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6. Repeated measures analyses of variance (serial position by subjects) for polynomial trends were used. (Unreported trends for functions tested were not significant, $P > 0.05$.) Significant linear trends for humans (H) and pigeons (P): H at 0 seconds, $F(1, 5) = 11.6$, $P = 0.019$; H at 100 seconds, $F(1, 5) = 23.3$, $P = 0.005$; P at 0 seconds, $F(1, 3) = 1003.7$, $P < 0.001$; P at 10 seconds, $F(1, 3) = 46.2$, $P = 0.006$. Significant quadratic trends: H at 10 and 25 seconds combined, $F(1, 4) = 11.4$, $P = 0.027$; P at 1 and 2 seconds combined, $F(1, 3) = 18.1$, $P = 0.024$. Because there were only two monkeys serial position by block was tested. Significant linear trends: 0 seconds, $F(1, 3) = 865$, $P < 0.001$; 30 seconds, $F(1, 3) = 156.9$, $P < 0.001$; significant quadratic trend, 2 and 10 seconds combined, $F(1, 3) = 60.1$, $P = 0.004$.
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Reduced Numbers of Somatostatin Receptors in the Cerebral Cortex in Alzheimer's Disease

Abstract. *Somatostatin receptor concentrations were measured in patients with Alzheimer's disease and controls. In the frontal cortex (Brodmann areas 6, 9, and 10) and temporal cortex (Brodmann area 21), the concentrations of somatostatin in receptors in the patients were reduced to approximately 50 percent of control values. A 40 percent reduction was seen in the hippocampus, while no significant changes were found in the cingulate cortex, postcentral gyrus, temporal pole, and superior temporal gyrus. Scatchard analysis showed a reduction in receptor number rather than a change in affinity. Somatostatin-like immunoreactivity was significantly reduced in both the frontal and temporal cortex. Somatostatin-like immunoreactivity was linearly related to somatostatin-receptor binding in the cortices of Alzheimer's patients. These findings may reflect degeneration of postsynaptic neurons or cortical afferents in the patients' cerebral cortices. Alternatively, decreased somatostatin-like immunoreactivity in Alzheimer's disease might indicate increased release of somatostatin and down regulation of postsynaptic receptors.*

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One approach to studying Alzheimer's disease is to examine its neurochemistry after death. Several neurochemical deficiencies related to the illness have thus far been identified. The best studied is a reduction in choline-acetyltransferase activity in the cerebral cortex, which has been attributed to a loss of cholinergic neurons in the basal nucleus of Meynert (*1*). Less marked cortical deficiencies of noradrenaline, serotonin, substance P, and γ-aminobutyric acid have been described (*2*). In addition, a widespread reduction in cortical somatostatin-like immunoreactivity (SLI) has been associated with Alzheimer's disease (*3*). Immunocytochemical studies have suggested that the alteration reflects degeneration of intrinsic somatostatin cortical neurons (*4*), and somatostatin appears to be a neurotransmitter or neuromodulator in the central nervous system having electrophysiologic effects on central neurons (*5*). The clinical importance and role of this peptide in the pathophysiology of Alzheimer's disease remains unknown.

Recently somatostatin receptors have been reported in the brain, the pituitary, and peripheral tissues (*6*). We have characterized somatostatin receptors in the human brain (*7*). Since the receptor is an integral component of the mechanism by which somatostatin activates or modulates neuronal activity, a study of somatostatin receptors may provide further insight into the role of somatostatin in Alzheimer's disease. In addition, an understanding of receptor changes associated with disease has implications for the development of drugs that modify receptor function. We have measured concentrations of somatostatin receptors in both control and patient tissue post-mortem and have related these findings to concentrations of SLI.

Postmortem brain tissue from 12 Alzheimer's patients (ten male, two female; mean age, 76.8 ± 2.3 years; range, 72 to 90 years) and from 13 controls (six male, seven female; mean age, 69.5 ± 3.9 years; range, 36 to 86 years) was dissected as previously described (*8*). The diagnosis in the Alzheimer's patients was confirmed by neuropathology in all cases, and other neuropathologic conditions were excluded. Neuropathologic examination showed the control tissues were normal in all cases except one that had some small lacunar infarcts in the white matter. Brain tissues from the patients and controls were handled identically. In all cases the time between death and storage of the tissue at −70°C was less than 24 hours (patients, 11.3 ± 2.0 hours; controls, 11.5 ± 1.6 hours); we have found that somatostatin receptors are stable for up to 24 hours in an animal model simulating human autopsy conditions.

