The 5-HT$_4$ Receptor Levels in Hippocampus Correlates Inversely With Memory Test Performance in Humans

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Abstract: The cerebral serotonin (5-HT) system is involved in cognitive functions such as memory and learning and animal studies have repeatedly shown that stimulation of the 5-HT type 4 receptor (5-HT$_4$R) facilitates memory and learning and further that the 5-HT$_4$R modulates cellular memory processes in hippocampus. However, any associations between memory functions and the expression of the 5-HT$_4$R in the human hippocampus have not been investigated. Using positron emission tomography with the tracer [^{11}C]SB207145 and Reys Auditory Verbal Learning Test we aimed to examine the individual variation of the 5-HT4R binding in hippocampus in relation to memory acquisition and consolidation in healthy young volunteers. We found significant, negative associations between the immediate recall scores and left and right hippocampal BP$_{ND}$, ($p = 0.009$ and $p = 0.010$ respectively) and between the right hippocampal BP$_{ND}$ and delayed recall ($p = 0.014$). These findings provide evidence that the 5-HT$_4$R is associated with memory functions in the human hippocampus and potentially pharmacological stimulation of the receptor may improve episodic memory. *Hum Brain Mapp 34:3066–3074, 2013.* © 2012 Wiley Periodicals, Inc.

Key words: cerebral 5-HT$_4$ receptor; memory; hippocampus; Positron Emission Tomography; Reys Auditory Verbal Learning Test; human experimentation

INTRODUCTION

Learning and memory is essential for normal human behavior and disabilities in these cognitive functions are characteristic for a variety of psychiatric and neurologic diseases such as addiction, anxiety, depression, schizophrenia, and neurodegenerative diseases. Current treatments of memory and learning deficits are far from optimal and substantial effort is undertaken to find therapeutic approaches for example for preventing Alzheimer's Dementia [Frautschy and

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Cole, 2010]. Because of its implications in memory and learning [Schmitt et al. 2006] one of the potential therapeutic targets is the serotonin (5-HT) system. Pharmacological intervention influencing the serotonin receptors can potentially improve memory and learning disabilities [Buhot et al., 2000; Cifariello et al., 2008; Perez-Garcia and Meneses, 2008].

Numerous animal studies have repeatedly shown that stimulation of the 5-HT type 4 receptor (5-HT_4R) facilitates memory and learning, and accordingly the receptor is an interesting pharmacologic target for treatment of memory deficits [Bockaert et al., 2004]. Pre-task systemic injections in animals of the relatively nonselective 5-HT_4R agonists BIMU-1 and BIMU-8 or the more selective partial agonists such as RS17017 and RS6733 improved performance in a variety of memory tasks such as olfactory associative learning [Marchetti et al., 2000, 2004; Marchetti-Gauthier et al., 1997], social memory [Letty et al., 1997], autoshaping [Meneses and Hong, 1997], place and object recognition [Lamirault and Simon, 2001], the Morris water maze [Lelong et al., 2001], and matching-to-sample [Terry et al., 1998]. Furthermore, the new highly selective and potent partial 5-HT_4R agonist, VRX-03011, also enhanced memory in the delayed spontaneous alternation task [Mohler et al., 2007]. In all these studies the agonists were injected immediately before the memory task and therefore 5-HT_4R agonism seems to improve memory acquisition. The evidence for the 5-HT_4R's involvement in memory consolidation is less clear: For example, post-task injection of a 5-HT_4R agonist impaired performance in an autoshaping task [Meneses and Hong, 1997], but improved performance in aged rats in an object recognition task [Lamirault and Simon, 2001]. In addition, administration of the 5-HT_4R antagonists SDZ205557 and GR125487 immediately after termination of the training session weakened passive avoidance memory [Galeotti et al., 1998]. With the radioligand [^{11}C]SB207145 and positron emission tomography (PET) it has recently become possible to visualize and quantify the human cerebral 5-HT_4R in vivo [Marner et al., 2009].

