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Neurobiology of Aging 26 (2005) 1073–1081

NEUROBIOLOGY OF AGING

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Aging and cholinergic deafferentation alter GluR1 expression in rat frontal cortex

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Received 23 March 2004; received in revised form 13 August 2004; accepted 13 September 2004

Abstract

Previously, we demonstrated that plasticity of frontal cortex is altered in aging rats: lesions of the nucleus basalis magnocellularis (NBM) produce larger declines in dendritic morphology in frontal cortex of aged rats compared to young adults. Cholinergic afferents from the NBM modulate glutamatergic transmission in neocortex, and glutamate is known to be involved in dendritic plasticity. To begin to identify possible mechanisms underlying age-related differences in plasticity after NBM lesion, we assessed the effect of cholinergic deafferentation on expression of the AMPA receptor subunit GluR1 in frontal cortex of young adult and aging rats. Young adult, middle-aged, and aged rats received sham or 192 IgG-saporin lesions of the NBM, and an unbiased stereological technique was used to estimate the total number of intensely GluR1-immunopositive neurons in layer II–III of frontal cortex. While the number of GluR1-positive neurons was increased in both middle-aged and aged rats, lesions markedly increased the number of intensely GluR1-immunopositive neurons in frontal cortex of young adult rats only. This age-related difference in lesion-induced expression of AMPA receptor subunit protein could underlie the age-related differences in dendritic plasticity after NBM lesions.
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Keywords: Acetylcholine; Aging; AMPA; Basal forebrain; Motor cortex; Glutamate

1. Introduction

Although originally considered to be a developmental phenomenon, neural plasticity extends into adulthood, underlying a variety of processes, including learning and memory and recovery from injury. While there is evidence that aging brains retain a certain amount of plasticity (e.g., [37]), recent evidence nonetheless suggests that plasticity may be reduced in the aged brain. For instance, aged patients are twice as likely as young patients to remain severely disabled following moderate head injuries [32,56]. Likewise, traumatic brain injury in aged rats produces a more prolonged suppression of such reflexes as escape, righting, and tail-flick or paw flexion in response to painful stimulation than does traumatic brain injury in young adult rats [11]. Furthermore, aged rats are differentially vulnerable to the effects of a noradrenergic neurotoxin: equivalent DSP-4 injections produced larger depletions of frontal cortical norepinephrine and more pronounced changes in cortical EEG in aged rats compared to young adults [35], and the axonal sprouting typically seen in hippocampal neurons following partial deafferentation via lesions of entorhinal cortex is reduced in aged rats [42]. Finally, lesions of the nucleus basalis magnocellularis attenuate benzodiazepine-induced potentiation of acetylcholine release in frontoparietal cortex in aged but not young adult rats [41].

The nucleus basalis magnocellularis (NBM) is a basal forebrain cholinergic nucleus that supplies specific and direct projections to frontal cortex [4,21]. Acetylcholine supplied by the NBM modulates neocortical function [23,34,47,48,50] and plays an important role in cortical plasticity [17,44]. To explore age-related alterations in the plasticity of frontal cortex, we assessed dendritic morphology of pyramidal neurons in layer II–III of frontal cortex after lesion of the NBM in

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0197-4580/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2004.09.005

young adult, middle-aged, and aged rats. Loss of afferents from the NBM, produced via either ibotenic acid lesion or the cholinergic immunotoxin 192 IgG-saporin, resulted in profound regressive changes in dendritic morphology in aging but not young adult rats [53,59]. The lesion-induced dendritic atrophy seen in aging rats is present as early as 2 weeks post-lesion [59], and remains until at least 3 months post-lesion [53]. The more pronounced dendritic atrophy in aging, lesioned rats suggests a fundamental change in plasticity.

Acetylcholine supplied by the NBM acts as a neuromodulator at glutamatergic synapses in the neocortex, increasing glutamate-evoked excitation when iontophoretically applied (e.g., [27,47]). Glutamate is the major excitatory neurotransmitter in neocortex [33], and glutamatergic transmission has been implicated in the development and maintenance of dendritic morphology [26,57]. Moreover, AMPA receptors appear to play an important role in mediating the effect of glutamate on dendritic morphology: blockade of AMPA/KA receptors alters morphology of developing neurons [28,58], and overexpression of the GluR1 subunit alters the number of primary dendrites, length of dendritic segments, and the number of branch points in mature motoneurons [18]. Together, these studies indicate that during development, the morphology of dendrites is in part regulated by the excitatory neurotransmitter glutamate and its AMPA receptors. If similar mechanisms regulate adult dendritic plasticity, then the age-related alteration we have found in dendritic plasticity in frontal cortical neurons may be mediated by changes in glutamatergic transmission at AMPA receptors.

