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Age-Related Changes of Strychnine-Insensitive Glycine Receptors in Rat Brain as Studied by In Vitro Autoradiography

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ABSTRACT Age-related changes of strychnine-insensitive glycine receptors in the rat brain were studied through quantitative in vitro autoradiography with ^{3}H-glycine. ^{3}H-glycine binding sites were most concentrated in the hippocampus, cerebral cortex, and olfactory tubercle, and moderate densities of binding sites were located in the striatum, nucleus accumbens, amygdala, and certain thalamic nuclei. Low densities of ^{3}H-glycine binding sites were observed in the lateral septal nucleus, midbrain nuclei such as the superior colliculus and central gray matter, and granule cell layer of the cerebellum. In aged animals, severe decline of ^{3}H-glycine binding sites was observed in the telencephalic regions including the hippocampus and cerebral cortex. On the other hand, decrease of binding sites in the midbrain nuclei was of lesser degree, and there were no changes in the cerebellum. These results suggest that the decrease of glycine receptors in particular brain regions has some relation with changes of neuronal functions associated with aging process in these areas. The glutamatergic neuronal system, particularly the N-methyl-D-aspartate (NMDA) subtype, has been considered to play an important role in learning and memory. Taking into consideration that strychnine-insensitive glycine receptors are contained in the NMDA receptor complex, the present study implies that the decrease of glycine receptors may be involved in impairments of learning and memory occurring in aged brains.

INTRODUCTION

The amino acid glycine has been suggested as important inhibitory neurotransmitter in the caudal regions of the mammalian central nervous system, particularly in the spinal cord (Aprison and Daly, 1978; Werman and Aprison, 1966). Strychnine, a glycine receptor antagonist, has been a useful tool for studing electrophysiological, pharmacological, and molecular properties of spinal glycine receptors. The density of ^{3}H-strychnine binding sites was the highest in the gray matter of the spinal cord and decreased progressively in more rostral regions, and no specific binding was observed in any of the forebrain areas (Probst et al., 1986; Zarbin et al., 1981).

Recently, the existence of strychnine-insensitive glycine receptors has been identified in higher centers of the rat central nervous system (Kishimoto et al., 1981; Monahan et al., 1989; Pycock and Kerwin, 1981), where the glycine receptors have been advocated to have a functional relationship with N-methyl-D-aspartate (NMDA) receptors, one subtype of glutamate receptors (Bertolino et al., 1988; Bonhaus et al., 1987; Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Snell et al., 1987). Receptor autoradiography with use of ^{3}H-glycine revealed that the distribution of strychnine-insensitive glycine receptors (Bristow et al., 1986) and that of NMDA receptors (Greenamyre et al., 1985a; Monaghan and Cotman, 1985) were almost identical. Their distributions were significantly different from that of ^{3}H-strychnine binding sites; that is, the highest densities were observed in the hippocampus, cerebral cortex, subiculum, and amygdala followed by the striatum, cerebellum, and olfactory areas.

It has been implied that glutamate receptors, particularly the NMDA subtype, are important in learning and memory (Collingridge, 1985; Harris et al., 1984; Morris et al., 1986). In addition, dysfunction of NMDA type neurotransmission seems to have some relation with neuropathogenesis of senile dementia of Alzheimer's type (SDAT) (Greenamyre et al., 1985b, 1987; Maragos et al., 1987a; Simpson et al., 1988). In normal aging, as well as in SDAT, impairments of learning and memory are found, and qualitatively same types of histological and neurochemical changes have been observed (Gottfries, 1980). In the present study, to elucidate a role of the NMDA receptor complex in the normal aging process, we examined age-related changes of

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strychnine-insensitive glycine receptors in the rat brain through quantitative in vitro autoradiography.

