Excitatory amino acid binding sites in the hippocampal region of Alzheimer's disease and other dementias

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Abstract

Quantitative receptor autoradiography was used to measure muscarinic cholinergic, benzodiazepine, kainate, phencyclidine (PCP), N-methyl-D-aspartate (NMDA) (measured in Tris acetate), quisqualate-sensitive, non-quisqualate-sensitive and total glutamate (measured in Tris chloride buffer) binding sites in adjacent sections of the hippocampal region of 10 Alzheimer's disease, nine control, and six demented, non-Alzheimer's disease postmortem human brains. The measurements were compared to the number of neurofibrillary tangles as revealed by Congo red staining of adjacent sections. All assays and measurements were done by observers blinded to the clinical diagnoses. Binding was decreased significantly for all ligands except quisqualate in stratum pyramidale of CA1 of the Alzheimer's disease brains. The binding loss was significantly greater for the non-quisqualate and NMDA sites than for the muscarinic, benzodiazepine and kainate sites with the total glutamate and PCP site losses being intermediate. Only the loss of benzodiazepine binding was significantly correlated with the number of neurofibrillary tangles. Lesser binding losses were seen in adjacent areas. This difference in the degree of binding decrease is consistent with the hypothesis that NMDA receptors are located on more distal dendrites of hippocampal neurons. There they may be relatively more vulnerable than the other receptors to the pathological process.

The traditional histopathological markers of Alzheimer's disease (AD), neurofibrillary tangles and senile plaques, are extremely prevalent in hippocampal formation. The density of plaques and tangles is highest in the entorhinal cortex which projects to the hippocampus via the perforant pathway, and in the CA1 region and the subiculum which project to other cortical regions.¹ The prominent memory disorder seen in AD patients is probably due to this hippocampal pathology.

Neurotransmitter studies suggest that glutamic acid or a glutamate-like substance is the neurotransmitter of all long intrahippocampal connections: the perforant pathway to the molecular layers of the dentate gyrus and CA1, the mossy fibre pathway from the dentate

granule cells to the CA4 and CA3 regions of Ammon's horn, the Schaeffer collateral pathway from CA3 to CA1, and the CA1 and subicular projection pathways.² Glutamate is known to interact with at least four different excitatory amino acid neurotransmitter receptors: the N-methyl-D-aspartate (NMDA) receptor, the quisqualic acid receptor, the kainate receptor and the metabotropic receptor.³⁻⁶ The first three of these appear to regulate ion channels. All three are present in hippocampal formation.^{3,4} The NMDA and quisqualate receptors are important in memory formation as blockade of these receptors alters long term potentiation (LTP), a presumed physiological analogue of learning in animals.⁷⁻¹⁰

There have been a number of studies of excitatory amino acid and other neurotransmitter receptors in the hippocampal formation of AD brains. These studies have employed a variety of techniques and have reported differing results. Serotonin receptors and GABA_B receptors are significantly decreased in hippocampal formation.^{11,12} Some studies report decreases in muscarinic cholinergic receptors.^{13,14} Others report little change in muscarinic or benzodiazepine receptors in the absence of significant cell loss.^{15,16} Greenamyre *et al*¹⁶ found marked decreases in glutamate receptors measured in the presence of chloride ion. Others¹⁷ found lesser changes when glutamate receptors were measured in the presence of acetate ion rather than chloride. Changes in postsynaptic glutamate receptors have been found in homogenate studies.¹⁸ There have also been conflicting results in measurements of the PCP receptor which is linked to the NMDA receptor. Monaghan *et al*¹⁹ found no significant changes in the absence of cell loss. In a blinded study comparing AD brains to normal controls and to demented patients without AD, Maragos *et al*²⁰ found a significant reduction in the number of PCP receptors. In a homogenate study of [³H]MK 801 binding to the PCP receptor, no changes were observed.²¹ It is not clear whether the differences in the results from the various studies of excitatory amino acid receptors in AD are due to differences in the degree of pathology in the brains, to differences in the methods used to measure the receptors, or to other differences.

