Journal of Neurochemistry
Raven Press, Ltd., New York

Laminar Alterations in γ-Aminobutyric Acid$_A$, Muscarinic, and β Adrenoceptors and Neuron Degeneration in Cingulate Cortex in Alzheimer's Disease

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Abstract: The laminar distribution of binding to a number of postsynaptic neurotransmitter receptors was assessed autoradiographically in postmortem samples of area 23a in posterior cingulate cortex from 13 Alzheimer and nine age-matched control cases. Specific binding in all Alzheimer cases was compared to that in control cases, and the following alterations were observed: reduced muscimol binding in most layers; no changes in pirenzepine binding; and elevated cyanopindolol binding in layers Ic, IIIc, and IV. The Alzheimer cases were classified further on the basis of neuronal degeneration: class 1, no neuron loss; class 2, greatest losses in layer II or III; class 3, greatest losses in layer IV; and class 4, greatest losses in layer V or VI. This classification uncovered further alterations in ligand binding patterns. First, muscimol binding was reduced in layers II and III only in class 2 cases and in layers V and VI only in class 4 cases. Second, pirenzepine binding was reduced in layers Ic, IIIa-b, and VI of class 1 cases and layers Va and VI of class 4 cases. In spite of neuron degeneration in classes 2 and 3, there was no change in pirenzepine binding in these classes. Third, elevated cyanopindolol binding occurred in classes 3 and 4, whereas classes 1 and 2 had normal levels of binding. These results suggest that cases of Alzheimer's disease express heterogeneities in neocortical pathology which are reflected in the laminar patterns of binding to postsynaptic receptors. Reductions in muscimol binding to the γ-aminobutyric acid$_A$ receptor had the closest relationship with neuron degeneration, whereas pirenzepine binding appeared to reflect a compensation in muscarinic receptors for changes in neuron densities.
Key Words: Neurotransmitter receptors—Autoradiography—Cerebral cortex—Neurodegenerative disease. **Vogt B. A. et al.** Laminar alterations in γ-aminobutyric acid$_A$, muscarinic, and β adrenoceptors and neuron degeneration in cingulate cortex in Alzheimer's disease. *J. Neurochem.* **57**, 282–290 (1991).

Alterations in neurotransmitter receptor binding in the neocortex of patients with dementia of the Alzheimer type (DAT) have been demonstrated for a number of systems. Whereas binding to γ-aminobutyric acid (GABA) (Reisine et al., 1978; Chu et al., 1987), M_2 acetylcholine (Mash et al., 1985), serotonin$_1$, and serotonin$_2$ (Cross et al., 1984; Reynolds et al., 1984; Middlemiss et al., 1986; Palmer et al., 1987) receptors is reduced in DAT, β_2 adrenoceptors increase in density (Kalaria et al., 1989*a*). Receptor binding studies generally presume that the neocortex is damaged uniformly based on consistent demonstration of neurofibrillary tangles and neuritic plaques in the hippocampus. However, Brun and Englund (1981) have shown that there can be a wide range of neuron loss in neocortex. The greatest variation among cases that they observed was between no loss and 80% loss in posterior cingulate cortex, and it was presumed that these losses reflected progressive stages or grades of a uniform disease process. We have proposed recently that laminar specificities in neuron degeneration in posterior cingulate cortex may reflect up to five distinct classes of DAT (Vogt

Received November 29, 1990; accepted December 31, 1990.
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Abbreviations used: DAT, dementia of the Alzheimer type; GABA, γ-aminobutyric acid.

et al., 1990*b*). Furthermore, these classes do not correspond to the deposition of neurofibrillary tangles or neuritic plaques in either the neocortex or hippocampus.

A number of recent experimental radioligand binding studies have localized classes of receptors to cortical neurons using selective ablation techniques. Thus, $GABA_A$, muscarinic, acetylcholine, and $serotonin_{1A}$ receptors are on both proximal and apical tuft dendrites of cortical neurons (Vogt and Burns, 1988; Vogt and Hedberg, 1988; Crino et al., 1990; Vogt, 1990). Although DAT is a chronic disease which is expressed clinically over a period of 5–15 years and possibly even longer (Jarvik, 1988), the simple hypothesis should be considered that alterations in postsynaptic receptor binding in DAT are related to neuron degeneration. No investigation of DAT has analyzed jointly alterations in receptor binding and neuron density in the same neocortical layers. The coverslip autoradiographic technique of Young and Kuhar (1979), in conjunction with neuron counting and thioflavin S staining in adjacent sections, provides the technical base for this approach.

The strategy of the present study was as follows. First, the laminar binding pattern for a number of ligands was determined in area 23a of posterior cingulate cortex for all DAT cases combined and compared to those in control cases. Second, the DAT cases were classified according to the layers in which most neurons had degenerated. Third, receptor binding data were reanalyzed by class to determine whether there were classes of cases which had changes in binding that were amplified beyond changes for all DAT cases and/or classes of cases in which binding was essentially normal. The following issues were also of interest: (a) what was the relationship between the layers with maximal changes in receptor binding and those with greatest neuron loss? and (b) were there changes in binding in layer I where there were essentially no neurons in either control or DAT cases?