MATERIALS AND METHODS

Fischer 344 strain male rats (Charles River Japan) were used in these experiments. Before autoradiographic procedures, initial binding experiments were performed on coronal sections containing the hippocampus of young adult rats (2-months-old) to ensure that ^{3}H-glycine bound to its receptors specifically. Rats were decapitated, and the brains were rapidly removed and frozen in dry-ice acetone. Serial cryostat sections with 10 μm thickness were thaw mounted on polyethyleneimine-coated slides and stored at −20°C. Sections were preincubated with 0.04% Triton X-100 for 10 min at 2°C to allow dissociation of possible endogenous inhibitors. After washing, sections were incubated with various concentrations of ^{3}H-glycine (New England Nuclear; 49.0 Ci/mmol) in 50 mM Tris acetate buffer, pH 7.4, for 15 min at 2°C. The specific binding was determined as the difference between binding in the presence and absence of 1 mM nonradioactive glycine. Then sections were washed three times in ice-cold fresh buffer for 10 sec. Binding to whole tissue sections was determined by scraping the tissue from the microscope slides, followed by liquid scintillation spectrophotometry.

Rats aged 2, 5, 13, and 21 months were used. Four animals were used for each age group and six to 20 cryostat sections per one discrete region were prepared. For labelling with ^{3}H-glycine, slide-mounted tissue sections were incubated with 80 nM ^{3}H-glycine under the above-mentioned conditions. After labelling, the slides were rapidly air-dried and apposed to a sheet of ^{3}H-sensitive film (Amersham). To quantify the receptor density, autoradiographic ^{3}H-microscales from Amersham were coexposed as standards. Autoradiograms were exposed for 4 weeks at 4°C. After exposure periods, the films were developed with Kodak Micodol X for 13 min at 20°C. The films obtained were placed in a photographic illumination apparatus, and optical densities of various brain regions and the ^{3}H-microscale were calculated using IBAS II from Zeiss. The molar quantities of bound ligands were determined from standards curves representing the relationship between optical density and the level of radioactivity (Unnerstall et al., 1982).

Because of the relatively low energy of β-rays produced by tritium, significant tissue quenching occurs in areas of high white matter content. This results in varying autoradiographic efficiency over a tissue section and an underestimation of the concentration of radioactivity in areas of high white matter density (Kuhar and Unnerstall, 1985; Rainbow et al., 1984).

RESULTS

To establish the incubation and rinse conditions most suitable for the detection of specific ^{3}H-glycine binding, sections were analyzed by liquid scintillation spectrometry. The binding of ^{3}H-glycine reached a plateau within 10 min after the initiation of incubation and was stable for 30 min at 2°C. The optimal rinse time was three periods of 10 sec each in the fresh buffer at 2°C. After this washing time, specific binding accounted for about 90% of total counts. Specific binding could be detected after 5 × 10 sec, but in this case specific binding was approximately 50% compared to 3 × 10 sec. Periods less than 3 × 10 sec reduced the specific:background ratio.

Figure 1 shows a saturation curve and Scatchard plots of ^{3}H-glycine binding on sections containing the hippocampus of a young adult rat. ^{3}H-glycine had a single high-affinity binding site whose Kd and Bmax values were 80.0 nM and 116.1 fmol/section, respectively. The binding was not inhibited by strychnine.

Autoradiographic distributions of ^{3}H-glycine binding sites in the rat brain are shown in Figure 2. In young adult rats (Fig. 2A–D), the highest density of ^{3}H-glycine binding sites occurred in the CA1 region of the hippocampus, especially in the stratum radiatum and stratum oriens. The adjacent stratum pyramidale had a few binding sites. The molecular layer of the dentate gyrus had a very high density of binding, and the granular layer exhibited a low level. The cerebral cortex showed a regional variation in the density of ^{3}H-glycine binding

