

Age-dependent effect of cholinergic lesion on dendritic morphology in rat frontal cortex

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Abstract

Previously, we demonstrated that plasticity of frontal cortex is altered in aging rats: 3 months after surgery, excitotoxic lesions of the nucleus basalis magnocellularis (NBM) produce larger declines in dendritic morphology in frontal cortex of aged rats relative to young adults. To determine whether the differential effect of the lesion was due specifically to loss of cholinergic input from the NBM, we assessed dendritic morphology in frontal cortex after specific cholinergic depletion in young adult, middle-aged, and aged male rats. Rats received unilateral sham or 192-IgG-saporin lesions of the NBM. Two weeks after surgery, brains were stained using a Golgi–Cox procedure. Dendritic morphology was quantified in pyramidal neurons in layers II–III of frontal cortex. Although lesions altered apical dendrites at all ages, these effects were most pronounced in aged rats. In addition, lesions produced marked atrophy of basilar dendrites in middle-aged and aged rats only. Thus, the differential dendritic atrophy resulting from NBM lesions in aged rats occurs within 2 weeks after lesion, and results specifically from loss of cholinergic innervation.

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1. Introduction

Plasticity has long been known to be a property of developing nervous systems. Although originally considered to be a developmental phenomenon, plasticity extends into adulthood as well, underlying a variety of processes, including learning and memory as well as recovery from injury. While there is evidence that aging brains retain a certain amount of plasticity (e.g. [48]), recent evidence suggests that this plasticity may be reduced in the aging brain. For instance, aged patients are twice as likely as young patients to remain severely disabled following moderate head injuries [41,66]. 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 [16]. 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 [46]. Similarly, the axonal sprouting typically seen in hippocampal neurons following partial deaf-ferentation via lesions of entorhinal cortex is reduced in aged rats [52]. Finally, lesions of the nucleus basalis magnocellularis (NBM) attenuate benzodiazepine-induced potentiation of acetylcholine release in frontoparietal cortex in aged but not young adult rats [51]. These differential responses of aging individuals to injury or lesion suggest that plasticity is altered in the aged, which has important consequences for both successful aging and treatment of neural disorders in the aged.

Normal aging results in a variety of functional and morphological changes in the frontal cortex of rats. Conduction velocity in fibers projecting from basal forebrain cholinergic nuclei to frontal cortex is decreased, apparently due to demyelination [3], and changes in frontal cortical EEG have also been documented [47]. Age-related increases in glial density [40] and decreases in synaptic density [50] have also been reported. Previously, we demonstrated age-related changes in several measures of the morphology of frontal cortex in rats [61]. For instance, the thickness of superficial layers was significantly reduced in aged rats. This reduction paralleled a significant decrease in total neuron number in the aged rats.

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These physiological and morphological changes are accompanied by neurochemical changes, including substantially reduced acetylcholine release [69] and choline acetyltransferase activity [2] in frontal cortex. This reduction in acetylcholine release may be due to downregulation of nicotinic autoreceptors: the facilitatory effect of the nicotinic agonist methylcarbamylcholine on spontaneous acetylcholine release in frontal cortical slices was reduced in aged rats. A subsequent autoradiographic assay in cortical homogenates suggests that this effect was due to decreased density of nicotinic receptors [2]. Similar experiments suggest a downregulation of M_1 and M_2 receptors as well [2,62]. Noncholinergic neurotransmitter systems have also been implicated. Ruano et al. [49] found decreased $GABA_A$ receptors but not benzodiazepine receptors in prefrontal cortex of aged rats; Nyakas et al. [37] documented decreases in serotonin ($5-HT_{1A}$) receptors in frontal cortex; and Porrás et al. [42] demonstrated decreased responsiveness of prefrontal cortical neurons to dopaminergic modulation of glutamate release in both middle-aged and aged rats. Finally, our previous work has demonstrated increased binding to noradrenergic reuptake sites in both middle-aged and aged rats [62]. Thus, a growing body of evidence demonstrates structural, functional, and neurochemical changes in frontal and prefrontal cortex in aging rats.