Tissue extraction and radioimmunoassay for SLI were carried out as described (*9*). The assay recognizes amino acids 6 to 10 of tetradecapeptide somatostatin. Somatostatin 14 and somatostatin 28 are recognized on an equimolar basis. Somatostatin receptors in human brain membranes were measured with ^{125}I-labeled [Leu^8, DTrp^{22}, Tyr^{25}] somatostatin 28 (*10*). The peptide was iodinated by the chloramine-T method and then purified by high-performance liquid chromatography (HPLC). It was eluted isocratically with 0.25*M* tetraethyl ammonium formate buffer, *p*H 3.5, with 17 percent *n*-propanol as an organic modifier on a μBondapak C18 column (Waters Associates, Milford, Massachusetts). The major radioactive peak eluted at 18 minutes and a minor peak, corresponding to less optimal binding, at 32 minutes. The binding of ^{125}I-labeled [Leu^8, DTrp^{22}, Tyr^{25}]somatostatin 28 to somatostatin receptors was performed in a final volume of 100 μl tris-HCl buffer

Table 1. Scatchard analysis of somatostatin binding sites in the cortices of Alzheimer's patients and controls.

	B_{max} (wet-weight, fmol/mg)	K_D (nM)
Middle temporal gyrus (Brodmann area 21)		
Controls (6)	1.53 ± 0.18	0.98 ± 0.13
Patients (6)	0.70 ± 0.07*	0.53 ± 0.10†
Superior temporal gyrus (Brodmann area 22)		
Controls (6)	1.63 ± 0.34	1.44 ± 0.46
Patients (6)	1.42 ± 0.15	1.14 ± 0.19
Frontal cortex (Brodmann area 9)		
Controls (5)	1.33 ± 0.21	1.22 ± 0.28
Patients (4)	0.69 ± 0.12†	1.06 ± 0.07

*$P < 0.01$. †$P < 0.05$.

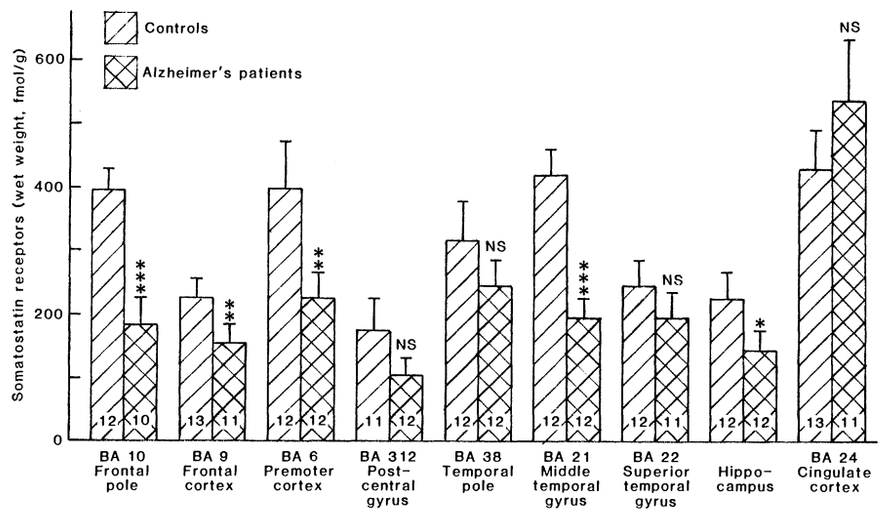

Fig. 1. Somatostatin-receptor binding (means ± standard error of the mean) in Alzheimer's patients and controls. The number of subjects tested is at the bottom of each column. Data were analyzed with the Mann-Whitney U test. Abbreviations: BA, Brodmann area; NS, not significant. *$P < 0.10$; **$P < 0.05$; ***$P < 0.001$.