No studies in Alzheimer's hippocampus have directly measured one neurotransmitter receptor using different methods or compared the changes to those of other receptors, to local pathology, or to similar measurements in other dementing illnesses. Therefore, we measured

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Table 1 Characteristics of the 25 brains

	Age	Post mortem delay (hours)	Months at -70°C	Chronic medications
Controls:				
C1	79	19	6	Carbidopa/levodopa
C2	67	20	23	Digoxin, propranolol
C3	74	25	47	Metoprolol, nifedipine, nitrate
C4	22	9	47	
C5	77	5	2	Amitriptyline, haloperidol
C6	64	17	48	Nifedipine
C7	69	6	5	Carbidopa/levodopa, bromocriptine, amitriptyline, aminophylline
C8	73	16	2	Lorazepam, verapamil, digoxin, prednisone
C9	55	21	48	
Mean	64.4	15.3	25.3	
SEM	5.8	2.4	7.3	
Alzheimer's disease:				
A1	73	40	31	Chlordiazepoxide, doxepine
A2	58	20	48	
A3	71	22	2	
A4	84	8	6	
A5	83	6	9	
A6	69	14	31	Hydroxyzine
A7	65	14	38	Haloperidol
A8	62	15	45	
A9	77	22	14	Carbidopa/levodopa, phenytoin, phenobarbital, dephenhydramine, amantadine
A10	67	1	10	Carbidopa/levodopa
Mean	70.9	16	23.4	
SEM	2.7	3.4	5.4	
Demented non-Alzheimer's disease:				
D1	68	7	24	
D2	73	12	20	
D3	69	9	10	Haloperidol
D4	68	12	55	
D5	63	15	25	Phenytoin
D6	69	17	5	Insulin, prednisone, aminophylline
Mean	68.3	12	23.3	
SEM	1.3	1.5	7.1	

markers for muscarinic cholinergic receptors, benzodiazepine receptors, the NMDA subtype of glutamate receptors (measured in acetate buffer), total quisqualate-sensitive and non-quisqualate sensitive glutamate receptors (measured in chloride buffer), PCP receptors and kainate receptors in serial sections from nine control, 10 AD and six demented non-AD brains. We compared these measurements to those of neurofibrillary tangles as revealed by Congo red staining of serial sections of the same brains. This report presents our results.

Methods

Human brains were obtained at necropsy and samples of brain material were stored as 1 inch slabs at -70°C. For this study, blocks of medial temporal lobe containing rostral hippocampus were removed from the slab by one of us (JBP) and presented as coded samples to the other investigators who performed all binding assays and data analysis. Only after the data analysis was complete, was the code broken and were statistical comparisons made. Nine non-demented, 10 AD and six non-AD brains were used (table 1). Two of the non-demented and two of the AD cases had coexistent Parkinson's disease. The other non-demented cases died of nonneurological disease. The Alzheimer's cases varied from early to late disease. The diagnosis of Alzheimer's disease was made if the criteria of Khachaturian *et al*²² were met for some region of cortex. The demented, non-AD brains included one case of Pick's disease, one of progressive aphasia and four of diffuse cortical atrophy and striatal and cortical gliosis without plaques or tangles.

The non-demented patients died of cardiac disease. Several had other diseases including the two with Parkinson's disease. Three had been chronically treated with calcium channel blockers, two each with carbidopa/levodopa, beta blockers, nitrates, and amitriptyline, and one each with haloperidol, aminophylline, bromocriptine, steroids and benzodiazepines. Terminally two each were treated with antibiotics, atropine, and dopamine while one each was treated with pancuronium, isoproterenol, heparin, furosemide and opiates. The demented patients had received fewer medications. Two of the Alzheimer's disease patients had received carbidopa/levodopa and one each had received haloperidol, benzodiazepines, doxepin, hydroxyzine, amantadine, phenobarbital, phenytoin and diphenhydramine. Terminally one each received epinephrine, isoproterenol and atropine. One of the non-Alzheimer's demented cases had received haloperidol, one aminophylline and one steroids. Terminally one had been given antibiotics and one furosemide.

From each block, serial, 20 micron thick sections were obtained. Two were stained for neurofibrillary tangles with Congo red, two for acetylcholinesterase, and the rest were processed for receptor autoradiography as described in table 2. Table 2 details the buffers, washes and blanks used in the assays. All assays were carried out on triplicate sections. Because of the number of sections to be cut and the number of assays to be performed, not all the brains could be assayed on the same day, nor could all the assays be performed at the same time. Because we have found that the number of measurable glutamate, but not of other, receptors declines over days when sections are stored at -20°C, glutamate receptor assays were carried out on the day of sectioning. Therefore, the brains were divided into two groups with roughly equal numbers of each clinical diagnosis in each group. The sections from the first group of brains were obtained for the NMDA, muscarinic, benzodiazepine, kainate, Congo red and cholinesterase assays. The NMDA assay was carried out on the day of sectioning. The next day the second group was sectioned for the same assays and the NMDA assay carried out. The other assays were done on subsequent days. After these assays were performed, additional sections were obtained from the first group of blocks for the assays of total glutamate and non-quisqualate-sensitive glutamate binding in chloride buffer. These assays were then performed on the day of sectioning. The same procedure was then used to run the same two glutamate binding assays on the other group of brains.

