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## Loss of hippocampal [<sup>3</sup>H]TCP binding in Alzheimer's disease

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**Key words:** [<sup>3</sup>H]*N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine ([<sup>3</sup>H]TCP); Alzheimer's disease; Receptor; CA<sub>1</sub>; Subiculum; *N*-methyl-D-aspartate (NMDA); Man

We have previously demonstrated a marked loss in *N*-methyl-D-aspartate (NMDA) receptors in the hippocampus and cerebral cortex of patients dying with dementia of the Alzheimer type (DAT). In addition, we have found that the dissociative anesthetic *N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine ([<sup>3</sup>H]TCP) binds to a site whose regional distribution is highly correlated with that of NMDA receptor sites. We studied the binding of [<sup>3</sup>H]TCP to sections of hippocampi from 8 controls, 12 patients with DAT and 7 patients with other dementias. [<sup>3</sup>H]TCP binding was significantly reduced in strata pyramidalia of CA<sub>1</sub>/CA<sub>2</sub>, CA<sub>3</sub> and subiculum of DAT hippocampal formation compared to that of control. Labelled dissociative anesthetics could potentially be used with positron emission tomography in the diagnosis of DAT.

Tritiated *N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine ([<sup>3</sup>H]TCP) is a derivative of the dissociative anesthetic phencyclidine. [<sup>3</sup>H]TCP has recently been shown to bind with high affinity to a distinct class of central nervous system binding sites which are most concentrated in the hippocampus and outer regions of cerebral cortex [20]. The pharmacology of [<sup>3</sup>H]TCP binding is most consistent with binding to the dissociative anesthetic site (PCP-site) [27] and not to the  $\sigma$ -opiate receptor [13]. Recent electrophysiological data suggest that the dissociative anesthetics block the excitatory action of *N*-methyl-D-aspartate (NMDA) on hippocampal [5] and spinal cord neurons [16], as well as on pyramidal cells in rat somatosensory cortex [24]. Behavioral data also support an interaction between the dissociative anesthetic receptor and the NMDA-sensitive glutamate receptor. The dissociative anesthetics produce similar behavioral changes to those induced by NMDA antagonists [12]. Moreover, we have recently shown that the regional distribution of [<sup>3</sup>H]TCP binding in rat forebrain is highly correlated with the regional distribution of [<sup>3</sup>H]glutamate binding to NMDA receptors [15].

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[<sup>3</sup>H]Glutamate binding to brain sections obtained from patients dying with dementia of the Alzheimer type (DAT) has been shown to be markedly reduced in cerebral cortex [9] and hippocampus [8]. Although all subtypes of glutamate receptors are affected in DAT, the most pronounced changes are in the NMDA subtype. We now report our studies of [<sup>3</sup>H]TCP binding to Alzheimer brain.

