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ORIGINAL INVESTIGATION

Differential involvement of hippocampal serotonin$_{1A}$ receptors and re-uptake sites in non-cognitive behaviors of Alzheimer's disease

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Abstract
Rationale Previous studies have shown extensive serotonergic deficits in the hippocampus of Alzheimer's disease (AD) patients. However, it is unclear whether such deficits play a role in non-cognitive, neuropsychiatric behaviors that occur frequently in AD and cause significant caregiver distress.
Objectives In this study, we aimed to correlate serotonergic markers in the AD hippocampus with neuropsychiatric behaviors.
Methods Using postmortem hippocampal homogenates from aged controls as well as a cohort of longitudinally assessed AD patients, measurements of 5-HT$_{1A}$ receptors, 5-HT$_{2A}$ receptors, and serotonin re-uptake (5-HTT) sites were performed by binding with ^{3}H-labeled 8-OH-DPAT, ketanserin, and citalopram, respectively.
Results Alterations of 5-HT$_{1A}$ receptors and 5-HTT were found to be differentially involved in neuropsychiatric behaviors, with loss of 5-HT$_{1A}$ receptors specifically correlated with depressive symptoms, while 5-HTT sites were preserved or up-regulated in patients with aggressive behaviors.
Conclusions Our data suggest that neuropsychiatric behaviors in AD share certain neurochemical features with psychiatric disorders like major depression and that serotonergic drugs used in psychiatric disorders may also be efficacious against behavioral symptoms in AD.

Keywords Dementia · Alzheimer's disease · Serotonin receptors · Serotonin transporter · Hippocampus · Depression · Psychosis · Anxiety · Aggression

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the commonest cause of dementia in the elderly, affecting more than 24 million people worldwide and set to quadruple by 2050 (Ferri et al. 2005; Brookmeyer et al. 2007). AD is characterized by neuropathologic hallmarks including senile plaques, neurofibrillary tangles, and neuronal degeneration. Besides progressive cognitive decline, AD patients frequently present with neuropsychiatric symptoms collectively termed Behavioural and Psychological Symptoms of Dementia (BPSD; IPA 1996). Because BPSDs, such as aggression, anxiety, psychosis, and depression, are more stress-inducing for caregivers than the cognitive decline,

behavioral problems are a major factor in patient institutionalization and the associated healthcare costs (Steele et al. 1990; Herrmann et al. 2006). Given that neurotransmission is well known to underlie both cognition and behavior, research efforts to uncover the pathologic basis and therapeutic targets of BPSD and dementia have focused on delineating patterns of neuronal loss as well as concomitant neurochemical alterations. Such approaches have led to the development of neurotransmitter system-acting therapeutics, namely, cholinesterase inhibitors and memantine, following studies that showed cholinergic and glutamatergic perturbations (Geerts and Grossberg 2006; Francis et al. 2010).