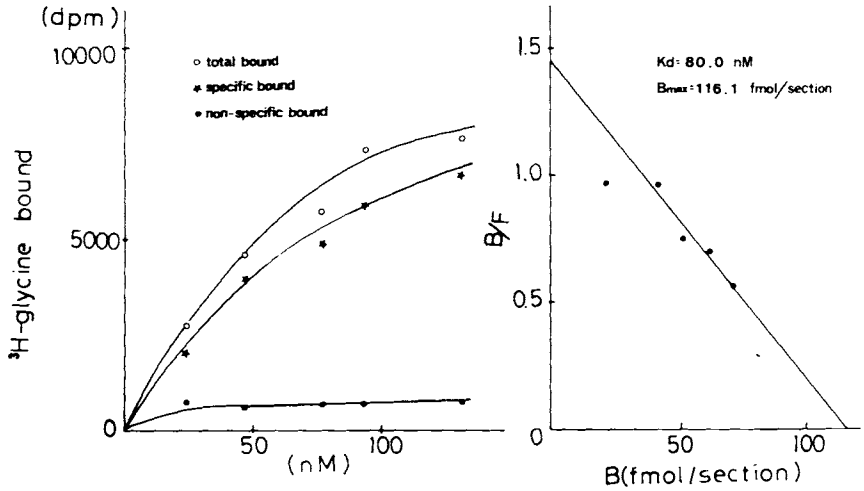

Fig. 1. Saturation curve and Scatchard plots of ^{3}H-glycine binding sites on cryostat sections containing the hippocampus of a young adult rat. Kd and Bmax values are the means of three experiments.