Postmortem brains from 6 persons dying without neurologic disease, 3 persons dying with other forms of dementia (two Huntington's disease and one case of diffuse cortical atrophy without DAT pathology) and 4 persons dying with dementia of the Alzheimer type were obtained through our Department of Pathology. After autopsy, one hemisphere of each brain was fixed in formalin and the other hemisphere was blocked, frozen over dry ice and sealed in containers at  $-70^{\circ}\text{C}$  until examination. Diagnosis of DAT was histologically verified in fixed sections according to the criteria outlined by Khachaturian [11]. Blocks of hippocampus (approximately 5 mm) were removed from each frozen hemisphere at the level of the lateral geniculate and equilibrated in a cyrostat at  $-15^{\circ}\text{C}$  for 0.5 h. Sections of 20  $\mu\text{m}$  were then thaw-mounted onto gelatin/chrom-alum coated glass slides. Sections were preincubated for 30 min in cold 50 mM Tris-acetate, pH 7.4 and dried. Sections were incubated for 45 min in the same buffer with 1 mM magnesium acetate and 20 nM [<sup>3</sup>H]TCP (52.9 Ci/mmol, New England Nuclear) in the presence or absence of 20  $\mu\text{M}$  PCP at  $4^{\circ}\text{C}$ . Sections were washed 3 times, 1 min each, in cold buffer with magnesium acetate and rapidly dried. Slides were subsequently mounted in stainless-steel X-ray cassettes and apposed to a piece of Ultrafilm-<sup>3</sup>H (LKB). After 3 weeks exposure, the films were developed in D-19 (Kodak). Autoradiograms were analyzed by computer-assisted densitometry according to the method of Pan et al. [18]. The amount of [<sup>3</sup>H]TCP bound, in pmol/mg protein, was determined by comparing the optical densities produced by the labelled tissue with densities produced by plastic standards containing known amounts of radioactivity [18]. Sections underlying the autoradiograms were stained with Cresyl violet to provide histological verification of hippocampus and subiculum as defined by Lorente de Nó [14]. A minimum of 20 readings were taken from each of several different areas on each section to determine total binding. Non-specific binding was determined similarly from adjacent sections. Specific binding was obtained by subtracting non-specific from total binding. The assays and all the densitometric readings were carried out by an experimenter blind to the patients' diagnoses.

In control hippocampi binding was highest in the strata pyramidalia of CA<sub>1</sub>/CA<sub>2</sub> (CA<sub>1</sub>) and the subiculum (SUB) (Figs. 1 and 2). High densities were also observed in the molecular layer of the dentate gyrus (DG). Binding was intermediate in stratum moleculare of CA<sub>1</sub> (SMCA<sub>1</sub>), outer layers of adjacent parahippocampal isocortex (PAR), stratum pyramidale of CA<sub>3</sub> (CA<sub>3</sub>) and quite low in pre- and parasubiculum. Because caudal levels of hippocampus were examined, little to no entorhinal cortex was present for examination.

[<sup>3</sup>H]TCP binding in brains from patients with non-DAT dementia were indistinguishable from those of controls (Fig. 2). However, [<sup>3</sup>H]TCP binding to DAT hippocampus appeared decreased relative to the other groups in all areas inspected

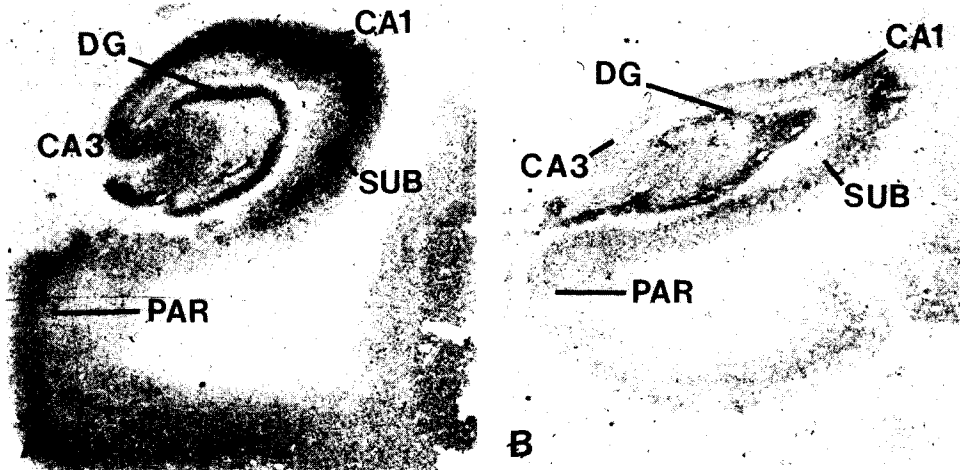


Fig. 1. Autoradiograms of [ $^3\text{H}$ ]TCP binding in human hippocampus. Tissue was incubated in 20 nM [ $^3\text{H}$ ]TCP for 45 min and processed for autoradiography as described in text. A: normal control. B: Alzheimer's disease. CA<sub>1</sub>, stratum pyramidale of CA<sub>1</sub>/CA<sub>2</sub>; CA<sub>3</sub>, stratum pyramidale of CA<sub>3</sub>; DG, molecular layer of dentate gyrus; PAR, parahippocampal cortex; SUB, subiculum.

