Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1352–1356, February 1990
Neurobiology

Differential alterations of cortical glutamatergic binding sites in senile dementia of the Alzheimer type

(glutamate receptor/Alzheimer disease/frontal cortex/neurotransmitter receptor plasticity)

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Communicated by Leslie L. Iversen, October, 30, 1989 (received for review March 29, 1989)

ABSTRACT **Involvement of cortical glutamatergic mechanisms in senile dementia of the Alzheimer type (SDAT) has been investigated with quantitative ligand-binding autoradiography. The distribution and density of Na^+-dependent glutamate uptake sites and glutamate receptor subtypes—kainate, quisqualate, and *N*-methyl-D-aspartate—were measured in adjacent sections of frontal cortex obtained postmortem from six patients with SDAT and six age-matched controls. The number of senile plaques was determined in the same brain region. Binding of D-[^{3}H]aspartate to Na^+-dependent uptake sites was reduced by ≈40% throughout SDAT frontal cortex relative to controls, indicating a general loss of glutamatergic presynaptic terminals. [^{3}H]Kainate receptor binding was significantly increased by ≈70% in deep layers of SDAT frontal cortex compared with controls, whereas this binding was unaltered in superficial laminae. There was a positive correlation (r = 0.914) between kainate binding and senile plaque number in deep cortical layers. Quisqualate receptors, as assessed by 2-amino-3-hydroxy-5-[^{3}H]methylisoxazole-4-propionic acid binding, were unaltered in SDAT frontal cortex compared with controls. There was a small reduction (25%) in *N*-methyl-D-aspartate-sensitive [^{3}H]glutamate binding only in superficial cortical layers of SDAT brains relative to control subjects. [^{3}H]Glutamate binding in SDAT subjects was unrelated to senile plaque number in superficial cortical layers ($r = 0.104$). These results indicate that in the presence of cortical glutamatergic terminal loss in SDAT plastic alterations occur in some glutamate receptor subtypes but not in others.**

Senile dementia of the Alzheimer type (SDAT) is characterized neuropathologically by neuronal cell loss, the presence of higher than normal densities of senile plaques, and the development of neurofibrillary tangles in neocortical and archicortical regions (1–3). Extensive neurochemical studies have indicated deficits in a number of neurotransmitters and neurotransmitter receptors in SDAT cerebral cortex (4–7). The relationship of these changes to the neuropathological progression of the disease, however, remains largely unknown.

Glutamate is putatively the major excitatory transmitter of cortical pyramidal neurons (8), which mediate cortico–cortical and corticofugal neurotransmission (9). A loss of cortical pyramidal cells is a prominent feature of SDAT pathology (2, 3, 10); thus, cortical glutamatergic dysfunction may be a contributory factor in the pathophysiological progression of the disease. In rodents, glutamate receptor-mediated excitotoxicity in the cerebral cortex induces retrograde degeneration of cholinergic neurons in the nucleus basalis (11), supporting the suggestion that cortical cholinergic axon degeneration in SDAT may be a consequence of a primary cortical pathology (12).

A deficit in cortical glutamatergic terminals, as assessed by Na^+-dependent D-[^{3}H]aspartate binding is a consistent finding of homogenate binding studies in SDAT brain (13–15). The integrity of cortical postsynaptic glutamate receptors in SDAT, however, remains largely unclear. Postsynaptic actions of glutamate are mediated by three receptor subtypes; quisqualate, kainate, and *N*-methyl-D-aspartate (NMDA), differentiated on the basis of antagonist potency (16). NMDA receptors have been extensively studied in SDAT brain, previously almost exclusively in hippocampus. However, results have been inconsistent and often contradictory (14, 15, 17–20).

Using quantitative ligand-binding autoradiography, we have measured the distribution and density of both high-affinity Na^+-dependent glutamate-uptake sites and the three glutamate receptor subtypes in adjacent sections of frontal cortex from SDAT patients and age-matched controls. These neuropharmacological measures could be directly related to the neuropathological severity of the disease by quantification of senile plaque numbers in the same brain region.