Using quantitative autoradiography, we demonstrated that in young adult rats, AMPA receptor binding was increased approximately 15% after lesion [52]. This effect is present as early as 1 week post-lesion [39] and remains at least 3 months later [52]. Given the well-documented role of the glutamatergic system in synaptic plasticity (e.g. [6,9,30]), it is likely that this increase in AMPA binding is a plastic, compensatory response to deafferentation.

In contrast with young adult rats, NBM lesions failed to significantly increase AMPA receptor binding in middle-aged and aged rats [52]. This differential age effect is consistent with the hypotheses that the lesion-induced change in AMPA receptor binding in young adults is a compensatory response to loss of cholinergic innervation, and that this response is lost with advancing age.

Lesion-induced changes in AMPA receptor binding as assessed by in vitro autoradiography could reflect changes in either receptor expression or affinity. Furthermore, changes identified using this technique are not localizable to specific cell populations (e.g. neurons versus glia). Thus, to begin to both localize lesion-induced changes in AMPA receptors and identify possible mechanisms underlying the change in binding, in the present study we assessed immunohistochemical labeling of the GluR1 subunit of the AMPA receptor in frontal cortex in young adult, middle-aged, and aged rats after either sham or 192 IgG-saporin lesions of the NBM. We focused on the GluR1 subunit because overexpression of this subunit has been shown to influence the dendritic morphology of mature neurons, suggesting a fundamental role for GluR1 in dendritic plasticity [18].

2. Methods

2.1. Animals

GluR1 subunit protein expression was assessed in young adult (3-months-old; $N = 17$), middle-aged (13-months-old; $N = 17$) and aged (24-months-old; $N = 14$) male Fischer 344 rats. These ages bracket a large portion of the adult life span of the Fischer 344 rat and represent ages both below and at the median mortality of this strain. Throughout the experiment, the rats were group-housed in cages equipped with filter tops. All experimental procedures were approved by the Bloomington Institutional Animal Care and Use Committee and carried out in accordance with NIH guidelines.

2.2. Surgery

Each rat was anesthetized with chlorapent (0.3 ml/100 g *ip*) and received either a unilateral cholinergic lesion of the NBM using the immunotoxin 192 IgG-saporin (RBI; Natick, MA) or a unilateral sham lesion. Previous work (e.g. [3,46,54]) has demonstrated that lesions produced using this procedure destroy cholinergic neurons of the NBM while leaving adjacent structures and noncholinergic neurons intact. Rats were placed in a stereotaxic instrument (Kopf) with the incisor bar set so that bregma and lambda were in the same horizontal plane. The scalp was incised and retracted, holes drilled, and unilateral immunolesions made at 0.8 mm posterior, 3.1 mm lateral, and 8.0 mm ventral to bregma (coordinates taken from the atlas of Paxinos & Watson [31]). For middle-aged and aged rats, the dorsal-ventral coordinate was adjusted to −8.1 mm to compensate for skull thickness. A cannula attached to a Hamilton microsyringe was lowered to the appropriate stereotaxic coordinates and left in place for 2 min prior to injection. 192 IgG-saporin (0.3 μl, 0.5 μg/μl) was pressure-injected in 0.1 μl steps at 1 min intervals, and the cannula slowly withdrawn 5 min after the final injection ($N = 9$ young adult, 8 middle-aged, and 7 aged rats). To produce sham lesions, a cannula was lowered to the appropriate stereotaxic coordinates and withdrawn after 5 min ($N = 8$ young adult, 9 middle-aged, and 7 aged).

2.3. Immunohistochemistry

Immunohistochemical labeling of the GluR1 subunit of the AMPA receptor was performed using a procedure similar to that of Kondo et al. [22]. Rats were deeply anesthetized with urethane and transcardially perfused with cold phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed, post-fixed 2 h, and cryoprotected in 20% sucrose in 0.1 M phosphate

buffer (pH 7.4). Frozen sections were cut coronally at 30 μm on a sliding microtome. For each brain, three series of equally spaced sections (saving ratio 1:6) from the anterior portion of the claustrum through the dorsal hippocampus were saved. One series was then processed free-floating for immunohistochemistry, while the other two series were processed for lesion verification (see below). After rinsing in 0.1 M phosphate buffer (pH 7.4) containing 1% bovine serum albumin and 0.1% Triton-X100 (IPB), sections were incubated in IPB containing 4% normal goat serum to block nonspecific binding and 0.5% H_2O_2 to block endogenous peroxidase activity. Sections were then incubated overnight at 4 °C in IPB containing 1% normal goat serum plus a polyclonal antibody to the GluR1 subunit of the AMPA receptor (1:500; Chemicon International, Temecula, CA). After rinsing in IPB, sections were incubated 1 h in IPB containing 4% normal goat serum and biotinylated goat anti-rabbit IgG (1:200; Vector, Burlingame, CA). After rinsing in 0.1 M phosphate buffer (pH 7.4), sections were incubated in phosphate buffer with ABC complex (Vector, Burlingame, CA) 1 h. Staining was visualized using a nickel-intensified DAB reaction (Fig. 1). After a final rinse, sections were mounted on chrome–alum subbed slides, dehydrated, cleared, and coverslipped. Control sections incubated without the primary antibody were generated and demonstrated virtually no staining.