Autoradiograms were generated by exposing the sections to Ultrosfilm³H (LKB). Quantitative densitometry was performed blindly by observers who compared readings from the regions of interest to those from the standards containing known amounts of radioactivity.²³ The number of quisqualate displaceable binding sites was calculated by subtracting binding remaining in the presence of 2.5 µM quisqualate (NMC) from total glutamate bind-

Table 2 Details of the Ligand Binding Assays

Abbreviation	Receptor	Ligand	Concentration	Blank	Buffer
QNB	Muscarinic cholinergic	[³ H]-quinuclidinyl benzilate	1 nM	*	Dulbecco's phosphate buffered saline, pH 7.4
FLU	Benzodiazepine	[³ H]-flunitrazepam	25 nM	*	50 mM Tris citrate, pH 7.0
TCP	PCP	[³ H]-N-(1-[2-thienyl]cyclohexyl)-3,4-piperidine	20 nM	10 μ M TCP	50 mM Tris acetate with 1 mM Mg acetate, pH 7.4
NMA	N-methyl-D-aspartate	[³ H]-glutamate	200 nM	1 mM glutamate	50 mM Tris acetate, pH 7.2 plus 2.5 μ M quisqualic acid
GLC	Total glutamate	[³ H]-glutamate	200 nM	1 mM glutamate	50 mM Tris HCl plus 2.5 mM CaCl ₂ , pH 7.2
NMC	Non-quisqualate	[³ H]-glutamate	200 nM	1 mM glutamate	50 mM Tris HCl, 2.5 mM CaCl ₂ , pH 7.2 + 2.5 μ M quisqualic acid
KA	Kainic acid	[³ H]-kainic acid	60 nM	100 μ M KA	50 mM Tris acetate, pH 7.2

*Blanks were not used for the [³H]-quinuclidinyl benzilate and [³H]-flunitrazepam assays as blank values have always been undetectable in our previous studies.

Details of the Ligand Binding Assays

Abbreviation	Prewash	Incubation	Rinse	Exposure time
QNB	2 $\times$ 5 min @ 0°C	3 hrs @ 20°C	2 $\times$ 5 min plus a H ₂ O dip	10 days
FLU	3 $\times$ 5 min @ 0°C	30 min @ 4°C	2 $\times$ 5 min	10 days
TCP	30 min @ 0°C	45 min @ 0°C	3 $\times$ 1 min	6 weeks
NMA	30 min @ 0°C	45 min @ 0°C	4 $\times$ 3 ml squirts of buffer, 2 $\times$ 2 ml squirts of 2.5% glutaraldehyde in acetone, total time < 10 secs	3 weeks
GLC	30 min @ 0°C	45 min @ 0°C	4 $\times$ 3 ml squirts of buffer, 2 $\times$ 2 ml squirts of 2.5% glutaraldehyde in acetone, total time < 10 secs	3 weeks
NMC	30 min @ 0°C	45 min @ 0°C	4 $\times$ 3 ml squirts of buffer, 2 $\times$ 2 ml squirts of 2.5% glutaraldehyde in acetone, total time < 10 secs	3 weeks
KA	30 min @ 2°C	45 min @ 2°C	4 $\times$ 3 ml squirts of buffer, 2 $\times$ 2 ml squirts of 2.5% glutaraldehyde in acetone, total time < 10 secs	10 weeks

ing (GLC) in each region of interest from each brain. The number of neurofibrillary tangles on the Congo red stained sections was counted in three fields from each of the five regions of interest of the hippocampal formation by one of us (ABY) who was also blind to the diagnoses of the patients.

In the statistical analyses the values from each region of interest from the different diagnostic groups were compared with one-way ANOVA and subsequent Newman-Keuls test to directly compare groups when the ANOVA indicated a significant result. For illustrative purposes the control and Alzheimer's groups were also compared using *t* tests. Correlations between receptor levels and the number of neurofibrillary tangles in each region were performed using the Pearson product-moment coefficient. To determine if performing the

assays on two different days affected the results, the different diagnostic groups were compared across the different days using a two-way ANOVA. Multiple regression analyses were used to determine which factors (disease state, age, post-mortem delay or freezer storage time) contributed to the differences in binding between the brains.