Besides the cholinergic and glutamatergic systems, research has suggested that the serotonergic system may be involved in cognition, mood, and emotive states (Barnes and Sharp 1999; Lanctôt et al. 2001; Perez-Garcia and Meneses 2008). The serotonergic system is extensively affected in AD. First, there is accumulating evidence of extensive losses of serotonin (5-hydroxytryptamine, 5-HT) synthesizing neurons in the dorsal and median raphe nuclei, which provide the major serotonergic innervation to the forebrain (Aletrino et al. 1992; Chen et al. 2000). In addition, we and others have reported alterations in several 5-HT receptor subtypes, some of which are associated with cognitive decline or manifestation of specific BPSDs. For example, there have been consistent findings of 5-HT_{2A} receptor deficits that correlated with cognitive decline (Cross et al. 1984a, 1986; Lai et al. 2005). Although 5-HT_{1A} receptors were initially thought to be relatively intact (Cross et al. 1984a, 1986), subsequent studies have shown that imbalances between 5-HT_{1A} receptor and 5-HT levels are associated with accelerated cognitive decline (Lai et al. 2002) and that a subset of AD patients with reduced 5-HT_{1A} receptors in the temporal cortex had higher incidence of aggressive behaviors (Lai et al. 2003). Similarly, deficits in neocortical 5-HT_4, 5-HT_6 receptors, and 5-HT transporters (5-HTT, also known as 5-HT re-uptake sites) have been correlated with various BPSDs, such as hyperphagia, overactivity, psychosis, and depression (Chen et al. 1996; Garcia-Alloza et al. 2004; Marcos et al. 2008; Tsang et al. 2010). Taken together, these findings indicate that serotonergic alterations may underlie both cognitive as well as neuropsychiatric features of AD. However, it is unclear whether other vulnerable brain regions beside the neocortex are involved in BPSD. Of the forebrain regions, the hippocampus is known to be extensively innervated by raphe projections, particularly those from the median raphe (Molliver 1987). Both pre-clinical and clinical studies have pointed to important roles of the hippocampal serotonergic system in cognitive function as well as in mood states, including depression and anxiety (Graeff et al. 1996; File et al. 2000; Manji et al. 2001; Santarelli et al. 2003). In AD, the hippocampus and related structures are known to be one of the earliest and most extensively affected brain regions (Haroutunian et al. 1998; Du et al. 2001). Furthermore, various serotonergic markers are altered in the AD hippocampus, including 5-HT receptors as well as 5-HTT (Cross et al. 1984b; Tejani-Butt et al. 1995; Kepe et al. 2006). However, it is unclear whether hippocampal serotonergic alterations are associated with BPSD in AD. The present study aimed to measure 5-HT_{1A}, 5-HT_{2A} receptors, and 5-HTT in the postmortem hippocampus of a cohort of behaviorally assessed AD patients and to correlate the neurochemical variables with clinical data.

Materials and methods

Patients and clinical information

The subjects were composed of the autopsied subset of a cohort of community-based dementia patients recruited for a longitudinal study of behavioral changes in dementia (Hope et al. 1997, 1999). Institutional review board approval for the recruitment and study of subjects had been obtained from the Oxford Psychiatric Sector Research. Subject selection for this study was based on postmortem tissue availability, neuropathologic diagnosis of AD, and a minimum follow-up period of 8 months. Subjects were not selected based on disease severity, gender, or presence of behavioral symptoms. Initial assessments of subjects included the Cambridge Mental Disorders of the Elderly Examination (Roth et al. 1986) and clinical diagnosis of dementia based on DSM IIIR criteria (1987). The disease duration at study baseline was estimated to be 5.3±0.4 years (mean ± SEM). Subjects were subsequently followed-up every 4 months till death (mean follow-up of 3.3±0.2 years) by trained personnel on home visits, who assessed cognitive function using the Mini-Mental State Examination (MMSE, Folstein et al. 1975) and behavioral changes using the Present Behavioural Examination (PBE, Hope and Fairburn 1992). The PBE is a standardized, semi-structured, caregiver-based interview that covers the subject's behavior and mental state over the previous 4 weeks in detail. Behavioural symptoms such as anxiety, depression, aggression and psychosis were as previously defined (Lai et al. 2001). Briefly, anxiety was assessed by behaviors and physical signs indicating anxiety or fright. Depression was assessed by apparent sadness of the patient, whereas aggressive behavior was indicated by acts of physical aggression (e.g., biting, kicking). Psychosis included delusions and/or hallucinations; with delusion being assessed by the subject expressing persecutory ideas, whereas hallucination was assessed by the patient's behaviors indicating they saw or heard things that were not really there. These behaviors were scored using a seven-point

scale (0–6) based on the frequency of occurrence reported by the caregiver (from zero, not present in the last 28 days; three, present in approximately 14 days of the last 28 days; to six, present every day). Behavioral change was considered significant if a minimum of two ratings for that behavior were more than three or if there was at least one rating of more than three and two ratings of 1-3, at any time during follow-up (Lai et al. 2001). Behavioral ratings were excluded from the study if changes were considered likely due to delirium or medications. Complete drug histories were collected for the subjects. Five AD patients were on sedative hypnotics and ten were on neuroleptics in the 8 months before death, whereas only one patient was on tricyclic antidepressants. Control subjects died from non-neurological causes and did not have any history of mental illness or neuropsychiatric behaviors.