2.4. Lesion verification

As in previous studies (e.g. [52]), to verify extent and placement of lesions, two series of sections were mounted on gelatin-coated slides and either stained with cresylecht violet or processed for AChE staining using a modification of the Karnovsky–Roots method [14].

To quantify the extent of the NBM lesions, AChE-positive fibers in frontal cortex were counted using a method similar to that of Stichel and Singer [45] and a computer-based morphometry system (Neurolucida; MicroBrightField, Williston, VT). A 5 × 5 counting grid consisting of 50 μm squares (for a total of 250 μm× 250 μm) was superimposed over the Fr1 area of frontal cortex (nomenclature of Zilles and Wree [61]) ipsilateral to the lesion and perpendicular to the pial surface in the middle of layer II–III, in which we have previously documented age-dependent dendritic changes after NBM lesions [59]. The number of crossings of fibers on the grid was then counted at 200×. For each rat, counts were made in each of three sections and averaged across samples. Density of AChE-positive fibers was expressed as number of fibers per counting grid, and compared across groups using a two-way ANOVA (age X lesion).

2.5. GluR1-immunopositive neuron counts

Because we have previously shown age-dependent effects of cholinergic deafferentation on dendritic plasticity of layers II–III neurons in frontal cortex [59], GluR1 subunit protein expression was assessed in layer II–III neurons of frontal

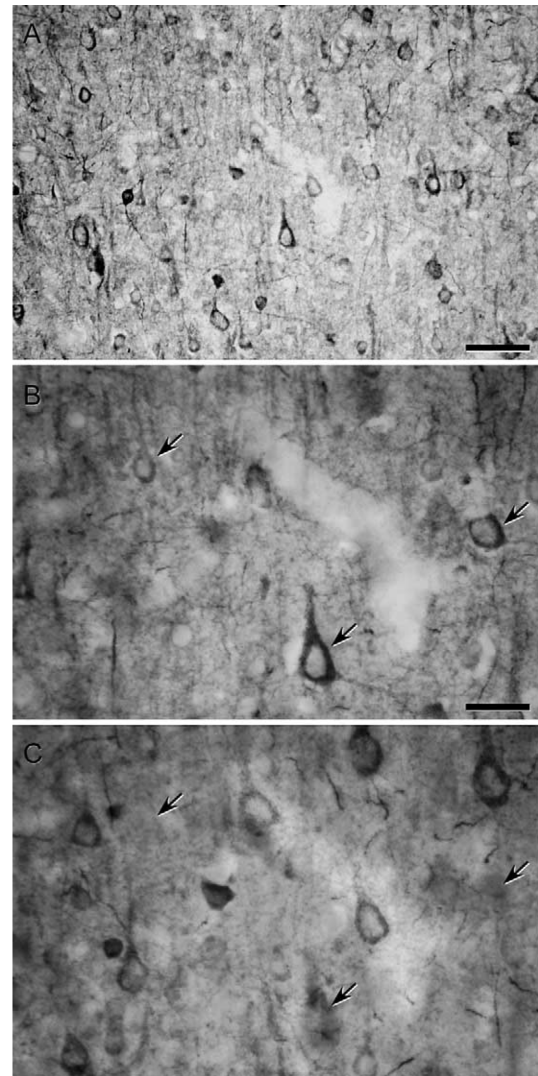

Fig. 1. (A) Low-power digital light micrograph of GluR1-immunopositive cells in frontal cortex in a sham-lesioned young adult rat, demonstrating robust cytoplasmic and dendritic staining of large numbers of neurons. Scale bar = 100 μm. (B) and (C) Higher-magnification images of the same section as in A captured at two different focal planes. Arrows in B indicate cells in first plane of focus; arrows in C indicate that these cells are no longer visible in the second plane of focus. Scale bar for B and C = 50 μm.

cortex. To quantify differences in GluR1 subunit protein expression, an unbiased stereological technique was used to estimate densities of labeled neurons. For each animal, one sample was taken in each of five randomly chosen sections per animal. For each section, the average optical density of the white matter in a 150 μm × 115 μm area directly below the Fr1-3 area of frontal cortex (nomenclature of Zilles and Wree [61]) was determined using a computerized image analysis system (StereoInvestigator; MicroBrightField, Williston, VT) interfaced with a microscope (Olympus BH-2) at a final magnification of 1400×. Optical density of each areal sample was expressed as average luminosity per pixel within that sample, with luminosity ranging from 0 (black) to 256 (white). For each section, neurons with optical

densities at least one standard deviation above this mean were then identified using the thresholding function of the image analysis system; these were considered labeled and counted as described below, with the experimenter blind to condition.

