Research article

Serotonin 5-HT\textsubscript{2A} and 5-HT\textsubscript{6} receptors in the prefrontal cortex of Alzheimer and normal aging patients

Dietrich E Lorke\textsuperscript{1,2}, Gang Lu\textsuperscript{2,3}, Eric Cho\textsuperscript{2} and David T Yew*\textsuperscript{2}

Address: \textsuperscript{1}Department of Anatomy, Faculty of Medicine and Health Sciences, UAE University, Box 17666, Al Ain, United Arab Emirates, \textsuperscript{2}Department of Anatomy, Chinese University of Hong Kong, Shatin, N.T., Hong Kong and \textsuperscript{3}Kunming Medical College, Kunming, Yunnan, China

Email: Dietrich E Lorke - lorke@uaeu.ac.ae; Gang Lu - lugang@surgery.cuhk.edu.hk; Eric Cho - eric-cho@ana.cuhk.edu.hk; David T Yew* - david-yew@cuhk.edu.hk

* Corresponding author

Published: 27 April 2006
BMC Neuroscience 2006, 7:36 doi:10.1186/1471-2202-7-36

Received: 12 December 2005
Accepted: 27 April 2006

This article is available from: http://www.biomedcentral.com/1471-2202/7/36

© 2006 Lorke et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Abstract

Background: It has been hypothesized that alterations of the serotonergic system contribute to neuropsychiatric symptoms in Alzheimer disease (AD). Cellular expressions of the two serotonergic receptors 5-HT\textsubscript{2A} and 5-HT\textsubscript{6} have therefore been determined by immunohistochemistry in the prefrontal cortex of patients with AD (n=6) and normal age-matched controls (n = 7).

Results: In normal aging patients, 5-HT\textsubscript{2A} label was mainly observed in large pyramidal cells, but to a lesser extent also in small pyramidal cells and in stellate cells of cortical layers II-VI. In AD, a similar distribution was observed, but density of positive cells was significantly reduced by 33%. In aging control patients, the 5-HT\textsubscript{6} receptor was expressed by pyramidal cells and occasional stellate cells, not only of layers II-V, but also of layer I, where a distinct label was observed in neurons and surrounding fibers. 5-HT\textsubscript{6} receptor expression in AD patients had the same pattern, but was significantly decreased by 40%.

Conclusion: Our results indicate that a decline in neurons expressing 5-HT\textsubscript{2A}, but also 5-HT\textsubscript{6} receptors may play a role in the etiopathology of neuropsychiatric symptoms in AD.

Background

Alzheimer disease (AD), the most common cause of dementia in the elderly, is clinically characterized by progressive cognitive impairment associated with severe neuropsychiatric disturbances. These behavioral and psychological symptoms of dementia (BPSD) include hallucinations, delusions, aggressive behavior, overactivity, anxieties and affective disturbances [1,2]. Whereas the decline in cognitive functions can be largely related to cholinergic dysfunction arising from disruption of basal forebrain cholinergic pathways (cholinergic hypothesis) [1,3,4], impaired balance between several neurotransmitters has been implicated in the pathogenesis of BPSP [2,5-7], with serotonin (5-HT) playing a pivotal role [2,8-10]. The actions of 5-HT are mediated through seven major receptors classes, 5-HT\textsubscript{1-7}, comprising a total of 14 distinct mammalian 5-HT receptor subtypes (for review, see [11]). The 5-HT\textsubscript{2A} receptor has attracted most interest because of its possible participation in behavioral alterations in AD. 5-HT\textsubscript{2A} is localized in the cortex and caudate and is involved in anxiety [10]. 5-HT\textsubscript{2A} receptors mediate the psychotomimetic effects of hallucinogens [11-13], and alterations in binding characteristics to this receptor have been observed in the prefrontal cortex of patients suffer-
ing from psychiatric diseases, e.g. schizophrenia [14,15], depression [16] and suicide [17]. Electrophysiological evidence suggests that 5-HT$_{2A}$ receptors are involved in the 5-HT$_{2A}$-induced increase in excitatory postsynaptic potentials [12,18] and play an important role in the working memory process [13]. There is indication that 5-HT$_{2A}$ also participates in the etiopathology of AD. Serotonin increases the secretion of amyloid precursor protein (APP) through activation of 5-HT$_{2A}$ receptors [19]. 5-HT$_{2A}$ receptor binding is decreased in AD (for review, see [9,10]), and polymorphic variations have been described for the 5-HT$_{2A}$ gene that may be risk factors for hallucinations [20], aggression [21] and major depression [22] in AD. Much less is known about the 5-HT$_{6}$ receptor, the most recent 5-HT receptor to be identified. Many antidepressants and antipsychotics are antagonists of the 5-HT$_{6}$ receptor [15,23]. It has been shown to influence acetylcholine release in the frontal cortex [24] and may play a role in cognition deficits and in some form of anxiety [23]. A recent autoradiographic study examining [125I]-SB-258585 binding in autopsy specimens of AD patients indicates lowered 5-HT$_{6}$ receptor density in the frontal and temporal cortices [5]. Association of a 5-HT$_{6}$ receptor gene polymorphic variant and late-onset AD has been observed in the Chinese population [25], but not in Germans [26].