Postmortem brain tissue processing and neuropathology

Postmortem brain tissues of the subjects were obtained from what is now known as the Thomas Willis Oxford Brain Collection, part of the Brains for Dementia Research network. At death, informed consent was obtained from next-of-kin before autopsy and harvesting of brains. One hemisphere was cut into 10-mm coronal slices and fresh frozen on steel slabs at −75°C. Subsequently, blocks of tissue from the hippocampal formation were removed from brain slices kept at −20°C, dissected free of white matter, then homogenized and washed in Tris-HCl buffer to obtain brain membrane homogenates as previously described for neocortex (Lai et al. 2003) before storage at −75°C. To maximize sampling consistency, only tissues from slices at the level of the lateral geniculate body (LGB) were taken, and the entire hippocampal formation (including CA1-3 and dentate gyrus) was used with no attempts to dissect individual hippocampal subfields. The contralateral hemisphere was processed for histological assessment, including confirmation of AD diagnosis by CERAD criteria (Mirra et al. 1991) and Braak staging (Braak and Braak 1991). In addition, 10-μm thick sections from paraffin-embedded blocks of hippocampal tissue at the level of LGB were stained with modified Palmgren/methanamine silver for the semi-quantitative assessment of senile plaques (SP) and neurofibrillary tangles (NFT) with scores of 0–3 equivalent to those described for neocortex (Chen et al. 1996). Lastly, determination of tryptophan hydroxylase (TH)-positive neuron density in the median raphe nucleus (MRN) by immunocytochemistry using a phenylalanine hydroxylase antibody known to cross-react with TH (Cotton et al. 1988) as previously reported (Chen et al. 2000) was used as a marker of serotonergic innervation. All subjects in this study were neuropathologically staged at Braak V–VI without significant cerebrovascular disease or hippocampal sclerosis.

Radioligand binding assays

All chemicals and reagents were from Sigma-Aldrich Co. USA unless otherwise stated. 5-HT_{1A}, 5-HT_{2A} receptors, and 5-HTT in the hippocampal membrane homogenates were measured by saturation binding assays using [^{3}H]8-OH-DPAT (specific activity 140–142 Ci/mmol, NEN Life Science Products, USA), [^{3}H]ketanserin (spec. act. 63 Ci/mmol, NEN), and [^{3}H]citalopram (spec. act. 83 Ci/mmol, Amersham Life Sciences, UK), respectively, according to previously published methods (see Table 1). Fluvoxamine maleate was obtained from Tocris Cookson Ltd., UK. Assay parameters for each radioligand are listed in Table 1. Briefly, brain membrane homogenates were thawed and diluted 1:6 *v*/*v* in specified assay buffer, then 100 μl aliquots of diluted homogenates were added to 1.0 ml macrowell tubes (Molecular Devices Inc. USA) containing six to seven concentrations of radioligand in triplicates in a total volume of 0.5 ml, followed by incubation at the specified temperature and duration. Non-specific binding was determined by setting up parallel series of tubes with the addition of specified unlabeled blockers and constituted less than 30% of total binding in all cases. Incubation was terminated by rapid filtration in a cell harvester (Molecular Devices Inc. USA) with ice-cold sodium phosphate buffer through 0.1% polyethylenimine-treated Whatman GF/B filters (Whatman BDS, UK). Filters were then dried and punched into scintillation vials, and membrane-bound radioactivity was measured by liquid scintillation spectrometry with a Wallac Beta counter. Scatchard transformation of data were performed using EBDA and LIGAND software (McPherson 1985) to calculate the binding affinity (K_D, in nM) and density (B_{max}, in fmol/mg protein) of the binding assays. Binding isotherms were found to be best fitted to single sites with Hill coefficients (N_H) around 1 for all cases. Finally, aliquots of diluted homogenate were used to determine protein concentration (Coomasie Plus, Thermo Fisher Scientific Inc. USA).