The numerical densities of GluR1-immunopositive neurons were obtained using an optical disector procedure similar to that of Srivastava et al. [43,51]. Shrinkage of sections due to immunohistochemical processing was measured by focusing through each section with a 100× oil-immersion objective and measuring the distance traveled using a stage-mounted microcator calibrated to a known standard. Shrinkage averaged $57 \pm .01\%$; thus the length of the disector was 12 μm. This length was adequate for visualizing neurons in multiple focal planes (see Fig. 1B and C). For each section, counts were made at a final magnification of 1400× in layer II–III of the Fr1-3 area within a 150 μm × 115 μm grid and unbiased counting frame (i.e., neuronal somata touching the lower and left edge of the frame were not counted) whose medial-lateral position within the Fr1-3 area was randomly selected. Cells were identified as neurons based on standard morphological criteria (large, multipolar cell body; small, oval, homogenously labeled cells were considered glia and therefore excluded). Neurons in the first focal plane ("tops") were not counted [7,55]. Neuronal counts were divided by the volume of the counting frame (150 μm × 115 μm × 12 μm) and densities expressed as neurons per mm^3. These estimates were averaged across the five samples within animals.

The volume of layer II–III was estimated using a computer-based image analysis system (MCID; Imaging Research, St. Catharines, Ontario). The area of layer II–III was measured in GluR1-labeled sections spaced evenly throughout the Fr1-3 area. Boundaries of the Fr1-3 area were determined based on its characteristic laminar structure and staining, which is apparent in Glur1-immunolabeled tissue. For instance, the lateral border of frontal cortex (the transition from Fr1-3 to Par1) is characterized by an increase in the thickness of the lightly-staining layer IV. Similarly, the medial border (the transition between the Fr1-3 and Fr2 areas) is characterized by an overall increase in the density of staining along with a narrowing of layer II–III. Laminar boundaries are similarly readily identifiable. Volumes were then calculated using the Cavalieri estimator [36].

To eliminate the possibility of spurious age effects due to differential effects of lesions across ages (see [52]), age effects were assessed by comparing the estimated number of GluR1-immunopositive neurons in young adult, middle-aged, and aged sham-lesioned rats using a one-way ANOVA followed by appropriate planned comparisons. Lesion effects were assessed by comparing the estimated number of GluR1-immunopositive neurons in young adult, middle-aged, and aged sham- and saporin-lesioned rats using a two-way ANOVA (age × treatment) followed by appropriate planned comparisons. For all analyses, planned comparisons consisted of F-tests done within the context of the overall ANOVA [13], comparing sham versus lesioned groups at each age.

3. Results

3.1. Lesion verification

Examination of cresylecht violet-stained sections revealed cannula tracts extending into the region of the NBM, and visual inspection revealed an absence of magnocellular neurons in the basal forebrain in all of the 192 IgG-saporin-lesioned rats. In addition, lesions significantly reduced AChE-stained fibers in frontal cortex by about 93% ($F(1,42) = 2276.31$, $p < 0.01$; see Fig. 2), and this effect was uniform across the three age groups (age × treatment interaction, $F(2,42) = 0.15$, ns). For young adult rats, the average number of AChE-positive fibers per counting grid was reduced from mean = 431.38 ± 14.02 S.E.M. in sham-lesioned animals to mean = 35.00 ± 8.75 in saporin-lesioned animals; for middle-aged rats, average density of AChE-positive fibers was reduced from mean = 416.45 ± 10.91 to mean = 25.71 ± 3.73; and in aged rats, average density of AChE-positive fibers was reduced from mean = 423.14 ± 8.79 to mean = 37.81 ± 10.07. Thus,