The profound changes in frontal cortex described above could either reflect or result in changes in plasticity. Previously, we used ibotenic acid lesions of the NBM to begin to investigate potential age-related changes in frontal cortical plasticity [61,64]. The NBM is a basal forebrain cholinergic nucleus, and projections from the NBM modulate cortical structure and function. Frontal cortex receives specific and direct projections from the NBM [7,22], and acetylcholine supplied by the NBM plays a fundamental role in modulating neocortical function [29,45,58–60]. In addition, the NBM plays an important role in cortical plasticity, both developmentally and in adulthood. For instance, in neonatal mice, NBM lesions disrupted morphogenesis of frontoparietal cortex, delayed differentiation of cortical layers, and temporarily reduced cortical cholinergic markers [20]. Others have shown that lesions of the NBM alter dendritic arborization of pyramidal cells in frontoparietal cortex of neonatal rats [55]. Finally, NBM lesion-induced deficits on a go/no go task were accompanied by gross morphological changes in neurons in frontoparietal cortex, including reduced soma size in particular cortical layers, resulting in decreased thickness of those layers. These effects were most pronounced in layers II–III [63]. Thus, projections from the NBM appear to play a role in frontal cortical plasticity, and manipulations of this system offer a model of cortical plasticity.

To explore age-related alterations in the plasticity of frontal cortex, we assessed dendritic morphology of frontal cortical neurons after lesion of the NBM in young adult, middle-aged, and aged rats. Loss of afferents from the NBM, produced via ibotenic acid lesion, produced profound

regressive changes in dendritic morphology in aging but not young adult rats [64]. The more pronounced dendritic atrophy in aging, lesioned rats suggests a fundamental change in plasticity, and may have important implications for understanding both successful aging and disorders associated with aging. Therefore, understanding the neurochemical substrate of this alteration in plasticity is critical. However, because a relatively nonspecific excitotoxin was used in the previous study, it is unclear whether the altered plasticity seen in the aged rats was specific to loss of cholinergic innervation from the NBM. Thus, to determine whether the differential effect of the lesion was due specifically to loss of cholinergic input from the NBM, we assessed dendritic morphology in frontal cortex after selective cholinergic depletion in young adult, middle-aged, and aged male rats. Preliminary results of this study have been presented previously in abstract form [68].

2. Materials and methods

2.1. Animals

Dendritic morphology was assessed in young adult, middle-aged, and aged male F344 rats (Harlan Laboratories, Indianapolis, IN) that received either 192-IgG-saporin or sham lesions of the NBM. Rats were 3 ($N = 12$), 13 ($N = 11$), and 24 ($N = 11$) months old at the time of surgery. These ages bracket a large portion of the adult life span of the F344 rat and represent ages both below and at the median mortality of this strain. Throughout the experiment, 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 chloralpent (0.3 ml/100 g i.p.) 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. [6,65]) 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 and Watson [39]). 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 μ l, 0.5 μ g/ μ l) was pressure-injected

in 0.1- μ l steps at 1-min intervals, and the cannula slowly withdrawn 5 min after the final injection ($N = 7$ young adult, 6 middle-aged, and 6 aged). To produce sham lesions, a cannula was lowered to the appropriate stereotaxic coordinates and withdrawn after 5 min ($N = 5$ per age group).