Episodic memory is the memory for events and experiences and involves three cognitive processes; encoding, consolidation, and retrieval [Lezak et al., 2004]. During encoding, the new information presented is acquired and learned. Consolidation is the processing of encoded information into long-term storage for later retrieval. The hippocampus is a crucial structure for both memory encoding and consolidation [Cipolotti and Bird, 2006; Desgranges et al., 1998]. In functional neuroimaging studies of humans, hippocampal activations are consistently observed during both memory encoding and retrieval [Nyberg et al., 2000]. The 5-HT_4R has a relatively high hippocampal density [Marner et al., 2010; Waeber et al., 1996] and plays a key role as a modulator of cellular memory processes in the hippocampus: 5-HT_4R agonists modulate long-term potentiation and long-term depression [Kemp and Manahan-Vaughan, 2002, 2004; Marchetti et al., 2004] and further facilitates the neuronal excitability of pyramidal cells through cAMP mediated closure of potassium channels and regulation of calcium release [Andrade and Chaput, 1991; Fagni et al., 1992; Mlinar et al., 2006; Torres et al., 1996].

Episodic memory functions in humans can be measured with verbal or visual memory tasks. In this study we used Reys Auditory Verbal Learning Test and Rey-Osterrieth's Complex Figure Test, to examine episodic memory function in relation to the individual variation of the in vivo 5-HT_4R binding in hippocampus in healthy young volunteers. On the basis of the experimental studies mentioned above, we hypothesized that the level of 5-HT_4Rs would be positively correlated with the immediate and delayed recall in the memory tasks.

MATERIALS AND METHODS

Participants

Thirty healthy adults (6 females, 24 males) were recruited through newspaper advertisements. Since the memory test scores are reported to decrease with age we only included participants below 45 years of age (mean age 27.2 $\pm$ 6.3 (s.d.) years; range, 20.0–44.7 years), to avoid confounding effects of aging. Written informed consent was obtained according to the Declaration of Helsinki II, and the Copenhagen Region Ethics Committee approved the study. Thirteen of the participants had previously been included in other studies of the 5-HT_4R in humans [Madsen et al., 2010a; Marner et al., 2009, 2010].

Exclusion criteria included significant medical history (including obesity), drug or alcohol abuse, psychiatric disorders or head trauma. All participants had a normal neurological examination and an unremarkable magnetic resonance imaging (MRI) scan of the brain. All participants had completed some education after high-school with a mean of 15.7 $\pm$ 2.1 (s.d.) years of education in total. IQ in the cohort was investigated with two subscales (the number series and verbal analogies) of the Intelligenz-Struktur-Test 2000 R [Amthauer 2001; Neubauer et al., 2005] and found to be a little over average for the general population (mean 104.4 $\pm$ 10.5 (s.d.); range, 90.5–123.5).

Memory Testing

Two experienced neuropsychologists conducted memory testing on a day separate from the day of the PET scan (mean time-interval: 45.1 $\pm$ 37.6 (s.d.) days).

Two memory tests were used: Rey Auditory Verbal Learning Test (RAVLT) to assess episodic verbal memory and Rey-Osterrieth's Complex Figure Test (ROCFT) to evaluate visual nonverbal memory [Lezak et al., 2004]. In RAVLT, a list of 15 emotionally neutral words was read aloud by the neuropsychologist with a 2-s interval in the same sequence five times. After each presentation of the list, the participant delivered a free immediate, verbal recall with a time limit of 60-s. These five presentations were followed by presentation and recall of an interference list and after a 30-min delay the participant was asked to recall the first list again. To reflect the cognitive process of

memory encoding we used the total number of words the individuals acquired during the five first trials (immediate recall, maximum score 75) and to reflect memory consolidation we used the number of recalled words after 30-min (delayed recall, maximum score 15) [Vakil et al., 2010]. In our set-up it was not possible to separate the cognitive process of retrieval from encoding and consolidation, since we did not have a recognition trial at the end of the test.

In ROCFT, participants copied a complex geometric figure and then reproduced it from memory after 3-min and after a delay of 30-min. A 36-point scoring system was used to evaluate the reproductions. To measure memory encoding the scores of 3-min were used (immediate recall) and to measure memory consolidation the delayed scores at 30-min were chosen (delayed recall). Again, in this test it was not possible to eliminate memory retrieval from memory encoding and consolidation.