Results

There were no significant differences between the groups in their ages, postmortem delays or storage times in the freezer (table 1). There was no significant effect on the results of performing the assays on different days.

The number of neurofibrillary tangles found in a 20 $\times$ field of the various subregions of the hippocampal formation in the brains is shown in table 3. The regions examined for tangles were the granule cell layer of dentate gyrus, the pyramidal layers of regions CA1 (SP1) and CA3 (CA3) of Ammon's horn, the subiculum (SUB) and the principal external layer of presubiculum (LPP). The Congo red staining revealed a wide range in the number of neurofibrillary tangles and senile plaques in the AD brains. Plaques and tangles were most common in subiculum and CA1.

Autoradiographic measurements of ligand binding were made in synaptic zones, including the molecular layers of dentate gyrus (DG) and CA1 (SM1), stratum pyramidale and (for kainate) stratum lucidum of CA3. Also, SP1, SUB, and LPP. Histograms showing the results of the measurements are shown in fig 1. Image-processing-computer generated pictures of muscarinic, benzodiazepine, PCP and NMDA binding to representative hippocampi are shown in fig 2.

In the normal hippocampus, muscarinic binding was highest in SP1 with high binding in SUB and DG. Benzodiazepine binding was highest in DG with slightly lower amounts in SP1 and the perihippocampal regions; there was very little benzodiazepine binding in CA3.

Table 3 Number of neurofibrillary tangles, per 20 $\times$ field in hippocampal subregions of the 25 brains

Brain	Dentate gyrus	CA3	CA1	Subiculum	Presubiculum
Controls:					
C1	0	0	1 found	0	0
C2	0	0	0	0	0
C3	0	0	0	0	0
C4	0	0	0	0	0
C5	0	—	0	0	0
C6	0	—	0	0	—
C7	0	0	0	0	0
C8	0	0	0	0	0
C9	0	0	0	0	0
Alzheimer's disease:					
A1	1-2	< 1	4-6	6-12	3-6
A2	0	0	2-5	15-30	< 1
A3	0	0	6-10	1-2	0
A4	—	—	8-10	2-4	< 1
A5	0	< 1	8-12	30-60	0-4
A6	0	—	20-40	60-80	< 1
A7	0	0	15-30	30-50	0-3
A8	0	Rare	0-2	0-2	0
A9	0	0	15-30	6-8	0
A10	0	0	0-2	2-4	Rare
Demented non-Alzheimer's disease:					
D1	0	0	0	0	0
D2	0	0	0	0	0
D3	0	—	0	0	0
D4	0	0	0	0	0
D5	0	0	0-2	0	0
D6	0	0	0	0	0

Fig 1 Histograms of binding to six subregions of hippocampus for each of the assays used. Error bars represent standard error of the mean, *: $p < 0.05$ by Newman-Keuls; **: $p < 0.01$ by Newman-Keuls; #: $p < 0.05$ in comparing Alzheimer's to control brains by t test when ANOVA is nonsignificant. Abbreviations of hippocampal subregions measured: DG: Stratum moleculare of dentate gyrus. CA3: Stratum pyramidale of CA3 (except stratum lucidum was measured in kainate assays). SP1: Stratum pyramidale of CA1. SM1: Stratum moleculare of CA1. SUB: Subiculum. LPP: Lamina principalis externa of presubiculum. PRO?: Prosubiculum? a: Muscarinic cholinergic receptors. b: Benzodiazepine receptors. c: PCP receptors. d: NMDA receptors measured in acetate buffer. e: Total glutamate binding in chloride and calcium. f: Non-quisqualate displaceable glutamate binding in chloride and calcium (NMDA and "other" binding sites). g: Quisqualate displaceable glutamate binding. h: Kainate receptors.

Fig 2 Computer generated images of receptor binding to adjacent sections from four representative hippocampi under the different assay conditions. The top left hippocampus is from a control brain (C9). The top right from a patient with severe dementia, diffuse cortical and basal ganglia atrophy and gliosis without senile plaques or neurofibrillary tangles (D4). The bottom left is from a brain with mild Alzheimer's disease changes (A8). The bottom right is from a brain with severe Alzheimer's changes (A7). Abbreviations as in fig 1. A: Muscarinic cholinergic receptors. B: Benzodiazepine receptors. C: PCP receptors. D: NMDA receptors measured in acetate buffer.

