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Mouse telencephalon exhibits an age-related decrease in glutamate (AMPA) receptors but no change in nerve terminal markers

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The central excitatory amino acid receptor selective for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was examined in brain tissue from mice at 3 and 25 months after birth. Antibodies against the rat GluR-A glutamate receptor subunit (selective for kainate and AMPA) labeled a mouse brain component of about M_r 100,000. Telencephalic tissue from the older group of mice exhibited 31% less immunoreactivity towards this component as compared with that from the young group. Binding of [^{3}H]AMPA also decreased with age in the telencephalon to an extent which was similar to the loss of receptor immunoreactivity. Scatchard analysis revealed that this reduction is due to a decrease in receptor density and not to a change in binding affinity. In contrast, there were only small age-related changes in AMPA receptor immunoreactivity and binding levels in the brain stem and cerebellum. Binding to dopamine, serotonin, or GABA receptors was not significantly reduced in the older mice. Since the nerve terminal markers synaptophysin and the SV2 glycoprotein were not detectably different in the two groups of mice, the age-related reduction in AMPA receptors is not likely to be due to a general decrease in synaptic density. These data suggest that glutamatergic neurotransmission mediated by AMPA-type receptors is selectively impaired with aging in the telencephalon.

INTRODUCTION

Release of glutamate from synapses at many sites in mammalian forebrain stimulates two classes of postsynaptic receptors usually referred to as AMPA/quisqualate and NMDA receptors. The first of these mediates a voltage independent fast excitatory postsynaptic current (the fast EPSC) while the NMDA receptor generates a voltage dependent, slow excitatory current. The AMPA receptor-mediated fast EPSC is by far the dominant component at most synapses so far studied. AMPA receptors are not evenly distributed across the brain but instead are largely restricted to telencephalon and cerebellum. They are found in high concentrations in the superficial layers of neocortex, in each of the major synaptic zones of hippocampus, and in the striatal complex[4,20,25]. Studies in animals and humans indicate that these structures organize complex perceptual–motor processes and provide the substrates for higher-order behaviors[26,27].

Given the above points, it is reasonable to ask if changes in AMPA receptors might be involved in age-related alterations in intellectual performance and memory. There is evidence that net binding is decreased in cortex of aged rats[28] but it is not known if this is due to a change in receptor properties, the balance of high vs. low affinity sites, the size of the receptor population, or a general loss of synaptic connections. The present study addressed these possibilities using ligand binding techniques and an antibody against one of the subunits (GluR-A) of the composite AMPA receptor to assay receptor density. The possibility that any age-related losses might be due to a decrease in the total number of synapses was tested with antibodies against other synaptic marker proteins. The results indicate that the density of AMPA receptors is

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Abbreviations: Single letter notation for amino acids are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

significantly reduced in the telencephalon but not in brain stem-cerebellum of the aged brain and that this effect is not likely to be due to a general loss of synapses. These observations suggest a possible contributor to the effects of aging on the performance of telencephalic networks.

MATERIALS AND METHODS

Materials

Rabbit antibodies (anti-GluR) developed with a fusion technique toward the sequence TEEGYRMLFQDLEKKKERLVVVDCES (amino acids 163–188) located in the large extracellular N-terminal domain of the 'GluR-1' receptor[13] were generously provided by Dr. K. Sumikawa (Department of Psychobiology, University of California, Irvine, CA). Partially purified monoclonal antibody against the synaptic vesicle SV2 glycoprotein from *Torpedo* electric organ[6] was provided by Drs. R. Kelly (Department of Biochemistry and Biophysics, University of California, San Francisco, CA) and S. Parsons (Department of Chemistry, University of California, Santa Barbara, CA). Nitrocellulose paper and the alkaline phosphatase-conjugated antibodies goat anti-rabbit IgGs were from Bio Rad Laboratories (Richmond, CA). Leupeptin was from Chemicon Inc., (Temecula, CA). DL-α-Amino-3-hydroxy-5-[methyl-^{3}H]isoxazole-4-propionic acid ([^{3}H]AMPA; 50–70 Ci/mmol), 3-[2-(4-fluorobenzoyl)-1-piperdinyl(ethyl-1,2-^{3}H)]-2,4(1H,3H)-quinazolinedione hydrochloride ([^{3}H]ketanserin; 60 Ci/mmol), 7-chloro-8-hydroxy-3-(methyl-^{3}H)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine ([^{3}H]SCH 23390; 85.6 Ci/mmol), and 2-[3-carboxy(propyl-2,3-^{3}H)]-3-amino-6-(4-methoxyphenyl)pyridazinium ([^{3}H]SR 95531; 50.5 Ci/mmol) were purchased from NEN/Du Pont Co. (Boston, MA). A monoclonal antibody against synaptophysin from rat synaptosomes was obtained from Sigma Chemical Co. (St. Louis, MO). Type GF/B glass microfibre filters were from Whatman Corp. (Hillsboro, OR). All other materials were from usual commercial sources, unless otherwise noted.