except the molecular layer of the dentate gyrus and pre- and parasubiculum (Figs. 1 and 2). Reductions in binding relative to control were 55% in CA<sub>1</sub> ( $F_{2,10} = 3.67$ ; not significant (n.s.)), 40% in SUB ( $F_{2,9} = 2.85$ ; n.s.), 40% in SMCA<sub>1</sub> ( $F_{2,7} = 1.80$ ; n.s.), 30%

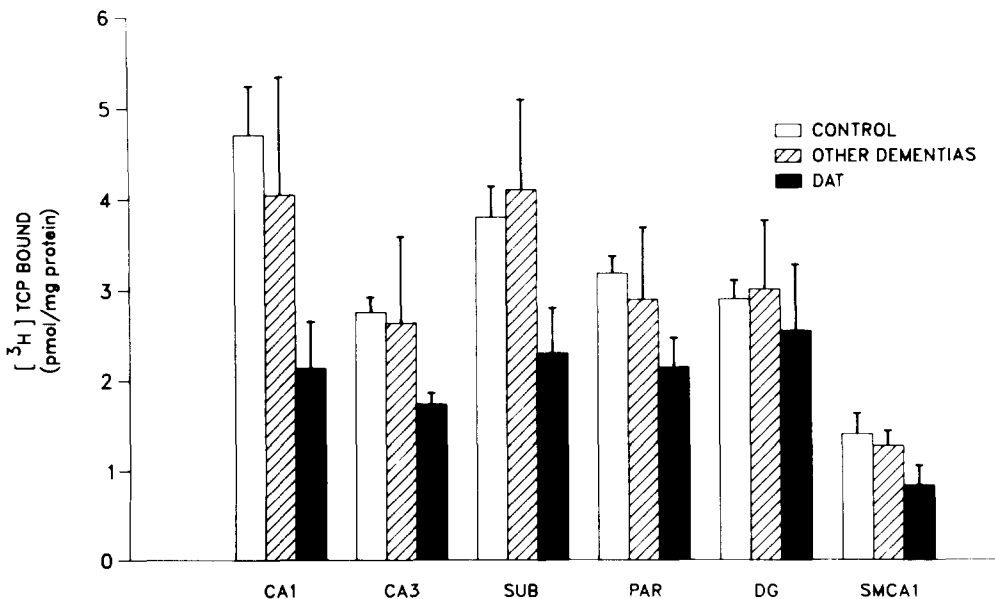


Fig. 2. Histogram of binding to hippocampal subregions of 6 control patients, 3 demented patients without Alzheimer's disease (other dementia) and 4 DAT patients. Error bars represent S.E.M. Abbreviations are as in text.

in CA<sub>3</sub> ( $F_{2,7}=2.01$ ; n.s.) and 33% in PAR ( $F_{2,8}=2.98$ ; n.s.) using one way ANOVA.

In order to increase our sample, an additional 14 hippocampi (2 controls, 4 other dementia (3 diffuse atrophy, 1 Pick's disease) and 8 DAT cases) were analyzed blindly and in a fashion identical to that described above except that 40 nM [<sup>3</sup>H]TCP was used instead of 20 nM [<sup>3</sup>H]TCP. As expected, assays carried out in the presence of increased concentrations of [<sup>3</sup>H]TCP significantly increased the amount of binding to all areas (data not shown). When the results from the two experiments were pooled and compared by two-way ANOVA using the BMDP statistical software package (Regents of the University of California), decreased binding to DAT brains was observed in CA<sub>1</sub> ( $F_{2,20}=4.94$ ;  $P=0.02$ ), CA<sub>3</sub> ( $F_{2,13}=4.19$ ;  $P=0.04$ ) and probably in the SUB ( $F_{2,18}=3.27$ ;  $P=0.06$ ). Since there were no differences in the amount of [<sup>3</sup>H]TCP binding in control and non-DAT dementia cases in both experiments, results from these two groups were combined and compared to DAT by two-way ANOVA. Significant decreases were found in CA<sub>1</sub> ( $F_{1,22}=9.27$ ;  $P=0.006$ ), SUB ( $F_{1,20}=7.22$ ;  $P=0.014$ ) and CA<sub>3</sub> ( $F_{1,15}=6.41$ ;  $P=0.02$ ).