PET and MR Imaging

[^{11}C]SB207145 was synthesized using a fully automated radio-synthesis system as previously described [Gee et al., 2008; Gillings and Larsen, 2005]. Immediately after an intravenous bolus injection of [^{11}C]SB207145, (mean 490.3 ± 143.0 (s.d.) MBq; range, 206–617 MBq) a 120 min dynamic 3D PET scan (6 × 5 s, 10 × 15 s, 4 × 30 s, 5 × 120 s, 5 × 300 s, and 8 × 600 s) was initiated using either an eighteen ring GE-Advance scanner (GE, Milwaukee, WI) with an approximate in-plane resolution of 6 mm ($n = 13$) or a High Resolution Research Tomograph (HRRT) with an approximate in plane resolution of 1.5 mm ($n = 17$) [Olesen et al., 2009]. The images from the Advance scanner were reconstructed with filtered back projection and corrected for attenuation, dead time and scatter. The HRRT scans were reconstructed using the iterative PSF reconstruction with attenuation map improvements [Comtat 2008; Sureau et al., 2008].

MRI was conducted on a 3T Siemens Magnetom Trio scanner (Erlangen, Germany). High-resolution 3D T1-weighted (matrix 256 × 256; 1 × 1 × 1 mm^3 voxels) and 2D T2-weighted sequences were acquired and corrected for spatial distortions and nonuniformity [Jovicich et al., 2006; Sled et al., 1998]. The T1-weighted brain MRIs were segmented into gray matter, white matter, and cerebrospinal fluid using SPM5 (Wellcome Department of Cognitive Neurology, London, UK) and each voxel was assigned to the tissue class with the highest probability and this segmentation was subsequently used for delineation of the region of interest. The T2 weighted images served for brain masking purposes.

Quantification of Hippocampal 5HT$_4$Rs

The frames of the dynamic PET scan were aligned to correct for head-motion artifacts of more than 3 mm using the AIR routines (version 5.2.5). A flow-weighted mean emission image was automatically aligned to the same individuals MRI using either AIR routines (GE Advance scans) or SPM5 (HRRT scans).

The quantitative analysis to obtain the binding potential (BP) of the 5-HT$_4$R was performed with the simplified reference tissue model (SRTM) [Lammertsma and Hume, 1996] using PMOD (PMOD Inc, Zürich, Switzerland) since it was found to be a reliable and reproducible method for quantification of [^{11}C]SB207145 receptor binding in humans [Marner et al., 2009]. The cerebellum was used as a reference region since blocking with a selective 5-HT$_4$R compound prior to radiotracer administration did not alter the cerebellar binding [Marner et al., 2009]. The model estimates the nondisplaceable BP (BP$_{ND}$), which is defined as:

$$BP_{ND} = f_{ND} * B_{max}/K_d$$

where f_{ND} is the free fraction of tracer in nondisplaceable tissue compartment, B_{max} is the receptor concentration, and K_d is the equilibrium dissociation constant for the tracer.

Regional Analysis

The regions of interest in the present study were left and right hippocampus. The gray matter tissue concentration of radioactivity in the regions of interest was obtained by automatic delineation on each participants MRI in a user-independent fashion with the Pvelab software package [Svarer et al., 2005].

Statistics

We modeled the association between the 5-HT$_4$R binding and memory functions using regression analyses. The four memory test scores used in the models were: (1) RAVLT immediate recall (sum of the five initial trials), (2) the RAVLT delayed recall, (3) the immediate recall in ROCFT, and (4) delayed recall score in ROCFT. These outcomes were one by one compared to the 5-HT$_4$R BP$_{ND}$ in left and right hippocampus. Ceiling effects were found for both RAVLT scores (immediate and delayed recall), which are often observed in young and well-educated cohorts [Uttl, 2005]. The ceiling effect causes bias in regression parameters in a standard linear regression (under-estimation of the association between memory score and 5-HT$_4$R BP$_{ND}$), and to limit this effect we conducted the analyses of RAVLT scores using Tobit regression [Tobin, 1958]. The Tobit model was designed to estimate linear relationships between censored variables. In this study we have a censoring, where individuals with a RAVLT memory score at or above the threshold of 15 words, take on the value of that threshold, so that the true memory score might be equal to the threshold of 15 words, but it might also be higher. In every regression analyses we adjusted for the influence of the type of PET scanner used by including the