MATERIALS AND METHODS

Case material

Thirteen cases diagnosed as DAT on clinical and neuropathological criteria (McKhann et al., 1984) were used in this study. A summary of the data for these cases and the nine neurologically intact, age-matched control cases is presented in Table 1. There was no evidence of neuropathological abnormalities in the control cases; this included a lack of any neurofibrillary tangles or neuritic plaques in cingulate cortex after staining with thioflavin S. Ages at death were closely matched at 68 ± 3 versus 71 ± 2 years for the control versus DAT cases, respectively. The postmortem interval to the time of freezing to −70°C was shorter for the DAT cases, but it should be noted that intervals as long as 24 h have virtually no influence on muscarinic acetylcholine receptor binding in rat brain (Syapin et al., 1987). Finally, brain weights of 1,151 ± 42 g for DAT cases were lower than those of 1,312 ± 43 g for control cases ($F = 6.9$, $p = 0.017$).

The drug histories of patients in this study were surveyed for compounds with CNS actions that were administered for 1 or more weeks during the 6 months prior to death. Six of the 13 DAT cases received compounds which have antagonistic actions at muscarinic acetylcholine receptors, including atropine, chlorpromazine, diphenhydramine, loxapine, metoclopramide, or thioridazine. Three DAT patients received benzodiazepine agonists, including oxazepam or temazepam, and two received morphine. Of the nine control cases, six received morphine, three benzodiazepine agonists (triazolam, oxazepam, or alprazolam), two the β-adrenoceptor antagonist atenolol, and one chlorpromazine.

Neuropathological assessment

The methods for neuropathological assessment of these cases have been described thoroughly elsewhere (Vogt et al., 1990*b*), and so will be restated only briefly here. Blocks of posterior cingulate cortex, including the corpus callosum (see Fig. 1), were dissected and frozen to −70°C, or 1–2-cm thick coronal slabs were frozen to this temperature and cingulate cortex later removed without intermediate thawing. For receptor binding studies, sections were cut at a 16-μm thickness and mounted on chrome-alum-coated slides, whereas sections for thionin and thioflavin S staining were cut at a 48-μm thickness.

In order to classify these cases according to neuron loss, the perikarya of thionin-stained neurons in area 23a which had nucleoli were drawn with a drawing tube attachment to a light microscope at a final magnification of 650×. Strips of neurons 160 μm in width were drawn extending from layer II to the white matter of area 23a (see Fig. 1 and Vogt, 1985, for the location and structure of this area). Adjacent sections were also stained with thioflavin S (Schwartz et al., 1964) and were analyzed for the distribution of neurofibrillary tangles and neuritic plaques with an epifluorescent microscope. The distribution of these structures was plotted with an *x–y* plotter attached to the microscope stage and the total number of neuritic plaques calculated for area 23a and in layer I of all cingulate areas. The total number of neurofibrillary tangles in area 23a was also calculated for each case.

Receptor binding protocols

Unlabeled muscimol was purchased from Research Biochemicals (Wayland, MA, U.S.A.). Radiolabeled ligands were purchased from New England Nuclear and included the following compounds: [^{3}H]muscimol (sp. act. 23.2 Ci/m*M*), [^{3}H]pirenzepine (sp. act. 84 Ci/m*M*), and [^{125}I]iodo-(−)-cyanopindolol (sp. act. 2,200 Ci/m*M*). Muscimol binding was evaluated as previously reported (Vogt and Hedberg, 1988): preincubation of sections in 50 m*M* Tris buffer for 40 min at 20°C, incubation in the same buffer with 2 n*M*, [^{3}H]muscimol for 15 min at 20°C, followed by two buffer washes of 2 min each at 4°C and one distilled water wash at 4°C. Nonspecific binding was determined in a parallel series with 100 μ*M* unlabeled muscimol. Nonspecific binding was 5.4 ± 0.3% of total binding. Pirenzepine binding was evaluated as previously reported (Vogt and Burns, 1988): incubation of sections in Krebs–Henseleit buffer with 12 n*M* [^{3}H]pirenzepine for 70 min at 25°C, followed by two washes in buffer for 3 min each at 4°C. Nonspecific binding was determined with coincubation of a series in 1 μ*M* atropine. Nonspecific binding was 17 ± 2.8% of total binding. Finally, (−)-cyanopindolol binding was evaluated according to the procedure described by Vogt et al. (1990*a*): incubation of the sections in 20 m*M* Tris buffer with 135 m*M* sodium chloride, 65 p*M* [^{125}I]iodo-(−)-cyanopindolol, and 1 μ*M* unlabeled serotonin for 120 min at 24°C, followed by three buffer washes