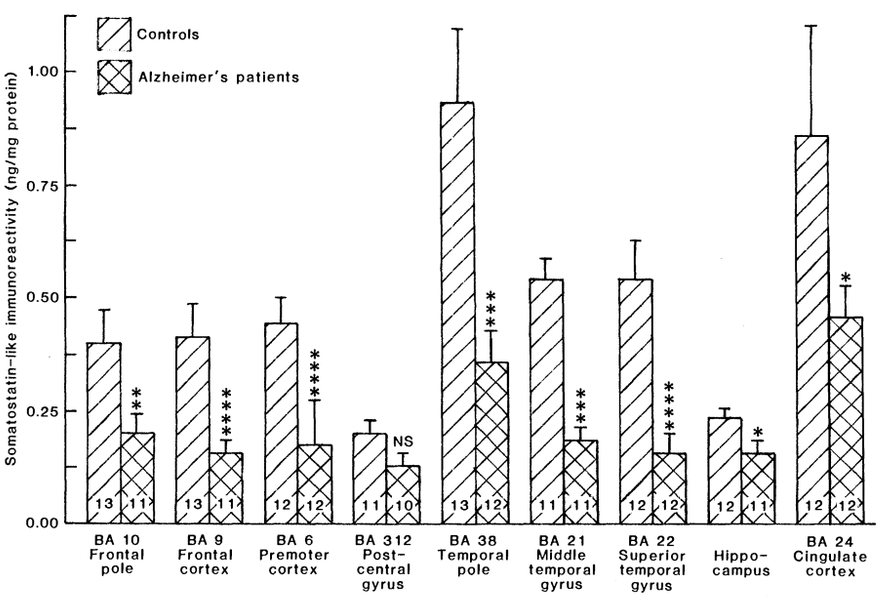

Fig. 2. Somatostatin-like immunoreactivity (means ± standard error of the mean) in the cortices of Alzheimer's patients and controls. The number of subjects tested is at the bottom of each column. Data were analyzed with the Mann-Whitney U test. Abbreviations: BA, Brodmann area; NS, not significant. *$P < 0.10$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$.

with 0.1 percent bovine serum albumin, 0.1 percent bacitracin, and 5 mM Mg^{2+}. Tissue (4 mg wet-weight membranes) was incubated with 0.08 nmol of radiolabeled ligand (corresponding to 60,000 counts per minute). Nonspecific binding was determined in the presence of $5 \times 10^{-7} M$ somatostatin 14. Reaction was allowed to proceed for 60 minutes at 27°C. The tissue was separated from the solution by filtration under suction onto GF/C filters that were presoaked in 0.3 percent polyethlyeneimine. The filtrate and filters were washed four times with 4 ml of ice-cold 150 mM tris-HCl buffer, pH 7.4, and then the radioactivity of the tissue and the filter was measured by a γ-counter (Beckman). Each experiment was performed in triplicate with the same sample. In routine experiments with human cortical membranes, the total binding is 4000 to 6000 counts per minute of which 2000 are attributed to nonspecific binding in the filtrate and filter. Degradation of the radio-ligand was negligible under the conditions of our experiment, as ascertained by extraction of iodinated ligand from the incubation solution and its purification with HPLC.

Scatchard analysis was performed by incubating cortical membranes with increasing concentrations of radiolabeled ligand from 0.04 to 1.6 nM. Nonspecific binding increased linearly with concentration, while specific binding approached saturation at 0.82 and 1.64 nM. Scatchard analysis showed a single class of binding sites. Statistical analysis was performed with the Mann-Whitney U test. All data are expressed as the mean ± the standard error of the mean.

We have shown that binding sites in the human brain for ^{125}I-labeled [Leu^8, DTrp^{22}, Tyr^{25}]somatostatin 28 have many of the properties expected from a receptor (*4*). The dissociation constant is in the nanomolar range. Binding is saturable with respect to tissue concentration and reversible as a function of time. There is an anatomic distribution of receptors that corresponds well to that reported in rats (*6*). Studies of binding and inhibition demonstrate that tracer is displaced by native somatostatin and its analogs but not by unrelated peptides. The binding affinity of somatostatin analogs is related to their ability to inhibit the release of growth hormone. Alanine-substituted analogs, which do not inhibit growth hormone release, are ineffective in competing for binding. These findings suggest that ^{125}I-labeled [Leu^8, DTrp^{22}, Tyr^{25}]somatostatin 28 binds to a physiologically relevant somatostatin receptor in the human cerebral cortex.