TABLE I. Results of Tobit regressions (RAVLT) and linear regressions (ROCFT) analyses of left and right hippocampus with the memory scores as outcome variable and 5-HT$_4$R binding and scanner type as explanatory variables

Memory score	Region	Estimate (β) ± SE	R^2	95% CI	*P*-value
RAVLT immediate recall	Left hippocampus	−37.9 ± 14.5 /BP_{ND}	0.23[a]	−66.4 to −9.4	0.009
	Right hippocampus	−37.3 ± 14.5 /BP_{ND}	0.21[a]	−65.8 to −8.8	0.010
RAVLT delayed recall	Left hippocampus	−4.6 ± 4.0 /BP_{ND}	0.05[a]	−12.5 to 3.3	0.25
	Right hippocampus	−9.6 ± 3.9 /BP_{ND}	0.17[a]	−17.2 to −1.9	0.014
ROCFT immediate recall	Left hippocampus	−1.9 ± 5.4 /BP_{ND}	0.02	−13.1 to 9.2	0.71
	Right hippocampus	−3.7 ± 5.7 /BP_{ND}	0.03	−15.4 to 8.0	0.52
ROCFT delayed recall	Left hippocamp−us	−5.1 ± 5.1 /BP_{ND}	0.05	−16.0 to 4.9	0.29
	Right hippocampus	−4.7 ± 5.4 /BP_{ND}	0.04	−15.8 to 6.3	0.39

[a]McKelvey-Zavoina Pseudo-R^2 defined as ratio of variance of prediction and the latent response [Veall and Zimmermann, 1994]. RAVLT, Reys Auditory Verbal Learning Test; ROCFT, Rey-Osterrieth's Complex Figure Test; SE, standard error; CI, confidence interval.

scanner type as a class variable. Age, gender and IQ were furthermore included in the regression models and eliminated if nonsignificant. Additionally, we used linear regressions to evaluate whether BP_{ND} correlated to the nonspecific binding in the brain (computed as the area-under-the curve in the reference region) or injected amount of cold dose/kg. Further, we evaluated with linear regressions if BP_{ND} or memory scores correlated with gender corrected grey matter volumes. A significance level of $P = 0.05$ was adopted throughout the analyses, and all tests were two-tailed. All analyses were carried out in SAS (v. 9.1 SAS Institute Inc.) or R (v. 2.1.1, The R Foundation for Statistical Computing).

Voxel-Based Analysis

In addition to the regional analyses we performed voxel-based analyses to reveal effects of interest within the structure of hippocampus. The parametric time-activity curves from the HRRT scanner and the GE Advance scanner were smoothed with a 6 mm and a 4 mm full-width-half-maximum (FWHM) Gaussian kernel respectively to obtain the same level of noise in the images. To generate parametric images of the BP_{ND} in each voxel, we used the basis function implementation of SRTM [Gunn et al., 1997]. Then the single-subject BP_{ND} parametric maps were warped to MNI space within SPM8 (http://www.fil.ion.ucl.ac.uk/spm) using the single-subject GM segmented MRI and the SPM8 apriori grey.nii image as the template. Final voxel-size was $2 \times 2 \times 2$ mm^2. The parametric images from the GE Advance scanner were additionally smoothed with a 12 mm FWHM Gaussian kernel after normalization. We defined hippocampus using the Wake Forest University Pickatlas (v3.0) [Maldjian et al., 2003, 2004]. A voxel-wise Tobit regression analysis was conducted with each of RAVLT immediate and delayed recall as dependent variable and 5-HT$_4$R BP_{ND} and scanner type as covariates. We corrected for multiple comparisons using Monte Carlo simulation in 3dClustSim within AFNI (http://afni.nimh.nih/gov/afni). In the hippocampal volume the method yielded a cluster extent significance threshold of $k \geq 30$ voxels ($P < 0.05$).