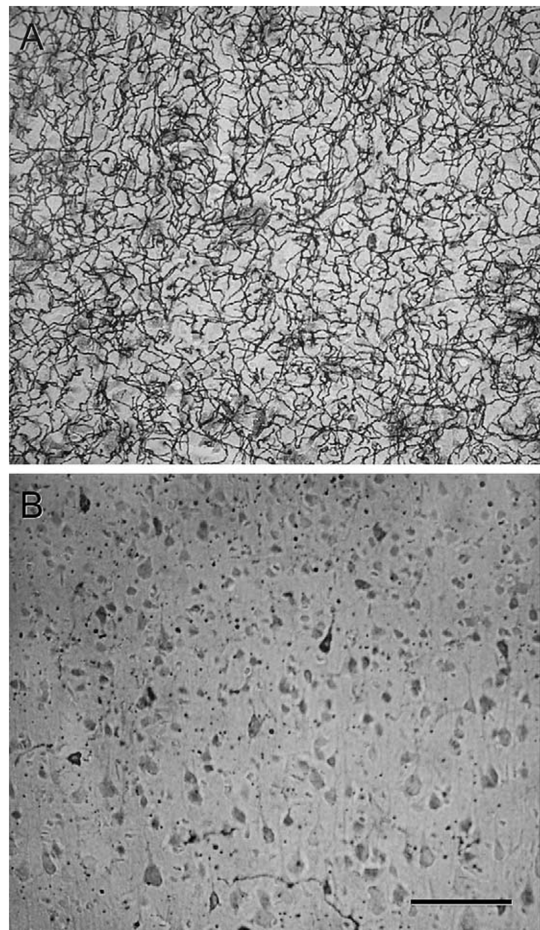

Fig. 2. Digital light micrographs of acetylcholinesterase-stained fibers in frontal cortex of a sham (A), and a 192 IgG-saporin-lesioned (B) rat. Reduction in staining is apparent. Scale bar = 200 μm.

lesions produced a profound reduction in cholinergic innervation across all age groups.

3.2. Stereological information

Average optical density of the white matter samples was 200.38 ± 2.62 S.E.M. The average number of objects counted per frame was 32.29 ± 1.49 S.E.M., and the total number of objects counted across the five frames averaged 151.67 ± 7.68. Within-subjects error for neuronal densities averaged 10% ± 1%. The average volume of frontal cortex varied significantly with age ($F(2,42) = 5.93$, $p \leq 0.01$), but was unaffected by treatment at any age (for main effect of treatment, $F(1,42) = 0.43$, ns; for interaction of age and treatment, $F(2,42) = 2.22$, ns). Planned comparisons demonstrated a small but significant decrease in the volume of layer II–III of frontal cortex in middle-aged sham-lesioned rats relative to young adults, from a mean of 2.69 ± 0.14 mm^3 to a mean of 2.25 ± 0.13 ($F(1,15) = 5.21$, $p \leq 0.05$).

3.3. GluR1-Immunopositive neuron counts

To assess whether expression of GluR1 subunit protein changed with age, the average number of intensely stained neurons was compared in young adult, middle-aged, and aged sham-lesioned rats. One-way ANOVA indicated a significant effect of age on the number of intensely stained neurons ($F(2,21) = 8.18$, $p \leq 0.01$; Fig. 3). Planned comparisons indicated that in middle-aged rats, the number of intensely stained neurons was increased approximately 59%, from mean = 21,439 ± 1361 neurons in young adult sham-lesioned rats to mean = 34,189 ± 2999 neurons in middle-aged sham-lesioned rats ($F(1,15) = 13.75$, $p \leq 0.01$). In addition, there was a less pronounced (25%) but significant increase in the number of intensely stained neurons in aged sham-lesioned rats (mean = 26,815 ± 1854 neurons; $F(1,13) = 5.46$, $p \leq 0.05$). Aged, sham-lesioned rats did not differ significantly from middle-aged sham-lesioned rats ($F(1,14) = 3.78$, ns).

To assess whether lesions of the NBM altered GluR1 expression, the average number of intensely stained neurons was compared in young adult, middle-aged, and aged rats with either sham or 192 IgG-saporin immunolesions of the NBM. Two-way ANOVA revealed significant effects of both treatment ($F(1,42) = 6.19$, $p \leq 0.02$; Fig. 4A) and age ($F(2,42) = 4.63$, $p \leq 0.02$; Fig. 3) on the number of intensely stained neurons, while the interactive effect of age and treatment approached significance ($F(2,42) = 1.95$, $p \leq 0.15$). Planned comparisons demonstrated a significant effect of treatment only in young adult rats. Whereas lesions significantly increased the number

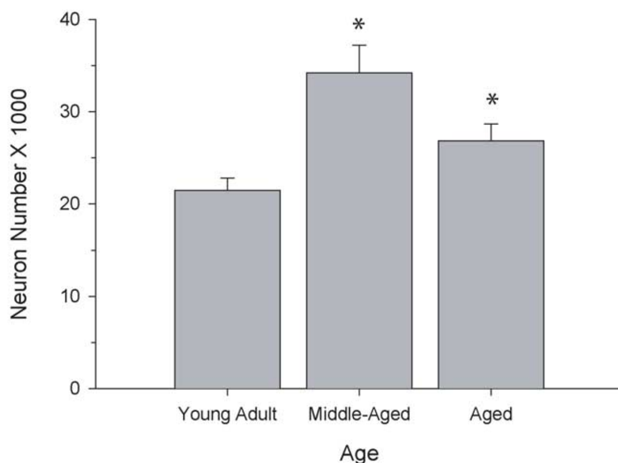

Fig. 3. Average number of intensely stained GluR1-positive neurons in layer II–II of frontal cortex ipsilateral to sham lesion in young adult, middle-aged, and aged rats. The number of GluR1-positive neurons is significantly increased in middle-aged rats. Vertical bars represent S.E.M. values; asterisk (*) indicates significant difference relative to young adult rats.

