptor, Alzheimer's Disease, Serotonin, Amyloid

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with high prevalence and incidence in people older than 60 years. AD is characterized by a decline in cognition, but emotional and behavioral disturbances are also frequently observed. The neuropathology is characterized by amyloid plaques and neurofibrillary tangles as well as neurodegeneration, with affection of several neurotransmitter systems. The cholinergic neurotransmitter system has been found to be impaired in AD [1, 2] which goes along with the notion that acetylcholine (Ach) enhances encoding memory and attention [3].

In contrast to the cholinergic system, the serotonin (5-HT) system has received less attention. The 5-HT system is, however, also profoundly affected in the course of AD: Marked loss of presynaptic raphe neurons [4] and reduction in particularly the 5-HT_{2A} receptors and to a lesser extent of the 5-HT transporter and the 5-HT_{1A} receptors has been shown in human post mortem studies [5] and in vivo positron emission tomography (PET) studies [6]. As serotonin is known to be involved in depressive and neuropsychiatric symptoms, the affection of this transmitter system may account for the emotional and behavioral symptoms that are common in AD. However, only few studies have been able to associate alterations in the serotonergic system in AD patients to cognitive function (a negative [7] as well as a positive [8] correlation has been described to the 5-HT_{1A} receptor) or depressive symptoms (a negative correlation is described with the 5-HT_{2A} receptor [9] and the serotonin transporter [10]).

Experimental studies have suggested that the 5-HT_4 receptors modulate Ach release: Global 5-HT_4 stimulation increase Ach release in frontal cortex [11] and local stimulation in hippocampus also increase Ach release [12]. In addition, experimental studies indicate that the 5-HT_4 receptor plays a role in memory acquisition and consolidation, and 5-HT_4 stimulation has mainly been found to improve performance [13]. Further, stimulation of 5-HT_4 receptors ameliorates the effect of scopolamine induced cognitive deficits [12, 14], and has even a beneficial synergistic interaction with acetylcholine esterase inhibitors (AchEI) [15-17].

The amyloid-β (Aβ) cascade model is currently a central hypothesis in AD pathogenesis [18] and new Aβ specific PET radiotracers now allow for quantitative in vivo measurements of the Aβ levels. Aβ is highly neurotoxic in its oligomeric form and is a possible product of the cleavage of the membrane bound amyloid-β precursor protein (AβPP). The accumulation of Aβ may start decades before the clinical stage of AD [19] and healthy elderly subjects with abnormal [^{11}C]PIB binding in are often referred to as *asymptomatic at risk for AD* or *preclinical AD*, as abnormal [^{11}C]PIB binding has been shown to associate with progression to AD [20].

Stimulation of the 5-HT_4 receptor has been found to activate a non-amyloidiogenic pathway of the AβPP cleavage with secretion of the non-amyloidogenic form of the amyloid precursor protein (sAβPPα) [21-23]. Further, 5-HT_4 receptor stimulation of cell cultures from Tg2576 transgenic mice inhibits the extracellular Aβ concentration in a concentration dependent manner and increases neuronal survival [24].

Data from two human postmortem studies in AD patients are available. The first found that AD was associated with decreased 5-HT_4 receptor binding in hippocampus and frontal cortex area 11, but not in temporal cortex area 22 and frontal cortex area 4 [25]. However, in the second, larger study no changes in 5-HT_4 receptor affinity and density were seen in frontal and temporal cortex [26]. Thus, post mortem studies have not clearly demonstrated how 5-HT_4 receptor levels change in patients with Alzheimer's disease in comparison with healthy subjects.

The development of the PET ligand [^{11}C]SB207145 has made it possible to measure the 5-HT_4 receptor in vivo. The aim of this study was for the first time to investigate the in vivo cerebral 5-HT_4 receptor binding in untreated early stage AD patients. Since around 25% of an elderly control

group may actually be AD patients at a preclinical stage [27] and the clinical diagnosis of AD has a moderate specificity, we additionally compared regional 5-HT_4 receptor binding between individuals with and without Aβ burden, as measured with [^{11}C]PIB PET scans, and investigated the correlation between 5-HT_4 receptor binding and Aβ burden. Further, the regional 5-HT_4 receptor binding in AD was related to cognitive function and depressive symptoms.

Materials and Methods

Subjects

Eleven patients newly diagnosed with AD (mean age 71 y, range 55-85 y, six males, mean MMSE 24, range 19-27) according to the NINCDS-ADRDA criteria [28] were recruited from the Rigshospitalet Memory Clinic, which is an out-patient clinic based at the Department of Neurology. Twelve healthy controls (HC) (mean age 67.2 y, range 54.6-86.2 y, six males) were recruited by public advertisements or extracted from the civil registration system in Denmark. The study was approved by The Copenhagen Region Ethics Committee (H-KF-274821 with amendments) and all subjects gave a written informed consent for participation according to the Declaration of Helsinki II.

Participants were extensively investigated with neurological and physical examination, laboratory screening tests and a neuropsychological examination. Exclusion criteria were a history of or present neurological or psychiatric disease (for the patients, except AD and depressive symptoms related to the AD diagnosis), severe concomitant somatic disease, abuse of alcohol or drugs (including sedatives), head trauma, use of a drugs known to act on the serotonergic/noradrenergic system within the preceding three months, or neurological signs suggestive of another neurological disorder. No subjects had significant focal pathology on magnetic resonance imaging (MRI).

PET scans were in all patients except for one done before initiation of AchEI treatment. In this single patient, PET scans were performed four weeks after treatment with AchEI was initiated.

MRI and Regions of Interest

Magnetic resonance imaging (MRI) was conducted with a Siemens Magnetom Trio 3T MR scanner. High-resolution 3D T1-weighted (matrix 256x256; 1x1x1mm voxels) and 2D T2-weighted sequences were acquired, and both T1 and T2 weighted images were corrected for spatial distortions and non-uniformity [29, 30]. The T1 weighted MRIs were segmented into grey matter, white matter and cerebrospinal fluid (CSF) using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, UK). The T2 weighted images served for brain masking purposes. Regions were automatically delineated on each subject's MRI in a user-independent fashion with the Pvelab software package [31] (freely available on www.nru.dk/downloads).