MATERIALS AND METHODS

Brains were obtained at postmortem from six subjects, mean age 84 ± 2 yr, who had no known neurological or neuropsychiatric disorders (two males, four females; postmortem delay 11–23 hr), and six clinically diagnosed SDAT patients, mean age 89 ± 2 yr (two males, four females; postmortem delay 3–15 hr). As part of an ongoing longitudinal study of SDAT, these patients were diagnosed antemortem according to the criteria set out in the Cambridge Diagnostic Examination for the Elderly (21). Eight of the twelve patients used in this study died of bronchopneumonia (three control, five SDAT), three died of malignant diseases (two control, one SDAT) and one died (control) of pulmonary thromboembolism. At death, three control patients and three SDAT patients had been receiving diamorphine.

At autopsy, brains were cut into 1-cm-thick coronal slabs, and the middle frontal gyrus (Brodmann area 9) was dissected out at the level of the genu, frozen in isopentane (−40°C), and stored at −80°C, in preparation for subsequent receptor autoradiography. The remaining undissected tissue was fixed in 10% formalin and processed for senile plaque quantification. Tissue from the left hemisphere was used in this study.

Senile plaque numbers were determined in sections taken from tissue blocks no more than 1 cm caudal to those used for receptor autoradiography. Sections 28-μm thick were stained with King's amyloid stain (45), and senile plaques were subsequently measured by using a light microscopic image analysis system. Mean plaque number was calculated from

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Abbreviations: SDAT, senile dementia of the Alzheimer type; NMDA, *N*-methyl-D-aspartate; AMPA, 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

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six individual readings (three on gyri, three on sulci) in both superficial (I–III) and deep (IV–VI) layers of frontal cortex. Senile plaque quantification was performed independently by an investigator who had no knowledge of the ligand-binding studies.

For receptor autoradiography, blocks of frontal cortex were cut into 20-μm-thick cryostat sections and mounted onto chrome alum/gelatin-coated glass slides. Serial sections were used for the determination of D-[^{3}H]aspartate, [^{3}H]kainate, 2-amino-3-hydroxy-5-[^{3}H]methylisoxazole-4-propionic acid ([^{3}H]AMPA) and NMDA-sensitive [^{3}H]glutamate binding in both SDAT and control brains. For each ligand, control and SDAT sections were incubated and washed simultaneously.

All sections were preincubated for 1 hr at 4°C followed by 15 min at 30°C in an appropriate buffer: 50 mM Tris·HCl, pH 7.4, for D-[^{3}H]aspartate binding, 50 mM Tris·acetate, pH 7.2, for [^{3}H]glutamate and [^{3}H]AMPA binding, and 50 mM Tris·citrate, pH 7.0, for [^{3}H]kainate binding. Incubations were at 4°C with 250 nM D-[^{3}H]aspartate (+300 mM NaCl) for 30 min, 150 nM [^{3}H]glutamate (+5 μM quisqualate and 100 μM 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid—a chloride channel blocker) for 10 min, 130 nM [^{3}H]AMPA (+100 mM KSCN) for 30 min, and 50 nM [^{3}H]kainate for 30 min. In both control and SDAT brains, levels of nonspecific binding for D-[^{3}H]aspartate, [^{3}H]kainate, [^{3}H]AMPA, and NMDA-displaceable [^{3}H]glutamate were determined in the presence of 250 μM D-aspartate, 50 μM kainate, 150 μM quisqualate, and 150 μM NMDA and represented 20%, 40%, 30%, and 45% of total binding, respectively. Postincubation all sections received four 5-sec washes in buffer at 4°C, one rinse in distilled water at 4°C, and one rinse of 2.5% gluteraldehyde in 1 ml of acetone solution while being dried in a stream of cold air. Sections were then placed in x-ray cassettes with [^{3}H]-microscales (Amersham) and apposed to [^{3}H]Hyperfilm (Amersham) for 4–6 weeks. The resulting receptor autoradiograms were analyzed by using a Quantimet 970 (Cambridge Instruments, Cambridge, England) image analysis system. Mean optical density measurements for each lamina were calculated from six individual readings (three on gyri, three on sulci) averaged over three sections. Binding to a discrete cortical lamina was determined by comparison of autoradiograms with adjacent cresyl violet-stained sections. Optical density values were converted to pmol/g of tissue with reference to the recalibrated [^{3}H]microscales and the specific activities of the ligands.

Statistical differences between control and SDAT cases were determined by using an unpaired, two-tailed Student's t test. Linear regression analysis was used to determine the relationship between ligand binding and senile plaque data.