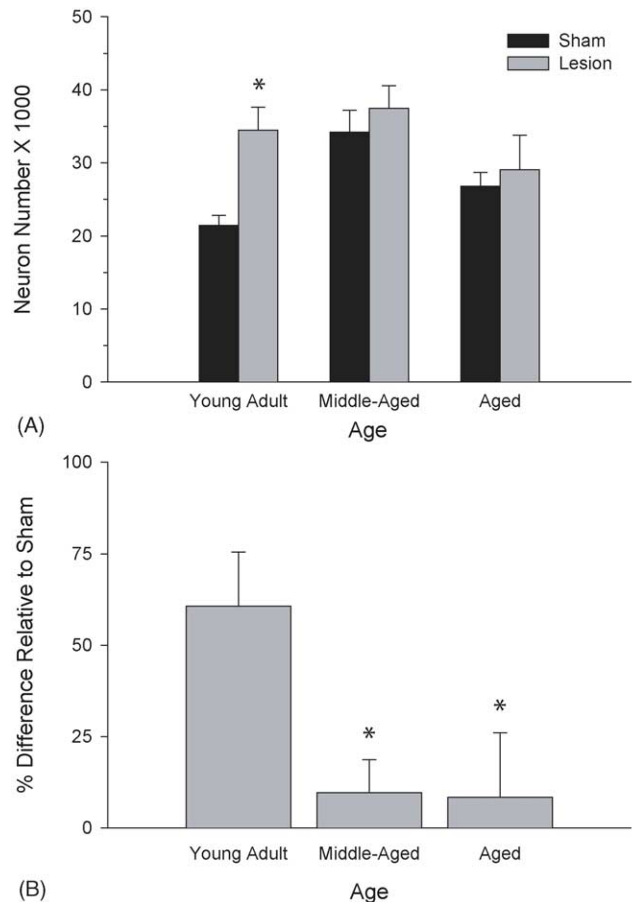

Fig. 4. (A) Average number of intensely stained GluR1-positive neurons in layer II–II of frontal cortex ipsilateral to sham and 192 IgG-saporin lesions in young adult, middle-aged, and aged rats. Lesions significantly increase the number of GluR1-positive neurons in young adult but not aging rats. Vertical bars represent S.E.M. values; asterisk (*) indicates significant difference relative to sham-lesioned rats. (B) Changes in number of intensely stained GluR1-positive neurons (expressed as a percentage relative to sham) in layers II–III of frontal cortex ipsilateral to 192 IgG-saporin lesion in young adult, middle-aged, and aged rats. Following lesion, the number of GluR1-positive neurons is significantly increased in young but not middle-aged or aged rats. Vertical bars represent S.E.M. values; asterisk (*) indicates significant difference relative to young adult rats.

of intensely stained neurons in young adult lesioned rats by approximately 61% (from mean = 21,439 ± 1361 neurons in sham-lesioned rats to mean = 34,461 ± 3147 neurons in saporin-lesioned rats; $F(1,15) = 13.91$, $p \leq 0.01$), they failed to significantly alter the number of intensely stained neurons in either middle-aged (for sham lesioned rats, mean = 34,189 ± 2999 neurons; for saporin-lesioned rats, mean = 37,470 ± 3115 neurons; $F(1,15) = 0.57$, ns) or aged rats (sham-lesioned, mean = 26,815 ± 1854 neurons; saporin-lesioned, mean = 29,074 ± 4710 neurons; $F(1,12) = 0.20$, ns).

In addition, because GluR1 expression varied across ages in sham-lesioned animals, to further investigate the potential age-dependence of cholinergic denervation, the percent difference in GluR1 expression in saporin-lesioned animals relative to sham-lesioned animals was calculated for each animal and compared across age groups. Consistent with the previous analysis, one-way ANOVA revealed a significant effect of age on lesion-induced changes in GluR1 expression ($F(2,42) = 4.77$, $p \leq 0.02$; Fig. 4B). Planned comparisons indicated that the approximately 61% increase in GluR1 expression in young adult lesioned rats was significantly different from the 10% and 8% increases in GluR1 expression seen in middle-aged ($F(1,15) = 8.24$, $p \leq 0.01$) and aged lesioned rats ($F(1,14) = 5.30$, $p \leq 0.04$). Lesion-induced changes in GluR1 expression did not differ between middle-aged and aged rats ($F(1,13) = 0.004$, ns).