Quantification of 5-HT_4 Receptor binding with PET Imaging

All subjects underwent a 120 minute dynamic [^{11}C]SB207145 PET scans starting with a bolus injection of mean 560 MBq (range 366-600 MBq) administered over 20 seconds. The injected mass dose was mean 3.2µg (range 0.6-5.3) which is within mass dose limits for [^{11}C]SB207145 [32]. The scan was performed in 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 and an approximate in-plane resolution of 6 mm. To minimize movement during the scan, a light headband fixation was used. The acquisition consisted of 38 time frames (6x5 s, 10x15 s, 4x30 s, 5x120 s, 5x300 s and 8x600 s). After acquisition, attenuation- and decay corrected recordings were

reconstructed by filtered back projection using a 6mm Hann filter. Frames were aligned using AIR 5.2.5 [33] to correct for movements during the scan. Before alignment, each frame was filtered with a 12 mm Gaussian filter, and the rigid transformation was estimated for each frame to a selected single frame with sufficient structural information (frame 26: 15-20 min. post injection) using the scaled least squares cost-function in AIR.

The [^{11}C]SB207145 PET scan was automatically co-registered to the MRI with the AIR algorithm using the mean of the first 20 min 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 was corrected for partial volume effects using Muller-Gartner method [34] with a point spread function of 6 mm.

Regional time activity curves (TAC's) were constructed from grey matter voxels only, and kinetic modeling of the non-displaceable binding potential (BP_{ND}) of the 5-HT$_4$ receptor was performed with the simplified reference tissue model (SRTM) using cerebellum as reference region as previously validated [35]. Kinetic modeling was done with the PMOD software version 2.95, build 2 (PMOD Technologies). A volume weighted average of five bilateral regions of interest for AD were computed, including parietal cortex, lateral prefrontal cortex (including dorsolateral and ventrolateral prefrontal cortex), lateral temporal cortex, posterior cingulate gyrus, and hippocampus.

The regional in vivo outcome measure, the binding potential, BP_{ND}, is defined as:

$$BP_{ND} = f_{ND} \frac{B_{avail}}{K_D} \qquad (1)$$

f_{ND} being the non-protein bound fraction of non-displaceable binding in brain tissue, B_{avail} the concentration of available receptors and K_D the dissociation constant.

Plasma Analysis of [^{11}C]SB207145: 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 as described previously [36]. Venous blood samples were drawn 32 and 55 minutes after injection and the parent [^{11}C]SB207145 compound and its radiolabeled metabolites were measured in plasma with high performance liquid chromatography (HPLC) [37].

Quantification of Amyloid-β with PET Imaging

After the [^{11}C]SB207145 PET scan, four HC did not want to participate in one additional scan with [^{11}C]PIB. Thus, all AD patients and eight HC additionally underwent a [^{11}C]PIB PET scan to measure the Aβ burden; this allowed us to classify subjects as Aβ-positive or Aβ-negative. [^{11}C]PIB PET scans were acquired at the same day as the [^{11}C]SB207145 PET scan, except for three of the [^{11}C]PIB scans in the HC group.
Subjects were injected with a 20 second bolus injection with an average of 565 MBq (range 301-601 MBq) [^{11}C]PIB. The scan was performed with a high resolution research tomography (HRRT) Siemens PET scanner. A static scan at 40-70 minutes after injection was acquired. To minimize movement during the scan, the head was fixated using a moldable pillow. The images were reconstructed with 3D-OSEM-PSF [38]. The mean voxel movement between frames (5-10 minutes) was measured using AIR 5.2.5 [33], and only when exceeding 3 mm, movement correction was applied. The [^{11}C]PIB PET scan was co-registered to the MRI and the quality of each co-registration was evaluated by visual inspection in three planes.

The regional PET standardized uptake value (SUV) was measured by summing the data acquired from regional grey matter voxels. Data was normalized to cerebellum SUV, resulting in a region to cerebellar ratio (SUVR) [27, 39]. The time window 40-70 min post injection was used, since it provides stability and an effective contrast between HC and AD patients [40]. The Aβ burden was expressed as a volume-weighted mean of SUVR of the following cortical regions that have shown high [^{11}C]PIB binding: posterior cingulate gyrus / precuneus, lateral prefrontal cortex (dorsolateral and ventrolateral part), parietal cortex and the lateral temporal cortex. No correction was applied for partial volume as the spatial resolution of the scanner is down to 2 mm [41]. A SUVR cut-of value at 1.5 to 1.6 have been generated in larger samples to categorize subjects as PIB-positive or PIB-negative [27, 42], therefore we used a cut-of value of 1.6.

Statistics

The difference between groups in 5-HT$_4$ receptor binding in the AD-region was investigated with a Welch t-test; this was done both regarding clinical status (AD patients vs. HC) and PIB binding (PIB-positive SUVR>1.6 vs. PIB-negative SUVR<1.6,). The association between PIB binding and 5-HT$_4$ receptor binding was further investigated with a linear regression analysis using [^{11}C]PIB binding as a quantitative variable (both standard and robust (sandwich) variance estimates were calculated). The dependent variable was the volume-weighted 5-HT$_4$ receptor binding. Sex, but not age, was entered as a co-variate since 5-HT$_4$ receptor binding is lower in the limbic system in women [43]. However, in this data set sex was not a significant co-variate in any analyses and was therefore excluded.
The clinical degree of dementia as measured with MMSE was correlated to 5-HT$_4$ receptor binding in a linear regression analysis.
To control for possible biases t-tests were employed to test for differences between AD and HC, in age, body mass index (BMI), plasma- and tracer data.