RESULTS

Neuropathology. All SDAT patients had postmortem neuropathological confirmation of the antemortem diagnosis of SDAT (22). All control and SDAT brains were free from gross morphological abnormalities such as tumors or infarcts. In SDAT frontal cortex, mean ± SEM senile plaque numbers were 35 ± 6 per mm^2 in superficial layers (I–III) and 21 ± 4 per mm^2 in deep layers (IV–VI). The number of senile plaques did not exceed 2 per mm^2 in any control subjects.

D-[^{3}H]Aspartate Binding. D-[^{3}H]Aspartate binding in frontal cortex exhibited an obvious laminar distribution in all brains from control subjects (Fig. 1*A*). In SDAT brains, there was a marked reduction of D-[^{3}H]aspartate binding throughout frontal cortex compared with controls (Fig. 2*A*) and a clear diminution in the prominence of the laminar pattern of binding (Fig. 1). D-[^{3}H]Aspartate binding in SDAT frontal cortex was unrelated to senile plaque number in both superficial and deep layers (r = 0.001 and r = 0.197, respectively).

[^{3}H]Kainate Binding. [^{3}H]Kainate binding in layers IV and V–VI of frontal cortex of SDAT patients was significantly greater (70%) than that of control subjects, whereas [^{3}H]-kainate binding in cortical layers I–III was similar in control and SDAT brains (Fig. 2*B*). The magnitude of the increase in kainate binding in SDAT frontal cortex was such that every SDAT brain could be readily distinguished from controls on visual inspection of the autoradiograms by the presence of a band of high optical density in the deep cortical laminae (Fig. 3*B*). Furthermore, [^{3}H]kainate binding was positively correlated with senile plaque number in layers IV (r = 0.901) and V–VI (r = 0.914) of SDAT frontal cortex. There was no correlation between kainate binding and senile plaque number in superficial laminae of SDAT brains (r = 0.089) (Fig. 4).

[^{3}H]AMPA Binding. [^{3}H]AMPA binding in SDAT frontal cortex was not significantly different from controls in any cortical layer (Fig. 2*C*), indicating preservation of AMPA-sensitive quisqualate receptors in this region in SDAT.

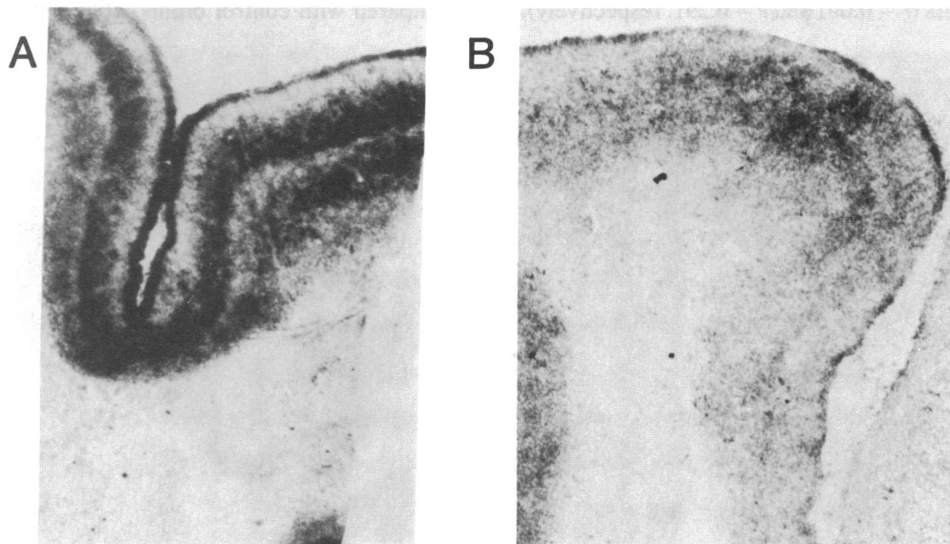

FIG. 1. Representative autoradiograms of D-[^{3}H]aspartate binding in sections of frontal cortex in control (*A*) and SDAT (*B*) brain. Note the reduction in D-[^{3}H]aspartate binding throughout SDAT frontal cortex in comparison with control frontal cortex and the significant diminution in the prominence of the laminar pattern of binding in SDAT frontal cortex.

FIG. 2. D-[^{3}H]Aspartate binding (*A*), [^{3}H]kainate binding (*B*), [^{3}H]AMPA binding (*C*), and NMDA-sensitive [^{3}H]glutamate binding (*D*) in control (□) and SDAT (■) frontal cortex. Data are means ± SEM, $n = 6$ for each group. Level of statistical significance was determined by the unpaired, two-tailed Student's t test; *, $P < 0.05$; **, $P < 0.005$.