Statistical analyses

Data were first tested for normality for the selection of parametric or non-parametric tests. The neurochemical variables were found to be normally distributed, whereas TH neuronal counts were not (Kolmogorov–Smirnov tests using SPSS 13.0 for Windows software, SPSS Inc. USA). The effects of demographic factors (age, sex, and postmortem delay) on serotonergic neurochemical variables (K_D and B_{max}) of all subjects were determined by Pearson's correlation or Student's *t* tests. Within the AD group, disease factors (dementia severity denoted by the mean of last five MMSE scores, duration of disease, SP and NFT scores, chronic sedative hypnotic or neuroleptic medica-

Table 1 Conditions for radioligand binding assays in hippocampal homogenates to serotonin receptors (5-HT_{1A}, 5-HT_{2A}) and re-uptake sites

	5-HT_{1A}	5-HT_{2A}	5-HTT
Radioligand[a]	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]citalopram
Concentration range, nM	0.05–5.0	0.05–5.0	0.1–12
Assay buffer	50 mM Tris–HCl, pH 7.7	50 mM Tris–HCl, pH 7.7	50 mM Tris–HCl, pH 7.4
Unlabeled blocker[a]	5-HT maleate, 10 μM	5-HT maleate, 10 μM	Fluvoxamine, 10 μM
Incubation time, min	30	30	60
Incubation temperature, °C	37	37	25
Reference	Lai et al. 2002	Lai et al. 2005	Tsang et al. 2003

[a] Chemical names: citalopram, (*RS*)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile; fluvoxamine, (*E*)-5-methoxy-1-[4-(trifluoromethyl) phenyl] pentan-1-one *O*-2-aminoethyl oxime; 5-HT maleate, 5-hydroxytryptamine maleate; ketanserin, 3-{2-[4-(4-fluorobenzoyl) piperidin-1-yl] ethyl}quinazoline-2,4(1*H*,3*H*)-dione; 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin

tion) effects on neurochemical variables were investigated by parametric/non-parametric correlations or Student's *t* tests as appropriate. The neurochemical variables of control and AD patients were compared by Student's *t* tests. The AD subjects were then grouped according to the presence or absence of specific behaviors and compared with controls by analyses of variance (ANOVA) followed by Bonferroni's post hoc tests. Possible associations between median raphe TH-positive neuron densities (as a marker of serotonergic innervation) and neurochemical measures were further studied by Spearman's rank correlation. Finally, stepwise multiple regression analyses were performed to control for concomitant behaviors in individual patients and possible interactions between cognition and behavior. For all analyses, the null hypothesis was rejected at $p<0.05$.

Results

Effects of demographic and disease factors on hippocampal serotonergic variables

The subjects for this study were a maximum of 24 AD patients and 14 neurologically normal controls matched for age, sex, and postmortem delay (Table 2). Not all assays were performed on all subjects due to limited tissue availability, and actual *N* values for each assay are listed in parentheses in the Tables. The hippocampal binding affinities (K_D) and binding densities (B_{max}) for [^{3}H]8-OH-DPAT (5-HT_{1A}), [^{3}H]ketanserin (5-HT_{2A}), and [^{3}H]citalopram (5-HTT) were not affected by any of the demographic factors in controls and AD (Pearson's correlation or Student's *t* tests, $p>0.05$). Among the AD group, neurochemical variables were not affected by disease factors (Pearson's [disease duration] and Spearman [mean of last five MMSE, SP, NFT scores] $p>0.05$). Furthermore, except for lower [^{3}H]citalopram K_D in patients on neuroleptics, the neurochemical variables were not different between patients on psychotropic medication compared to patients not on such medication in the 8 months before death (Supplementary Table S1). Because of these findings, neuroleptic medication was included as a covariate in subsequent regression analyses.

Hippocampal serotonergic variables of controls vs. AD

Binding affinity (K_D) values of 5-HT_{1A} and 5-HT_{2A} receptors and 5-HTT sites in the hippocampal homogenates of AD patients were not significantly different from the controls (Table 2). For binding densities, the B_{max} values for 5-HT_{1A} receptors and 5-HTT in hippocampal homogenates were significantly reduced in AD by around 50% and 30%, respectively, compared to controls (Table 2). In contrast, reduction of 5-HT_{2A} receptor density did not reach statistical significance (Table 2).