Immunoblot analysis

Male Balb/c mice of 3 or 25 months of ages were received from the National Institute of Aging and housed for three days to observe for health. Animals in good condition were sacrificed by cervical dislocation, and the brains rapidly cooled, removed, dissected, and homogenized in ice-cold buffer consisting of 0.32 M sucrose, 5 mM HEPES (pH 7.4), 1 mM EDTA, 1 mM EGTA, and the following protease inhibitors: 4 μg/ml antipain, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 2 μg/ml pepstatin A. Aliquots of homogenate samples were lysed on ice for 60 min in hyposmotic buffer containing 6 mM Tris-HCl (pH 8.1), 1 mM EDTA, 1 mM EGTA, and the protease inhibitors mentioned above. After centrifugal pelleting at 25,700× *g* for 15 min, membranes were resuspended at 1–3 mg protein per ml in the lysis buffer. For Western blot analysis, homogenate or lysed samples (40–80 μg protein) were treated with 2.5% (w/v) sodium dodecyl sulphate (SDS) and in the presence of 3% (v/v) 2-mercaptoethanol at 100°C for 5 min, then subjected to polyacrylamide gel electrophoresis based on the method developed by Laemmli[16]. Linear acrylamide gradient gels (3 to 17% w/v) were used to separate proteins which then were transferred to nitrocellulose (0.2-μm pore size) for 6–12 h as described[8]. Incubation of the nitrocellulose with anti-GluR antibodies diluted 1:900, anti-synaptophysin diluted 1:1000, or anti-SV2 diluted 1:100 in Tris-buffered saline, pH 7.4, with 0.1% (v/v) Tween-20 and 1.5% (w/v) non-fat dry milk was carried out at 4°C with agitation for 12–16 h. Secondary antibody incubation and color development utilized goat anti-rabbit IgG–alkaline phosphatase conjugate and the 5-bromo-chloro-3-indolyl phosphate and nitro blue tetrazolium substrate system. Color development of immunoreactive bands was terminated well before maximal intensity was reached in order to avoid saturation and allow comparative studies within each blot. Quantitative immunoreactivity was measured as the immunoblot band area (arbitrary units) determined with scanning laser densitometry using a Zeineh SLR-504-XL densitometer (BioMed Instruments, Inc.; Fullerton, CA) and a Var-