[^{3}H]AMPA binding in SDAT frontal cortex was unrelated to senile plaque number in both superficial (I–III) and deep (IV–VI) cortical layers ($r = 0.001$ and $r = 0.291$, respectively).

NMDA-Sensitive [^{3}H]Glutamate Binding. [^{3}H]Glutamate binding in layers I–II of SDAT frontal cortex was reduced compared with control brains, although the difference only

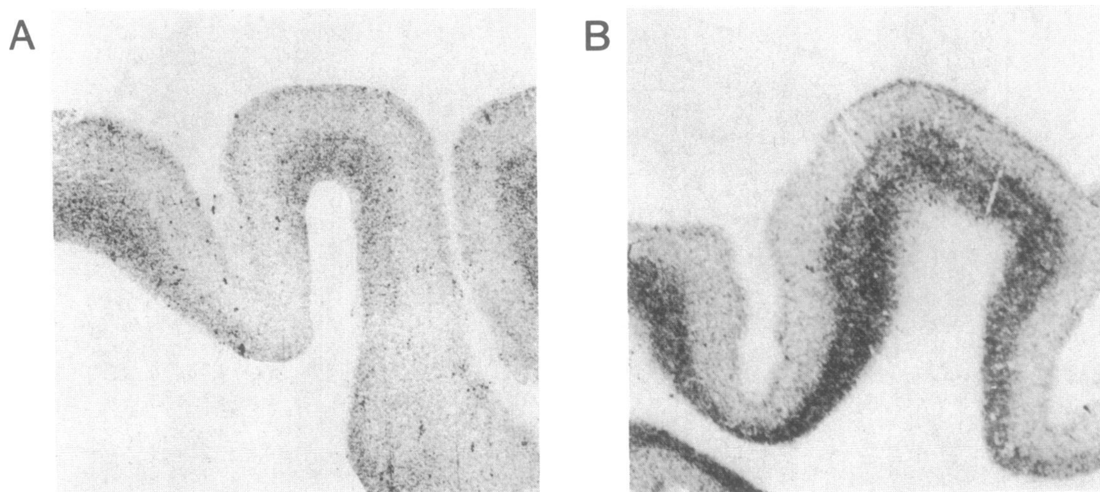

FIG. 3. Representative autoradiograms of [^{3}H]kainate binding in sections of frontal cortex in control (*A*) and SDAT (*B*) brain. In SDAT frontal cortex, [^{3}H]kainate binding is increased in deep cortical layers and unaltered in superficial cortical layers in comparison with control frontal cortex.

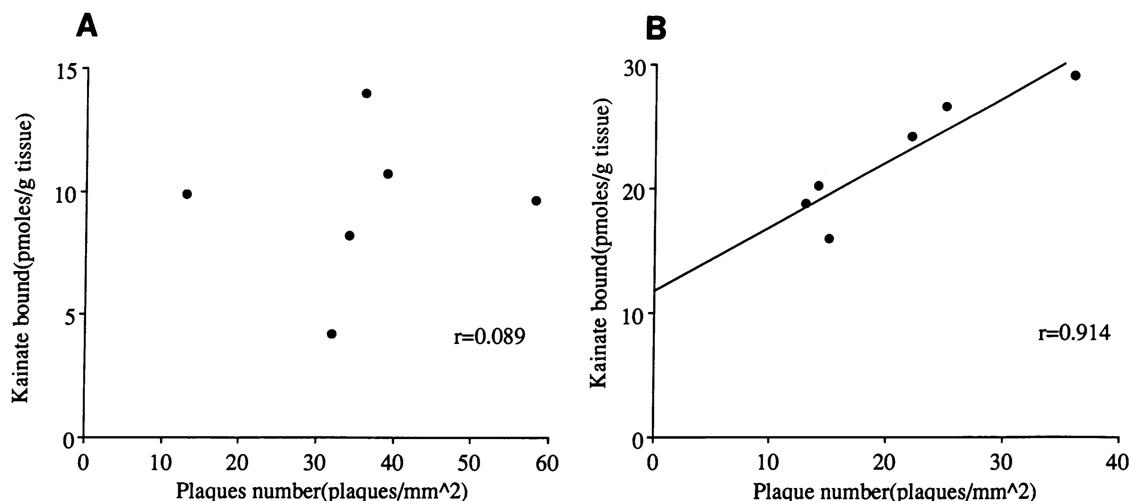

FIG. 4. Relationship between kainate receptor binding and senile plaque number in superficial layers (I–II) (*A*) and deep layers (IV–VI) of SDAT frontal cortex (*B*). Correlation of kainate receptor binding with senile plaque number was significant ($P < 0.02$) in deep layers ($r = 0.914$) but not in superficial layers ($r = 0.089$).