Hippocampal serotonergic variables of controls vs. behavioral subgroups of AD

The K_D values for [^{3}H]8-OH-DPAT, [^{3}H]ketanserin, and [^{3}H]citalopram binding were not significantly different among controls and behavioral subgroups of AD patients (i.e., those rated "present" for a particular behavior and those rated "absent", one-way ANOVA, $p>0.05$, Table 3). For B_{max} values, Table 3 shows that 5-HT_{1A} receptors are unchanged between AD subgroups rated "present" versus "absent" for studied behaviors except for "apparent sadness", where receptor levels in "present" were significantly lower than "absent" as well as controls. In contrast, 5-HTT sites were unchanged in AD behavioral subgroups except for "physical aggression", where it was reduced in AD without aggression, but preserved in AD with aggression (Table 3). No changes were found in any AD behavioral subgroups for 5-HT_{2A} receptor densities (Table 3).

Table 2 Demographic, disease, and serotonergic neurochemical variables of controls and AD patients

Characteristics	Controls (n=14)	AD (n=24)
Demographic variables		
Age, years	76.4±3	81.7±2
Sex,% male	67%	50%
Postmortem delay, hours	47±10	40±5
Disease variables		
Disease duration, years	–	9.1±1
MMSE5	–	6.3±2
BPSD prevalence,%[a]		
Apparent sadness	–	58 (n=14)
Anxiety	–	33 (n=8)
Delusion/hallucination	–	33 (n=8)
Physical aggression	–	33 (n=8)
Hippocampal serotonergic variables		
5-HT_{1A} receptor		
K_D	1.3±0.2	1.5±0.2
B_{max}	220±18	124±15[b]
5-HT_{2A} receptor		
K_D	1.6±0.2	1.3±0.1
B_{max}	82±12	67±7
5-HTT		
K_D	2.5±0.4	2.9±0.4
B_{max}	121±13	87±9[b]

Values are mean ± SEM

B_{max} binding density in fmol/mg protein, *BPSD frequency* the percentage of patients within the cohort with significant behavioral symptoms (see text), *5-HTT* serotonin transporters (re-uptake sites), K_D binding affinity in nM, *MMSE5* mean of last five MMSE scores used as an indicator of predeath dementia severity (Lai et al. 2001), *n* maximum numbers of subjects

[a] Thirteen patients were rated positive for two or more behaviors

[b] Significantly different from controls (Student's t test, $p<0.05$)

Correlations of median raphe neuron densities with neurochemical variables

We have previously shown that TH-positive MRN densities are reduced in AD (Chen et al. 2000), with MRN data for the subjects comprising the current study presented in Supplementary Figure S1. Because MRN efferents are the main source of serotonergic input to the hippocampus (Molliver 1987), our data indicate a loss of serotonergic innervation of the hippocampus. K_D values of 5-HT_{1A}, 5-HT_{2A} and 5-HTT binding did not correlate with MRN densities (defined as the number of TH-positive neurons per square millimeter by immunohistochemistry as previously described by Chen et al. 2000). For B_{max} values, only 5-HTT sites were positively correlated with MRN densities (Fig. 1).

Multiple regression analyses

Stepwise multiple regression analyses showed that of the disease factors studied (presence of behaviors, neuroleptic medication, dementia severity, disease duration, SP and NFT scores), only "apparent sadness" predicted the variability of hippocampal 5-HT_{1A} receptor density (B_{max}; adjusted R^2=0.225, β=−0.51, p=0.017). Furthermore, only "neuroleptic medication" predicted the variability of 5-HTT binding affinity (K_D; adjusted R^2=0.162, β=−0.45, p=0.04). None of the other neurochemical variables were significantly correlated with disease factors (stepwise multiple regression, $p>0.05$).