TABLE 1. *Summary of case data*

	Control (n = 9)	All DAT (n = 13)	Class 1 (n = 3)	Class 2 (n = 3)	Class 3 (n = 4)	Class 4 (n = 3)
Age at death (yr ± SEM)	68 ± 3	71 ± 2	71 ± 4	72 ± 6	69 ± 2	71 ± 4
Age at disease onset (yr ± SEM)	—	64 ± 2	67 ± 6	66 ± 6	61 ± 3	61 ± 2
Length of disease (yr ± SEM)	—	7.5 ± 0.7	6.3 ± 0.9	6.7 ± 0.7	7.8 ± 1.3	9.3 ± 1.9
Postmortem interval (h ± SEM)	13 ± 1	6 ± 1[a]	8 ± 5	8 ± 4	4 ± 1[a]	5 ± 2[a]
Brain weight (g ± SEM)	1,312 ± 43	1,151 ± 42[b]	1,240 ± 38	1,095 ± 4	1,131 ± 123	1,127 ± 39
Neuron density (perikarya ± SEM/160 μm wide layer)						
Layer II	27 ± 3	22 ± 2	27 ± 6	16 ± 4	21 ± 3	24 ± 4
Layer IIIa–b	45 ± 6	34 ± 4	46 ± 7	19 ± 3[a]	36 ± 6	36 ± 4
Layer IIIc	34 ± 4	30 ± 4	41 ± 6	19 ± 3	31 ± 7	31 ± 7
Layer IV	19 ± 2	14 ± 3	27 ± 6	12 ± 2	8 ± 1[b]	12 ± 4
Layer Va	39 ± 3	27 ± 3[b]	41 ± 3	29 ± 4	24 ± 2[b]	17 ± 5[a]
Layer Vb	18 ± 2	20 ± 3	33 ± 9[b]	16 ± 4	18 ± 4	11 ± 5
Layer VI	70 ± 5	62 ± 8	101 ± 10[a]	62 ± 13	51 ± 11[a]	38 ± 3[a]

[a] $p < 0.01$; [b] $p < 0.05$, compared to control values.

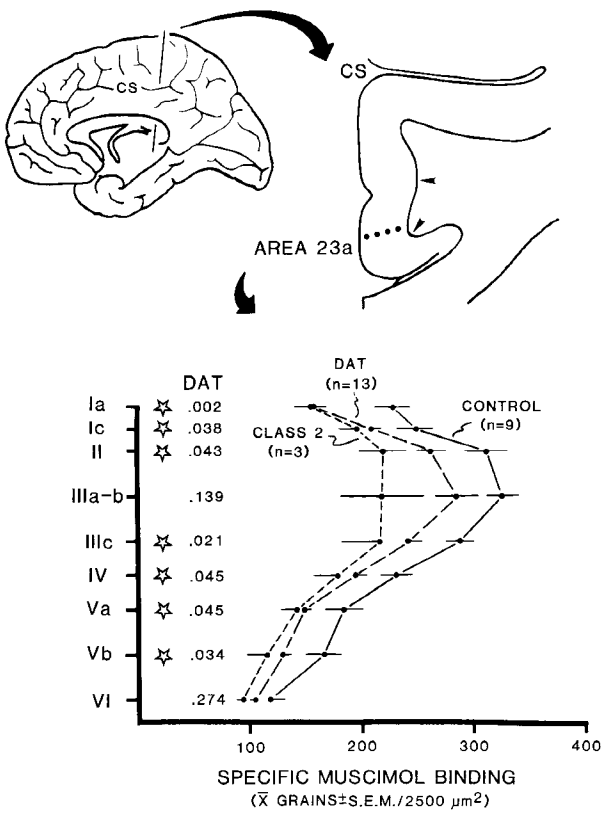

FIG. 1. Specific binding and neuron density were assessed in posterior cingulate cortex at the level indicated on the medial surface (CS, cingulate sulcus). Analysis was performed in area 23a as designated by the dots in the coronal section. Specific binding of [^{3}H]muscimol in all DAT cases was reduced significantly in comparison with control cases as shown by the stars next to individual *p* values for each layer. An amplified reduction in muscimol binding occurred in layers II, IIIa-b, and IIIc in class 2 cases, but not in other cases.

for 20 min each at 4°C and one 5-s water wash at 4°C. Serotonin was included routinely in this assay to assure that no cyanopindolol binding occurred to serotonin receptors. Nonspecific binding was determined in a parallel series with 1 μM propranolol. Nonspecific binding was 39 ± 3.4% of total binding.

Autoradiographs were prepared according to the method of Young and Kuhar (1979). Coverslips were acid-cleaned and dipped in Kodak NTB-2 nuclear tract emulsion. The dried coverslips were attached to slides with cyanoacrylate and exposed in the dark at −20°C for 3 weeks to 3 months for tritiated compounds and 3–5 days for iodocyanopindolol. Autoradiographs were developed to the point at which grains just began to overlap in the layer with highest binding. They were developed in Kodak D-19 without hardener, fixed in Kodak Rapid Fixer, and then counterstained with thionin.

Quantification of autoradiographs

Because the classification of DAT cases was based on neuron degeneration in area 23a of posterior cingulate cortex, autoradiographs were quantified in this region (see Fig. 1). Each cortical layer or sublayer was first identified with bright-field illumination, and then dark-field illumination was used so that a computerized image-analysis system (Image Technology model 1000, Donsanto Corp., Natick, MA, U.S.A.) could be used to count grain densities. Grain counts were corrected visually for miscounts due to overlapping grains, and cases were often counted by two individuals who were unaware of case classification to assure accuracy in terms of both cytoarchitectural localization of specific layers and absolute grain densities. Grains were quantified per 2,500 μm^2 of a cortical layer in three sections from each case, incubated with or without an unlabeled blocker to determine nonspecific and total binding, respectively. Specific binding was determined by subtracting these two values. Mean ± SEM values were calculated for control, all DAT, and each class of DAT.