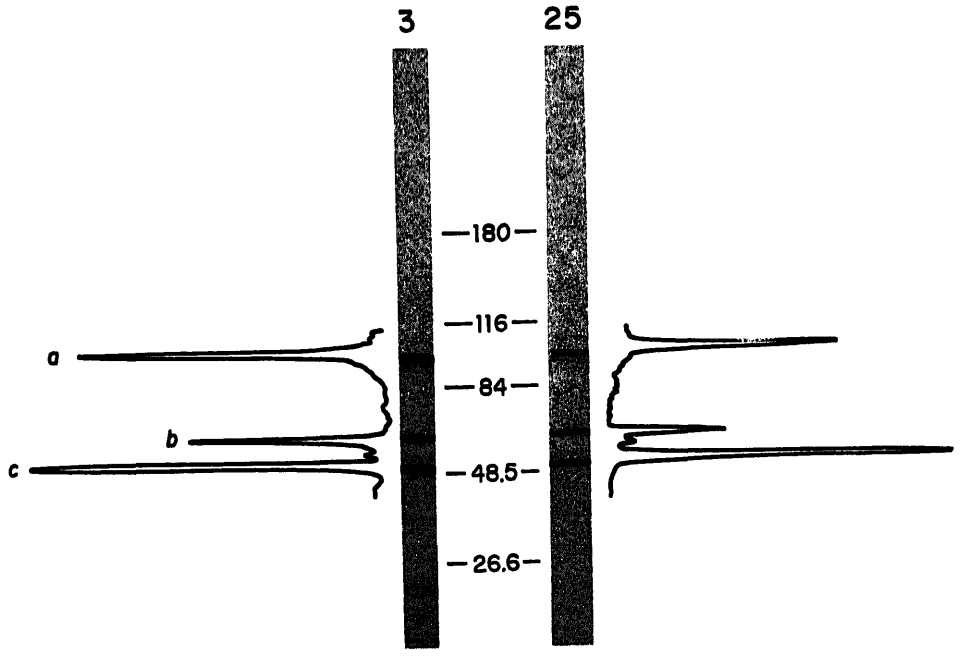

Fig. 1. Age-related loss of polypeptides recognized by antibodies against GluR-A. Representative telencephalic material from 3- (left) and 25- (right) month-old mice were homogenized and subjected to SDS-PAGE and Western blot analysis with anti-GluR as described in Materials and Methods. Scanning laser densitometry was used to determine the optical density of immunoreactive bands of 99 (a), 61 (b), and 52 (c) kDa. Scan areas for the 99 kDa antigen in the young and old samples shown are 23.1 and 15.4, respectively; this is an age-related reduction of 33%. Electrophoretic positions of protein standards from 26.6 to 180 kDa are indicated with lines.

ian 4270 integrator (Palo Alto, CA). Calibration of immunoblots using prestained protein molecular weight standards allowed the determination of the M_r for pertinent species. All protein determinations used the assay developed by Bradford[5].

Receptor binding assay

[^{3}H]AMPA binding to lysed membranes was studied by using modifications of both the filtration and centrifugation methods previously described[29]. Typically 10–40 μg protein in a final volume of 50–100 μl was equilibrated with 50 nM [^{3}H]AMPA in the presence of 60 mM Tris-HCl (pH 7.5), 5 mM EDTA, 50 mM KSCN, and 0–100 mM NaCl, for 60 min in an ice-water bath. Subsequently, bound ligand was determined by either: (i) rapidly diluting each membrane suspension with 4 ml ice-cold Buffer A consisting of 50 mM Tris-acetate (pH 7.2) and 80 mM KSCN, and immediately filtering it through a polyethyleneimine-coated (0.03% aqueous for approximately 1 h) glass-fiber filter and washing with two sequential 4 ml volumes of ice-cold Buffer A; or (ii) centrifuging the samples at 48,000× g for 15–20 min at 4°C, superficially rinsing the pellet with ice-cold Buffer A, and resuspending the membranes in 10 μl Beckman Tissue-Solubilizer-450 (San Ramon, CA). The filters and pellet suspensions were assayed for ^{3}H content by liquid scintillation spectroscopy with a counting efficiency of 0.40 in 2 ml of aqueous counting scintillant (Liquiscint; National Diagnostics, Manville, NJ). Nonspecific binding is defined as the amount of bound [^{3}H]AMPA in the presence of 2.5 mM non-radiolabeled L-glutamate. Results were always expressed as specifically bound [^{3}H]AMPA which equals the difference between the total bound and non-specifically bound ligand. The Scatchard equation was fit to the equilibrium binding of 0.01–5 μM [^{3}H]AMPA to different pools of lysed membranes using the filtration method, and the data subjected to linear regression whose quoted errors give the 95% confidence limits. Binding of other radioligands were assessed in the absence of KSCN with the filtration method.