It has previously been demonstrated for the 5-HT$_{2A}$ receptor that there is a strong correlation between regions of 5–HT$_{2A}$ receptor binding, indicating that a general level, rather than the regional levels, explains the variance between subjects [44]. This is also in agreement with PET studies of Aβ levels where one cortical level is the general accepted measurement. Therefore, as a supplement to the traditional statistical analysis (comparing groups with t-tests and using linear regression analysis) of volume-weighted averages in regions of interest, we also tested the fit of the structural equation model that instead directly models the suggested underlying general level of PIB load and of 5–HT$_4$ receptor binding ($\eta_{5\text{–HT4}}$ and η_{PIB}, respectively) in the regions of interests, and the association between the two latent variables [45]. To take measurement error on these variables into account, we defined a measurement model for 5–HT$_4$ as

$$\text{ROI}_i(5-\text{HT}_4) = \mu_i + \lambda_i \eta_{5-\text{HT}4} + \varepsilon_i \qquad (2)$$

where μ_i is the intercept for the *i*th region, ε_i is the residual-term, $\eta_{5\text{–HT4}}$ is the latent 5–HT$_4$ level, and λ_i is the loading parameter on the *i*th region. The variables $\eta_{5\text{–HT4}}$ and ε_i are assumed to be independent and zero-mean normal distributed, and the residuals are furthermore assumed to be pairwise independent. Similarly, a measurement model was set up for the PIB measurements

$$\text{ROI}_i(\text{PIB}) = \nu_i + \gamma_i \eta_{\text{PIB}} + \xi_i \qquad (3)$$

with intercept ν_i, latent PIB level given by η_{PIB}, and residual term ξ_i. To allow estimation of the linear association of the two latent variables, we set up a joint model where the correlation between the bivariate normal distributed latent variables $\eta_{5\text{–HT4}}$ and η_{PIB} were estimated. The proposed model has been summarized in a path diagram in figure 1. Parameters were obtained by maximum likelihood estimation and incomplete observations were included in the analysis under a missing at random assumption (MAR). The p-value for the hypothesis that the covariance between $\eta_{5\text{–HT4}}$ and

η_{PIB} was zero was calculated with a likelihood ratio test, and a 95% confidence limit for the correlation coefficient was calculated with Fishers z-transform. The conditional independence assumption was tested with score tests and the likelihood ratio test against the unstructured (saturated) multivariate model was used as an omnibus goodness-of-fit test.
A bio-statistician (co-author Klaus Holst) is responsible for the statistical analysis.

Results

Demographic data and tracer values are listed in table 1; no statistically significant differences were found between AD patients and HC.
When comparing groups defined by their clinical status, there was no difference in 5-HT_4 receptor binding between HC and AD patients (p-value=0.54). With a cut-off value at 1.6, two HC were PIB-positive and one AD patient was PIB-negative (figure 2). The mean PIB SUVR in the PIB positive group was 2.3 (range 1.8-2.8) and in the PIB-negative group 1.2 (range 1.1-1.3). There was a 13% higher 5-HT_4 receptor binding in the PIB-positive group compared to the PIB-negative (p-value=0.02) (example in figure 3). A post hoc investigation of the 5 brain regions that were included in the volume-weighted outcome showed that the difference was highest in hippocampus (28%, p-value=0.02) followed by parietal cortex (15%, p-value=0.01) and lateral temporal cortex (12%, p-value=0.02) (figure 4).

In addition, we found a positive correlation between PIB binding and 5-HT_4 receptor binding (robust variance estimates: p-value=0.03, estimate=0.09 ± 0.04, R^2=0.27; standard variance estimates: p-value=0.02, estimate=0.09 ± 0.04) (figure 5) and a negative correlation between 5-HT_4 receptor binding and MMSE score in the patients diagnosed with AD (p=0.02, estimate=-0.03 ± 0.01, R^2 = 0.50) (figure 6).

For the structural equation model, each measurement model was fitted separately and validated. The score tests indicated a significant improvement in model for η_{5-HT4} if the assumption of conditional independence between lateral temporal cortex and hippocampus was removed. This additional correlation was therefore added to the model, though this did not substantially alter the other parameters of the model which was also the case in the joint model for PIB and 5-HT_4 receptor binding. No other additions to the model were statistically significant. The omnibus goodness-of-fit test led to a χ^2 value of 26.34 on 25 degrees of freedom (p-value=0.39) indicating an acceptable fit. For the full model (figure 1) the positive correlation between the latent variables η_{5-HT4} and η_{PIB} was found (p-value=0.02, estimate=0.55, 95% confidence limits (0.23; 0.76)). A complete-case analysis showed only minor difference in estimates and standard errors compared to the MAR analysis.

Discussion

In this study we found a positive correlation between cerebral 5-HT_4 receptors and Aβ accumulation. Importantly, the upregulation of 5-HT_4 receptors was associated to PIB status and MMSE, but not to clinical status (AD or HC) and the importance of correct disease classification cannot be overemphasized in this context. The patients were all diagnosed with AD according to NINCDS-ADRDA criteria which have been shown to have moderate specificity as other disorders also fulfill the criteria [46]. Inclusion of HC at a preclinical AD stage in the control group may contribute to discrepant results in studies of AD. In line with our finding other studies of elderly healthy subjects have revealed that around 20 % of elderly in their sixties actually have abnormal [^{11}C]PIB binding, increasing up to 65 % in the eighties [47], corresponding to the delayed prevalence of 25% of dementia at age 85 [48]. Further, abnormal [^{11}C]PIB binding in healthy elderly subjects has been shown to associate with progression to AD [20] and the level of [^{11}C]PIB

binding is correlated to episodic memory impairment [27]. Therefore, it is likely that the two PIB positive HC may represent preclinical AD patients.

The upregulation of 5-HT_4 receptors in PIB-positive individuals contrasts the decrease of other serotonergic markers [5, 6]. There are several possible explanations for our observation of an upregulation of cerebral 5-HT_4 receptor binding in PIB- positive individuals: Firstly, it may be a selective response to compensate for the decreased levels of interstitial 5-HT levels in AD, since stimulation of 5-HT_4 receptors facilitates 5-HT release [49] and subchronic 5-HT depletion increases the 5-HT_4 receptor binding in the dorsal hippocampus [50]. Alternatively, upregulation of 5-HT_4 receptors may be an attempt to increase Ach release [11, 12], improve cognitive function [13] or counteract Aβ accumulation [24]. Our study implies that at least the latter suggestion is plausible: We found a positive correlation between Aβ accumulation and 5-HT_4 receptor binding and the between group differences were only found when including the two PIB-positive HC (*preclinical AD*) together with the AD group. This indicates that the upregulation is initiated at a preclinical stage and follows the increase in Aβ accumulation, which is known to start decades before clinical symptoms occur in AD [51]. Interestingly, a post hoc investigation of the 5 brain regions that were included in the volume-weighted outcome showed that the largest upregulation in 5-HT_4 receptor binding (28%) was found in hippocampus (p=0.02) where no local increase in [^{11}C]PIB binding was seen, and similarly, no significant upregulation was found in posterior cingulate gyrus (p=0.14) and lateral prefrontal cortex (p=0.23) where the highest local [^{11}C]PIB binding was found. This could be interpreted as the upregulation of 5-HT_4 receptors successfully counteracted Aβ accumulation *locally*, despite the *general positive* correlation between [^{11}C]PIB and [^{11}C]SB207145 binding. This is in line with the finding in cell cultures from Tg2576 transgenic mice where 5-HT_4 agonism inhibits the extracellular Aβ concentration [24].

