

BRE 23025

Is senile dementia of the Alzheimer type associated with hippocampal plasticity?

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(Accepted 26 April 1988)

Key words: Human hippocampus; Aging; Senile dementia of the Alzheimer type; Autoradiography; Excitatory aminoacid binding site; Mossy fiber plasticity

The density of binding sites for excitatory amino acids in the hippocampus of SDAT (senile dementia of the Alzheimer type) patients has been investigated. This was compared to that found in younger and old non-dement cases. In the SDAT hippocampi there was a significant decrease in the glutamate and kainate binding. This reduction correlated positively with the degree of the severity of neurological and pathological disease. In spite of an extensive denervation of SDAT hippocampi, there was no sign of sprouting of mossy fibers; this observation indicates a loss of plasticity in the hippocampus of SDAT patients.

Senile Dementia of the Alzheimer Type (SDAT)¹⁷ is associated with a degeneration of the temporal lobe including, in the hippocampal formation, neuronal loss and a great number of neurofibrillary tangles (NFT) and senile plaques (SP)^{1,2,9}. In view of the role of the hippocampus in memory, these lesions are likely to contribute to the memory deficit seen in SDAT patients. Furthermore, the lesion of the entorhinal cortex⁹, as well as the degeneration of the cholinergic septo-hippocampal system observed in SDAT patients^{13,19}, largely denervate the hippocampus from its major inputs. Experimental observations indicated that a similar deafferentation of the hippocampus in the rat induces a reactive synaptogenesis notably of the mossy fibers, which innervate the dentate granule cells from which they originate⁶.

In the present study we have used a multidisciplinary approach to investigate whether reactive synaptogenesis occurs in the hippocampi of SDAT patients in response to lesions. This included a psychometric and pathological evaluation of the cases as well as an autoradiographic study of the subtypes of glutamate

binding sites: kainate (KA), which is intimately associated with the mossy fiber terminals¹⁵ and NMDA.

We studied 6 cases from the Charles Foix longitudinal study¹⁴; this series involved very old women (82–97 years) either normal (old non-dement group) or with SDAT. The mental status was assessed by the Blessed et al. test score (BTS)² which was performed during the year preceding the death. High BTS values mean good mental efficiency. Two additional old cases (76–79 years) who died without neuropathological disease were used in the old non-dement group. Finally, 6 younger adult cases (27–64 years) who died without neurological disease were used as younger control group. The neuropathological data have been observed with routine hematoxylin eosin staining and silver impregnation according to Bodian's method³ to reveal the presence of neurofibrillary tangles (NFTs) and senile plaques (SPs) and by anti-NFT antisera⁴. The results (see Table I) have already been reported in more detail elsewhere⁴. Alternate hippocampal sections (30 μ m) were assayed for either KA- or sodium-independent glutamate using

TABLE I

Densities of histopathological lesions and KA and glutamate binding sites (in fmol/mg tissue) in normal and SDAT hippocampi

For each case at least 9 separate sections were quantified. Lu, lucidum; Ra, radiatum; mol and sg, respectively molecular and supra-granular layers of the fascia dentata (FD); T, temporal cortex; BTS, test score of Blessed et al.²; NFT, neurofibrillary tangles; SP, senile plaques.

	Case	BTS	Age	Lesions/mm ²				L-[³ H]glutamate		[³ H]kainate	
				H ₁		T		H ₁ /Ra	FD/mol	H ₃ /Lu	FD/sg
				SP	NFT	SP	NFT				
Controls	3		27					120	91	39	30
	23		30					128	89	49	17.4
	21		40					/	/	32	22.5
	11		57					150	105	32	8.5
	12		58					102	45	32	13.6
	7		64					/	/	40	21
\bar{X}								125 + 9	82.5 + 13	38.4 + 3	20.9 + 3
Old non-dement	5		76					/	/	26	15
	8		79					81	63	/	/
	2634	28	87	0	1	0	0	74	48	28	9.7
	2809	26	94	1	63	1	0	62	54	27	21.5
\bar{X}								72.3 + 5*	55 + 4.3	27.1 + 0.4*	15.4 + 3
SDAT	2857	19	87	5	62	0	0	33	34	10.3	7.2
	2916	16	97	0	23	0	3	/	/	27	21.5
	2942	6	93	4	48	8	5	53	40	23	9
	2812	2	82	28	75	15	29	37	33	12.5	7
\bar{X}								41 + 6*	35.6 + 2*	18 + 4**	7.1 + 0.6*

* and ***P* = 0.05 and 0.02 respectively, Mann-Whitney U-test.