PCP and NMDA binding (measured in Tris-acetate buffer) were highest in DG and SP1 with lower values in the other regions. In Tris-chloride buffer, total glutamate binding and binding in the presence of quisqualate tended to be enhanced in all regions compared to binding in Tris-acetate. The amount of binding displaced by quisqualate was high only in DG. Kainate binding was highest in stratum lucidum of CA3 and DG. There was a narrow band of high kainate binding in a region adjacent to SP1 that was probably prosubiculum.

In this group of Alzheimer's disease brains there were decreases in all receptors measured (figs 1 and 2). The degree of reduction was most marked in SP1 with lesser but consistent reductions in the subiculum. No significant reductions were found in DG. Only a few ligands were reduced in CA3, SM1 or LPP.

The only drugs the patients had been given that might have interacted with the receptors measured in this study were amitriptyline, benzodiazepines and phenobarbital. The benzodiazepines and phenobarbital had no apparent effect on flunitrazepam binding since binding in the treated cases did not differ from binding in the untreated ones. However, in the two nondemented cases that had been treated with amitriptyline, muscarinic cholinergic binding was significantly increased compared with that in the other cases ($F_{1,5,34} = 18.09$; $p < 0.001$ by 2 way ANOVA). If these two cases were excluded from analysis of muscarinic binding then muscarinic binding to DG was 1.25 pmol/mg protein (SEM 0.11), binding to CA3 was 1.063 (0.16), binding to SP1 was 1.79 (0.09), binding to SM1 was 1.07 (0.11), binding

to SUB was 1.39 (0.07) and binding to LPP was 1.15 (0.09). With the two cases excluded the only significant decreases of muscarinic binding in the diseased brains were to SP1 of Alzheimer's brain ($p < 0.05$ by Newman-Keuls) and to SUB of the non-Alzheimer's brains ($p < 0.05$ by Newman-Keuls).

The only binding levels that correlated significantly with the number of neurofibrillary tangles within the Alzheimer's brains were benzodiazepine binding in SP1 ($r = 0.804$; $p < 0.01$) and muscarinic binding in LPP ($r = 0.630$; $p < 0.05$). Multiple linear regression

Fig 3 Reanalysis of the data of Greenamyre et al. NMDA receptors were defined as glutamate binding displaced by 100 μ M NMDA. Quisqualate receptors were defined as glutamate binding displaced by 2.5 μ M quisqualate. "Other" binding is defined as -glutamate binding displaced by 1 mM glutamate which is not displaced by 100 μ M NMDA plus 2.5 μ M quisqualate. Abbreviations: CON: Control; AD: Alzheimer's disease.

analysis was performed for DG and SP1 to determine which factors (disease state, age, post mortem delay or storage time) might contribute to the differences in ligand binding between the brains. Only disease state, not age, post mortem delay or storage time was found to significantly affect binding for all ligands.

In SP1, binding of all the ligands, except quisqualate-sensitive glutamate binding, to Alzheimer's disease brains was significantly below control values. The percentages of decline from mean control binding levels was compared among the ligands using one-way ANOVA. A significant difference between the ligands was found ($F_{6,63} = 3.010$, $p = 0.012$). In pairwise comparisons using the Duncan multiple range test, the declines in NMDA and non-quisqualate-sensitive binding were significantly greater than the muscarinic, benzodiazepine, quisqualate and kainate declines ($p < 0.05$ for each comparison). There were no other significant differences between pairs of ligands.

Discussion

The distribution of hippocampal binding was very similar for the different ligands with the exception of quisqualate and kainate. Binding was high in the synaptic zones of the dentate gyrus, CA1, subiculum and presubiculum. For most ligands it was highest in a broad band over stratum pyramidale of CA1. In humans this region contains the CA1 pyramidal cell bodies and many of their apical and basal dendrites, thus corresponding to the strata pyramidale, radiatum and oriens of rodents.²⁴ Quisqualate-sensitive glutamate binding was high only in the dentate molecular layer. For kainate, binding was highest to stratum lucidum of CA3 and the inner portion of the dentate molecular layer.