The positive correlation between severity of dementia (measured with MMSE) in early AD and 5-HT_4 receptor binding (see figure 6) indicate that 5-HT_4 receptors are progressively upregulated with increasing cognitive dysfunction, at least while dementia is still at a mild stage. This indicates that the upregulation may not only be related to Aβ accumulation, as follow-up studies have shown limited further increase in Aβ accumulation in the course of AD [51, 52]. However, different pathogenic patterns may emerge at a later stage of AD. Keeping the limitations of postmortem studies in mind, these studies indicate that the 5-HT_4 receptor levels may be unchanged [26] or even downregulated in late stages of AD [25]. Further, no correlation to cognitive or behavioral data was found [26] - maybe because compensatory mechanisms collapse when neurodegeneration becomes more severe. There was a borderline significant correlation between PIB and MMSE in the AD patients (p=0.06), but after correcting for 5-HT_4 receptor binding, this association disappeared (p=0.23), thus, a possible association between PIB and MMSE may be mediated through the 5-HT_4 receptor.

One limitation of this study is the small sample size. In a sensitivity test, when excluding the subject with the highest 5-HT_4 binding, no overall change of significance in t-tests was found in group comparisons (neither in the larger volume-weighted region nor in analysis of subregions), but the linear regression was only borderline significant (p=0.10). The correlation was also insignificant if we only included PIB-positive (p=0.38) or AD patients (p=0.18), presumably because of the smaller dispersion and size of the sample. Another limitation is the partial volume correction based on the segmentation of the MRI into grey and white matter. The partial volume effect, caused by the low resolution in the PET image, results in an underestimation of the BP_{ND}, and this underestimation will be more pronounced in atrophic brains. Thus, partial volume correction is necessary to obtain valid results, and is commonly used in AD brain PET studies [9, 53-57].

In conclusion, upregulation of cerebral 5-HT_4 receptor binding was associated to Aβ accumulation but not to clinical status. Our data suggests that the cerebral 5-HT_4 receptor upregulation starts at a preclinical stage and continues, at least as long as dementia is still at a mild stage. Locally, the upregulation of 5-HT_4 receptors may counteract Aβ accumulation and our results suggest a preserved 5-HT_4 receptor pool for possible pharmacological treatment of AD patients with 5-HT4 receptor agonists. Further studies of 5-HT_4 receptors in AD are highly interesting in later stages of the disease and for confirmation of results.

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Disclosures

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References

[1] Farlow MR, Evans RM (1998) Pharmacologic treatment of cognition in Alzheimer's dementia. *Neurology* **51** Suppl, S36-44; discussion S65-67.

[2] Herholz K (2008) Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. *Eur J Nucl Med Mol Imaging* **35 Suppl 1**, S25-29.

[3] Hasselmo ME, Giocomo LM (2006) Cholinergic modulation of cortical function. *J Mol Neurosci* **30**, 133-135.

[4] Aletrino MA, Vogels OJ, Van Domburg PH, Ten Donkelaar HJ (1992) Cell loss in the nucleus raphes dorsalis in Alzheimer's disease. *Neurobiol Aging* **13**, 461-468.

[5] Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ, Reynolds CF, 3rd (1998) Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacology* **18**, 407-430.

[6] Nagren K, Halldin C, Rinne JO (2010) Radiopharmaceuticals for positron emission tomography investigations of Alzheimer's disease. *Eur J Nucl Med Mol Imaging* **37**, 1575-1593.

[7] Kepe V, Barrio JR, Huang SC, Ercoli L, Siddarth P, Shoghi-Jadid K, Cole GM, Satyamurthy N, Cummings JL, Small GW, Phelps ME (2006) Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc Natl Acad Sci U S A* **103**, 702-707.

[8] Lai MK, Tsang SW, Francis PT, Keene J, Hope T, Esiri MM, Spence I, Chen CP (2002) Postmortem serotoninergic correlates of cognitive decline in Alzheimer's disease. *Neuroreport* **13**, 1175-1178.

[9] Hasselbalch SG, Madsen K, Svarer C, Pinborg LH, Holm S, Paulson OB, Waldemar G, Knudsen GM (2008) Reduced 5-HT2A receptor binding in patients with mild cognitive impairment. *Neurobiol Aging* **29**, 1830-1838.

[10] Chen CP, Alder JT, Bowen DM, Esiri MM, McDonald B, Hope T, Jobst KA, Francis PT (1996) Presynaptic serotonergic markers in community-acquired cases of Alzheimer's disease: correlations with depression and neuroleptic medication. *J Neurochem* **66**, 1592-1598.

[11] Consolo S, Arnaboldi S, Giorgi S, Russi G, Ladinsky H (1994) 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* **5**, 1230-1232.

[12] Matsumoto M, Togashi H, Mori K, Ueno K, Ohashi S, Kojima T, Yoshioka M (2001) Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. *J Pharmacol Exp Ther* **296**, 676-682.

[13] King MV, Marsden CA, Fone KC (2008) A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. *Trends Pharmacol Sci* **29**, 482-492.

[14] Galeotti N, Ghelardini C, Bartolini A (1998) Role of 5-HT4 receptors in the mouse passive avoidance test. *J Pharmacol Exp Ther* **286**, 1115-1121.

[15] Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, Rutkowski J, Marantz Y, Dumuis A, Bockaert J, Gold PE, Ragozzino ME (2007) VRX-03011, a novel 5-HT4 agonist, enhances memory and hippocampal acetylcholine efflux. *Neuropharmacology* **53**, 563-573.