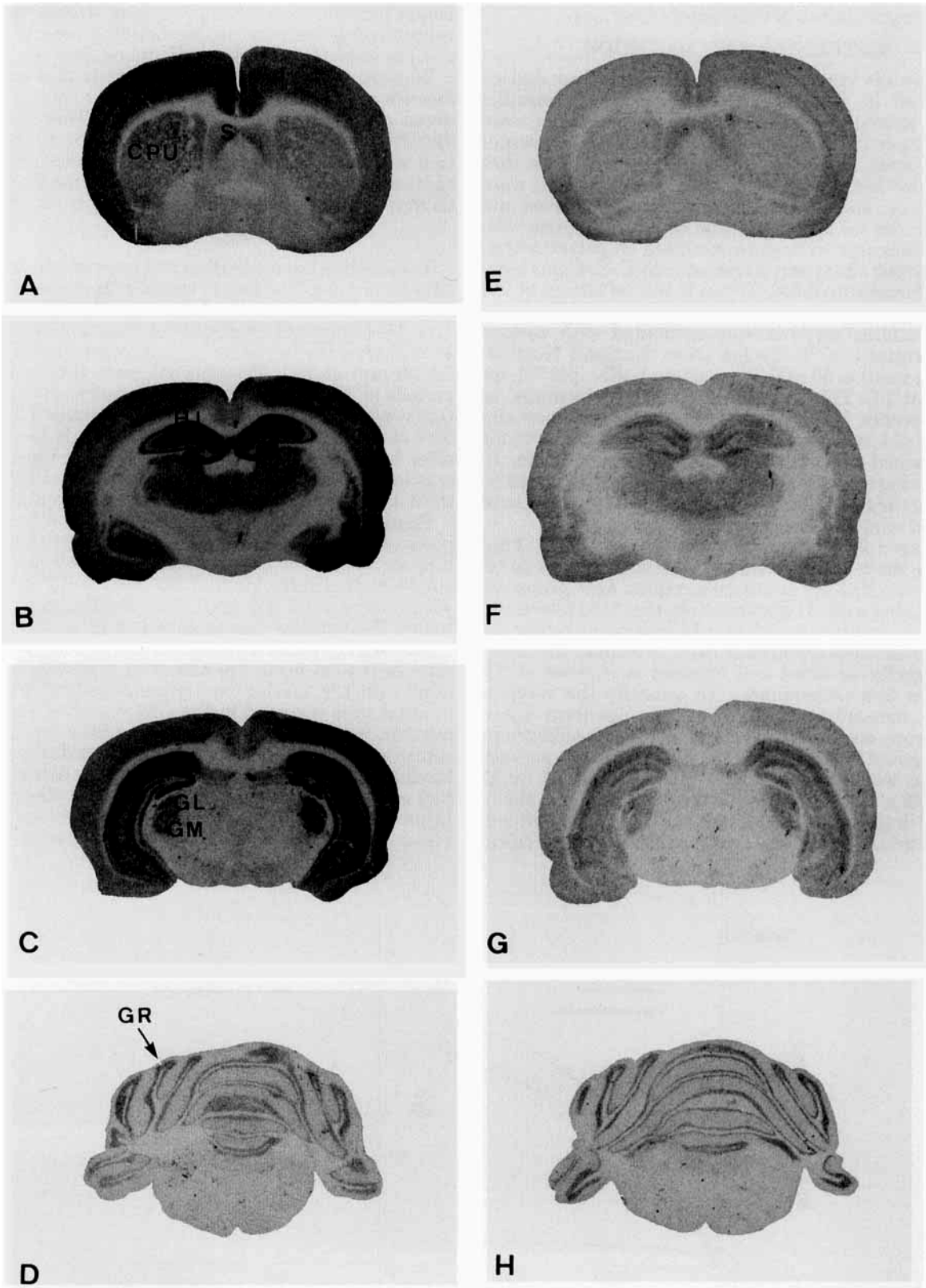

Fig. 2. Autoradiographic distributions of ^{3}H-glycine binding sites in the rat brain. A–D (2 months old) and E–H (21 months old) present representative brain sections in a rostral to caudal progression. A. CPU, caudate-putamen; S, lateral septal nucleus. B. HI, hippocampus; TH, thalamus. C. GM, medial geniculate body; GL, lateral geniculate body. D. GR, granule cell layer of the cerebellum.

sites, though an apparent laminar distribution was not observed. Within the basal forebrain the olfactory tubercle, nucleus accumbens, and striatum had relatively high levels of binding. In the amygdala, the lateral and basal nuclei were rich in ^{3}H-glycine binding sites, whereas the cortical and medial amygdaloid regions had low concentrations. The thalamus showed moderate levels of binding, as opposed to the hypothalamus, in which few binding sites were observed. Within the thalamus the lateral, dorsomedial, ventroposterior, and midline nuclei exhibited higher levels of binding than other nuclei. In general, there were few binding sites in the midbrain regions. Among these regions, the superficial gray layer of the superior colliculus and central gray matter showed slightly higher levels. In the cerebellum, the granule cell layer had a significantly higher density than the molecular layer. There was no apparent ^{3}H-glycine binding in the lower brain stem.

Figure 2E–H shows autoradiograms of ^{3}H-glycine binding sites in the brain of aged rats (21 months old). In telencephalic regions, ^{3}H-glycine binding sites were severely decreased in the aged rat brain. On the other hand, decreases of binding sites in the midbrain regions were less and there were no changes in the cerebellum of aged animals.

Table I shows a summary of age-related changes of ^{3}H-glycine binding sites in various areas of the rat brain. In the hippocampus proper, ^{3}H-glycine binding sites were decreased by up to about half in 21-month-old animals. Comparing to the hippocampus proper, decreases in the dentate gyrus were relatively of lesser degree. ^{3}H-glycine binding sites in other telencephalic regions were also reduced by about half in 21-month-old rats, although the aging pattern of binding sites varied with brain region. On the other hand, in midbrain regions such as the superior colliculus, central gray matter, geniculate bodies, substantia nigra, and interpeduncular nucleus, decreases of ^{3}H-glycine binding sites in parallel with age remained less than in the telencephalon. ^{3}H-glycine binding sites in both granule cell and molecular layers of the cerebellum were not significantly changed.