binding procedures described in detail elsewhere^{10, 18}. For KA binding the incubation was performed at 3 °C with 20 nM vinylidene-[³H]KA (NEN, 60 Ci/mmol) in the presence (for non-specific binding) or absence (for total binding) of an excess of 10 μM cold KA. Na⁺, Cl⁻ and Ca²⁺ independent glutamate binding was performed at 3 °C for 45 min with 100 nM L-[³H]glutamate (Amersham, 41.5 Ci/mmol) with or without 500 μM cold L-glutamate to determine non-specific and total binding, respectively. Additional sections from younger controls and SDAT hippocampi were also incubated in the presence of 100 μM NMDA to study the NMDA-sensitive glutamate binding sites. Quantification of labeling was performed on the films with a computer-assisted image analyser (IMSTAR). The comparison of binding values was performed statistically with the Mann-Whitney U-test, because of the relatively small number of available samples. The postmortem delays were less than 24 h in all cases; a previous study has shown that the densities and distribution of KA¹⁸ and glutamate (unpublished observations) binding sites are not reduced with these postmortem delays.

The cases from the Charles Foix longitudinal study¹⁴ were classified according to the BTS, as old non-dementia (2634, 2809) or dementia cases (2857, 2916, 2942, 2812. See Table I). In the histopathological study performed in these cases, senile plaques (SP) and neurofibrillary tangles (NFT) were quantified in the temporal cortex, subiculum and H₁ and H₂ fields of Ammon's horn; the densities and distribution of NFT and SP (see Table I) are in keeping with earlier studies^{1,2,9,17}. The neuropathological observations⁴ indicated that SDAT hippocampi were largely denervated, due to the extensive lesions found in the entorhinal cortex, which constitutes the main afferent source to the dentate gyrus. As shown in Table I, there was also a good correlation between the BTS and the pathological features in hippocampus and temporal cortex.

In the old normal cases, there was a clear-cut reduction of glutamate binding sites in the H₁ region of Ammon's horn compared to younger cases (42%, see Table I). In the SDAT hippocampi, the decrease of glutamate binding sites compared to younger normal cases was even more severe (Table I). Thus we found