2.3. Histology and lesion verification

Two weeks after surgery, all rats were deeply anesthetized and transcardially perfused with 0.9% saline. Brains were removed and the rostral portion of each brain (from the olfactory bulb to approximately the level of the medial septum) dissected and processed using Glaser and Van der Loos' modified Golgi stain [14], which allows visualization of whole neurons including processes. The rostral forebrain was immersed in Golgi–Cox solution (a 1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate). When staining was complete (14 days; determined in pilot animals by developing test sections at regular intervals and assessing the presence of dendrites trailing off into a series of dots; see [10]), brains were dehydrated in 1:1 absolute ethanol/acetone (3 h), followed by absolute ethanol and then 1:1 ethanol/ether (30 min each). Brains were then infiltrated with a graded series of celloidins before being embedded in 8% celloidin (8% (w/v) parlodion in 1:1 absolute ethanol/ether). Coronal sections were cut at 145 μ m on a sliding microtome (American Optical 860). Free-floating sections were then alkalized in 18.7% ammonia, developed in Dektol (Kodak), fixed in Kodak Rapid Fix (prepared as per package instructions with Solution B omitted), dehydrated through a graded series of ethanols, cleared in xylene, mounted, and coverslipped (see [14]).

The remainder of the brain was immersed in 4% paraformaldehyde, cryoprotected, and frozen sections (40 μ m) taken coronally through the region of the NBM. To verify extent and placement of lesions, two series of sections were mounted and either stained with cresylecht violet or processed for AChE staining using a modification of the Karnovsky–Roots method [17].

To estimate the efficacy of NBM lesions, AChE-positive fibers in frontal cortex were counted using a method similar to that of Stichel and Singer [56] and a computer-based morphometry system (NeuroLucida; MicroBrightField, Colchester, VT). A 5 \times 5 counting grid consisting of 50 μ m squares (for a total of 250 μ m \times 250 μ m) was superimposed over the FR1 area of frontal cortex (nomenclature of Zilles and Wree [70]) ipsilateral to the lesion and perpendicular to the pial surface in the middle of either layers II–III or V–VI. The number of crossings of fibers on the grid was then counted at 200 \times . For each rat, counts were made in three sections, for a total of six samples per animal. Density of AChE-positive fibers was expressed as number of fibers per counting grid, and compared across groups using a two-way ANOVA (age \times lesion).

2.4. Dendritic analyses

Previous research demonstrated excitotoxic lesion-induced dendritic changes in layer II–III neurons in frontal cortex [64]. Thus, layer II–III pyramidal neurons in the FR1-3 area of frontal cortex (nomenclature of Zilles and Wree [70]) ipsilateral to either 192-IgG-saporin or sham lesions were drawn. Pyramidal neurons were defined by the presence of a basilar dendritic tree, a distinct, single apical dendrite, and dendritic spines (Fig. 2). Neurons with somata in the middle third of sections were chosen to minimize the number of truncated branches. For each animal, 7–10 neurons were drawn; this number yielded a within-animal error of approximately 13% (mean within-animal S.E.M. for total branch number and length = $13.68 \pm 0.41\%$), and thus was considered to provide a representative sample of layer II–III pyramidal neurons in frontal cortex. All neurons were drawn at 600 \times and morphology of apical and basilar arbors was quantified in three dimensions using a computer-based neuron tracing system (NeuroLucida; MicroBrightField), with the experimenter blind to condition.

Several aspects of dendritic morphology were examined. To assess overall changes in dendritic morphology, total length and number of basilar and apical dendrites were compared across groups using two-way ANOVA (age \times lesion). To assess differences in the amount and location of dendritic material, a three-dimensional version of a Sholl analysis [53] was performed. A Sholl analysis estimates the amount and distribution of dendritic material by counting the number of intersections of dendrites with an overlay of concentric rings centered on the soma. In the present study, the number of intersections of dendrites with concentric spheres at 10- μ m intervals was assessed; these numbers were then summed over 20- μ m intervals. These data were compared using three-way ANOVA (age \times lesion \times distance from soma). Finally, terminal branches may be more plastic than other parts of the arbor [10,48], and thus may be more sensitive to the effects of cholinergic deafferentation. Therefore, the length and number of terminal dendritic branches were also calculated and compared using two-way ANOVA (age \times lesion).