The regions of medial temporal lobe with the largest degree of cell loss, neurofibrillary tangles and senile plaques in Alzheimer's disease are the entorhinal cortex (layers 2 and 4), CA1 and subiculum.^{1,25} Unfortunately, entorhinal cortex was not present on the sections removed from many of the brains examined in this study so no attempt was made to analyse data from that region. Although no direct measure of cell loss was made, the counts of tangles (table 3) suggests that the pathological severity in these brains ranged from mild to severe. The areas in which there were binding losses were generally those in which there was also the most marked pathology. Areas with severe pathology, however, did not always display receptor loss. The subiculum, for instance, had severe histological pathology but relatively little loss of excitatory amino acid binding. There were no significant losses in dentate gyrus and only muscarinic and benzodiazepine binding were down slightly in LPP.

The magnitude of the muscarinic and benzodiazepine binding changes found in these brains was similar to that found by Greenamyre *et al.*¹⁶ However, in the current study the changes were significant. The decreases seen are unlikely to reflect simple cell damage as

reflected in the number of neurofibrillary tangles. Only flunitrazepam binding loss in SP1 correlated with the number of neurofibrillary tangles. This correlation may reflect a localisation of benzodiazepine receptors on proximal dendrites of hippocampal neurons while the other receptors may be located on more distal dendrites where the pathological process begins.²⁶⁻²⁹ However, even for flunitrazepam, there was a significant decrease in the presubiculum, a region with little cellular pathology.

Quisqualate displaceable binding was high in dentate gyrus and low in the other regions measured. There were no significant changes in the Alzheimer's disease brains.

Kainate receptor binding was highest in CA3 and dentate gyrus. However, even though kainate binding in SP1 was low in all the brains, there was a significant decrease in binding to SP1 of the Alzheimer's disease brains. In addition in normal brains there was a more dense band of binding along the lateral border of CA1 that was much reduced or missing in the AD brains. It may be that this represents binding in the prosubiculum, a region of severe pathology in Alzheimer's disease [BT Hyman, personal communication]. Geddes *et al.*³⁰ described expansion in width of the band of [³H]kainate binding in dentate gyrus in Alzheimer's disease. We did not specifically study that issue in this study.

In other studies, we have found an extremely high correlation between the density of NMDA binding sites and PCP binding sites in adult rats.³¹ There was a good correlation between NMDA and PCP in the normal brains in this study ($r = 0.616$, $p < 0.001$). Such a correlation is expected considering the biochemical data suggesting that the two sites exist on different parts of the same receptor ion channel complex.

Previously, we demonstrated large decreases in NMDA receptor binding in Alzheimer's hippocampus.¹⁶ In that study, we assayed [³H]glutamate binding in Tris-chloride buffer. We assessed quisqualate-sensitive and quisqualate-insensitive glutamate binding as measures of quisqualate and NMDA receptors respectively. However, quisqualate-insensitive glutamate binding is probably not a selective measure of NMDA receptors. Quisqualate-insensitive glutamate binding is much greater in chloride buffer than in acetate buffer and not all the binding in chloride buffer that remains in the presence of 2.5 μ M quisqualate is blocked by 500 μ M NMDA. We have reanalysed the data of Greenamyre *et al.* (fig 3) using only measures of quisqualate-sensitive and NMDA-sensitive glutamate binding. This reanalysis confirms the presence of the large decreases in NMDA receptors reported in that study and suggests that the reported changes were not substantially influenced by methodology. The cases in that previous report, however, were all affected with severe pathology. It is likely, when comparing the results of this study with those of the previous report, that the degree of receptor loss reflects the degree of cellular pathology.

The greater degree of loss of non-quisqualate and NMDA receptors than of muscarinic, benzodiazepine and kainate receptors may reflect a distal dendritic localisation of NMDA/PCP receptors, since the distal dendrites and spines of pyramidal cells are thought to be affected first in AD.²⁶⁻²⁹ Overall, those areas with greatest glutamate receptor losses were those in which there is significant pathology in AD. However, subiculum receptors were less affected than those in SP1 but pathologically these regions are similarly affected.

The regional specificity of pathology in AD has similarities with the density of NMDA receptors in the brain.^{17,32} However, there are several areas in which there are dense NMDA receptors and very little pathology. These include primary visual, auditory and sensory cortices (unpublished observations). These discrepancies suggest that excitotoxicity is unlikely to play a primary role in AD. Cells which have compromised metabolic function as a result of the disease process could become more vulnerable to excitotoxic damage as a secondary phenomenon.^{32,33}

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