Surprisingly, although 5-HT$_{2A}$ and 5-HT$_{6}$ receptor binding in AD has been studied extensively [2,5,9,10], there is no information on the expression of these receptors at the cellular level. We have therefore examined the expression of the 5-HT$_{2A}$ and 5-HT$_{6}$ receptors in the brains of AD and of normal aging control patients by immunohistochemistry. Because the prefrontal cortex is of particular importance for the etiopathology of BPSD [1,8,12,14,16,27-31] and because neuropsychiatric symptoms in AD are associated with reduced metabolism [32,33] and perfusion [34] in Brodmann area 10, we have chosen this brain region for examination.

**Methods**

**Tissue samples**

The brains were obtained from a total of 13 autopsy cases with the consent of a close relative and with full approval by the ethical committees of Guangzhou hospital authorities and of the Chinese University of Hong Kong. Six of the specimens were from individuals with clinically and pathologically diagnosed senile dementia of the Alzheimer type (mean age: 88 years, see table 1), seven specimens were from individuals, matched for age, gender and postmortem delay, who had no history of neurological diseases (normal control). Drug history was recorded for all patients, who had only received antibiotic treatment, but no psychopharmacological medication. Alzheimer Disease was clinically diagnosed according to the "National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association" (NINCDS-ADRA) criteria and the "Diagnostic and Statistical Manual of Mental Disorders", Fourth Edition, (DSM-IV-R) criteria.

Whole brains were removed with an average postmortem delay of six hours, and tissue samples of the anterior prefrontal cortex (Brodmann’s area 10) were dissected. Specimens were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4), dehydrated in graded ethanol, cleared with xylene and embedded in paraffin. 6-μm-thick serial sections were cut in the coronal plane, 90° perpendicular to the surface to avoid sectioning artifacts. Two sets of slides were obtained for each individual.

**Immunohistochemistry**

All steps were carried out at room temperature unless stated otherwise. Mounted sections were dewaxed, rehy-