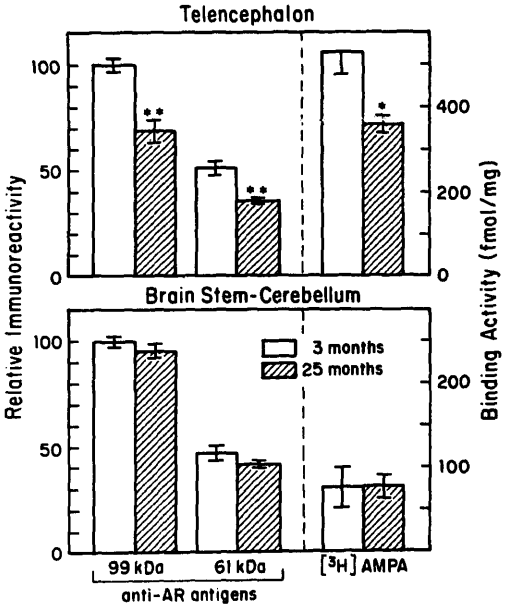

Fig. 2. AMPA receptor markers decrease with age in telencephalic but not brainstem-cerebellar samples. Tissue samples from three- ($n = 9$) and twenty-five- ($n = 7$) month-old mice were either homogenized and subjected to electrophoresis for Western blotting or lysed and membranes prepared for binding assays. The 99 and 61 kDa antigens recognized by anti-GluR were quantitatively compared within each immunoblot as in Fig. 1, and the relative immunoreactivity levels are expressed as percent of the 99 kDa band area from the young animals. Lysed membrane samples (20 μg protein) were incubated with [^{3}H]AMPA to determine the binding activity with the filtration method described. Similar binding results were obtained with the alternative centrifugation method. Each error bar represents the respective S.E.M.. Old to young data comparisons were made with two-tailed t-tests: ** $P < 0.002$, * $P < 0.02$.

RESULTS

Antibodies against an extracellular domain of the rat GluR-A glutamate receptor subunit (anti-GluR) intensely labeled three protein bands of 99 ± 4 (mean ± S.D.), 61 ± 2, and 52 ± 2 kDa in the telencephalon and brain stem-cerebellum from three- ($n = 9$) and twenty-five- ($n = 7$) month-old mice; typical immunoblots and densitometric scans are shown in Fig. 1. No bands were detected with secondary antibody alone, but only the 99 and 61 kDa bands were labeled by immunogen-affinity purified antibodies. The molecular mass of the upper band (99 kDa) closely approximates that expected for the GluR-A subunit on the basis of its amino acid sequence[13]. This immunoreactive band in rat brain co-purifies with AMPA binding sites and is photoaffinity labelled by the glutamate receptor antagonist [^{3}H]CNQX[30], confirming that it is a rodent brain AMPA receptor. According to amino acid sequences reported by Keinanen et al.[15], the antigenic domain of the GluR-A subunit used here is similar to the corresponding domains in GluR-B (65.4%), GluR-C (19.2%), and GluR-D (34.6%); therefore, the 99 kDa mouse antigen may consist of more than one subunit type. The nature of the two smaller bands is unknown. The 61 kDa band may be related to a kainate-binding protein of similar size recently characterized from chick cerebellum[11], whereas the 52 kDa band appears to be unrelated to the GluR-A 163–188 domain but recognized intrinsically by rabbit anti-serum. Fig. 2 (top frame) summarizes the density of the 99 kDa band, as measured by densitometric scans, in homogenates of telencephalon taken from 3- and 25-month-old mice. The immunolabelling was decreased 31 ± 6% (mean ± S.E.M.) in the aged group as compared to the younger animals ($P < 0.001$, two-tailed t-test). Interestingly enough, the smaller band of 61 kDa was also reduced by this amount (31 ± 5%, $P < 0.002$) as illustrated in Fig. 2. In membranes collected by centrifugation of hyposmotically lysed homogenates, the 99 kDa band exhibited an age-related decrease (32.5 ± 11%, $P < 0.05$) that was comparable to that found in samples of the homogenates (Table I). The 99 kDa polypeptide was less abundant in the lysed membrane fractions as compared to homogenates and was associated with a slightly smaller immunoreactive band (92–97 kDa) possibly as a result of proteolysis or deglycosylation (data not shown). The 61 kDa band was also less abundant in

TABLE I

Age-related changes in GluR-A immunoreactivity levels in lysed telencephalic membranes

Lysed membrane samples were analyzed as in Fig. 1, and the optical densities (arbitrary units) of immunoreactive bands were determined with laser densitometry. Each value represents the mean densitometric scan area ± S.E.M. and the values in parentheses are numbers of animals used. Two-tailed *t*-test: * $P < 0.05$ vs. 3-month-old mice.

Age (months)	*Antigen molecular weight*	
	99 kDa	*61 kDa*
3	46.8 ± 5.6 (9)	9.9 ± 1.5 (8)
25	31.6 ± 3.2 (7) *	7.5 ± 1.1 (7)

the lysed membranes; its age-related decrease (24.5%) did not approach statistical significance (Table I).