4. Discussion

In the present study, we have demonstrated effects of both aging and cholinergic deafferentation on expression of the GluR1 subunit of the AMPA receptor in layer II–III neurons of frontal cortex. Middle-aged rats showed a transient increase in neuronal GluR1 expression, with number of intensely immunopositive neurons increasing by an average of 59%, while the number of intensely immunopositive neurons in aged rats was increased by an average of 25% relative to young adult rats. In addition, we have demonstrated an interactive effect of aging and cholinergic deafferentation on neuronal GluR1 expression. Two weeks after lesion of the NBM with the cholinergic immunotoxin 192 IgG-saporin, young adult rats showed an average 61% increase in intensely immunopositive neurons in layer II–III. On the other hand, in middle-aged and aged rats, despite comparable extent of deafferentation, cholinergic lesions failed to significantly alter counts of GluR1-positive neurons. Thus, the upregulation of this AMPA receptor subunit induced by cholinergic lesions in young adult rats was attenuated in aging animals.

4.1. Age effects

Our present finding that GluR1 expression in layer II–III neurons in frontal cortex is markedly increased in middle-aged rats but only modestly increased in aged rats complements previous studies demonstrating nonlinear age effects. For instance, middle-aged rats are more impaired on a radial arm maze task than are older animals (e.g., [52]). In addition, there are nonlinear age-related changes in morphology of frontal cortex: layer II–III neuronal somata in middle-aged rats are hypertrophied relative to both young adult and aged animals [51]. Finally, nonlinear effects of aging on neurochemical parameters in frontal cortex have also been demonstrated. For example, middle-aged rats show different patterns of changes in the noradrenergic and cholinergic systems in frontal cortex [52], and have elevated levels of the dopamine metabolite HVA and the serotonin metabolite 5-HIAA in medial prefrontal cortex relative to both younger and older animals [60].

In addition, a growing body of evidence suggests a general age-related decline in cortical synaptic connectivity as reflected in concentration of the synaptic vesicle membrane protein synaptophysin [40] and density of dendritic spines [12,19,24,25,49]. Over-activation of hippocampal neurons in culture has been shown to decrease spine density [8,20,29], suggesting that in adults, spine density is down-regulated in response to increased afferent input. The increased GluR1 expression we observed in middle-aged rats suggests a possible mechanism for this downregulation of synaptic connectivity. The increased expression of the GluR1 subunit, which mediates Ca^{2+} permeability in AMPA receptors [10], may result in increased excitation. Therefore, the age-related loss of synaptic connectivity might be due to increased expression of the GluR1 subunit in middle-aged rats.

Finally, previous studies have shown a small but significant decrease in the number of frontal cortical neurons in aged rats [51]. It is possible that the decrease in intensely GluR1-positive neurons in the aged relative to middle-aged rats reflects a selective loss of GluR1-positive neurons, rather than decreased GluR1 expression in the remaining neurons.

4.2. Effects of cholinergic deafferentation

In the present study we have shown an upregulation of neuronal GluR1 expression in frontal cortex induced by cholinergic deafferentation, which is consistent with previous studies demonstrating increased AMPA receptor binding in frontal cortex after excitotoxic lesions of the NBM in young adult rats [38,52]. A major disadvantage of the in vitro quantitative autoradiography used in previous studies is that it does not allow assessment of the cellular site of potential receptor changes. Given that both glia and neurons bear glutamate receptors, it is important to discriminate between these two cell types to be able to make more direct inferences about mechanism and to ensure that potential differences in neuronal receptors are not obscured by a lack of effect in glia. While the present results do not rule out lesion-induced changes in glial GluR1 expression, they nonetheless indicate that the alteration in AMPA receptor binding occurs in neurons and may be due at least in part to an upregulation of the GluR1 subunit of the AMPA receptor. However, it should be noted

that GluR1 is one of several subunits that can make up the AMPA receptor; in order to obtain a more complete understanding of lesion-induced changes in the receptor, further studies should examine other subunits as well.

Acetylcholine supplied by the NBM acts as a neuromodulator at glutamatergic synapses in the neocortex, increasing glutamate-evoked excitation when iontophoretically applied (e.g., [27,47]). Our previous results suggest that acetylcholine from the NBM is involved in the maintenance of cortical dendritic arbor [59]. Previously, we found age-dependent effects of cholinergic lesions on dendritic arbor of pyramidal neurons in layer II–III of frontal cortex. Whereas NBM lesions produced minor alterations of dendrites of cortical neurons in young adult rats, lesions produced a profound atrophy of dendrites in middle-aged and aged rats. Our present data suggest that acetylcholine may exert its effects on dendritic plasticity via its modulatory effects on glutamate. Glutamate is the major excitatory neurotransmitter in neocortex [33], and glutamatergic transmission has been implicated in the development and maintenance of dendritic morphology. For example, dendritic morphology in hippocampal pyramidal cells is altered by exposure to glutamate [26,57]. In addition, overexpression of the GluR1 subunit of the AMPA receptor via viral transfection has been shown to influence the dendritic morphology of mature motoneurons, suggesting a fundamental role for the GluR1 subunit in dendritic plasticity [18]. The present finding is consistent with both our previous results and a role for AMPA receptors in cholinergic lesion-induced dendritic plasticity. Thus, the increase in GluR1 expression that we observed in young adult rats may be a compensatory response to cholinergic deafferentation, which might prevent lesion-induced dendritic atrophy.