[16] Cachard-Chastel M, Devers S, Sicsic S, Langlois M, Lezoualc'h F, Gardier AM, Belzung C (2008) Prucalopride and donepezil act synergistically to reverse scopolamine-induced memory deficit in C57Bl/6j mice. *Behav Brain Res* **187**, 455-461.
[17] Moser PC, Bergis OE, Jegham S, Lochead A, Duconseille E, Terranova JP, Caille D, Berque-Bestel I, Lezoualc'h F, Fischmeister R, Dumuis A, Bockaert J, George P, Soubrie P, Scatton B (2002) SL65.0155, a novel 5-hydroxytryptamine(4) receptor partial agonist with potent cognition-enhancing properties. *J Pharmacol Exp Ther* **302**, 731-741.
[18] Walsh DM, Selkoe DJ (2007) A beta oligomers - a decade of discovery. *J Neurochem* **101**, 1172-1184.
[19] Price JL, Morris JC (1999) Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol* **45**, 358-368.
[20] Morris JC, Roe CM, Grant EA, Head D, Storandt M, Goate AM, Fagan AM, Holtzman DM, Mintun MA (2009) Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Arch Neurol* **66**, 1469-1475.
[21] Cachard-Chastel M, Lezoualc'h F, Dewachter I, Delomenie C, Croes S, Devijver H, Langlois M, Van Leuven F, Sicsic S, Gardier AM (2007) 5-HT4 receptor agonists increase sAPPalpha levels in the cortex and hippocampus of male C57BL/6j mice. *Br J Pharmacol* **150**, 883-892.
[22] Robert SJ, Zugaza JL, Fischmeister R, Gardier AM, Lezoualc'h F (2001) The human serotonin 5-HT4 receptor regulates secretion of non-amyloidogenic precursor protein. *J Biol Chem* **276**, 44881-44888.
[23] Lezoualc'h F, Robert SJ (2003) The serotonin 5-HT4 receptor and the amyloid precursor protein processing. *Exp Gerontol* **38**, 159-166.
[24] Cho S, Hu Y (2007) Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp Neurol* **203**, 274-278.
[25] Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM, Wong EH (1995) 5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br J Pharmacol* **114**, 993-998.
[26] Lai MK, Tsang SW, Francis PT, Esiri MM, Hope T, Lai OF, Spence I, Chen CP (2003) [3H]GR113808 binding to serotonin 5-HT(4) receptors in the postmortem neocortex of Alzheimer disease: a clinicopathological study. *J Neural Transm* **110**, 779-788.
[27] Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, Mathis CA, Klunk WE, Masters CL, Rowe CC (2007) Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain* **130**, 2837-2844.
[28] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939-944.
[29] Jovicich J, Czanner S, Greve D, Haley E, van der Kouwe A, Gollub R, Kennedy D, Schmitt F, Brown G, Macfall J, Fischl B, Dale A (2006) Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. *Neuroimage* **30**, 436-443.
[30] Sled JG, Zijdenbos AP, Evans AC (1998) A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* **17**, 87-97.
[31] Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM (2005) MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* **24**, 969-979.

[32] Madsen K, Marner L, Haah M, Gillings N, Knudsen G (2010) Tracer-dose limits and in vivo 5-HT4 receptor affinity in human brain PET studies with [11C]SB207145. *NeuroImage* **52 Suppl 1**, 193.

[33] Woods RP, Cherry SR, Mazziotta JC (1992) Rapid automated algorithm for aligning and reslicing PET images. *J Comput Assist Tomogr* **16**, 620-633.

[34] Muller-Gartner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ (1992) Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. *J Cereb Blood Flow Metab* **12**, 571-583.

[35] Marner L, Gillings N, Comley RA, Baare WF, Rabiner EA, Wilson AA, Houle S, Hasselbalch SG, Svarer C, Gunn RN, Laruelle M, Knudsen GM (2009) Kinetic modeling of 11C-SB207145 binding to 5-HT4 receptors in the human brain in vivo. *J Nucl Med* **50**, 900-908.

[36] Kornum BR, Lind NM, Gillings N, Marner L, Andersen F, Knudsen GM (2009) Evaluation of the novel 5-HT4 receptor PET ligand [11C]SB207145 in the Gottingen minipig. *J Cereb Blood Flow Metab* **29**, 186-196.

[37] Gillings N (2009) A restricted access material for rapid analysis of [(11)C]-labeled radiopharmaceuticals and their metabolites in plasma. *Nucl Med Biol* **36**, 961-965.

[38] Sureau FC, Reader AJ, Comtat C, Leroy C, Ribeiro MJ, Buvat I, Trebossen R (2008) Impact of image-space resolution modeling for studies with the high-resolution research tomograph. *J Nucl Med* **49**, 1000-1008.

[39] Lopresti BJ, Klunk WE, Mathis CA, Hoge JA, Ziolko SK, Lu X, Meltzer CC, Schimmel K, Tsopelas ND, DeKosky ST, Price JC (2005) Simplified quantification of Pittsburgh Compound B amyloid imaging PET studies: a comparative analysis. *J Nucl Med* **46**, 1959-1972.

[40] McNamee RL, Yee SH, Price JC, Klunk WE, Rosario B, Weissfeld L, Ziolko S, Berginc M, Lopresti B, Dekosky S, Mathis CA (2009) Consideration of optimal time window for Pittsburgh compound B PET summed uptake measurements. *J Nucl Med* **50**, 348-355.

[41] Olesen OV, Sibomana M, Keller SH, Andersen F, Holm JJS, Svarer C, Højgaard L (2009) Spatial Resolution of the HRRT PET Scanner Using 3D-OSEM PSF Reconstruction. *In 2009 IEEE Nuclear Science Symposium Conference Record (MIC), IEEE*. 3789-3790.

[42] Jack CR, Jr., Lowe VJ, Senjem ML, Weigand SD, Kemp BJ, Shiung MM, Knopman DS, Boeve BF, Klunk WE, Mathis CA, Petersen RC (2008) 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain* **131**, 665-680.

[43] Madsen K, Haahr MT, Marner L, Keller SH, Baaré W, Svarer C, Hasselbalch SG, Knudsen GM (2011) Age and sex effects on 5-HT4 receptors in the human brain: a [11C]SB207145 PET study. *J Cereb Blood Flow Metab*. doi:10.1038/jcbfm.2011.11

[44] Erritzoe D, Holst K, Frokjaer VG, Licht CL, Kalbitzer J, Nielsen FA, Svarer C, Madsen J, Knudsen G (2010) A nonlinear relationship between cerebral serotonin transporter and 5-HT(2A) receptor binding: an in vivo molecular imaging study in humans. *J Neurosci* **30**, 3391-3397.