Evidence for a loss of AMPA receptors in the telencephalon of aged mice was also obtained using binding of [^{3}H]AMPA to lysed membranes. As shown in Fig. 2 (top frame), binding assayed with 50 nM [^{3}H]AMPA was 32 ± 8% (mean ± S.E.M.) less in the 25-month group compared to the 3-month animals ($P < 0.02$). Scatchard analyses (Fig. 3) indicated that this reduction in the telencephalic samples of the older mice was primarily due to a decrease (33 ± 6%, $P < 0.005$) in the density of binding sites (B_{max} for the 3- and 25-month mice were 13.8 ± 0.9 and 9.2 ± 0.5 pmol/mg, respectively). The apparent affinity in pooled samples from the young brains was 306 ± 47 nM and 239 ± 31 nM in samples from the 25-month group, a difference that was not statistically significant.

To test the selectivity of the age-related difference in AMPA receptors, binding to dopamine, serotonin, and γ-amino-*n*-butyric acid (GABA) receptors was assayed in telencephalic samples from 3- and 25-month-old mice. There was only a marginal decrease (16%, $P < 0.1$) in the binding of the D-1 dopamine receptor ligand [^{3}H]SCH 23390[3,14] to lysed membranes in the older animals (Table II). Surprisingly, binding of the S_2 serotonin receptor ligand [^{3}H]ketanserin[17] was significantly greater (18%, $P < 0.05$) in the older samples. $GABA_A$ receptor binding assayed with [^{3}H]SR 95531[12] was not detectably different in the two groups.

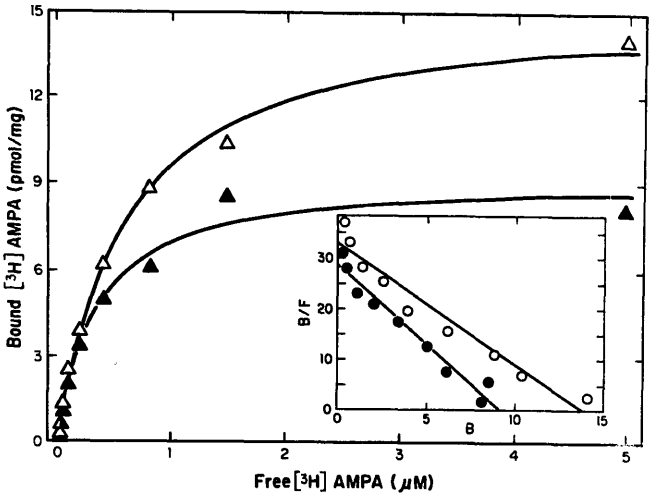

Fig. 3. Equilibrium binding of [^{3}H]AMPA in young and old mouse telencephalon. Aliquots (40 μg protein) of pooled lysed membranes from five 3-month (△, ○) and four 25-month (▲, ●) mice were equilibrated with different concentrations of ligand and assayed by filtration as described in Materials and Methods. Nonspecific binding was measured in the presence of excess L-glutamate and subtracted from the total binding. Each datum is an average of three determinations. The *Insert* shows the old and young data sets plotted in the Scatchard format of bound (B) vs. bound/free (B/F) [^{3}H]AMPA. The linear correlation coefficients for the 3- and 25-month groups were 0.971 and 0.978, respectively. Similar results were obtained with another group of animals consisting of four 3-month and three 25-month mice.

In marked contrast to the results with telencephalic tissue, no evidence for a significant age-related loss of glutamate receptors was obtained in the brainstem-cerebellar samples using either immunoblotting or binding assays (Fig. 2, bottom frame). Scatter plots comparing brain-stem cerebellar vs. telencephalic density values for the 99 and 61 kDa bands in individual animals from the two groups are shown in Fig. 4. As is

TABLE II

Binding of radioligands to three types of neurotransmitter receptors in mice of different ages

Lysed membrane samples (10–15 μg protein) were incubated with the indicated concentration of ligand in the absence or presence of excess non-radioactive ligand in order to determine the specific binding activity with the filtration method described. Each value represents the mean pmol bound radioligand per mg protein ± S.E.M., and the number of animals used in each study is shown in parentheses. Two-tailed *t*-test: * $P < 0.05$ vs. 3-month-old mice.