---

**Table 1: Profiles of Alzheimer (AD) and matched normal aging patients**

| Subject | Diagnosis | Sex | Age | Cause of death          |
|---------|-----------|-----|-----|-------------------------|
| 1       | Normal    | F   | 83  | Pneumonia               |
| 2       | Normal    | F   | 86  | Gastrointestinal bleeding |
| 3       | Normal    | M   | 82  | Pneumonia               |
| 4       | Normal    | M   | 93  | Sudden death            |
| 5       | Normal    | F   | 72  | Sudden death            |
| 6       | Normal    | F   | 85  | Pneumonia               |
| 7       | Normal    | F   | 81  | Pneumonia               |
| 8       | Alzheimer | M   | 82  | Subarachnoid hemorrhage  |
| 9       | Alzheimer | F   | 91  | Septicemia              |
| 10      | Alzheimer | F   | 96  | Pneumonia               |
| 11      | Alzheimer | F   | 82  | Pneumonia               |
| 12      | Alzheimer | F   | 86  | Pneumonia               |
| 13      | Alzheimer | F   | 89  | Sudden death            |
drated and predigested with 0.1% trypsin (BDH Laboratory Supplies, Poole, U.K.) in 0.05M tris buffered saline containing 0.1% CaCl₂ (pH 7.6; 37°C; 20 min) followed by two rinses (5 min) in 0.01M PBS for antigen retrieval. The endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in absolute methanol for 30 min, followed by another three rinses in PBS (5 min each). To suppress non-specific binding, sections were then incubated for 1h in 2 % normal blocking serum (Vectastain® ABC Kit, Vector Laboratories, Burlingame, CA) in 0.3 % triton/PBS. Thereafter, sections were incubated overnight with the primary polyclonal antibodies: either goat anti-human serotonin 2A receptor (Santa Cruz sc-15073, Santa Cruz Biotechnology Inc., Sta. Cruz, CA) or goat anti-human serotonin 6 receptor (Santa Cruz sc-26668). The sections were then washed with three rinses of PBS containing 0.05 % Tween 20 (5 min each) and incubated with biotinylated secondary antibody in blocking solution (1:200) for 30 min (PK6105, anti-goat IgG, Vectastain® ABC Kit, Vector Laboratories, Burlingame, CA). Sections were washed three times in PBS again (5 min) and incubated with ABC Reagent (Vectastain® ABC Kit, Vector Laboratories, Burlingame, CA) for 30 min. The immunocytochemical staining signals were induced by incubating the sections in 0.05% of the substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) in PBS containing 0.01% H₂O₂. All stainings included negative controls with omission of the primary antibody, which did not show any immunoreaction. The sections were washed with distilled water and then counterstained with 0.5% cresyl fast violet in 0.1M sodium acetate (pH 3.5, 5 min). They were differentiated with 95% ethanol, dehydrated, cleared and covered in Permount® (Fisher Scientific, Hampton, VA). Additional sections were stained with routine methods, including H&E, Nissl and Bielschowsky silver impregnation for tangle staging [35,36].

Statistics
For qualitative and quantitative evaluation, sections were observed under a photomicroscope (Axioplan 2 Photomicroscope, Zeiss, Germany). The number of 5-HT₂A receptor immunoreactive cells was counted in 30 random selected 700 µm² fields of normal aged and AD patients. 4–5 sections per individual were evaluated, sparing areas containing amyloid plaques. It has been shown in AD patients [38] that astrocytes in and around Aβ plaques are strongly positive for 5-HT₂A receptor protein, whereas astrocytes in control patients do not display any 5-HT₂A immunoreaction. Amyloid plaques were therefore excluded from the quantitative assessment, because the aim of our study was to count neurons expressing 5-HT receptors. Measurements were expressed as means ± SEM. For statistical comparison, the values were subjected to a one-way analysis of variance (ANOVA), demonstrating that the four data groups exhibited equal variance, but were not distributed normally. We have then applied the One Way non-parametric ANOVA (Kruskal-Wallis test) and the Mann Whitney Rank sum test, yielding the same results. A p value below 0.05 was considered significant.

Results
In the prefrontal cortex of normal aging patients, 5-HT₂A receptor immunoreaction was observed in cortical layers II-VI. Large pyramidal neurons of layer V constituted the majority of immunoreactive cells, characterized by a strongly labeled cytoplasm and a moderately stained proximal part of the apical dendrite (Figs. 1a, c). In addition, smaller pyramidal cells and scattered stellate-shaped cells in the other layers were immunoreactive. Stellate cells had a strongly stained cytoplasm and labeled thin processes radiating to the side of the ovoid cell bodies and most likely represented interneurons. A few multipolar cells in layer VI were also 5-HT₂A receptor positive. In the underlying white matter, occasional 5-HT₂A receptor immunoreactive fiber bundles were observed. AD patients showed a similar distribution of 5-HT₂A receptor label in the prefrontal cortex, i.e. staining in the cell bodies and apical dendrites of large pyramidal cells and in the soma and tender processes of a few scattered interneurons (Figs. 1b, d). However, numerical density of 5-HT₂A receptor immunoreactive cells was significantly (p=0.001) decreased by 33% in the frontal cortex of AD patients (Fig. 2), as compared to normal aging patients. Reduction affected both pyramidal cells and cells of stellate morphology. In addition, labeled cells were also seen within and close to amyloid plaques. An association between 5-HT₂A receptors and NFT was not observed.
Figure 1
5-HT$_{2A}$ receptor. Immunoreaction for the 5-HT$_{2A}$ receptor in the prefrontal cortex of a normal aging (a, c) and an Alzheimer patient (b, d), cresyl violet counterstain. Both large pyramidal cells (arrowheads) and small interneurons (arrows) are stained. Note the reduction in labeled cells in the cortex of the Alzheimer patient (b, d). a, b: x200; c, d: x400.
was no association between 5-HT6 receptors and NFT. 