There were inherent sources of variation in developing the autoradiographic series, such as exposure time, temperature of the D-19 developer, and development time. In order to

control for these factors, groups of two or three control cases were used to standardize series of DAT cases. Thus, if binding was higher in one set of control cases than another, the former set was reduced by an appropriate factor, as was the binding for DAT cases processed in the same series. In some instances, control and DAT cases were reprocessed to check absolute grain densities. There were four muscimol series, all of which were adjusted to the first. The grain densities for specific muscimol binding were highest in layer IIIa-b of control cases. The grain densities in this layer and correction factors were as follows: (a) 335 ± 19 grains (mean ± SEM); (b) (875 ± 31) ÷ 2.6; (c) (370 ± 25) ÷ 1.1; and (d) (259 ± 6) × 1.3. There were four pirenzepine series, all of which were adjusted to the first based on binding in layer II where it was highest: (a) 383 ± 19 grains; (b) (288 ± 24) × 1.33; (c) (283 ± 31) × 1.33; and (d) (437 ± 31) × 0.87. There were two iodocyanopindolol series which were corrected based on highest binding in layer IIIa-b. The first series had 643 ± 48 grains and the second had 380 ± 32 grains, and so the latter series was multiplied by 1.7.

Because grain density was evaluated by a unit of area (2,500 μm^2), reductions in the thickness of a layer would result in an underestimation of changes in binding associated with DAT. The thickness of each layer was calculated from the cortical strips on which neuronal perikarya were drawn. All layers had the same vertical thickness in controls and classes 1, 2, and 3 cases. However, layers Va and VI of class 4 cases had a reduced thickness: layer Va thickness was 407 ± 24 μm in controls and 273 ± 76 μm in class 4 cases, and layer VI thickness was 957 ± 70 μm in controls and 633 ± 96 μm in class 4 cases. Thus, grain densities in layers Va and VI of class 4 cases were reduced according to the percentage to which each of these layers shrank in each case.

Specific binding for control and all DAT cases was compared for each layer with a one-way analysis of variance. Subsequently, the DAT cases were separated into classes, and the binding was calculated for each class and plotted graphically. Protected *t* tests for multiple comparisons (Couch, 1982; software produced for IBM-AT computers by Dynamic Microsystems, Inc., Silver Springs, MD, U.S.A.) were performed and $p < 0.05$ accepted as statistically significant. Protected *t* tests were also used for analysis of case data, neuron, neurofibrillary tangle, and neuritic plaque densities, and laminar thickness. Finally, the Pearson product moment correlation coefficient was used to determine the level to which muscimol binding was correlated with clinical and neuropathological features of DAT.

RESULTS

Case classification

An average number of neurons was calculated for each layer of the nine control cases, as shown in Table 1. The number of neurons in a cortical layer was also determined for each DAT case and percentage differences calculated between the control means and that for individual DAT cases. If losses were less than 40%, the case was characterized as class 1. The remaining cases were classified according to the layer in which there was the greatest percentage of neuron loss, i.e., class 2 with losses in layer II or III, class 3 with greatest losses in layer IV, and class 4 with greatest losses in layer V or VI. There were no examples in this series of class 5 cases in which severe neuron degeneration occurred throughout all layers (Vogt et al., 1990*b*). Average neuron density by layer for these cases is presented in Table 1. When all DAT cases were considered as a group, there was only a small loss of neurons in layer Va. When evaluated by class, class 1 cases had more neurons on average in the deep layers than did control cases. Class 2 cases had a very selective loss of neurons in layer IIIa-b. Class 3 cases had a loss of neurons in layers IV and Va, as well as in layer VI; the latter loss was not as large as it was for class 4 cases. Finally, class 4 cases had extensive neuron loss in layers Va and VI.

There were no differences among these classes in terms of the age at which the disease became clinically apparent or the length of the disease. There were also no differences among these classes in terms of the total number of neuritic plaques or neurofibrillary tangles in area 23a.

Muscimol binding

The laminar distribution of specific muscimol binding in control and DAT cases is shown in Fig. 1 and Table 2. Binding in control cases was highest in layers II and IIIa-b, moderate in layers I, IIIc, and IV, and low in layers V and VI. Muscimol binding in all DAT cases was reduced significantly in layers I, II, IIIc, IV, and V. Classification of the DAT cases showed that binding in layer I was reduced to the same extent in all classes, except in class 3 cases, which had the lowest binding in layer Ia (149 ± 30 grains/2,500 μm^2) and essentially normal binding in layer Ic (230 ± 19 grains/2,500 μm^2). The ratio of muscimol binding in layer Ia to that in layer Ic was 0.64 ± 0.09 for class 3 cases and, therefore, distinguished this class from all others: class 1, 0.81 ± 0.04; class 2, 0.82 ± 0.13; class 4, 0.78 ± 0.14. The ratio for control cases was 0.91 ± 0.02. Figure 2 shows an example of muscimol binding in layer I for a control and class 1 case which were processed in the same series. Reduced binding was particularly pronounced in layer Ia.

Muscimol binding in layers II, IIIa-b, and IIIc was reduced to a greater extent in class 2 than that for all DAT cases, as shown in Figs. 1 and 3. Interestingly, this class had greatest neuron losses in layer IIIa-b (Table 1). A relationship between reduced muscimol binding and neuron densities was also evident in class 4 cases, because binding was lower in layers Va, Vb, and VI than it was in any other class or in control cases (Table 2). Without the correction for laminar shrinkage, binding in class 4 was 145 ± 20 in layer Va and 92 ± 5 in layer VI. Once again, neuron losses were greatest in class 4 cases in layers V and VI (Table 1).