RESULTS

Our regional Tobit regression analyses of left and right hippocampus revealed significant, negative associations between the RAVLT immediate recall scores and left and right hippocampal $BP_{ND,}$ ($P = 0.009$ and $P = 0.010$, respectively) (Table I and Fig. 1). Correspondingly, the right hippocampal BP_{ND} and RAVLT delayed recall showed a significant, negative association ($P = 0.014$), whereas this relationship was not found for the left hippocampus ($P = 0.25$) (Table I and Fig. 1). The model assumptions of linearity and distribution were found reasonable when assessed by inclusion of polynomial terms of the predictor and by graphical comparison of the Kaplan-Meier estimator and the model-specific distribution of the residuals. Linear regression analyses between ROCFT immediate and delayed recall scores and binding potentials in left and right hippocampus were not significant ($P > 0.29$). Age, gender, and IQ were included in the regression models, but eliminated from the analyses, as they did not contribute significantly to the model (All *P*-values > 0.23). The voxel-level Tobit analyses of associations between left hippocampal BP_{ND} and RAVLT immediate recall revealed two inversely associated clusters, which were significant after corrections for multiple comparisons (MNI coordinates −30, −16, −16, $z = 3.23$, 51 voxels, $P < 0.05$; MNI coordinates −30, −32, −8, $z = 3.72$, 31 voxels, $P < 0.05$) (Fig. 2). We also identified two clusters within the right hippocampus where RAVLT immediate recall was inversely associated with 5-HT$_4$R binding, however these did not survive correction for multiple comparisons (MNI coordinates 32, −16, −18, $z = 3.58$, 24 voxels, $P > 0.05$; MNI coordinates 30, −26, −12, $z = 3.36$, 14 voxels, $P > 0.05$). No clusters in left or right hippocampus were associated with RAVLT delayed recall after corrections for multiple comparisons. We found zero positively associated