DISCUSSION

At the molecular level, the NMDA receptor complex possesses an L-glutamate recognition site, a strychnine-insensitive glycine modulatory site, and a voltage-dependent cation channel. In the present paper, strychnine-insensitive glycine binding sites were detected in the rat brain. It has been reported that these binding sites are disclosed by Triton X-100 treatment of brain synaptic membrane (Ogita and Yoneda, 1988; Ogita et al., 1989). We applied this method to brain cryostat sections and performed quantitative in vitro autoradiography. As a result, a single high-affinity binding site was found on brain cryostat sections. The distribution of ^{3}H-glycine binding sites in the adult rat brain was

TABLE I. Age-related changes of ^{3}H-glycine binding sites in the rat brain (fmol/mg tissue)[1]

Brain region	Age (months)			
	2	5	13	21
Cerebral cortex				
Frontal cortex	98.9 ± 9.0	71.1 ± 17.7**	47.3 ± 10.9**	40.2 ± 8.9**
Parietal cortex	57.8 ± 10.1	41.3 ± 6.5**	40.6 ± 9.3**	28.4 ± 8.4**
Striatum	64.4 ± 7.8	45.1 ± 9.1**	32.9 ± 5.7**	21.4 ± 5.6**
Septal nucleus	46.4 ± 6.9	41.4 ± 10.5	26.9 ± 9.8**	27.1 ± 8.0**
Nucleus accumbens	73.5 ± 5.8	53.6 ± 4.6**	48.1 ± 4.6**	23.1 ± 8.4**
Olfactory tubercle	84.1 ± 13.0	71.8 ± 8.2	42.5 ± 11.4**	35.2 ± 7.9**
Hippocampal proper				
Stratum oriens	86.4 ± 15.3	65.4 ± 8.2**	53.6 ± 10.6**	44.4 ± 6.4**
Stratum pyramidale	34.3 ± 7.3	29.8 ± 6.7	26.5 ± 6.0*	16.2 ± 3.4**
Stratum radiatum	130.6 ± 35.8	89.5 ± 9.6**	86.2 ± 18.0**	68.7 ± 16.8**
Dentate gyrus				
Granular layer	32.9 ± 8.8	25.7 ± 5.8*	24.0 ± 8.7**	21.3 ± 8.0**
Molecular layer	96.0 ± 14.3	79.7 ± 9.1*	76.6 ± 16.5**	71.1 ± 16.5**
Amygdaloid complex				
Corticomedial group	33.5 ± 6.6	25.5 ± 2.6*	23.1 ± 5.4**	16.6 ± 3.6**
Basolateral group	68.2 ± 10.3	57.2 ± 9.1*	40.9 ± 10.3**	34.9 ± 8.5**
Thalamus				
Lateral nucleus	66.0 ± 11.3	46.7 ± 8.2**	36.8 ± 5.8**	21.7 ± 3.7**
Dorsomedial nucleus	82.9 ± 12.2	55.4 ± 5.4**	44.6 ± 9.4**	29.4 ± 8.3**
Ventral posteromedial nucleus	70.8 ± 9.6	47.4 ± 8.4**	39.6 ± 5.5**	25.0 ± 8.0**
Ventral posterolateral nucleus	50.0 ± 7.4	34.6 ± 3.6**	29.8 ± 5.3**	18.6 ± 4.6**
Rhomboid nucleus	85.4 ± 13.1	59.0 ± 7.9**	51.9 ± 7.2**	26.8 ± 6.3**
Reuniens nucleus	61.0 ± 7.6	43.9 ± 5.0**	36.7 ± 4.3**	23.8 ± 5.3**
Nucleus gelatinosus thalami	78.8 ± 14.3	52.8 ± 4.4**	43.0 ± 7.8**	28.2 ± 6.5**
Lateral geniculate body	51.9 ± 6.1	54.0 ± 5.8	48.9 ± 11.3	43.2 ± 6.7
Medial geniculate body	55.1 ± 10.0	45.9 ± 8.9	44.1 ± 10.7	43.9 ± 11.4*
Superior colliculus				
Superficial layer	27.0 ± 5.7	22.9 ± 4.0	18.6 ± 4.4*	22.2 ± 5.2
Deeper layer	16.5 ± 4.4	14.9 ± 4.8	11.7 ± 4.5	13.1 ± 4.8
Central gray matter	20.2 ± 6.9	14.6 ± 3.6	11.1 ± 4.2*	12.1 ± 4.3*
Substantia nigra	16.8 ± 4.6	14.8 ± 3.6	11.8 ± 2.4	12.5 ± 3.5
Interpeduncular nucleus	17.6 ± 6.0	13.2 ± 3.4	10.5 ± 2.8*	11.3 ± 2.8
Cerebellar cortex				
Molecular layer	12.2 ± 2.1	13.3 ± 1.7	13.9 ± 1.8	14.9 ± 3.9
Granular layer	38.5 ± 5.5	49.6 ± 2.2	55.3 ± 5.8	55.7 ± 3.9