dal and stellate cells were decreased by about 40%. There compared to normal aging patients (Fig. 2). Both pyramidal reactive cells was again significantly (p=0.001) reduced, as different from that of the 5-HT2A receptor. As for the 5-HT2A receptor, the most prominent label was observed in the cell bodies and the proximal apical dendrites of large pyramidal neurons, and occasionally, the soma and fine processes of scattered stellate cells were stained as well (Figs. 3a, c). In addition, 5-HT6 receptor immunoreaction was also detected in neurons of the molecular layer (layer 1). These cells had a bipolar strongly labeled cell body and numerous immunoreactive processes radiating into the molecular layer (Fig. 3d). In contrast, hardly any 5-HT6 receptor immunoreactive cells were observed in layer VI. Occasional immunoreactive fiber bundles were seen in the underlying white matter. In summary, density of 5-HT6 receptor positive neurons was significantly higher than that of 5-HT2A receptor immunoreactive cells (p=0.05). In the prefrontal cortex of AD patients, 5-HT6 receptor label showed the same overall distribution (Figs. 3b, e), but numerical density of 5-HT6 receptor immunoreactive cells was again significantly (p=0.001) reduced, as compared to normal aging patients (Fig. 2). Both pyramidal and stellate cells were decreased by about 40%. There was no association between 5-HT6 receptors and NFT.

Discussion

The present study has been undertaken in order to determine changes in cellular distribution of two serotonin receptors, 5-HT2A and 5-HT6, in the prefrontal cortex of AD patients as compared to normal age-matched individuals. Our observation in aging (control) patients, showing 5-HT2A immunoreactivity in the cell bodies and the apical dendrites of pyramidal cells as well as in stellate-shaped cells, is consistent with previous studies in young humans [17], primates [18,39] and rodents [40,41]. However, we have detected relatively few labeled cells, and we are currently testing if the paucity in 5-HT2A receptors is attributable to the very advanced age of our patients or to the technique employed. A considerable age-related reduction in the number of cortical 5-HT2A binding sites in the frontal lobe has been described by numerous authors (see [9] for review). In contrast to 5-HT2A, relatively little is known on the distribution of the 5-HT6 receptor in the mammalian neocortex. 5-HT6 receptor binding sites have been detected by receptor autoradiography in the gray matter of the prefrontal cortex of healthy human adults [5,42], but there are no studies on the cellular level. We have been able to show that the 5-HT6 receptor is expressed both by pyramidal cells and by stellate-shaped cells located in cortical layers I-V. We have observed very little 5-HT6 immunoreaction in layer VI, but a distinct 5-HT6 label in layer I, with staining detected not only in a dense network of fibers, but also in neurons. This is somewhat at variance with a study on the rat neocortex, where 5-HT6 receptor mRNA has been detected by in-situ hybridization in layers II-VI, but not in layer I [43]. This discrepancy is, however, not surprising, given the marked interspecies differences described [44].