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 98% ($F(1, 27) = 2052.17$, $P < 0.05$; see Fig. 1), and this effect was uniform across the three age groups (age \times treatment interaction: $F(2, 27) = 2.93$, ns). Thus,

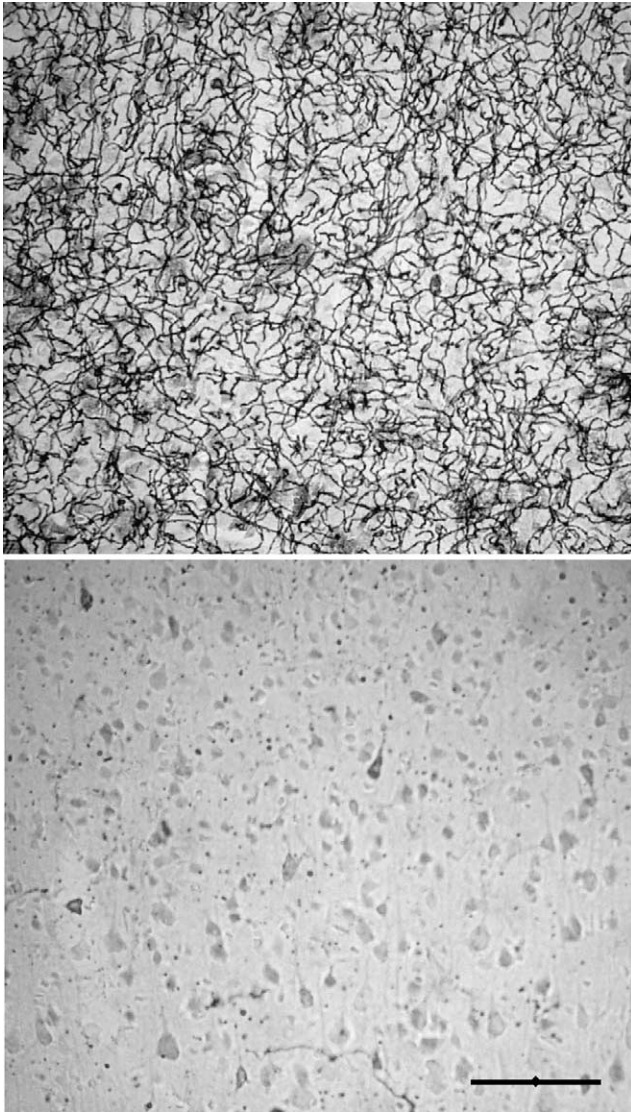


Fig. 1. Digital light micrographs of acetylcholinesterase-stained fibers in frontal cortex of a sham- (top) and a 192-IgG-saporin-lesioned (bottom) rat. Reduction in staining is apparent. Scale bar = 200 μm .

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

3.2. Dendritic analyses

In all treatment groups, complete impregnation of numerous cortical pyramidal neurons was apparent (Fig. 2), and layer II–III was readily identifiable. Because relatively thick sections were taken through frontal cortex, the apical and basilar arbors of essentially all neurons selected were completely contained within a single section.

To rule out potential artifactual differences in dendritic morphology due to differential sampling in layers II–III, the distance from the soma to the pial surface of cortex was measured for each neuron. Average distance to pia was then

compared across groups using a two-way ANOVA (age \times treatment). Average distance to pia did not vary with either age or treatment (for main effect of age: $F(2, 28) = 1.11$, ns; for main effect of treatment: $F(1, 28) = 0.31$, ns; for age \times treatment interaction: $F(2, 28) = 2.80$, ns). Thus, neurons were sampled from equivalent laminar depths across groups.