Discussion

To date, at least 13 subtypes of 5-HT receptors have been characterized, making the serotonergic system one of the most anatomically and functionally diverse transmitter systems in the CNS (Hoyer et al. 2002). Previous preclinical and clinical studies have pointed to roles in affect, learning, and memory for many of these subtypes (Barnes and Sharp 1999), and we hypothesized that alterations of several receptors, including the 5-HT_{1A}, 5-HT_{2A} subtypes as well as serotonin re-uptake sites (5-HTT), may underlie the cognitive and behavioral features of the disease. In this study, we uncovered losses of 5-HT_{1A} receptors and 5-HTT sites in the hippocampus of AD, which are differentially involved in BPSD. Firstly, alterations of hippocampal 5-HT_{1A} receptors seem to be a specific neurochemical substrate of depression, as it is significantly reduced only in the subgroup of patients with significant depression (Table 3). This finding is comparable to reported reductions of 5-HT_{1A} receptors in the forebrain (including the limbic system) of patients with major depressive disorder (Savitz et al. 2009). Furthermore, people with lifelong depression have increased risk of developing AD (Sierksma et al. 2010), suggesting that different (but overlapping) disease processes leading to a similar state of disturbed 5-HT_{1A} receptor-mediated neurotransmission in the forebrain may likewise manifest clinically as depressive symptoms. In contrast, loss of 5-HTT is significant only in AD without aggression, but levels are unchanged in patients rated positive for aggression, suggesting a preservation or up-regulation of 5-HTT sites analogous to that reported in temporal cortex for anxiety in AD (Tsang et al. 2003). We have previously shown that homozygosity for the long variant of the serotonin transporter promoter region (5-HTTPR *L/*L) correlated with increased 5-HTT expression in AD temporal cortex, which was thought to account for the relative preservation of 5-HTT in the anxious AD subgroup under conditions of presynaptic serotonergic

Table 3 Serotonergic neurochemical variables in hippocampal homogenates of controls and behavioral subgroups of AD

Behavior	5-HT$_{1A}$		5-HT$_{2A}$		5-HTT	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
Control[a]	1.3±0.2 (13)	220±18 (13)	1.7±0.2 (13)	82±12 (13)	2.5±0.4 (13)	121±13 (13)
Apparent sadness						
Absent	1.4±0.3 (10)	173±26 (10)	1.5±0.2 (10)	70±7 (10)	2.8±0.7 (10)	79±10 (10)
Present	1.6±0.2 (14)	88±11 (14)[c]	1.2±0.2 (13)	66±11 (13)	3.0±0.6 (14)	93±14 (14)
Anxiety						
Absent	1.6±0.2 (16)	133±20 (16)[b]	1.4±0.2 (15)	65±6 (15)	2.8±0.5 (16)	90±10 (16)
Present	1.4±0.2 (8)	104±20 (8)[b]	1.0±0.1 (8)	73±16 (8)	3.2±1.0 (8)	81±20 (8)
Delusion/Hallucination						
Absent	1.4±0.2 (16)	119±21 (16)[b]	1.3±0.2 (15)	66±8 (15)	2.7±0.3 (16)	91±11 (16)
Present	1.8±0.4 (8)	132±19 (8)[b]	1.2±0.1 (8)	70±12 (8)	3.4±1.1 (8)	78±18 (8)
Physical aggression						
Absent	1.8±0.2 (16)	137±21 (16)[b]	1.2±0.2 (15)	71±9 (15)	3.1±0.6 (16)	77±11 (16)[b]
Present	1.1±0.1 (8)	96±16 (8)[b]	1.4±0.2 (8)	62±10 (8)	2.6±0.4 (8)	106±14 (8)

Values are mean ± SEM (n, number of subjects). The maximum n of available subjects in each behavioral category are listed in Table 2

B_{max} binding density in fmol/mg protein, *5-HT$_{1A}$* serotonin 5-HT$_{1A}$ receptors, *5-HT$_{2A}$* serotonin 5-HT$_{2A}$ receptors, *5-HTT* serotonin transporters (re-uptake sites), *K_D* binding affinity in nM

[a] Neurochemical variables for controls are compared to behavioral subgroups of each behavior (apparent sadness, anxiety, delusion/hallucination and physical aggression)

[b] Significantly different from control group (one-way ANOVA with Bonferroni post hoc, $p<0.05$)