For the two experiments average postmortem delay was  $22 \pm 6$  h for the controls,  $14 \pm 2$  h for the non-Alzheimer's demented cases and  $14 \pm 3$  h for patients dying with DAT ( $F_{2,21}=0.895$ ; n.s.). Average age of the patients was  $60 \pm 6$  for the controls,  $62 \pm 5$  for the non-Alzheimer's cases and  $74 \pm 3$  for the DAT cases ( $F_{2,24}=3.18$ ; n.s.). Multiple regression analysis revealed no significant relationship of age or postmortem delay with [<sup>3</sup>H]TCP binding to any of the measured regions. One control patient was taking a calcium channel blocker, two DAT cases were taking low doses of haloperidol, two DAT cases were taking benzodiazepines, one DAT case and two non-DAT dementia cases were taking phenytoin and one non-DAT case was taking anti-parkinsonian medication. The control cases had sudden cardiac or traumatic deaths. The DAT and non-DAT cases died of prolonged illnesses and several in both categories required artificial respiration for several days prior to death.

The hippocampus is a major site of pathology in DAT. In DAT brain, the hippocampus contains the greatest numbers of neurofibrillary tangles [1] and senile plaques [4] and has significant neurochemical changes including cholinergic [3], monoaminergic [2] and glutamatergic deficits [6, 8]. Functionally, the hippocampus has been associated with learning and memory. Bilateral lesions to this region produce severe memory deficits [19] as well as impairments on spatial learning tasks [23]. Anatomically, the hippocampus, via the parahippocampal gyrus, reciprocally connects the limbic cortex with a number of neocortical regions [26]. Loss of these connections due to the observed structural and chemical abnormalities may thus underlie the cognitive and memory deficits so prominent in DAT.

The areas of hippocampus which have the largest changes in dissociative anesthetic binding (CA<sub>1</sub> and SUB) are those which contain significant anatomical pathology in DAT [10]. These areas are known to receive glutamatergic input [21, 28] and contain high densities of NMDA-sensitive receptors [7]. Furthermore, it is these regions in which the greatest losses of NMDA receptors in DAT were observed [8]. The CA<sub>3</sub> and molecular layers have less neuroanatomical pathology in DAT and, in this study, had lesser changes in dissociative anesthetic binding. The CA<sub>3</sub> neurons receive input

from the dentate granule cells [22] but much of the granule cells' actions are thought to be mediated by glutamate receptors sensitive to kainic acid [25]. The dentate gyrus is known to contain a large number of NMDA binding sites [7, 17] yet is relatively spared of plaques and tangles. Since we observed no changes in [<sup>3</sup>H]TCP binding in this region it is possible that plaque and/or tangle formation may antedate reductions in the glutamate/PCP receptor complex.

The reductions in NMDA receptor binding in hippocampus [8] are more marked than the losses in dissociative anesthetic binding but the pattern of binding loss is similar for the two ligands. Whether TCP also binds to channels associated with other receptors is a possibility that needs further investigation. Nevertheless, the large decreases observed in [<sup>3</sup>H]TCP binding might also be observed with a positron-labelled analog of TCP using positron emission tomography. Unlike glutamate and its analogs, the dissociative anesthetics enter brain rapidly and thus would be ideal potential ligands for in vivo imaging.

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