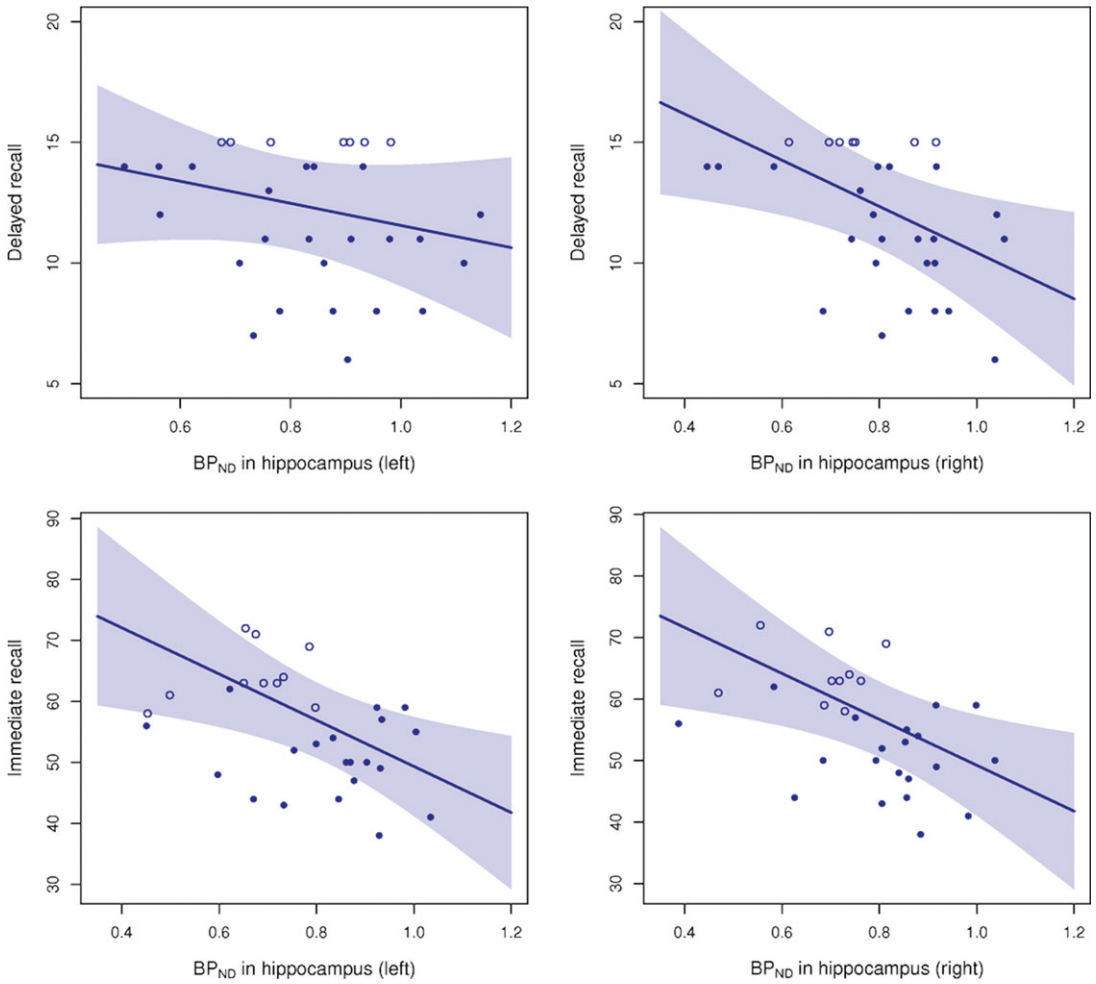

Figure 1.

Four plots showing estimated linear associations between RAVLT immediate recall and delayed recall and the left and right regional BP_{ND} in hippocampus. BP_{ND} values are adjusted for the estimated scanner effect. The shaded region is the pointwise 95% confidence limits. The censored observations—the unfilled circles—all scored 15 in the delayed recall or in one or more of the five immediate recall sessions.

voxels between RAVLT immediate or delayed recall and 5-HT_4R binding.

Since the BP_{ND} value of the hippocampus was associated with verbal but not nonverbal memory performance, we examined the interrelationships between these two kinds of memory test scores. In a univariate linear regression immediate and delayed recall scores were strongly correlated in both RAVLT ($P < 0.001$) and ROCFT ($P < 0.001$). Further, we observed strong correlations between verbal and nonverbal immediate recall scores ($P < 0.001$) as well as verbal and nonverbal delayed recall scores ($P < 0.001$).

We did not see any significant correlation between (1) gray matter volumes and BP_{ND} or memory scores or (2) BP_{ND} and nonspecific binding or cold dose/kg injected (All P-values > 0.26).

DISCUSSION

This is the first human in vivo study to examine relationships between 5-HT_4R-binding and memory functions. In 30 young and healthy individuals we found a significant negative correlation of the 5-HT_4R expression in left and right hippocampus and immediate recall and further a significant negative correlation between the right hippocampal 5-HT_4R expression and delayed recall. Our results suggest that the 5-HT_4R in humans is involved in memory encoding and consolidation and that fewer hippocampal 5-HT_4Rs are representative of a better episodic memory function. As receptor stimulation according to the animal literature generally has a facilitatory effect on memory acquisition and as receptor expression is augmented when rats undergo memory training [Manuel-Apolinar et al.,

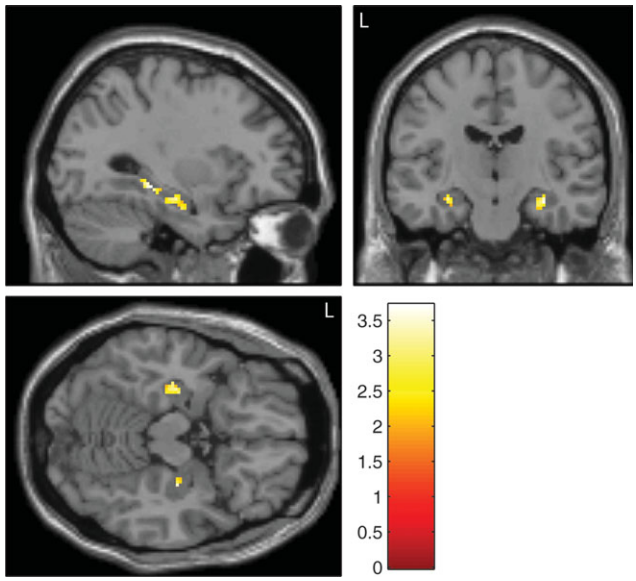

Figure 2.