To our knowledge, this is the first immunohistochemical study of 5-HT2A receptor expression in AD. The reduction observed corroborates the results of previous 5-HT2A receptor binding studies showing decreased binding of [3H] ketanserin [45-47] and [3H] spiperone [48] in post-mortem specimens of the frontal cortex of AD patients and in PET imaging studies [49]. Contrasting reports describing unaltered [3H] ketanserin binding [50], which are at variance with our results, may be attributable to recent psychotropic medication, which had not been administered to our patients. Reduced 5-HT2A receptor binding in AD has been explained by a loss of interneurons [51]. In contrast, our immunohistochemical data indicate that both 5-HT2A immunoreactive pyramidal and stellate-shaped cells, i.e. interneurons, are affected in AD. Moreover, we have demonstrated that the density of neurons expressing the 5-HT6 receptor is reduced to a similar extent, corroborating autoradiographic studies revealing decreased binding to 5-HT6 receptors in the prefrontal cortex of AD patients [5]. Our observation of reduced density of neurons expressing 5-HT2A and 5-HT6 receptors, which is not accompanied by similar alterations in GABAergic markers [6], points at a selective vulnerability of neurons receiving serotonergic input. This decrease is associated with several other abnormalities of the serotonergic system in AD. A marked depletion in 5-HT and its metabolite 5-Hydroxyindole Acetic Acid (5-HIAA) has been
described in the frontal and temporal cortices of AD patients [2,28,52], which is most likely attributable to a reduction in serotonergic projection fibers. The underlying cause appears to be a significant loss of neurons in the dorsal and median raphe nuclei [53,54], which are the source of serotonergic nerve terminals and which, in addition, are a preferential site for neurofibrillary tangle formation [55]. It is very likely that the decrease in neurons expressing 5-HT_{2A} and 5-HT_{6} receptors is related to this loss of serotonergic fibers. However, it may either reflect a process of primary cortical degeneration [4,56] leading to presynaptic disturbance of the serotonergic system, or be secondary to the decrease in serotonergic afferents.

Several lines of evidence indicate that serotonergic dysfunction in AD has important functional consequences. In a study using retrospective data, loss in 5-HT_{2A} binding has been confined to AD patients with aggressive symp-
toms [57]. When cognitive function is assessed antemortem, 5-HT<sub>2A</sub> receptors are lost in the frontal and temporal cortex only in patients with severe, but not with mild to moderate dementia [47]. The decrease in the expression of 5-HT<sub>6</sub> receptors in the prefrontal cortex of AD patients can be correlated to the extent of aggressive behavior [5]. The number of 5-HT uptake sites is significantly reduced in the frontal and temporal cortex of AD patients with persistent depression, anxiety and overactivity, compared with AD patients without these symptoms [52], and 5-HT levels in the prefrontal cortex (Brodman 10) of AD correlate with overactivity [2].

Conclusion

Our results of normal aging patients, showing that 5-HT<sub>2A</sub> and 5-HT<sub>6</sub> receptors are expressed by both pyramidal cells and stellate-shaped neurons, indicate that serotonergic fibers exert their influence upon these two neocortical cell types not only through the 5-HT<sub>2A</sub> but also the 5-HT<sub>6</sub> receptor. The significant 33–40% reduction in cells immunoreactive for 5-HT<sub>2A</sub> and 5-HT<sub>6</sub> receptors observed in AD patients, affecting both large pyramidal cells and interneurons, points at a severely compromised serotonergic system in AD, involving not only serotonergic projection fibers, but also their corresponding receptors. This decline in receptors most likely contributes to the development of neuropsychiatric symptoms in AD.

Authors’ contributions

Dietrich E. Lorke performed the evaluation of the slides, interpreted the results and wrote the manuscript. Gang Lu performed the quantitative and statistical analyses. Eric Cho performed the immunohistochemistry and documented the results. Dietrich E. Lorke performed the neuropathological examination and evaluated the slides. Dietrich E. Lorke conceived and planned the study, performed the pathological examination and evaluated the slides.

Abbreviations

AD: Alzheimer disease
BPDS: behavioral and psychological symptoms of dementia
5-HT: serotonin
5-HT<sub>2A</sub>: serotonergic receptor 2A
5-HT<sub>6</sub>: serotonergic receptor 6
NFT: neurofibrillary tangles
PBS: phosphate-buffered saline
SEM: standard error of the mean

Acknowledgements

The authors wish to thank Samuel Wong for skilful assistance in preparing the photographs and Stephanie Yeung for typing the manuscript.