[1]Significant difference from the values of 2-month old rats when examined by Student's t test: *$P < 0.05$, ** $P < 0.01$.

consistent with that previously observed by the same method (Bristow et al., 1986).

^{3}H-glycine binding sites showed age-dependent reduction in telencephalic regions of the rat brain. However, the decline of ^{3}H-glycine binding sites did not extend to the midbrain and cerebellum. These results show that the decrease of strychnine-insensitive glycine receptors in particular brain regions has some relation with changes of neuronal functions associated with aging in these areas. The glutamatergic neuronal system, particularly NMDA receptors, has been considered to play a role in learning and memory; that is, the NMDA subtype is involved in the development of long-term potentiation in vitro (a model of memory formation; Collingridge, 1985; Harris et al., 1984; Morris et al., 1986). In a pathological state, excitotoxicity by excess glutamate can cause neuronal cell death, probably leading to impairments of learning and memory (Maragos et al., 1987b). In this regard, loss of both NMDA binding sites and cation channel sites in the cerebral cortex and hippocampus of patients with SDAT has been reported (Greenamyre et al., 1985b, 1987; Maragos et al., 1987a; Simpson et al., 1988). However, other researchers have not found these declines in the brain in SDAT (Cowburn et al., 1988a,b; Geddes et al., 1986; Monaghan et al., 1987; Mouradian et al., 1988). Therefore, it is still controversial whether the NMDA site has a primary importance in impairments of learning and memory (Hardy and Cowburn, 1987; Maragos et al., 1987c).

Procter et al. (1989) reported that significantly less binding of ^{3}H-MK-801, an NMDA-associated cation channel blocker (Huettner and Bean, 1988; Wong et al., 1986), was observed in the frontal cortex of SDAT under glutamate- and glycine-stimulated conditions, whereas ^{3}H-MK-801 binding without added amino acids was not altered. They suggested that neither the agonist recognition site nor the cation channel site in the NMDA receptor complex were altered in the brain of SDAT but that their coupling to a strychnine-insensitive glycine recognition site was impaired. Their result together with data from the present study implies that abnormalities of regulation of NMDA receptors by strychnine-insensitive glycine receptors may play an important role in degeneration process of neurons occurring in normal aging and SDAT.

Although it has been advocated that glycine modulates NMDA-associated channel opening, the physiological function of the amino acid is not yet known. However, the present study indicates the pathophysiological importance of glycine in the neuronal aging process.

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REFERENCES

Aprison, M.H., and Daly, E.C. (1978) Biochemical aspects of transmitter at inhibitory synapses: the role of glycine. Adv. Neurochem., 3:203–294.

Bertolino, M., Vicini, S., Mazzetta, J., and Costa, E. (1988) Phencyclidine and glycine modulate NMDA-activated high conductance cationic channels by acting at different sites. Neurosci. Lett., 84:351–355.