Statistical parametric map overlaid on a T1-weighted MR image showing the negative associations between $5HT_4R$ BP_{ND} and RAVLT immediate recall in hippocampus ($P < 0.05$). Two clusters in the left hippocampus were significant after corrections for multiple comparisons while two clusters within the right hippocampus were below the cluster extent threshold. The color bar depicts the T values. MNI coordinates for the sagittal image is $X = -30$, the coronal image is $Y = -16$ and the axial image is $Z = -16$.

2005], the result does not call for a straightforward interpretation. However, most of the animal studies examined functional effects of agonism or antagonism of the receptor, which could potentially explain the difference between the present clinical study and the experimental studies.

As shown in the methods section the binding potential of the 5-HT_4R is a composite measure of receptor expression and affinity [Lammertsma and Hume, 1996]. Since [^{11}C]SB207145 has not been found sensitive to acute endogenous serotonin release [Marner et al., 2010] and since the affinity is assumed to be equivalent in hippocampus and the reference region, the BP_{ND} in this study is proportional to the receptor density in hippocampus. The 5-HT_4R is expressed in hippocampus in central locations, where both intrinsic hippocampal and extrinsic cortical and subcortical circuits are modified such as the CA1, the dentate gyrus and the subiculum [King et al., 2008]. The precise hippocampal neuronal localization has not yet been specified, but lesion studies indicate that the receptor is present on glutamatergic neurons [Vilaro et al., 2005]. Furthermore, stimulation of the 5-HT_4R leads to modulation of the acetylcholine and GABA release in hippocampus, so the receptor is likely to be localized on these neurons, even though indirect effects cannot be excluded [Bianchi et al., 2002; Bockaert et al., 2006; Matsumoto et al., 2001]. As the in vivo binding of [^{11}C]SB207145 cannot differentiate the type of neurons or the specific location of the 5-HT_4R within hippocampus, a low receptor density in hippocampus cannot simply be linked to low neuronal activity and functions. Further, stimulation of the receptor is also not in a simple way linked to better memory. For example, in healthy volunteers a low dose of selective serotonin reuptake inhibitors (SSRI) improved short-term memory, while a high dose impaired it [Dumont et al., 2005] and, as mentioned in the introduction, 5-HT_4R agonists impaired memory function in young rats while it improved memory functions in old rats [Lamirault and Simon, 2001]. Therefore, various explanations of the results in this study can be proposed. Acute tryptophan depletion impairs episodic memory [Mendelsohn et al., 2009; Sambeth et al., 2007], and therefore efficient serotonergic innervation and adequate 5-HT interstitial levels in hippocampus seem important for memory functions. Since 5-HT_4R binding was found to be down-regulated after chronic SSRI treatment [Licht et al., 2009; Vidal et al., 2009], high serotonin levels in hippocampus may reduce the levels of the 5-HT_4R. This might explain the paradoxical finding that the participants in this study with a high memory performance have lower levels of the receptor. On the other hand, since subchronic serotonin depletion was found to cause an up-regulation of the 5-HT_4R [Licht et al., 2009] subjects with an inefficient memory function may improve the functioning of the serotonin system by up-regulating the 5-HT_4R, that both may improve memory

directly and indirectly by stimulating 5-HT release in hippocampus [Ge and Barnes 1996; Licht et al., 2010]. However, hypothetically this up-regulation is insufficient to boost memory function to the level of the better performing subjects. This is in line with a recent study of Alzheimer's disease from our group, where accumulation of β-amyloid and thereby possible decline of interstitial serotonin levels was associated with an up-regulation of the 5-HT_4Rs [Madsen et al., 2010b]. In this context we also speculate that the relation between the serotonin system and memory may be inversely u-shaped as it is assumed for dopaminergic modulation of cognitive functions [Cools and D'Esposito, 2011]: both low and high 5-HT_4R binding may reflect levels of serotonin outside the optimal range for memory function. However, this study lacks variation in memory scores, with few low scoring subjects, to find evidence for this hypothesis.