just achieved statistical significance ($P = 0.05$), (Fig. 2*D*). [^{3}H]Glutamate binding in these cortical layers did not correlate with senile plaque number in superficial layers of SDAT cortex ($r = 0.104$).

DISCUSSION

Frontal cortex, the focus of this study, has widespread reciprocal connections with other cortical areas (23) and is fundamental to the integration of higher mental function (24). Hitherto, temporal cortex and hippocampus have been the anatomical foci of many studies of SDAT brain because of their role in learning and memory, a central and early deficit in SDAT (25). However, patients with Alzheimer disease may also show patterns of cognitive dysfunction that are similar to those found in patients with focal frontal lesions (26). Moreover, the extent of neuropathological change (e.g., plaques, neuronal loss) seen in SDAT frontal cortex is similar to that found in temporal cortex (2, 3). Glutamate is the major excitatory transmitter in mammalian cerebral cortex putatively involved in intracortical and corticofugal information processing (8, 27, 28) and cortical plasticity (29, 30). Furthermore, the excitotoxic effects of glutamate (31, 32) have been the basis for speculative hypotheses implicating glutamatergic mechanisms in the etiology of SDAT (33, 34).

The present study represents a comprehensive examination of multiple elements of glutamatergic transmission in the SDAT brain and their relationship to the severity of neuropathology. There are three important elements to the present study. (*i*) The spatial resolution of autoradiography has allowed the nonprejudicial examination of alterations in neurotransmitter receptors in single cortical layers. The study is further strengthened by performing ligand-binding autoradiography and senile plaque quantification in adjacent tissue sections. (*ii*) An increase (as opposed to reduction) in the binding of a specific neurotransmitter receptor (kainate) was seen in SDAT cerebral cortex, and this response was positively related to the severity of neuropathology in the same region. (*iii*) The integrity of two postsynaptic glutamate receptor subtypes (quisqualate and NMDA) was maintained even in the presence of a major loss of presynaptic glutamatergic input.

In the present study, the marked reduction in D-[^{3}H]aspartate binding in all layers of frontal cortex in SDAT subjects (Fig. 2*A*) confirms and extends previous findings based largely on homogenate preparations (13–15). D-Aspartate is a substrate for the high-affinity glutamate uptake system (35) and has been proposed to be a suitable marker of glutamate-releasing nerve terminals in human brain (36). The present data, therefore, indicate a marked loss of glutamatergic terminals in SDAT frontal cortex. However, a reduction in D-[^{3}H]aspartate binding to nonneuronal sites, which also possess the capacity for high-affinity uptake (28), could make a small contribution to the observed reduction in D-[^{3}H]-aspartate binding. In view of the significant loss of cortical pyramidal neurons in SDAT (2, 3, 10), which are the cells of origin for intracortical projection fibers (9), reduced D-[^{3}H]aspartate binding may reflect the degeneration of excitatory cortico–cortical association fibers (10). A deficit in excitatory input to frontal cortex from other cortical regions may represent the neuropharmacological basis for the breakdown of organized intrahemispheric function in SDAT patients, as detected by positron emission tomography (37).

Numerous ligand-binding studies of the glutamatergic system in SDAT, to date, have concentrated on NMDA receptors with minimal examination of quisqualate or kainate receptors. In this respect, the most striking finding of the present study has been the substantial increase in kainate receptor binding in deep layers of SDAT frontal cortex (Figs. 2*B* and 3*B*). Of the control and SDAT subjects used in this study, full autoradiographic saturation analysis of this response was only possible in one control and two SDAT brains in which sufficient tissue remained. Calculated B_{max} values in deep cortical layers of both SDAT brains (69 and 63 pmol/g of tissue) were substantially greater than that in the control case (41 pmol/g of tissue), although K_d values were similar in all three brains (19 and 21 nM in SDAT brains, 16.5 nM in control). B_{max} and K_d values in superficial cortical layers were similar in both control and SDAT brains. In addition, inhibition of kainate receptor binding by a range of excitatory amino acid analogues was similar in both the control and SDAT brains examined and in agreement with previous reports in which both rat (38) and human (39) membrane preparations were used; the order of potency was domoate $\simeq$ kainate $\gg$ glutamate $\simeq$ quisqualate, whereas AMPA and NMDA produced negligible inhibition. Although by no means conclusive, these results, taken together, support the conclusion that the increase in kainate binding in deep layers of SDAT frontal cortex reflects a change in kainate receptor numbers, rather than a change in receptor recognition properties.