[45] Bollen KA ed. (1989) *Structural equations with latent variables. Wiley Series in Probability and Mathematical Statistics: Applied Probability and Statistics.* , Wiley-Interscience Publication, New York.

[46] Varma AR, Snowden JS, Lloyd JJ, Talbot PR, Mann DM, Neary D (1999) Evaluation of the NINCDS-ADRDA criteria in the differentiation of Alzheimer's disease and frontotemporal dementia. *J Neurol Neurosurg Psychiatry* **66**, 184-188.

[47] Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, Jones G, Fripp J, Tochon-Danguy H, Morandeau L, O'Keefe G, Price R, Raniga P, Robins P, Acosta O, Lenzo N, Szoeke C, Salvado O, Head R, Martins R, Masters CL, Ames D, Villemagne VL (2010) Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol Aging* **31**, 1275-1283.

[48] Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M (2005) Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**, 2112-2117.

[49] Ge J, Barnes NM (1996) 5-HT4 receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo. *Br J Pharmacol* **117**, 1475-1480.

[50] Licht CL, Marcussen AB, Wegener G, Overstreet DH, Aznar S, Knudsen GM (2009) The brain 5-HT4 receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. *J Neurochem* **109**, 1363-1374.

[51] Jack CR, Jr., Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, Shiung MM, Gunter JL, Boeve BF, Kemp BJ, Weiner M, Petersen RC (2009) Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. *Brain* **132**, 1355-1365.

[52] Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, Wall A, Ringheim A, Langstrom B, Nordberg A (2006) Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. *Brain* **129**, 2856-2866.

[53] Truchot L, Costes N, Zimmer L, Laurent B, Le Bars D, Thomas-Anterion C, Mercier B, Hermier M, Vighetto A, Krolak-Salmon P (2008) A distinct [18F]MPPF PET profile in amnestic mild cognitive impairment compared to mild Alzheimer's disease. *Neuroimage* **40**, 1251-1256.

[54] Truchot L, Costes SN, Zimmer L, Laurent B, Le Bars D, Thomas-Anterion C, Croisile B, Mercier B, Hermier M, Vighetto A, Krolak-Salmon P (2007) Up-regulation of hippocampal serotonin metabolism in mild cognitive impairment. *Neurology* **69**, 1012-1017.

[55] Lanctot KL, Hussey DF, Herrmann N, Black SE, Rusjan PM, Wilson AA, Houle S, Kozloff N, Verhoeff NP, Kapur S (2007) A positron emission tomography study of 5-hydroxytryptamine-1A receptors in Alzheimer disease. *Am J Geriatr Psychiatry* **15**, 888-898.

[56] Marner L, Frokjaer VG, Kalbitzer J, Lehel S, Madsen K, Baare WF, Knudsen GM, Hasselbalch SG (2010) Loss of serotonin 2A receptors exceeds loss of serotonergic projections in early Alzheimer's disease: a combined [(11)C]DASB and [(18)F]altanserin-PET study. *Neurobiol Aging*.

[57] Meltzer CC, Price JC, Mathis CA, Greer PJ, Cantwell MN, Houck PR, Mulsant BH, Ben-Eliezer D, Lopresti B, DeKosky ST, Reynolds CF, 3rd (1999) PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. *Am J Psychiatry* **156**, 1871-1878.

Tables

		Healthy Controls N=12	Alzheimer's Patients N=11	t-test p-value
Demographic data	Age [yrs]	67 (55-86)	71 (55-85)	0.36
	Sex	6M / 6F	6M / 5F	
	BMI [kg/m2]	27 (21-40)	25 (17-33)	0.41
[^{11}C]SB207145	Injected mass per kg [ng/kg]	50 (14-93)	38 (6-82)	0.28
	Injected radioactivity [MBq]	552 (366-600)	567 (376-598)	0.58
	f_P [%]	23 (16-41), (N=10)	24 (10-55), (N=9)	0.82
	Parent compound fraction 32 min post-injection [%]	26 (13-64), (N=10)	26 (16-42), (N=8)	0.93
	Parent compound fraction 55 min post-injection [%]	14 (7-27), (N=10)	17 (9-30), (N=7)	0.39
[^{11}C]PIB	Injected radioactivity [MBq]	551 (301-600)	559 (383-601)	0.84
	Specific activity GBq/µmol	99 (19-351)	228 (66-1135)	0.30

Table 1 Demographic and radiotracer data

	Grey Matter Volume [ml]		[^{11}C]SB207145 BP_{ND}	
	HC N=12	**AD N=11**	**HC N=12**	**AD N=11**
Parietal cortex	51 ± 5	44 ± 5	0.85 ± 0.13	0.89 ± 0.14
Lateral temporal cortex	67 ± 8	60 ± 9	0.79 ± 0.08	0.80 ± 0.12
Lateral prefrontal cortex	25 ± 3	23 ± 3	0.69 ± 0.07	0.72 ± 0.12
Posterior cingulate gyrus	3.9 ± 0.7	3.7 ± 0.4	0.72 ± 0.11	0.79 ± 0.15
Hippocampus	5.1 ± 0.5	4.5 ± 0.9	0.83 ± 0.15	0.89 ± 0.26

Table 2 Regional grey matter volume and PV corrected 5-HT_4 receptor binding in patients with Alzheimers Disease (AD) and clinically healthy controls (HC).