Although layer Ia muscimol binding was reduced by an average of 30% for each class, there were cases with up to a 62% reduction in this layer. The possibility was considered that other clinical or pathological factors might be relevant to cases with particularly low muscimol binding in layer Ia. For example, one case with

TABLE 2. *Muscimol binding*

Layer	Control (n = 9)	All DAT (n = 13)	Class 1 (n = 3)	Class 2 (n = 3)	Class 3 (n = 4)	Class 4 (n = 3)
Ia	226 ± 17	156 ± 12[a]	156 ± 11[a]	158 ± 20	149 ± 30[a]	160 ± 36
Ic	247 ± 17	207 ± 9[a]	194 ± 17[a]	196 ± 17	230 ± 19	200 ± 16
II	311 ± 21	261 ± 13[a]	252 ± 21	218 ± 24[a]	286 ± 18	279 ± 38
IIIa-b	330 ± 15	284 ± 20	300 ± 36	217 ± 39[a]	316 ± 30	290 ± 5
IIIc	287 ± 12	240 ± 13[a]	256 ± 28	215 ± 36[a]	261 ± 22	221 ± 23[a]
IV	230 ± 15	194 ± 10[a]	207 ± 24	177 ± 22	194 ± 14	197 ± 29
Va	183 ± 18	138 ± 10[a]	159 ± 9	140 ± 15	146 ± 13	101 ± 36[a]
Vb	166 ± 16	129 ± 8	153 ± 12	123 ± 19	126 ± 5	114 ± 21
VI	118 ± 14	104 ± 5	120 ± 12	102 ± 16	101 ± 7	62 ± 13[a]

[a] $p < 0.05$, compared to control cases.

low binding had a large number of layer I neuritic plaques and, therefore, muscimol binding was correlated with a number of measures of neuropathology in these cases. There were no correlations between the following factors: layer I neuritic plaque density and muscimol binding in layer I (binding in layers Ia and Ic combined); neuritic plaque density and muscimol binding in layer Ia; neurofibrillary tangle density in layer III and muscimol binding in layer III (binding in layers IIIa-b and IIIc combined); age at disease onset and muscimol binding in layer I; age at disease onset and muscimol binding in layer III; length of the disease and muscimol binding in layer I; length of the disease and muscimol binding in layer III. Thus, neuropathological factors other than neuron density and clinical features of DAT were not associated with muscimol binding.

Pirenzepine binding

Specific binding of pirenzepine in control cases was highest in layers II and IIIa-b and moderate in other layers (Table 3). Pirenzepine binding in all DAT cases did not differ from that in control cases. Analysis of pirenzepine binding by class showed that in classes 2 and 3 binding was equivalent to control values in spite of significant neuron degeneration in layers IIIa-b and IV, respectively. However, in class 1 cases, pirenzepine binding was reduced by 26%, 24%, and 32% in layers Ic, IIIa-b, and VI, respectively. Pirenzepine binding was similar to control levels in layers I–IV and Vb of class