3.2.1. Apical dendrites

To examine age-related changes in dendritic arbor without potential spurious effects due to lesions, total length and number of apical dendrites were compared across young adult, middle-aged, and aged sham-lesioned rats using one-way ANOVA. While total number of apical branches did not vary significantly with age ($F(2, 12) = 1.71$, ns), total length of the apical arbor varied significantly with age ($F(1, 12) = 4.70$, $P < 0.05$; Fig. 3). Planned comparisons revealed a significant increase in apical dendritic length of about 34% in aged sham-lesioned rats compared to young adult sham-lesioned rats ($F(1, 8) = 9.34$, $P < 0.05$); apical dendritic length in middle-aged sham-lesioned rats was not significantly different from either younger or older animals ($F(1, 8) = 0.23$ and 2.80 , respectively, ns).

To assess overall changes in apical dendritic morphology after lesion, total length and number of apical dendrites were compared across groups using two-way ANOVA (age \times treatment). Overall apical branch number and length did not vary across ages ($F(2, 28) = 1.66$, ns). However, two-way ANOVA revealed a significant effect of treatment on apical branch number and length ($F(1, 28) = 6.76$ and 10.38 , respectively, $P < 0.05$; Fig. 3). Although the interactive effects of age and treatment were not significant ($F(2, 28) = 0.96$ and 2.52 , respectively, ns), planned comparisons revealed a significant effect of treatment only in aged animals, in which NBM lesions resulted in a 25% decrease in apical branch number and a 32% decrease in apical branch length (for aged sham versus lesion: $F(1, 9) = 4.90$ and 9.16 , respectively, $P < 0.5$; for young adult sham versus lesion: $F(1, 10) < 0.84$, ns; for middle-aged sham versus lesion: $F(1, 9) < 2.06$, ns).

To assess differences in the amount and location of dendritic material, a Sholl analysis [53] was performed. While the amount and distribution of dendritic material did not vary with age (for main effect of age: $F(2, 28) = 2.71$, ns; for interaction of age and distance from soma: $F(16, 224) = 1.59$, ns), lesions significantly altered apical dendritic material ($F(1, 28) = 20.89$, $P < 0.05$), and this effect varied with both age and distance from the soma (for treatment \times distance from soma interaction: $F(8, 224) = 6.77$, $P \leq 0.05$; for age \times treatment \times distance from soma interaction: $F(16, 224) = 1.84$, $P < 0.05$). Planned comparisons indicated that while lesions significantly decreased apical dendritic material at all ages, the pattern and extent of this effect differed dramatically with age (Fig. 4). In young adult rats, lesion effects were restricted to the distal portion of the apical arbor, decreasing dendritic material by 38–88% (for 110–20,