In the present study we have found that somatostatin receptors are reduced in the frontal cortex (Brodmann areas 6, 9, and 10) and temporal cortex (Brodmann area 21) (Fig. 1). The cingulate cortex, postcentral gyrus, temporal pole, and superior temporal gyrus showed no significant changes. The concentrations of receptors were reduced approximately 40 percent in the hippocampus, but this change was not statistically significant ($P < 0.10$). Scatchard analysis indicates that the reduction in receptor binding reflects a decrease in receptor number (B_{max}) rather than a change in receptor affinity (K_D). The value of B_{max} was reduced in Brodmann areas 9 and 21 in Alzheimer's patients. In Brodmann area 21 the receptor affinity was increased in the Alzheimer's patients, as reflected by the reduced value of K_D (Table 1). In Brodmann area 22, where there was no significant reduction in receptor binding, there was no significant difference between the values of B_{max} and K_D in the patients and in the controls. The value of K_D for all three regions was in the nanomolar range, and Scatchard analysis indicated a single class of binding sites. There was no significant relation between the postmortem interval and receptor binding for any brain region in the patients or the controls.

The SLI concentrations were significantly reduced in the frontal cortex (areas 6, 9, and 10) and temporal cortex (areas 21, 22, and 38). There was no significant change in the cingulate cortex, hippocampus, and postcentral gyrus (Fig. 2). This distribution of reduced cortical SLI is consistent with previous studies (*3*). In addition there was a relation between the number of receptors and cortical concentrations of SLI. The concentrations of both receptors and SLI were highest in the cingulate cortex and lowest in the postcentral gyrus and hippocampus. There was a significant linear relation between mean SLI concentrations and somatostatin receptor concentration in the patients' cortices (slope = 0.011, $P < 0.01$) but not in the controls'. Within individual regions in the patients' cortices, significant linear relations between SLI and somatostatin-receptor concentrations were found only in Brodmann areas 9 and 22.

Several explanations could account for our results. Reduced SLI and reduced somatostatin receptors in the same regions could reflect loss of both presynaptic (somatostatin) and postsynaptic (somatostatinceptive) neurons in areas predisposed to the pathologic process. The greatest neuronal loss in Alzheimer's disease occurs in the frontal and temporal cortex where we see the largest alterations in SLI and somatostatin receptors (*11*). If somatostatin neurons are selectively vulnerable in Alzheimer's disease, some of the reduction in somatostatin receptor binding might also reflect a loss of autoreceptors. In addition, some somatostatin receptors could be located on cortical afferents, such as the cholinergic system, which degenerate in Alzheimer's disease (*1*). Other evidence has demonstrated interactions between acetylcholine and somatostatin. Acetylcholine can cause release of somatostatin in vitro, and somatostatin neurons in the cerebral cortex may receive a cholinergic input (*12*).

Since SLI is reduced in the cerebral cortex in Alzheimer's disease and somatostatin cortical neurons are intrinsic to the cerebral cortex these neurons may selectively degenerate in the disease, accounting for the reduced concentrations of SLI (*3*). Recent immunocytochemical studies support this hypothesis (*4*); however, reduced concentrations of SLI in Alzheimer's disease could also reflect either altered processing or degradation of SLI or increased release of SLI. If the release of SLI were increased in Alzheimer's disease, one might expect a down regulation of somatostatin receptors in the same regions. This hypothesis could explain the present findings.

The present results contrast with observations in the cholinergic system in which loss of presynaptic cholinergic markers is not accompanied by loss of muscarinic receptors in the cerebral cortex (*13*). Concentrations of α- and β-adrenergic, benzodiazepine, and γ-aminobutyric acid receptors are also unaltered in Alzheimer's disease (*14*). Serotonin receptors on the other hand, are reduced concomitantly with reductions in 5-hydroxyindoleacetic acid in patients' cortices (*15*). The reduction in serotonin receptors is related to concentrations of SLI in the frontal and temporal cortex, which suggests that the receptors could be localized on somatostatin neurons in these regions. Since somatostatin receptors are reduced in the cerebral cortex, replacement therapy might not be successful. The loss of receptors is not absolute, however, and empirical trials will be necessary to determine the success of pharmacological intervention. Further studies of somatostatin and its receptors will be needed to define the precise role of this peptide in Alzheimer's disease and its significance in the pathophysiology of the illness.

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