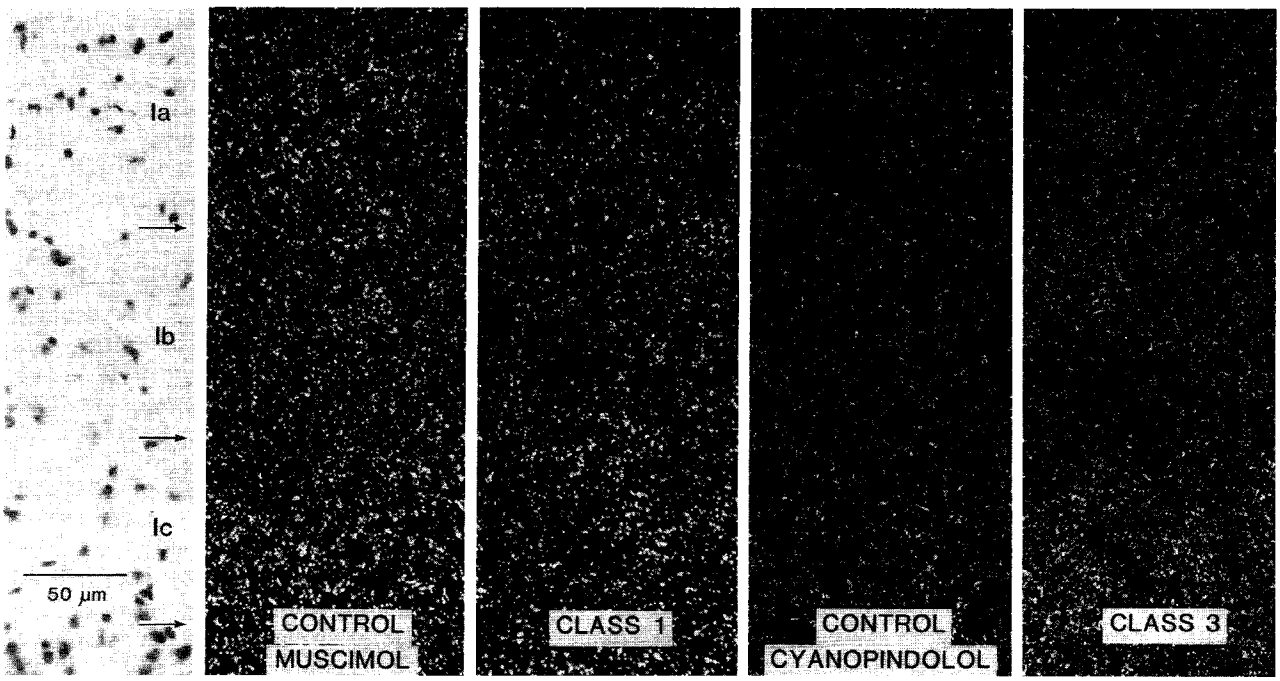

FIG. 2. Examples of layer I ligand binding from control and Alzheimer cases which were processed in the same autoradiographic series. The subdivisions of layer I are indicated in the bright-field photomicrograph (left). The dark-field micrographs show control and reduced class 1 muscimol binding and control and elevated class 3 cyanopindolol binding.

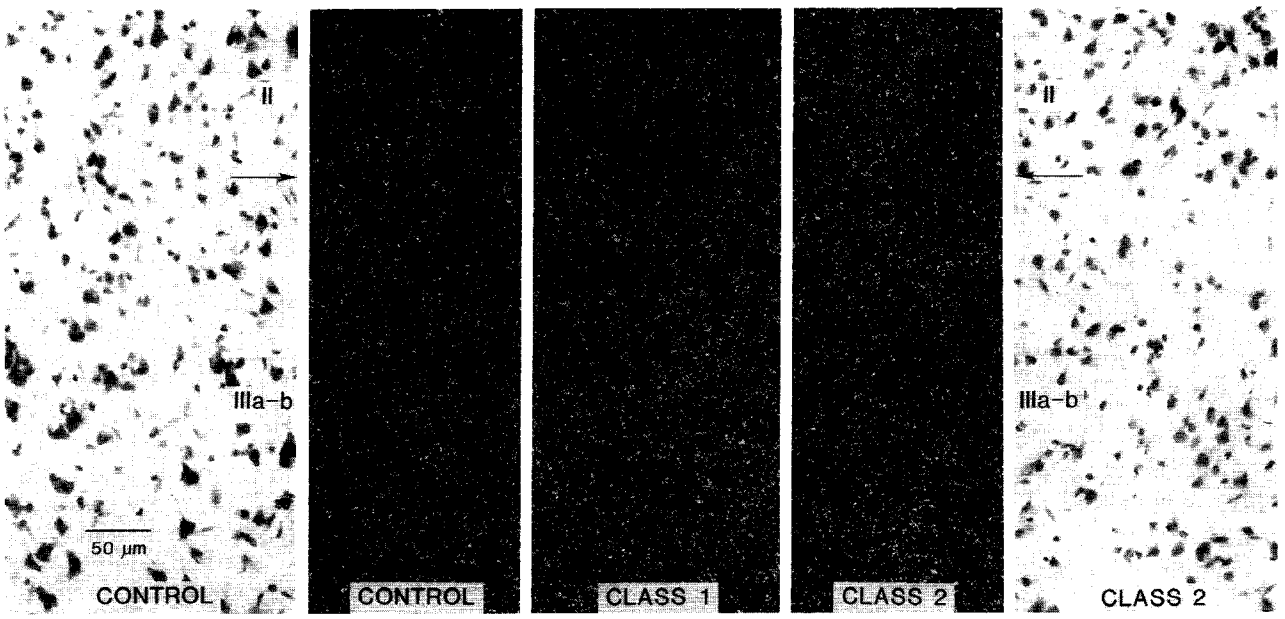

FIG. 3. The bright-field micrographs show neurons in layers II and IIIa-b in a control and a class 2 case. Muscimol binding in these and a class 1 case processed in the same autoradiographic series are shown with dark-field micrographs of the same layers. Muscimol binding in control and class 1 cases was essentially the same; however, there was a pronounced reduction in binding in layer IIIa-b in the class 2 case, which was associated with neuron degeneration in the same layer.

4 cases, but it was reduced in layers Va and VI by 50% and 55%, respectively. Before correcting for shrinkage, binding in layer Va was 246 ± 51, and in layer VI it was 219 ± 50. These latter cases had the greatest percentage loss of neurons in layers Va and VI.

Cyanopindolol binding

Table 4 presents specific cyanopindolol binding for seven control and 10 DAT cases. Specific cyanopindolol binding in the control cases was highest in layers II and IIIa-b and moderate in all other layers. Binding for all DAT cases was elevated over control values in layers Ic, IIIc, and IV. Classification of the DAT cases according to neuron losses, however, showed that increased cyanopindolol binding was not uniform. There were no differences in cyanopindolol binding between control and class 1 or class 2 cases. It was clear that increased cyanopindolol binding among all DAT cases was accounted for by that which occurred in the class 3 and 4 cases. As shown in Table 4, binding of cyanopindolol in class 3 cases was elevated in relation to control cases in all layers; only binding in layer II was not increased significantly. An autoradiograph of cyanopindolol binding in a class 3 case is compared with that in a normal case in Fig. 2.