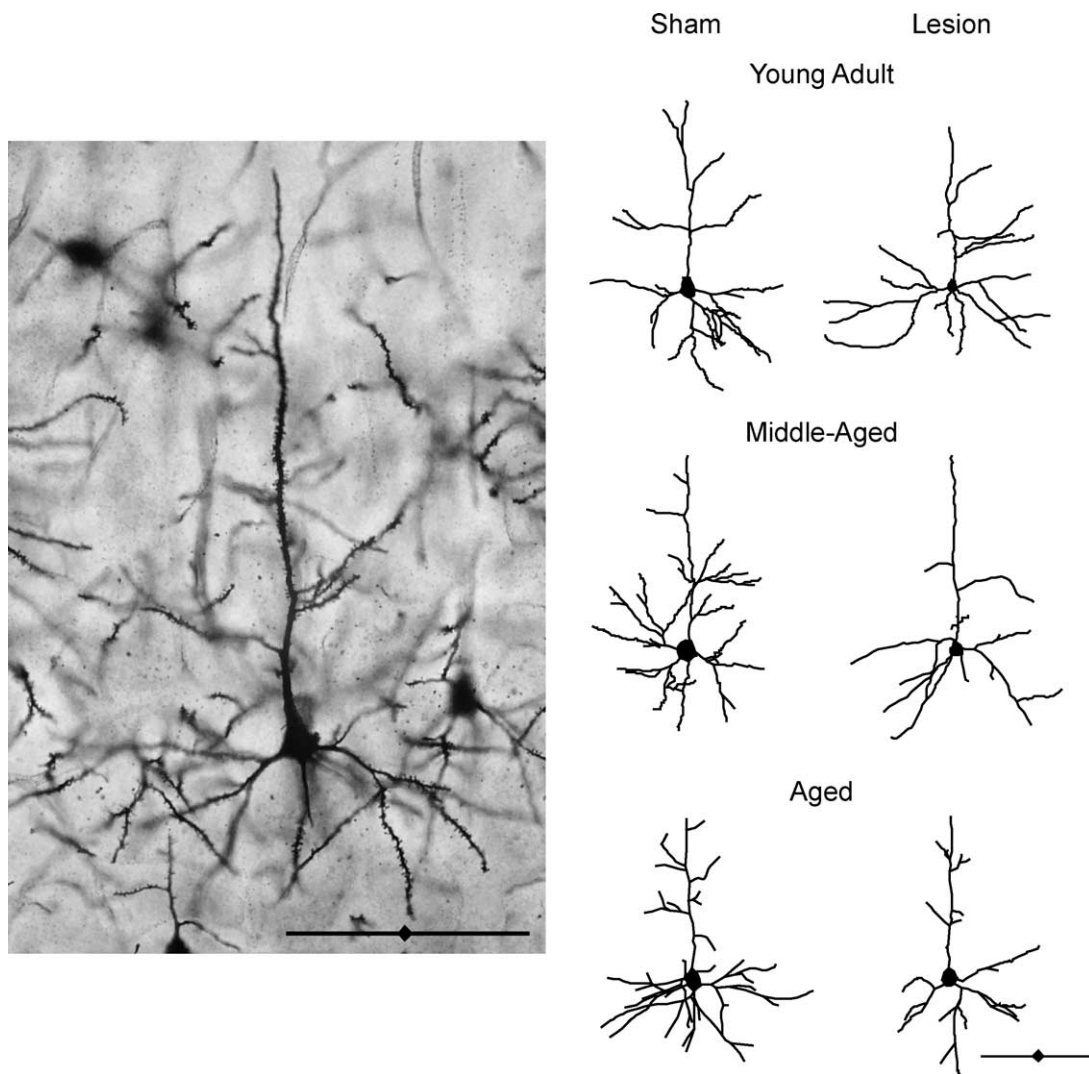


Fig. 2. *Left*: Digital light micrograph of Golgi-stained neuron in layers II–III of frontal cortex in a sham-lesioned rat. Scale bar = 100 μm . *Right*: Computer-assisted reconstructions of Golgi-stained neurons in layers II–III of frontal cortex ipsilateral to sham (left) and 192-IgG-saporin lesions of the NBM in young adult (top), middle-aged (middle), and aged (bottom) rats. Scale bar = 100 μm . These neurons were selected because they are representative of their respective group apical and basilar dendritic lengths.

130–40, 150–60, and ≥ 170 μm from soma, all $F_s(1, 10) \geq 5.62$, $P_s < 0.05$; all other $F_s(1, 10) \leq 1.31$, ns). For middle-aged rats, lesions essentially failed to alter the distribution of apical dendritic material, with a lesion-induced reduction in dendritic material only reaching significance at 70–80 μm from the soma ($F(1, 9) = 6.85$, $P < 0.05$; all other $F_s(1, 9) \leq 4.72$, ns). On the other hand, in aged rats, lesions resulted in a marked reduction in dendritic material, ranging from 34 to 72%, throughout most of the apical arbor (for 70–80, 90–100, 110–120, 130–140, and 150–160 μm from soma, all $F_s(1, 9) \geq 7.73$, $P_s < 0.05$; for 10–20, 30–40, and ≥ 170 μm from soma, all $F_s(1, 9) \leq 4.23$, ns).