References

1. Cummings JL, Back C: The cholinergic hypothesis of neuropsychiatric symptoms in Alzheimer’s disease. Am J Geriatr Psychiatry 1998, 6(Suppl 1):S64-S78.
2. Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M, Chen CP, Francis PT, Lasheras B, Ramirez MJ: Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer’s disease. Neuropsychologia 2005, 43:442-449.
3. Giacobini E: Cholinergic function and Alzheimer’s disease. Int J Geriatr Psychiatry 2003, 18(Suppl 1):S1-S5.
4. Arendt T: Synaptic plasticity and cell cycle activation in neurons are alternative effector pathways: the ‘Dr. Jekyll and Mr. Hyde concept’ of Alzheimer’s disease or the yin and yang hypothesis of Alzheimer’s disease. J Mol Psychiatry 2002, 7:1-13.
5. Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT, Ramirez MJ: Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer’s disease. Neuropsychopharmacology 2004, 29:410-416.
6. Yew DT, Li WP, Webb SE, Lai HW, Zhang L: Neurotransmitters, peptides, and neural cell adhesion molecules in the cortices of normal elderly humans and Alzheimer patients: a comparison. Exp Gerontol 1999, 34:117-133.
7. Francis PT, Palmer AM, Snape M, Wilcock GK: The cholinergic hypothesis of Alzheimer’s disease: a review of progress. J Neurol Neurosurg Psychiatry 1999, 66:137-147.
8. Marek GJ, Aghajanian GK: The electrophysiology of prefrontal serotonin systems: therapeutic implications for mood and psychosis. Biol Psychiatry 1999, 44:1118-1127.
9. Maritz CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kuperf DJ, Reynolds CF II: Serotonin in aging, late-life depression, and Alzheimer’s disease: the emerging role of functional imaging. Neuropsychopharmacology 1998, 18:407-430.
10. Barnes NM, Herrmann N, Mazzotta P: Role of serotonin in the behavioral and psychological symptoms of dementia. J Neuropsychiatry Clin Neurosci 2001, 13:5-21.
11. Barnes NM, Sharp T: A review of central 5-HT receptors and their function. Neuropharmacology 1999, 38:1083-1152.
12. Aghajanian GK, Marek GJ: Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Brain Res Rev 2000, 31:302-12.
13. Williams GV, Rao SG, Goldman-Rakic PS: The physiological role of 5-HT2A receptors in working memory. J Neurosci 2002, 22:2843-2854.
14. Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, Opeskin K, Copolov DL: Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J Neurochem 1999, 72:1593-1599.
15. Meltzer HY, Li Z, Kaneda Y, Ichikawa J: Serotonin receptors: their key role in drugs to treat schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003, 27:1159-1172.
16. Celada P, Puig MV, Amargó-Bosch M, Adell A, Artigas F: The therapeutic role of 5-HT(1A) and 5-HT(2A) receptors in depression. Rev Psychiatr Neurosci 2004, 29:232-265.
17. Pandey GN, Dwivedi Y, Rizvi HS, Ren X, Pandey SC, Pesold C, Roberts RC, Conley RR, Tamminga CA: Higher expression of serotonin 5-HT(2A) receptors in the postmortem brains of teenage suicide victims. Am J Psychiatry 2002, 159:419-429.
18. Jakab RL, Goldman-Rakic PS: 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. Proc Natl Acad Sci U S A 1998, 95:735-740.
19. Nitsch RM, Deng M, Growdon JH, Wurtman RJ: Serotonin5-HT2A and 5-HT2C receptors stimulate amyloid precursor protein ectodomain secretion. J Biol Chem 1996, 271:4188-4194.
20. Holmes C, Arranz MJ, Powell JF, Collier DA, Lovestone S: 5-HT2A and 5-HT2C receptor polymorphisms and psychopathology in late onset Alzheimer’s disease. Hum Mol Genet 1998, 7:1507-1509.
21. Assal F, Alarcon M, Solomon EC, Masterman D, Geschwind DH, Cummins JL: Association of the serotonin transporter and receptor gene polymorphisms with neuropsychiatric symptoms in Alzheimer disease. Arch Neurol 2004, 61:1249-1253.

22. Holmes C, Arranz M, Collier D, Powell J, Lovestone S: Depression in Alzheimer’s disease: the effect of serotonin receptor gene variation. Am J Med Genet B Neuropsychiatr Genet 2003, 119:40-43.