Neuropathological and/or clinical factors may have contributed to the selective elevation of cyanopindolol binding in class 3 and 4 cases. Data for classes 1 and 2 were pooled, as well as data for classes 3 and 4. Although age at disease onset, duration of the disease, and densities of neurofibrillary tangles and neuritic plaques did not differ between these two groups, neuron

TABLE 3. *Pirenzepine binding*

Layer	Control (n = 9)	All DAT (n = 13)	Class 1 (n = 3)	Class 2 (n = 3)	Class 3 (n = 4)	Class 4 (n = 3)
Ia	320 ± 24	323 ± 19	237 ± 17	349 ± 33	374 ± 17	315 ± 41
Ic	355 ± 12	349 ± 18	263 ± 21[a]	405 ± 41	375 ± 8	345 ± 27
II	389 ± 15	384 ± 18	323 ± 32	454 ± 42	390 ± 21	366 ± 9
IIIa-b	378 ± 16	342 ± 17	287 ± 17[a]	374 ± 24	373 ± 32	322 ± 39
IIIc	335 ± 12	318 ± 17	265 ± 26	344 ± 27	350 ± 33	302 ± 41
IV	322 ± 21	284 ± 16	244 ± 13	330 ± 32	295 ± 27	264 ± 43
Va	314 ± 22	254 ± 21	236 ± 12	314 ± 20	298 ± 26	152 ± 23[a]
Vb	301 ± 17	266 ± 15	230 ± 5	310 ± 31	282 ± 23	236 ± 35
VI	279 ± 19	221 ± 21	189 ± 6[a]	278 ± 23	275 ± 30	125 ± 20[a]

[a] $p < 0.05$, compared to control cases.

TABLE 4. *Cyanopindolol binding*

Layer	Control (n = 7)	All DAT (n = 10)	Class 1 (n = 3)	Class 2 (n = 2)	Class 3 (n = 4)	Class 4 (n = 1)
Ia	472 ± 52	663 ± 52	578 ± 82	551 ± 9	745 ± 94[a]	819
Ic	507 ± 41	706 ± 54[a]	611 ± 74	596 ± 21	784 ± 96[a]	900
II	579 ± 49	748 ± 52	662 ± 99	683 ± 29	813 ± 98[a]	880
IIIa-b	638 ± 59	772 ± 47	634 ± 83	760 ± 73	867 ± 61[a]	832
IIIc	513 ± 51	704 ± 37[a]	576 ± 50	691 ± 21	791 ± 43[a]	767
IV	460 ± 51	647 ± 46[a]	506 ± 45	593 ± 13	749 ± 67[a]	765
Va	446 ± 45	605 ± 44	484 ± 23	516 ± 2	690 ± 60[a]	408
Vb	428 ± 47	586 ± 42	479 ± 29	528 ± 7	653 ± 74[a]	756
VI	388 ± 48	552 ± 45	450 ± 32	455 ± 29	624 ± 70[a]	520

[a] $p < 0.05$, compared to control cases.

densities in layers IV, Va, and VI were reduced significantly in classes 3 and 4 (see also Table 1).

DISCUSSION

The present study demonstrates that alterations in neurotransmitter receptor binding in Alzheimer's disease can be assessed accurately with a combination of laminar autoradiographic and neuropathological analyses. When receptor binding in all DAT cases is compared to that in control cases, muscimol binding is reduced in most layers, there are no changes in pirenzepine binding, and there is an elevation in cyanopindolol binding in layers Ic, IIIc, and IV. Although these results are interesting, they presuppose that DAT is a uniform neuropathologic entity. However, classification of DAT cases according to laminar alterations in neuron density reveals subgroups of cases (Vogt et al., 1990*b*) which can have unaltered, increased, or decreased ligand binding beyond changes in averages for all DAT cases. For example, class 1 cases have no neuron losses and muscimol and cyanopindolol binding is normal in most layers, whereas pirenzepine binding is altered in a few layers. Class 2 cases have neuron losses mainly in layer IIIa-b, as well as reduced muscimol binding in layers II and III and unaltered pirenzepine and cyanopindolol binding. Class 3 cases have greatest neuron degeneration in layer IV, reduced muscimol binding only in layer Ia, and elevated cyanopindolol binding in all layers. Class 4 cases have greatest neuron degeneration in deep layers which also have significantly reduced muscimol and pirenzepine binding. In contrast to separation of cases according to neuron losses, no classical measures of DAT neuropathology, such as thioflavin S-stained neurofibrillary tangles and neuritic plaques, distinguished among groups of cases which had significantly different changes in receptor binding.

Muscimol binding: a measure of neuropathological class

One of the goals of the present study was to identify a population of receptors to which binding was differentially altered in each neuropathological class of DAT. Although muscimol binding to $GABA_A$ receptors was altered in layer Ia of all classes, it came the closest to differentiating among the four classes. Thus, in addition to the layer Ia reduction, class 1 also had reduced muscimol binding in layer Ic, class 2 had reduced muscimol binding in layers II and III, class 3 muscimol binding was not altered beyond layer Ia, and class 4 had major muscimol binding losses in layers V and VI. Previous studies of GABA or muscimol binding in homogenized cortical tissue showed a decrease (Reisine et al., 1978) or no change (Bowen et al., 1983; Bowen and Davison, 1986) in binding, respectively. Chu et al. (1987) did not observe altered $GABA_A$ binding in autoradiographs of frontal cortex in DAT. The lack of agreement among these and the present study may be because subtle laminar differences in binding were lost in homogenized tissue preparations, there were differences in the cytoarchitectural areas analyzed, and no case classification was attempted in previous studies. Although differences in muscimol binding between classes 1 and 3 were not striking, binding of cyanopindolol was at control levels in class 1, but extremely high in class 3 cases. Thus, ligand binding observations support the neuropathological classification scheme proposed by Vogt et al. (1990*b*).