Age (months)	*D-1 dopamine receptor*	*S_2 serotonin receptor*	*γ-Aminobutyric acid$_A$ receptor*
	16 nM [^{3}H]SCH 23390	*10 nM [^{3}H]Ketanserin*	*300 nM [^{3}H]SR 95531*
Telencephalon			
3	0.75 ± 0.05 (6)	0.56 ± 0.03 (5)	2.66 ± 0.11 (5)
25	0.63 ± 0.03 (6)	0.66 ± 0.03 (5) *	2.40 ± 0.26 (5)
Brainstem-cerebellum			
3	0.33 ± 0.03 (5)	0.73 ± 0.09 (6)	–
25	0.33 ± 0.01 (5)	0.70 ± 0.10 (6)	–

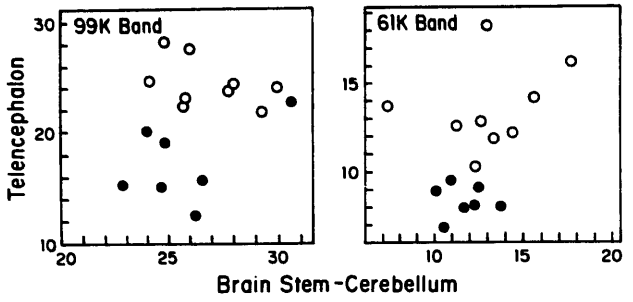

Fig. 4. Scatter plots of GluR-A immunoreactivity levels in telencephalon vs. brainstem-cerebellum. Densitometric scan areas (arbitrary units) of the 99 (left frame) and 61 (right frame) kDa antigens labeled by anti-GluR were measured as in Fig. 1. The areas measured in the telencephalic and brainstem-cerebellar samples from each animal of 3 (○) or 25 (●) months were plotted against each other. Note, the two frames have different scales on both axes in order to compensate for the lower immunoreactivity of the 61 kDa antigen.

evident, any tendencies for reduced immunolabelling in the brainstem-cerebellum from older mice cannot account for the reliable decreases seen in the telencephalic material. It can thus be concluded that the apparent loss of AMPA receptors is selective to the forebrain. Scatchard analyses of [^{3}H]AMPA binding to brainstem-cerebellar samples exhibited an insignificant difference in B_{max} (3-month: 2.8 ± 0.2 pmol/mg, 25-month: 2.5 ± 0.2 pmol/mg; mean ± S.E.M.) and a marginal difference in apparent affinity (3-month: 250 ± 30 nM, 25-month: 360 ± 50 nM; $P < 0.1$) between the two age groups (data not shown). Binding to dopamine and serotonin receptors was also unchanged in the aged vs. young brainstem-cerebellar samples (see Table II, bottom).

In order to determine whether the loss in telencephalic receptors represents age-related degeneration of synapses, the concentration of the nerve terminal markers synaptophysin and the vesicular glycoprotein SV2 were measured in tissue samples from the above groups. Differences in the immunoreactivity levels of the markers between telencephalic samples of 3- and 25-month-old mice were small (3 to 5%) and did not approach statistical significance (Table III). This strongly suggests that the total number of telencephalic synapses was not different in the two age groups. Brain stem-cerebellar samples exhibited no age-related differences in synaptophysin content but a slight decrease in the SV2 antigen (Table III).

TABLE III

Synaptophysin and SV2 immunoreactivity levels in 3- vs. 25-month-old mice brains

Tissue samples from three- ($n = 9$) and twenty-five- ($n = 7$) month-old mice were homogenized and subjected to electrophoresis for Western blotting. The 38 and 100 kDa antigens recognized by anti-synaptophysin and anti-SV2, respectively, were quantitatively compared within single immunoblots using scanning laser densitometry as described in Materials and Methods. Each value represents the mean densitometric scan area (arbitrary units) ± S.E.M.

Age (months)	*Anti-synaptophysin*	*Anti-SV2*
Telencephalon		
3	41.5 ± 0.9	92.0 ± 4.2
25	43.5 ± 1.2	89.6 ± 5.4
Brainstem-cerebellum		
3	18.3 ± 0.8	38.8 ± 7.3
25	19.1 ± 0.5	36.7 ± 6.8