23. Liberski TP, Blackburn TP: 5-HT6 receptors as emerging targets for drug discovery. Annu Rev Pharmacol Toxicol 2000, 40:319-334.

24. Riemer C, Borrioni E, Levett-Traff T, Martin JR, Poli S, Porter RB, Bos M: Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyridinyl-1)-ypridylidine-4-sulfonyl)phenylamine, a potent and selective 5-HT6 receptor antagonist. J Med Chem 2003, 46:1273-1276.

25. Kan R, Wang B, Zhang C, Yang Z, Ji Shun, Lu Z, Zheng C, Jin F, Wang L: Serotonin receptor C267T with late-onset Alzheimer disease in Chinese. Neurosci Lett 2004, 372:27-29.

26. Thome J, Retz W, Baader M, Pesobil O, Hu M, Cowen M, Durany N, Adler G, Henn FA, Rosler M: Association analysis of HTR6 and HTR2A polymorphisms in sporadic Alzheimer’s disease. J Neurotransm 2001, 108:1175-1180.

27. Arango V, Underwood MD, Mann JJ: Serotonin brain circuits involved in major depression and suicide. Proc Brain Res 2002, 1036:443-452.

28. Palmer AM, Francis PT, Benton JS, Sims NR, Mann DM, Neary D, Stern DR: Neurotransmitters and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. J Comp Neurol 1991, 303:51-82.

29. Marek GJ, Wright RA, Schoepf DD, Monn JA, Aghajanian GK: Postsynaptic currents in layer V neurons of the rat medial prefrontal cortex. J Physiol 2000, 292:76-87.

30. Stuttmann GE, Marek GJ, Aghajanian GK: Adenosine preferentially suppresses serotonin2A receptor-enhanced excitatory postsynaptic currents in layer V neurons of the rat medial prefrontal cortex. Neuroscience 2001, 105:55-69.

31. Puig MV, Celada P, Artigas F: Serotoninergic control of prefrontal cortex. Rev Neurol 2004, 39:539-547.

32. Li D, Lu C, Mandalia MA, Mahler ME, Mendez MF, Chen ST: Delusional thoughts and regional frontal/temporal cortex metabolism in Alzheimer’s disease. Am J Psychiatry 2003, 160:341-349.

33. Hohlfeld VA, Beuthien-Baumann B, Kalbe E, Lüdecke S, Lenz O, Zün-

34. Arango V, Underwood MD, Mann JJ: Serotonin brain circuits involved in major depression and suicide. Proc Brain Res 2002, 1036:443-452.

35. Riemer C, Borrioni E, Levett-Traff T, Martin JR, Poli S, Porter RB, Bos M: Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyridinyl-1)-ypridylidine-4-sulfonyl)phenylamine, a potent and selective 5-HT6 receptor antagonist. J Med Chem 2003, 46:1273-1276.

36. Harding AJ, Kril JJ, Halliday GM: Fusion correlates of the apathy inventory dimensionsof Alzheimer type. J Neurol Sci 2004, 227:193-200.

37. Curcio CA, Kemper T: Nucleus raphes dorsalis in dementia of the Alzheimer type: neurofibrillary changes and neuronal packing density. J Neuropath Exp Neurol 1984, 43:359-368.

38. Li WP, Chan WY, Lai HW, Yew DT: Terminal dUTP nick end labeling (TUNEL) positive cells in the different regions of the brain in normal aging and Alzheimer patients. J Mol Neurosci 1997, 8:75-82.

39. Curcio CA, Kemper T: Nucleus raphes dorsalis in dementia of the Alzheimer type: neurofibrillary changes and neuronal packing density. J Neuropath Exp Neurol 1984, 43:359-368.

40. Li WP, Chan WY, Lai HW, Yew DT: Terminal dUTP nick end labeling (TUNEL) positive cells in the different regions of the brain in normal aging and Alzheimer patients. J Mol Neurosci 1997, 8:75-82.

41. Curcio CA, Kemper T: Nucleus raphes dorsalis in dementia of the Alzheimer type: neurofibrillary changes and neuronal packing density. J Neuropath Exp Neurol 1984, 43:359-368.