The number and length of apical branch terminals did not vary significantly with age (for terminal branch number: $F(2, 28) = 1.01$, ns; for terminal branch length: $F(2, 28) = 0.01$, ns). However, lesions significantly altered both number

and length (for terminal number: $F(2, 28) = 6.52$, $P < 0.05$; for terminal length: $F(2, 28) = 10.80$, $P < 0.05$), and planned comparisons indicated that this effect varied across ages (Fig. 5). Whereas lesions failed to significantly alter apical branch number or length in either young adult or middle-aged rats (for young adults: $F_s(1, 10) = 1.34$ and 1.80; for middle-aged rats: $F_s(1, 9) = 1.16$ and 2.07, all ns), lesions reduced both number and length of apical terminal branches, by 21 and 32%, respectively, in aged rats (for terminal branch number: $F(1, 9) = 5.54$; for terminal branch length: $F(1, 9) = 7.26$, $P < 0.05$).

3.2.2. Basilar dendrites

To examine age-related changes in dendritic arbor without potential spurious effects due to lesions, total length and number of basilar dendrites were compared across

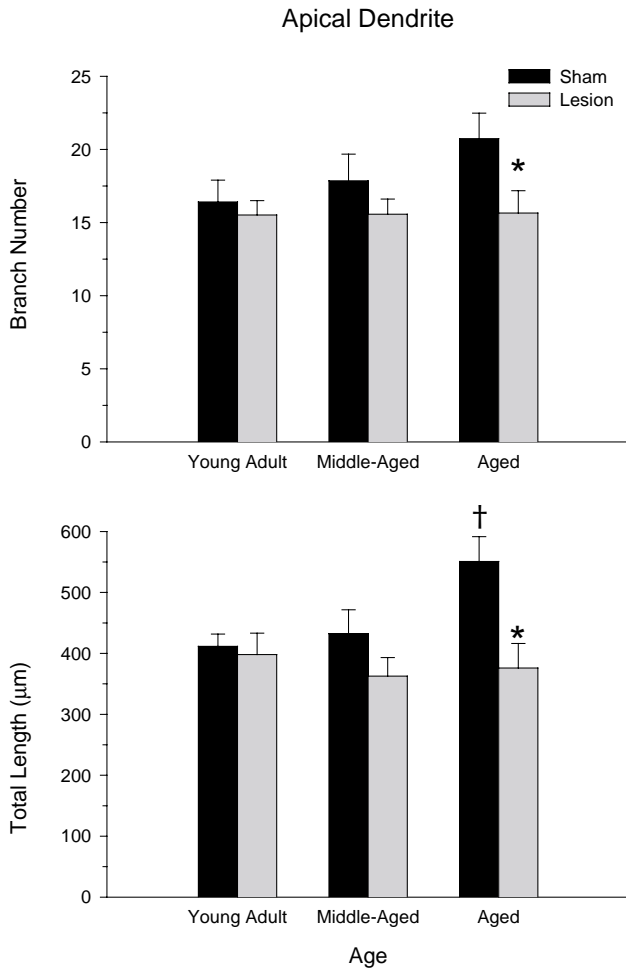


Fig. 3. Mean apical branch number (top) and length (bottom) ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Vertical bars represent S.E.M. values; dagger (†) indicates significantly different from young adult sham-lesioned rats; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

young adult, middle-aged, and aged sham-lesioned rats using one-way ANOVA. Total length and number of basilar dendrites did not vary across ages ($F_s(1, 12) = 1.66$ and 0.19 , respectively, ns; Fig. 6).