A remaining question, however, is site of action. In the present study, we did not discriminate between GluR1-positive interneurons and pyramidal cells; thus, the lesion-induced increase in GluR1 expression may be localized to interneurons, pyramidal cells, or both. Thus, if the lesion-induced changes in GluR1 expression are preventing dendritic atrophy in pyramidal cells, they could be doing so either indirectly, for instance, via inhibitory interneurons synapsing on the pyramidal cells (see [1]), or directly at the pyramidal cells. Finally, while we have interpreted these results as a direct effect of loss of cholinergic innervation, it is possible that some other effect, such as loss of co-transmitters in NBM neurons, loss of cholinergic input to cortically projecting GABAergic basal forebrain neurons, cortical glial or interneuronal responses, or changes in trophic factor release may play a role in the effect of cholinergic deafferentation on cortical GluR1 expression.

4.3. Altered plasticity in aging

Whereas cholinergic lesions increased GluR1 expression in young adults, they failed to significantly alter GluR1 expression in middle-aged and aged rats. This differential age effect is consistent with the hypothesis that the lesion-induced increase in GluR1 is a compensatory response that is lost with advancing age. This hypothesis is consistent with a growing body of evidence for age-related alterations in neuronal plasticity. For instance, aged rats show slower behavioral recovery from traumatic brain injury [11] and ischemia [5], reduced axonal sprouting of hippocampal neurons following lesions of entorhinal cortex [42], and more pronounced attenuation of induced acetylcholine release in frontoparietal cortex after basal forebrain lesions [41].

While the present data demonstrate an age-dependent effect of lesion and are consistent with the hypothesis that plasticity in frontal cortex is altered in aging rats, alternative interpretations must be considered. For example, the attenuated increase in GluR1 expression after lesioning in middle-aged animals could potentially reflect a ceiling effect. GluR1 expression is elevated in middle-aged rats over young adult levels, and this elevation could prevent a further increase post-lesion. However, this seems unlikely, given that aged animals show even less lesion-induced GluR1 upregulation, despite the fact that baseline levels of GluR1 expression in the aged rats are lower than that seen in middle-aged animals. Another consideration is that the design of this study, in which we assessed changes in GluR1 immunolabeling at only one time point post-lesion, prevents an assessment of the precise nature of the age-related alteration in plasticity. Our data are consistent with the hypothesis that neurons ipsilateral to lesions in aging rats are less able to upregulate GluR1. Alternatively, the time course of cortical neurons' response to cholinergic deafferentation may simply be slower in aging rats. It is possible that at a later time point, GluR1 expression in the older lesioned animals may reach the magnitude seen in young adults. This hypothesis is consistent with previous studies demonstrating age-dependent effects on the time course but not extent of reactive synaptogenesis following deafferentation of hippocampal subregions [2,15,16]. For example, while deafferentation of the dentate gyrus via removal of entorhinal cortex results in reactive synaptogenesis that is comparable in magnitude in young adult and aged rats, the increase in synaptic density is delayed in the older animals [15], apparently due to slower clearing of degenerative material [16]. Similarly, partial deafferentation of hippocampal area CA1 in aged rats elicits reactive synaptogenesis that is comparable in magnitude but delayed compared to that seen in young adult rats [2]. Examination of the time course of changes in GluR1 expression in cortical neurons following NBM lesions in aging rats would determine whether GluR1 upregulation is attenuated or if the response is simply slowed.

Finally, the present results complement our previous studies [53,59] demonstrating that both ibotenic-acid lesions and specific cholinergic lesions of the NBM resulted in marked regressive changes in the dendrites of layer II–III pyramidal neurons in frontal cortex in middle-aged and aged, but not young adult, rats. As described above, our previous results suggest that acetylcholine from the NBM is involved in the maintenance of cortical dendritic arbor via its modulatory effects on glutamate. The increased GluR1 expression

after cholinergic deafferentation in young adult rats may provide the neurochemical substrate for increased transmission at AMPA receptors necessary to maintain dendritic arbor; the attenuation of this response in middle-aged and aged rats may thus underlie the lesion-induced dendritic atrophy seen in aging rats.

Acknowledgements

We thank Joy E. Buse for her excellent technical assistance and Dale R. Sengelaub for his helpful comments on the manuscript. This research was supported by NIH-NIA P30AG10133 to C.L.W.

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