An increase in kainate receptor numbers in SDAT contrasts with the great majority of reports, in which reductions of many neurotransmitters and their receptors, including

glutamate, have been described (4–7, 15, 18, 20). A rare exception, however, reports an expansion of the kainate receptor field in the hippocampus of SDAT patients (40). On the basis of animal experiments, this receptor response was interpreted as being due to axon sprouting of both commissural and associational fibers in the dentate gyrus as a consequence of reduced excitatory input from entorhinal cortex (40). However, the possibility of regenerative changes in SDAT cerebral cortex in response to loss of excitatory input remains to be investigated.

The loss of a presynaptic glutamatergic marker (D-[^{3}H]aspartate) together with an increase in the postsynaptic kainate receptor may, at a simplistic level, be considered as evidence for denervation supersensitivity in SDAT frontal cortex. Increased numbers of neurotransmitter receptors have been reported in other neurodegenerative diseases such as Parkinson disease (41) and Huntington chorea (42). However, in the present study, the laminar-specific increase in kainate receptor contrasts with the widespread loss of presynaptic glutamatergic terminals from all cortical layers. Quantitative autoradiography was crucial in the detection of increased kainate receptor binding restricted to deep cortical layers. A recent study that used homogenate preparations of SDAT frontal cortex failed to demonstrate any significant alteration in kainate receptor binding (39). The neuroanatomical basis for the laminar-specific increase in kainate binding in SDAT frontal cortex remains to be established. Although this response was intimately associated with an index of neuropathological severity of SDAT (Fig. 4), there were no changes in kainate receptors in other cortical layers (I–III) in which similar or more severe neuropathological changes were present. Pyramidal cells in cortical layers V–VI are the source of corticofugal projections to the caudate putamen, thalamus, and brainstem (9). Mechanistic hypotheses relating the binding data in the present study to the preservation of corticofugal information processing, in the presence of intracortical excitatory dysfunction, must await critical evaluation.

Although the quisqualate receptor, like the kainate receptor, is involved in fast excitatory postsynaptic transmission (43), quisqualate receptor binding in SDAT frontal cortex, as assessed by [^{3}H]AMPA, did not differ from control values. The absence of changes in quisqualate receptors in SDAT frontal cortex contrasts with the marked reduction of these sites, as labeled with [^{3}H]glutamate, in SDAT hippocampus (18). The differential regulation of quisqualate receptors in neocortical and archicortical regions of the SDAT brain may reflect either neuroanatomically distinct physiological roles or differences in the populations of quisqualate receptors labeled by using [^{3}H]AMPA or [^{3}H]glutamate (44).

The NMDA receptor has been the most extensively studied glutamate receptor subtype in SDAT. Although excellent ligands are available for the NMDA receptor and its related ion channel, no consistent alterations of this receptor complex have emerged in SDAT brain, even in the hippocampus, which has been the subject of the majority of studies (17–20). The limited information available for frontal cortex indicates a moderate reduction in NMDA receptors in this region (15). Our demonstration of a small reduction in NMDA receptor binding in SDAT frontal cortex contributes minimally to the clarification of the general status of the NMDA receptor in the SDAT brain. However, the small reduction in NMDA receptors in superficial layers of SDAT frontal cortex contrasts with the marked alterations in other recognition sites in the same region and was unrelated to neuropathological measurements.

In the present study both the strong association of the kainate receptor response with local neuropathology and the direction of the receptor response (an increase, as opposed to a decrease) indicate the importance of this change to the pathophysiology of SDAT.

We thank Dr. B. Murphy, Gartnavel Royal Hospital, Glasgow, for clinical diagnoses of patients; Mr. A. Shand and Mrs. A. Lynch, Department of Neuropathology, Institute of Neurological Sciences, Glasgow, for neuropathological data, and Mrs. L. Graham for typing the manuscript. This work was supported by the Wellcome Trust.

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