DISCUSSION

The results from binding assays and Western blot analyses are in good agreement that the telencephalon of aged mice contains a lower density of AMPA-type glutamate receptors than does that of young adult animals. This age-related loss appears to be selective since other receptor types (dopamine, serotonin, and GABA) in the older tissue were shown not to be reduced to a substantial degree. Age-related reductions in dopamine[22], adrenergic[7,19], and serotonin[22] receptors have been reported for rat brain. These decreases may be reflections of the synaptic loss that becomes detectable in rat telencephalon at 18–22 months postnatal and increases at older ages[18]. Anatomical studies of comparable detail have not been conducted for mouse but our biochemical results suggest that this species does not experience an equivalent age-related effect. Within the limits of Scatchard analysis it does not appear that a change in affinity contributes to the age-related decrease in AMPA receptors. Previous studies[10,23] (as well as binding data in the present results) estimate that 80–90% of telencephalic synapses are glutamatergic, therefore a 20–30% reduction in total synaptic density would be evident if synaptic degeneration was the major cause of receptor loss. However, the loss of AMPA receptors is not likely to reflect a generalized decrease in synapses (i.e. synapses per unit protein) because universal markers for nerve terminals (synaptophysin and SV2) were not detectably different in the two groups of mice. Presumably then, the observed effect is due either to a decreased rate of receptor synthesis or an increased rate of degradation. A decreased rate of transcription and/or translation is a distinct possibility though this would have to be somewhat selective given the absence of changes in the concentrations of the two pre-synaptic markers. Studies using probes for mRNAs encoding AMPA receptor subunits should provide evidence on this point. Increases in the activity of proteolytic processes involving the receptor also deserve consideration as an explanation. Previous work[1] established that the levels of a

breakdown product resulting from proteolysis of spectrin increase linearly with age in the mice strain used in the present study and are twice as high at 20 months than at 3 months of age. Similar to the present study, the age-related degradation of spectrin was most evident in the telencephalon. Spectrin is a primary constituent of the membrane cytoskeleton and is present in high concentrations in the postsynaptic density[9]. The breakdown product that increases with age is known to appear following activation of endogenous calpain[24] and recent work suggests that the GluR-A subunit is a substrate for this protease[30]. It will be of interest to test if manipulations that produce spectrin breakdown in situ (e.g. ischemia, denervation, prolonged stimulation of NMDA receptors) also result in a reduction in the density of AMPA receptors. Finally, it is possible that aging in the mouse causes generalized changes in telencephalic synapses resulting in altered densities of a variety of postsynaptic constituents. The report of Peterson and Cotman[21] showing that glutamate binding to NMDA receptors is reduced by 55% in the Balb/c mouse telencephalon is of interest in this regard. There is evidence for co-localization of NMDA and AMPA receptors[2] and the age-related reductions for the two classes is sufficiently similar to raise the possibility of a common cause.

An intriguing aspect of the present results is that age-related changes in the density of AMPA-type glutamate receptors were not obtained in brainstem-cerebellum. This does not imply a lack of age-related alterations in these brain regions but rather that brain stem-cerebellar AMPA receptors are not differentially affected by such changes. It is not readily apparent why telencephalic AMPA receptors should be more susceptible to the aging process than those in brainstem-cerebellum although it is the case that a variety of forebrain areas are unusually vulnerable to pathogenic conditions (e.g. ischemia, kainic acid excitotoxicity, Alzheimer's disease). It is of interest that certain of the processes thought to be involved in cell pathology (e.g. stimulation of NMDA receptors, increases in postsynaptic calcium levels) have also been linked to the production of long-term changes in synaptic strength in hippocampus and cortex. It is thus conceivable that age-related deterioration of postsynaptic mechanisms associated with plasticity result in local changes in the synaptic environment and secondarily in receptor numbers.

The functional consequences of the reduction in receptor density will depend on whether the effect is broadly distributed or concentrated in particular regions of the telencephalon, an issue not addressed by the present experiments. Mathematical analyses incorporating diffusion constants, affinity of glutamate receptors, and estimates of the number of receptors per synapse suggest that a release event involving one or two quanta of transmitter would not begin to saturate the AMPA receptor pool. However, the number of receptors stimulated, and hence the magnitude of the synaptic current, is directly related to the number of receptors (Ambros-Ingerson et al., unpublished). Hence, a generalized loss of receptors would likely result in functionally 'weaker' synapses. If the observed reduction of receptor density resulted from profound decreases in a subset of forebrain areas, then it could produce substantial and to a degree predictable impairments. Thus, interpretation of the present results in terms of age-related changes in behavior will depend upon the outcome of studies measuring receptor density across telencephalic regions.

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