Bonhaus, D.W., Burge, B.C., and McNamara, J.O. (1987) Biochemical evidence that glycine allosterically regulates an NMDA receptor-coupled ion channel. Eur. J. Pharmacol., 142:489–490.

Bristow, D.R., Bowery, N.G., and Woodruff, G.N. (1986) Light microscopic autoradiographic localisation of [^{3}H]glycine and [^{3}H]strychnine binding sites in rat brain. Eur. J. Pharmacol., 126:303–307.

Collingridge, G.L. (1985) Long term potentiation in the hippocampus: Mechanisms of initiation and modulation by neurotransmitters. Trends Pharmacol. Sci., 6:407–411.

Cowburn, R., Hardy, J., Roberts, P., and Briggs, R. (1988a) Regional distribution of pre- and postsynaptic glutamatergic function in Alzheimer's disease. Brain Res., 452:403–407.

Cowburn, R., Hardy, J., Roberts, P., and Briggs, R. (1988b) Presynaptic and postsynaptic glutamatergic function in Alzheimer's disease. Neurosci. Lett., 86:109–113.

Geddes, J.W., Chang-Chui, H., Cooper, S.M., Lott, I.T., and Cotman, C.W. (1986) Density and distribution of NMDA receptors in the human hippocampus in Alzheimer's disease. Brain Res., 399: 156–161.

Gottfries, C.G. (1980) Biochemistry of dementia and normal ageing. Trends Neurosci., 3:55–57.

Greenamyre, J.T., Olson, J.M.M., Penney, J.B., and Young, A.B. (1985a) Autoradiographic characterization of N-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. J. Pharmacol. Exp. Ther., 233:254–263.

Greenamyre, J.T., Penney, J.B., D'Amato, C.J., and Young, A.B. (1987) Dementia of the Alzheimer's type: Changes in hippocampal L-[^{3}H]glutamate binding. J. Neurochem., 48:543–551.

Greenamyre, J.T., Penney, J.B., Young, A.B., D'Amato, C.J., Hicks, S.P., and Shoulson, I. (1985b) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. Science, 227:1496–1499.

Hardy, J., and Cowburn, R. (1987) Glutamate neurotoxicity and Alzheimer's disease. Trends Neurosci., 10:406.

Harris, E.W., Ganong, A.H., and Cotman, C.W. (1984) Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res., 323:132–137.

Huettner, J.E., and Bean, B.P. (1988) Block of N-methyl-D-aspartate activated current by anticonvulsant MK-801: Selective binding to open channels. Proc. Natl. Acad. Sci. USA, 85:1307–1311.

Johnson, J.W., and Ascher, P. (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature, 325:529–531.

Kishimoto, H., Simon, J.R., and Aprison, M.H. (1981) Determination of the equilibrium dissociation constants and number of glycine binding sites in several areas of the rat central nervous system, using a sodium-independent system. J. Neurochem., 37:1015–1024.

Kleckner, N.W., and Dingledine, R. (1988) Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science, 241:835–837.

Kuhar, M.J., and Unnerstall, J.R. (1985) Quantitative receptor mapping by autoradiography: Some current technical problems. Trends Neurosci., 8:49–53.

Maragos, W.F., Chu, D.C.M., Young, A.B., D'Amato, C.J., and Penney, J.B. (1987a) Loss of hippocampal [^{3}H]TCP binding in Alzheimer's disease. Neurosci. Lett., 74:371–376.

Maragos, W.F., Greenamyre, J.T., Penney, J.B., and Young, A.B. (1987b) Glutamate dysfunction in Alzheimer's disease: An hypothesis. Trends Neurosci., 10:65–67.

Maragos, W.F., Greenamyre, J.T., Penney, J.B., and Young, A.B. (1987c) Reply from W.F. Maragos and colleagues. Trends Neurosci., 10:407.

Monaghan, D.T., and Cotman, C.W. (1985) Distribution of N-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. J. Neurosci., 5:2909–2919.