[c] Significantly different from control and "absent" groups (one-way ANOVA with Bonferroni post hoc, $p<0.01$)

deficits (Tsang et al. 2003). Interestingly, Sukonick et al. (2001) reported an association of 5-HTTPR *L/*L with aggressive behaviors in AD, and further work is needed to determine if this association is mediated via preserved or up-regulated 5-HTT expression in the hippocampus. In contrast with previous studies on other forebrain regions (see "Introduction"), hippocampal 5-HTT and 5-HT$_{1A}$ receptor changes were not associated with behaviors like anxiety and psychosis, suggesting regional specificity in the neurochemical correlates of behaviors. For 5-HT$_{2A}$ receptors, we found non-significant trends toward decreased levels in the hippocampus, in agreement with a previous study using [^{3}H]ketanserin binding (Cross et al. 1984a). This is contrasted with the neocortex, where there are consistent findings of substantial 5-HT$_{2A}$ loss (Cross et al. 1984a, 1986; Lai et al. 2005). One reason for this difference, besides regional specificity of receptor changes, could be the relatively low levels of 5-HT$_{2A}$ receptors in the hippocampus compared to neocortex (Cross et al. 1984a; Lai et al. 2005), which may lead to reduced sensitivity in detecting significant receptor changes. This may also explain the absence of association between cognition or dementia severity and 5-HT$_{2A}$ receptors in the hippocampus, in contrast to findings in the temporal cortex (Lai et al. 2005). Another reason could be the inability of the present study to detect hippocampal subfield-specific changes since the entire hippocampal formation (CA1–CA3 and dentate gyrus) was used in the homogenate binding studies.

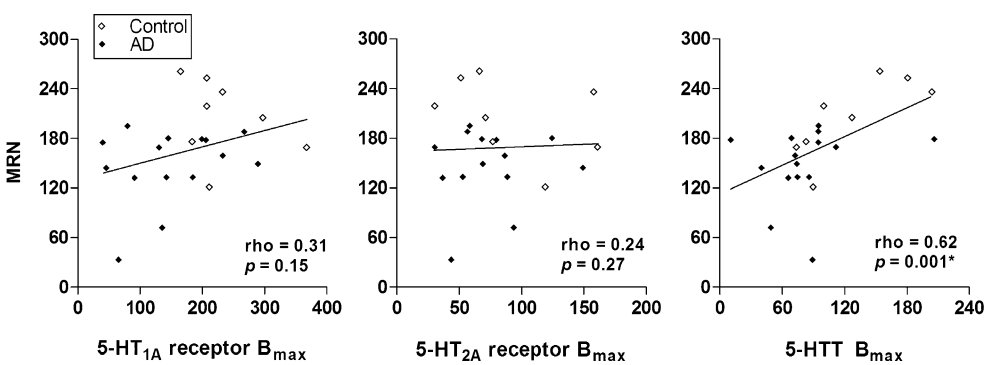

Fig. 1 Correlations of TH-positive median raphe neuronal densities (MRN, in neuron counts per mm^2) with hippocampal serotonergic receptor and transporter binding densities (B_{max}, in fmol/mg protein). Linearly regressed best-fit trend lines are given for all available subjects (controls, n=8 and AD, n=15). *Significant Spearman's correlation

Therefore, further studies using autoradiographic or imaging approaches may be needed to address current limitations.