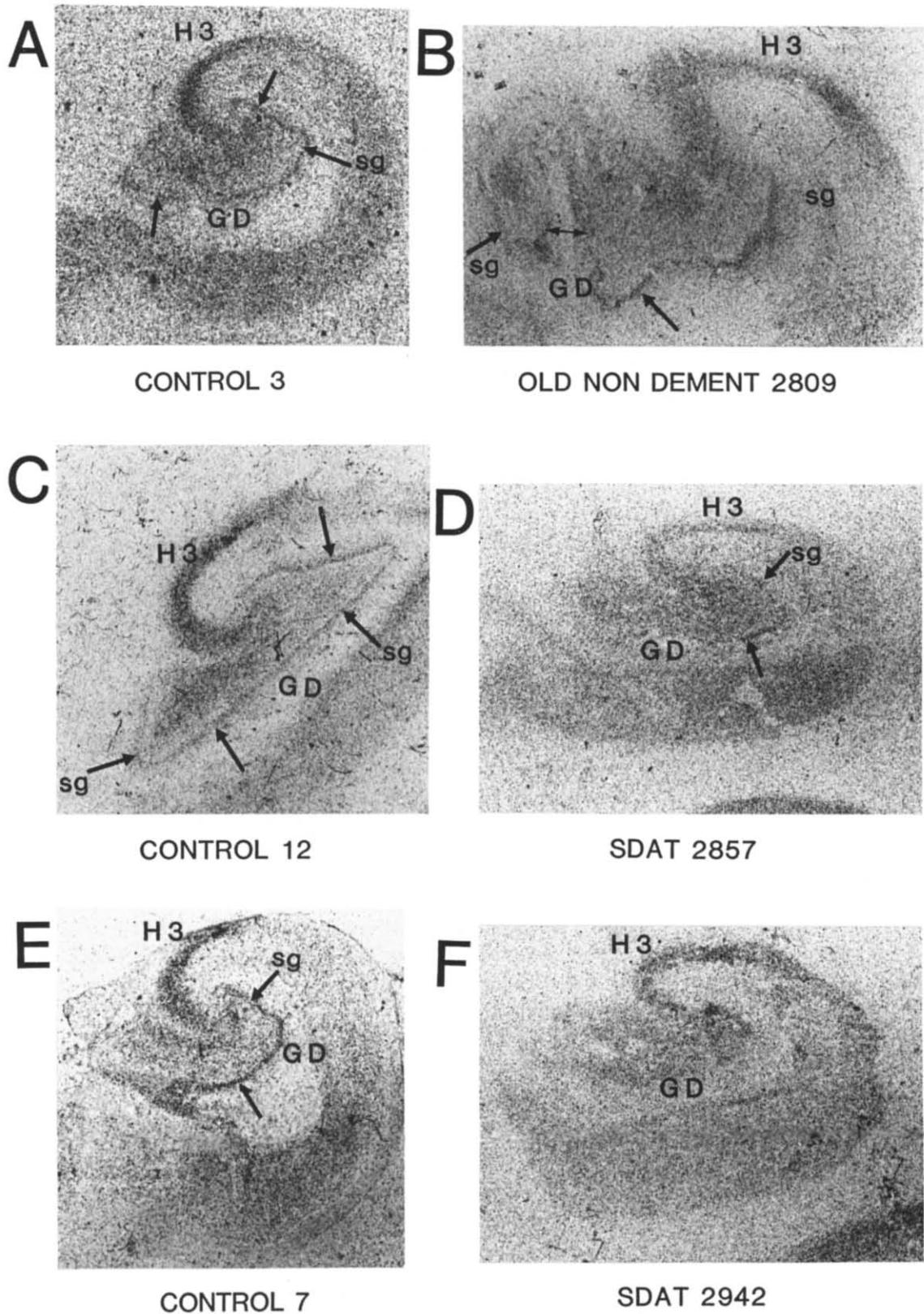


Fig. 1. Photomicrographs depicting the KA binding sites in the hippocampus in young (A, C, E) and old non-dement cases (B) and their disappearance in the supragranular layer (sg, arrows) of the gyrus dentatus (GD), in SDAT cases (D, F, also see Table).

a loss of glutamate binding sites not only in H₁ (56%) but also in H₃ (53%) and dentate gyrus (57%). There was a good correlation between the extent of the decrease in H₁ and that of the neurofibrillar tangles and senile plaques in the same region; thus the highest decrease of glutamate binding was found in cases 2857 and 2812, for which this zone was the most affected. The NMDA subtypes of binding sites were significantly decreased compared to normal younger cases (by 80 + 12% and 70 + 9% in CA₁ and DG, respectively). This loss of binding is probably due to the cellular degeneration and loss of pyramidal and granular dendritic spines which occur during the aging process and appear to be more extensive in Alzheimer's patients^{5,16}. Accordingly, we found that the loss of sites in SDAT cases was significantly greater than in old non-demented ones ($P = 0.05$ in each case), indicating that it does not only reflect age-related changes. In SDAT patients there was a good correlation between the loss of NMDA sites in H₁ and the severity of the neuropathological changes.

In old non-demented cases there was a loss of KA binding values in H₃ region (by about 70%) compared to younger control cases. In SDAT cases (see Fig. 1 and Table I) there was a marked and significant decrease in the binding of KA compared to younger control cases in both, the H₃ region of Ammon's horn (by 53%) and the supragranular layer of dentate gyrus (by 53%). In the latter zone, the labeling of KA was significantly lower than in the old non-demented group (by 52%, $P = 0.04$). In fact, KA labeling was virtually absent from this region in the more severe cases (see Fig. 1D,F).

The first conclusion of the present study is that NMDA binding was reduced in the hippocampus of SDAT patients compared to old-normal and younger cases. The present results are in good agreement with those of Young et al.²⁰ and Penney J.B. et al.¹². In contrast, Geddes et al.⁷ generally found no change in these sites and suggested that the discrepancies between their results and those of Young et al.²⁰ probably reflect differences in either the methods or the severity of the disease. Since we have worked in very

similar experimental conditions as Geddes et al.⁷ (low temperature, lack of calcium or chloride ions in the medium to avoid high-affinity uptake), it is not the method but the severity of the neuropathology which explains these discrepancies. It bears stressing that of 7 SDAT patients studied by Geddes et al.⁷, there was at least one case with a decrease (60%) in glutamate binding sites in H₁, and this case had the highest hippocampal cell loss.

Several experimental observations suggest a role for NMDA receptors in the memory process¹¹. Therefore, the general reduction of NMDA-sensitive glutamate binding in Alzheimer patients reported here (also see Young et al.²⁰) probably contributes to the loss of memory characteristic of SDAT patients¹⁷.

The second conclusion is that in SDAT hippocampi, there was a significant lack of KA binding in the terminal field of mossy fibers (stratum lucidum of CA₃ and supragranular of dentate gyrus). This indicates that mossy fibers fail to sprout in response to the denervation of the dentate gyrus. Our results are in contrast to those of Geddes et al.⁸, who found an expansion (73%) of the KA binding in the inner part of the dentate molecular layer (supragranular zone) of SDAT cases. For these authors this expansion was due to a sprouting of commissural-associational fibers; this interpretation is somewhat difficult to reconcile with experimental evidence suggesting that KA binding sites are exclusively associated with the terminals of the mossy fibers^{15,18}. Furthermore, the observations of Geddes et al.⁸ are based on qualitative autoradiography and quantitative information on the severity of their disease (BTS score values and number of NFT and SP) was not provided.

To conclude, in agreement with previous studies^{5,16}, our results suggest that the hippocampal plasticity which normally occurs after lesioning to compensate for the innervation deficits is lost in the senile forms of Alzheimer's disease. The mechanism by which this loss of plasticity occurs remains however to be determined.

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