Monaghan, D.T., Geddes, J.W., Yao, D., Chung, C., and Cotman, C.W. (1987) [^{3}H]TCP binding sites in Alzheimer's disease. Neurosci. Lett., 73:197–200.

Monahan, J.B., Corpus, V.M., Hood, W.F., Thomas, J.W., and Compton, R.P. (1989) Characterization of a [^{3}H]glycine recognition site as a modulatory site of the N-methyl-D-aspartate receptor complex. J. Neurochem., 53:370–375.

Morris, R.G.M., Anderson, E., Lynch, G., and Baudry, M. (1986) Selective impairment of learning and blockade of long term potentiation by an N-methyl-D-aspartate receptor antagonist, AP-5, Nature, 319:774–776.

Mouradian, M.M., Contreras, P.C., Monahan, J.B., and Chase, T.N. (1988) [^{3}H]MK-801 binding in Alzheimer's disease. Neurosci. Lett., 93:225–230.

Ogita, K., Suzuki, T., and Yoneda, Y. (1989) Strychnine-insensitive binding of [^{3}H]glycine to synaptic membranes in rat brain, treated with Triton X-100. Neuropharmacology, 28:1263–1270.

Ogita, K., and Yoneda, Y. (1988) Disclosure by Triton X-100 of NMDA-

sensitive [^{3}H]glutamate binding sites in brain synaptic membranes. Biochem. Biophys. Res. Commun., 153:510–517.
Probst, A., Cortes, R., and Palacios, J.M. (1986) The distribution of glycine receptors in the human brain. a light microscopic autoradiographic study using [^{3}H]strychnine. Neuroscience, 17:11–35.
Procter, A.W., Wong, E.H.F., Stratmann, G.C., Lowe, S.L., and Bowen, D.M. (1989) Reduced glycine stimulation of [^{3}H]MK-801 binding in Alzheimer's disease. J. Neurochem., 53:698–704.
Pycock, C.J., and Kerwin, R.W. (1981) The status of glycine as a supraspinal neurotransmitter. Life Sci., 28:2679–2686.
Rainbow, T.C., Biegon, A., and Berck, D.J. (1984) Quantitative receptor autoradiography with tritium-labeled ligands: comparison of biochemical and densitometric measurements. J. Neurosci. Methods, 11:231–241.
Simpson, M.D.C., Royston, M.C., Deakin, J.F.W., Cross, A.J., Mann, D.M.A., and Slater, P. (1988) Regional changes in [^{3}H]D-aspartate and [^{3}H]TCP binding sites in Alzheimer's disease brains. Brain Res., 462:76–82.
Snell, L.D., Morter, R.S., and Johnson, K.M. (1987) Glycine potentiates N-methyl-D-aspartate-induced [^{3}H]TCP binding to rat cortical membranes. Neurosci. Lett., 83:313–317.
Unnerstall, J.R., Niehoff, D.L., Kuhar, M.J., Palacios, J.M. (1982) Quantitative receptor autoradiography using [^{3}H]Ultrofilm: Application to multiple benzodiazepine receptors. J. Neurosci. Methods, 6:59–73.
Werman, R., and Aprison, M.H. (1966) Glycine: the search for a spinal cord inhibitory transmitter. In: Structure and Function of Inhibitory Neuronal Mechanisms. C. von Euler, S. Skoglund, and U. Soderberg, eds. Pergamon Press, New York, pp. 473–486.
Wong, E.H.F., Kemp, J.A., Priestley, T., Knight, A.R., Woodruff, G.N., and Iversen, L.L. (1986) The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc. Natl. Acad. Sci. USA, 83:7104–7108.
Zarbin, M.A., Wamsley, J.K., and Kuhar, M.J. (1981) Glycine receptor: Light microscopic autoradiographic localization with [^{3}H]strychnine. J. Neurosci., 1:532–547.