Figures

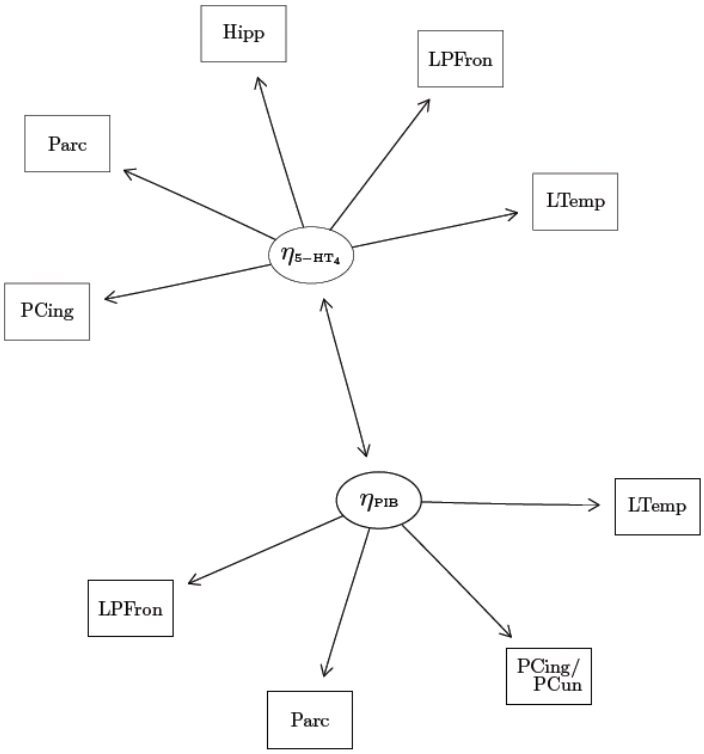

Figure 1 Path diagram for the structural equation model describing the joint distribution of 5–HT_4 and PIB. The ellipsoids are the latent variables which are measured by the manifest variables drawn with rectangles. The double-headed arrows illustrate covariance between residual terms and the single-head arrows describe direct (regression) associations between variables.

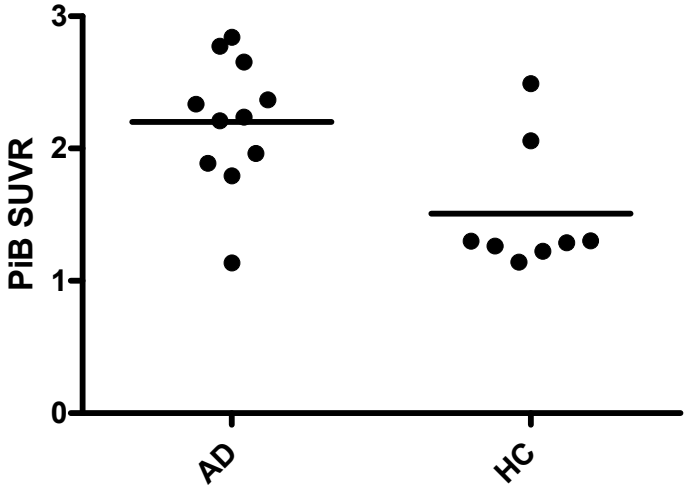

Figure 2 [^{11}C]PIB binding in AD patients and healthy controls. Two healthy controls were categorized as PIB-positive and one subject clinically diagnosed with AD was PIB-negative (cut-of value 1.6).

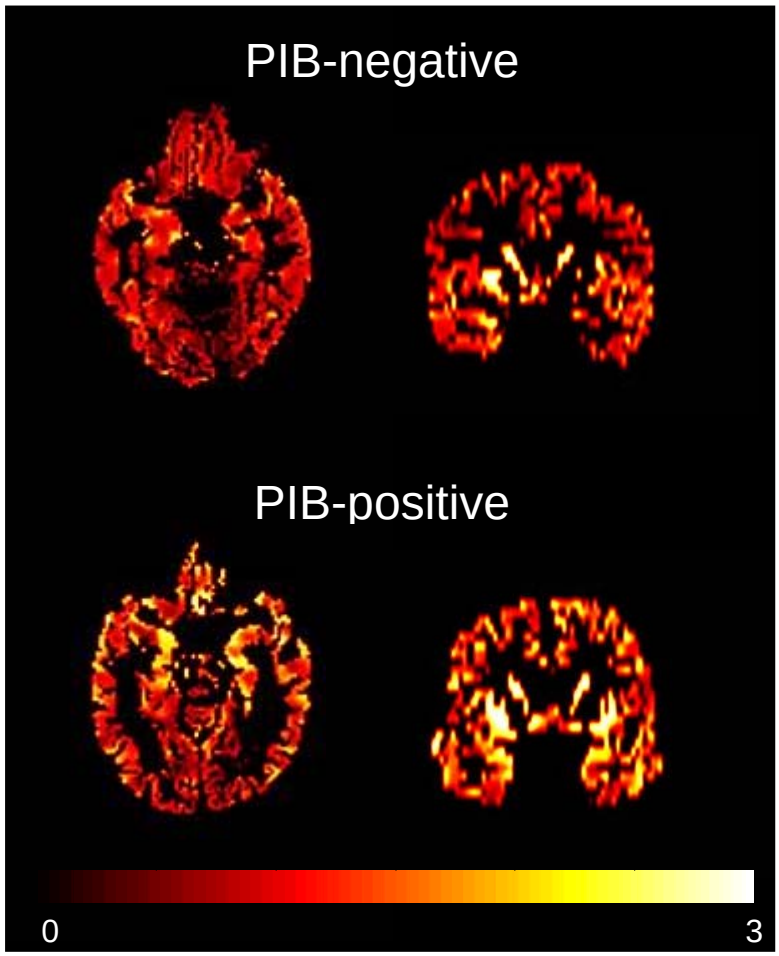

Figure 3 Parametric images of grey matter partial volume corrected 5-HT_4 BP_{ND} in a PIB-negative healthy control and a PIB-positive AD patient.