Receptor binding in layer I

An interesting feature of muscimol binding is that binding in layer Ia is reduced to exactly the same extent in all classes of DAT. Experimental radioligand studies support the notion that $GABA_A$ receptors in layer I are on apical tuft dendrites of cortical pyramidal neurons (Vogt and Hedberg, 1988; Vogt, 1991). It is possible that reduced muscimol binding in layer I is due to degeneration of apical tufts, altered synthesis or transport of the $GABA_A$ receptor, and/or a reduced affinity of the receptor for ligand. Patent apical tuft degeneration is unlikely, because class 1 cases had no neuron losses and showed the same reduction in layer Ia muscimol binding as in other classes of DAT, and pirenzepine binding was unaltered in layer Ia in any class. Normal muscimol binding in layers below layer I, such as layers II–IV of class 1 cases, suggests that $GABA_A$ receptor synthesis is intact. However, it is pos-

sible that in DAT there is a failure to transport receptors to the apical tuft dendrites secondary to disruption of the cytoskeleton. It is also possible that in layer I there is a reduction in the affinity of $GABA_A$ receptors for ligand due to an alteration in their phosphorylation state (Gyenes et al., 1988; Stelzer et al., 1988).

Pirenzepine binding and classes of DAT

Many early studies of quinuclidinyl benzilate binding in neocortex suggested that muscarinic receptors were stable in DAT (e.g., White et al., 1977; Perry et al., 1978; Rinne et al., 1985). Recent studies have also shown that pirenzepine and carbachol binding is stable in DAT (Smith et al., 1987; Flynn et al., 1991). From a neuropathological perspective, Lang and Henke (1983) observed that muscarinic receptor binding in cingulate cortex does not appear to be influenced by the presence of neurofibrillary tangles or neuritic plaques. Furthermore, in the present study, many layers with extensive neuron degeneration, e.g., layer IIIa-b of class 2 cases, had control levels of pirenzepine binding. One interpretation of these findings is that pirenzepine binding sites are up-regulated by surviving neurons. These receptors may not be functional, however, because they may be primarily in a low-affinity state (Smith et al., 1987; Flynn et al., 1991)

Elevated cyanopindolol binding

Neuropathological studies have demonstrated an involvement of the locus coeruleus in DAT (Tomlinson et al., 1981; Bondareff et al., 1982). In view of these studies, Kalaria et al. (1989*a*) analyzed β-adrenoceptor binding in prefrontal and hippocampal cortices where they observed a substantial increase in the number of β_2 adrenoceptors. Although the present study confirms an elevation in β-adrenoceptor binding, not all classes of DAT were influenced to the same extent. Increases in cyanopindolol binding occurred in classes 3 and 4, whereas the cases with less severe neuronal damage, i.e., classes 1 and 2, had normal levels of cyanopindolol binding. Kalaria et al. (1989*a*) have discussed the possibility that up-regulation of β adrenoceptors was caused by denervation supersensitivity or glial proliferation. We propose another mechanism for this up-regulation: proliferation of the sympathetic innervation of blood vessels and capillaries. β adrenoceptors are present on cerebral blood vessels (Janowsky and Sulser, 1987; Kalaria et al., 1989*b*), and Stenevi and Björklund (1978) showed that cholinergic denervation of the hippocampus and posterior cingulate cortex resulted in sprouting of axons from blood vessels into the neuropil. It is possible, therefore, that sprouting of noradrenergic innervation into the cortical neuropil occurs in class 3 and 4 cases and that this accounts for the increased receptor binding in these classes.

Conclusion

The neocortical layer in which most neurons degenerate can be used as the basis for neuropathological subtyping of DAT and provides an important dimension along which to study alterations in neurotransmitter receptor binding. It should be possible to expand the combined disease classification and receptor binding analysis to other neurodegenerative disorders, as well as to other neurochemical assays. In terms of DAT, it now appears warranted to reevaluate the cholinergic system from this perspective, as well as the serotoninergic, glutamatergic, and GABAergic systems.

Acknowledgment: We thank Dr. Thomas Kemper for his comments and suggestions on aspects of this research. We also thank the following individuals for their assistance in providing samples of cingulate cortex for these studies: Drs. Devay Lathi and Chompol Mahasaen at the Veterans Administration Hospital (Bedford, MA, U.S.A.); Dr. Edward Bird at the Brain Tissue Resource Center, McLean Hospital (Belmont, MA, U.S.A.); Dr. Judith Marquis at Boston University School of Medicine, Department of Pharmacology (Boston, MA, U.S.A.); and Dr. J. Ulrich at the Institut für Pathologie der Universität Basel (Basel, Switzerland). This research was supported by NIH-NINDS grant no. NS 18745 and the Department of Veterans Affairs.

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