Given that 5-HT_{1A} receptors are localized presynaptically, as somatodendritic autoreceptors, and postsynaptically (Barnes and Sharp 1999), it may be worthwhile to further consider its status in depressed AD patients and hypothesize on the potential mechanisms of the observed receptor deficits. In the hippocampus, 5-HT_{1A} receptors are found to be mainly postsynaptic, where their activation results in inhibition of CA1 pyramidal neurons (Jacobs and Azmitia 1992; Burnet et al. 1995; DeFelipe et al. 2001). In our study, we investigated the status of serotonergic innervation using MRN neuronal densities, as axonal projections from MRN are known to form the bulk of the serotonergic input in hippocampus (Molliver 1987). In concurrence with this postulate, measurements of dorsal raphe neuronal densities did not correlate with any of the measured serotonergic variables (data not shown). In contrast, MRN densities expectedly correlated with presynaptic 5-HTT, but not postsynaptic 5-HT_{2A} receptor levels in the postmortem hippocampus (Fig. 1). In the case of 5-HT_{1A} receptors, a lack of significant correlation with serotonergic innervation from MRN (similar to 5-HT_{2A} receptors) suggests that at least a proportion of labeled receptors may likewise be postsynaptic, which may be altered through mechanisms of synaptic plasticity. Furthermore, changes in raphe neuronal densities per se were not associated with BPSD in AD (Chen et al. 2000). Therefore, 5-HT_{1A} reduction in AD hippocampus does not merely reflect the loss of serotonergic innervation but may be a specific neurochemical alteration associated with increased risk of significant depressive symptoms in a subgroup of patients. It should also be noted that because binding densities are measured per unit of brain protein, the observed receptor losses are unlikely to be simply a result of atrophy but represent relative loss of receptor binding in remaining neurons. Interestingly, functional imaging and postmortem studies on patients with major depression have found decreased 5-HT_{1A} receptor binding both in raphe nuclei as well as in the neocortex and hippocampus, along with reduced 5-HT_{1A} messenger RNA in the prefrontal cortex and hippocampus (Drevets et al. 1999; Francis et al. 1993; López-Figueroa et al. 2004; Sargent et al. 2000). There is evidence that 5-HT_{1A} receptor gene expression is down-regulated by cortisol hypersecretion, another well-established finding in major depression (Meijer et al. 1997). Interestingly, hypercortisolemia is also evident in AD and correlates with clinical progression (Giubilei et al. 2001; Weiner et al. 1997). Thus, it is possible that cortisol dysregulation may underlie 5-HT_{1A} receptor alteration in AD. However, recent work suggest that the status of 5-HT_{1A} receptors in AD brain is rather complex, as overexpression of 5-HT_{1A} receptors is found in minimal cognitive impairment, a condition generally thought to be prodromal for AD, followed by decreases as AD ensues (Truchot et al. 2007). Furthermore, injection of β-amyloid peptides into rodent dorsal hippocampus leads to transient increases in 5-HT_{1A} receptors, likely expressed by astroglial cells to compensate for local neuron loss (Verdurand et al. 2009). Lastly, Sharp et al. (2008) found increased binding to 5-HT_{1A} receptors in the temporal cortex of depressed Parkinson's disease dementia/dementia with Lewy Bodies patients via a mechanism that may involve alterations of receptor binding affinity. Because our approach is limited by the use of postmortem tissue, which allows a one-time measurement usually at late stages of disease, further work is needed to delineate the various disease-specific mechanisms, which may interact with 5-HT_{1A} receptor expression and function in AD and other dementias.

Conclusion

By using postmortem tissues and clinical data from a well-characterized cohort of prospectively assessed, longitudinally followed-up AD patients, we have found decreases in hippocampal 5-HT_{1A} receptors and 5-HTT sites that are differentially associated with neuropsychiatric behaviors. Specifically, losses of 5-HT_{1A} receptors are correlated with depressive symptoms, whereas 5-HTT sites are preserved or up-regulated in patients with aggressive behaviors. Our data suggest that neuropsychiatric behaviors in AD share certain neurochemical features with psychiatric disorders, such as major depression and that distinct or overlapping disease processes leading to similar neurochemical alterations (e.g., 5-HT_{1A} receptor deficits) may manifest clinically as behavioral symptoms in both conditions. The implication of our findings in terms of rational therapeutic strategies would be that serotonergic drugs used in psychiatric orders may also be efficacious against BPSD, for example, 5-HT_{1A} ligands and selective serotonin re-uptake inhibitors for depressive symptoms and aggression in AD. Indeed, there is already some evidence supporting the use of such compounds (Ballard and Waite 2006; Thompson et al. 2007). However, the status of serotonergic changes in AD is complex anatomically as well as chronologically and may be subject to genetic or hormonal regulatory processes, necessitating further longitudinal imaging (e.g., positron emission tomography), gene polymorphism, and endocrine studies to delineate serotonergic changes in the AD brain as well as the underlying mechanisms.

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