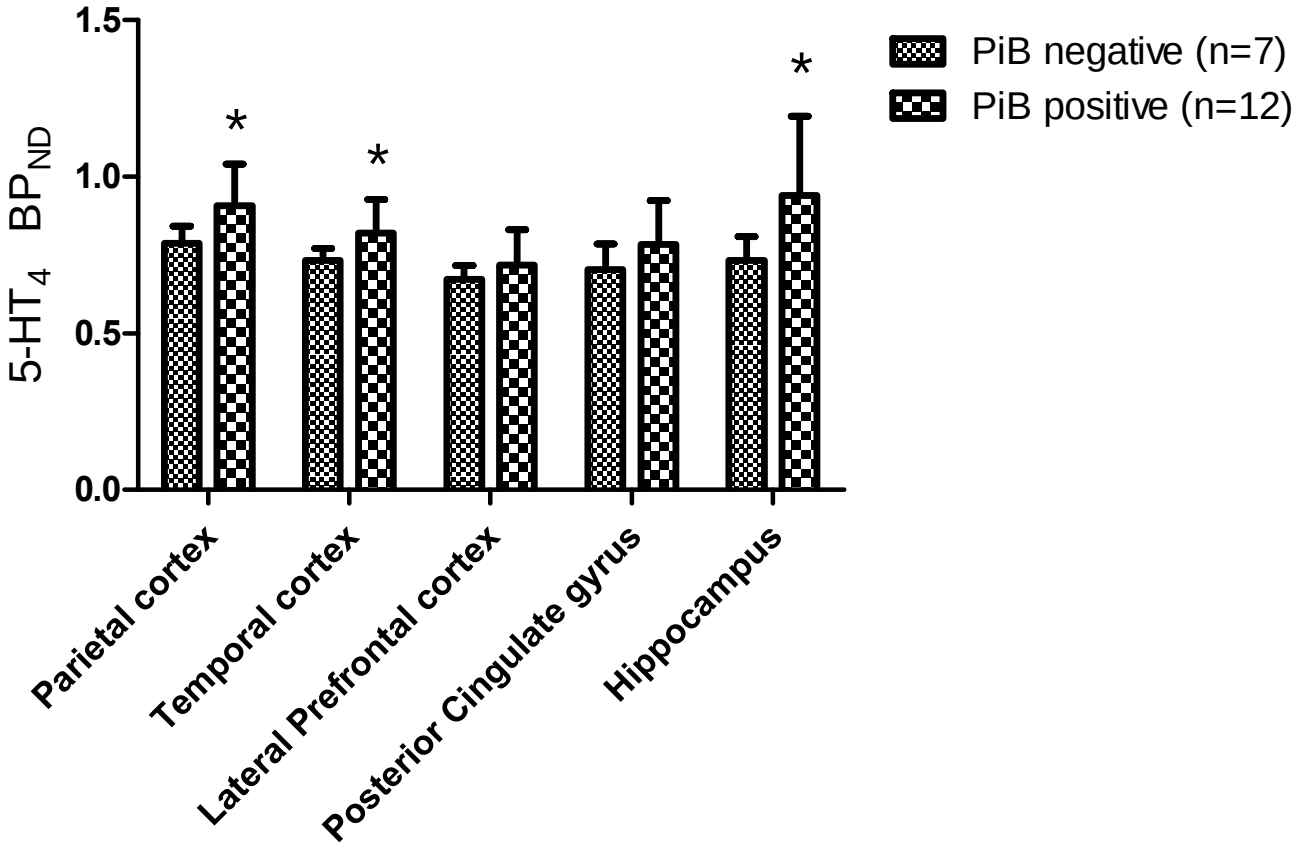

Figure 4 Regional 5-HT_4 receptor binding according to PIB-status. Statistically significant larger 5-HT_4 receptor binding was seen in PIB-positive individuals in parietal cortex (p=0.01), hippocampus (p=0.02) and temporal cortex (p=0.02), but not in posterior cingulate gyrus (p=0.14) and lateral prefrontal cortex (p=0.23). T-tests are not corrected for multiple comparisons.

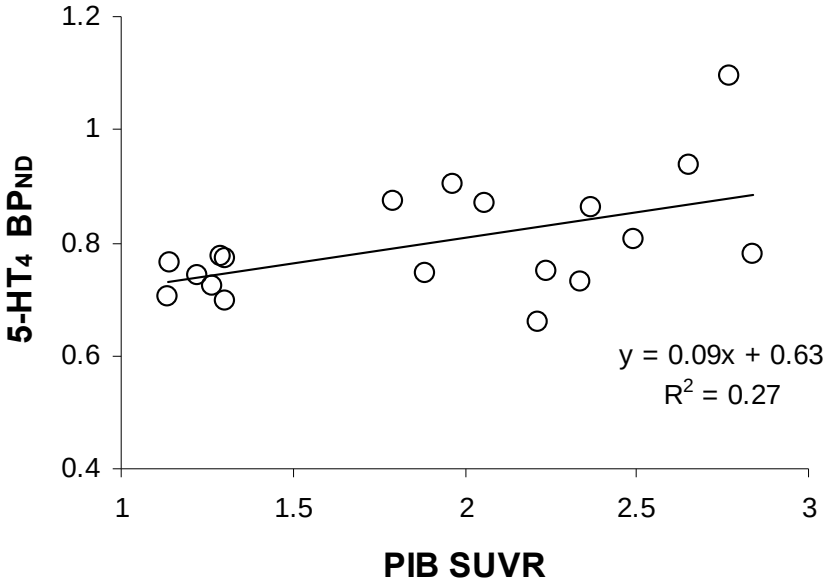

Figure 5 Correlation between amyloid-β load and the volume weighted average 5-HT_4 receptor binding (representing parietal cortex, lateral prefrontal cortex, lateral temporal cortex, posterior cingulate gyrus and hippocampus). A statistically significant positive correlation was found, ($p = 0.03$).

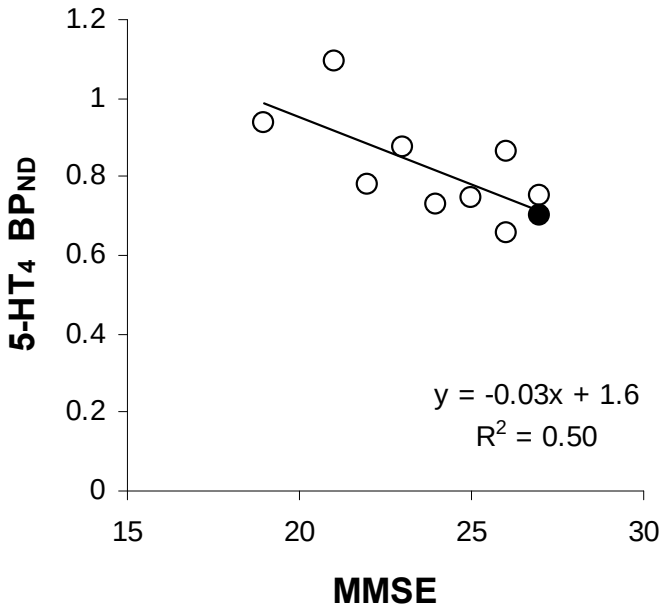

Figure 6 Correlation between MMSE scores of AD patients (N=10) and volume weighted average 5-HT_4 receptor binding (representing parietal cortex, lateral prefrontal cortex, lateral temporal cortex, posterior cingulate gyrus and hippocampus). The filled circle represents the PIB-negative patient, clinically diagnosed with AD. A statistically significant negative correlation was found, ($p = 0.02$).