We previously investigated the relation between the RAVLT memory scores and the serotonin transporter (SERT) and found no correlation [Madsen et al., 2011]. The reason for these differing results may be that SERT is considered to be a marker of general serotonergic innervation with complex postsynaptic actions, while the 5-HT_4R is likely to be directly involved in memory function as previously show in the introduction.

It was clear from the voxel-based analysis within hippocampus that the association between memory functions and receptor expression was found both in ventral and dorsal hippocampus, which have been assigned to dissimilar functional roles: Dorsal hippocampus is mainly participating in cognitive functions while ventral hippocampus is mainly involved in emotions, stress and affect [Fanselow and Dong, 2010]. This is in line with the roles of the 5-HT_4R in the brain. As shown both from the previous animal literature and this study the receptor is likely to play a role in memory and learning processes, however, the receptor is also likely to be involved in emotional processes as studies has linked the receptor to regulation of the hedonic part of food intake [Compan et al., 2010; Francis et al., 2010; Jean et al., 2007] and emotional processes such as stress [Compan et al., 2004].

It was evident from the overlapping confidence intervals (Table I) that the difference between left and right binding potentials would not statistically reach a 5% significance level. However, the RAVLT delayed recall scores were only significant in the right hippocampus. This finding could be due to differences in cognitive processes in the left and right hippocampus as some studies suggest that encoding processes may be left-lateralized and recall processes right-lateralized [Desgranges et al., 1998]. Another explanation may be that the 5-HT_4Rs differ in functionality with regard to memory encoding and consolidation as the previously mentioned animal literature suggest. This may result in differential regulation of the receptor dependent on the cognitive process.

In contrast to the verbal memory performance we did not find significant correlations between the nonverbal memory task and hippocampal 5-HT_4Rs even though the memory tests correlated strongly. These results could be explained by the specific functions of the 5-HT_4R. It has been shown that neuronal networks of encoding and retrieval can be distinguished by their reaction to specific stimuli (e.g., verbal vs. visual) even though interactions between the systems are evident [Nyberg et al., 2000]. Our results suggest that the 5-HT_4R primarily affects the verbal memory network and not the visual. It is not possible to confirm this result in the existing literature, as 5-HT_4R agonism seems to improve all the various animal memory tests, at least in the acquisition phase. However, in animals it is difficult to precisely imitate the visual and verbal memory tasks used in humans, as species differences may be considerable. Thus, rather than drawing any specific conclusions, we recommend that future human studies seek to clarify these associations between serotonergic tone, 5-HT_4Rs and verbal and visual memory.

One should consider the limitations of this study. We found ceiling effects in RAVLT, which in young and well-educated cohorts is a prevalent finding with the applied testing procedures [Uttl, 2005; Van der Elst et al., 2005]. We corrected for this bias by using a censored regression model to avoid inconsistent estimates present in an ordinary linear regression in these kinds of data. However, if the data was analyzed using an ordinary linear regression model, we still found significant correlations between the immediate recall scores and the 5-HT_4R levels in left hippocampus ($P = 0.04$) and right hippocampus ($P = 0.02$) and between RAVLT delayed scored and the receptor level in right hippocampus ($P = 0.03$). Another issue was that the subjects were scanned on two different PET scanners. We compensated for this by including scanner type in the regressions models, but it is not possible to exclude that some scanner effects were still present. However, we would point out that scanner type was insignificant in the regression models in all analyses and we did not observe any tendencies toward main effects of scanner type on immediate or delayed memory scores.

In conclusion, this is the first study in humans to examine the association between the 5-HT_4R in hippocampus and memory functions. The study provides evidence that the 5-HT_4R is associated with memory functions in hippocampus and supports an association between 5-HT_4R and the previously reported asymmetry of hippocampal function in memory. Since 5-HT_4R stimulation generally enhances memory performance across various domains in animals, it was an unexpected finding that the 5HT_4R level correlated negatively with measures of memory function. This finding may be explained by the complex interactions between the intrinsic serotonergic tonus and receptor functions in the hippocampus. Further studies in humans are needed to elucidate the functional significance of this study; however, we speculate that stimulation of the human 5-HT_4R could improve memory functions.

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