To assess lesion-induced overall changes in basilar dendritic morphology, total length and number of basilar dendrites were compared across groups using two-way ANOVA (age \times treatment). Overall basilar dendritic length and number did not vary significantly across ages ($F_s(2, 28) = 0.75$, ns). However, two-way ANOVA revealed a significant effect of treatment on basilar branch number and length ($F_s(1, 28) = 7.38$ and 10.02 , respectively, $P < 0.05$; Fig. 6). Although the interactive effects of age and treatment were not significant ($F_s(2, 28) = 1.70$ and 2.72 , respectively, ns), planned comparisons revealed a significant effect of treatment only in middle-aged and aged animals. In aged rats, lesions reduced basilar branch length by 37% ($F(1, 9) = 7.65$, $P < 0.05$), while lesions re-

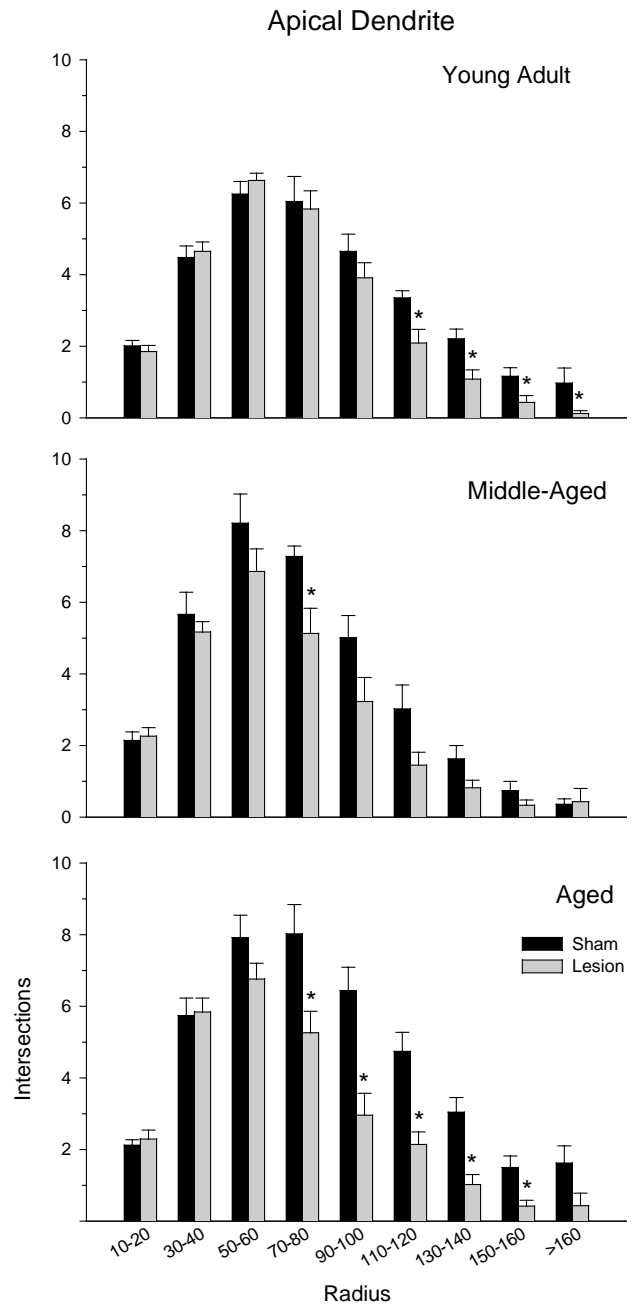


Fig. 4. Mean intersections of apical dendrites with 10-µm concentric spheres ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Data have been summed into nine bins. Vertical bars represent S.E.M. values; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

duced basilar branch number by 23% in middle-aged rats ($F(1, 9) = 4.89$, $P < 0.05$). All other planned comparisons were nonsignificant (for young adult rats: $F_s(1, 10) \leq 0.02$, ns; for middle-aged and aged rats: $F_s(1, 9) \leq 3.88$, ns).

Three-way ANOVA revealed that the distribution of basilar dendritic material did not vary significantly across age groups ($F(2, 28) = 0.33$, ns). However, lesions significantly altered the distribution of basilar dendritic material

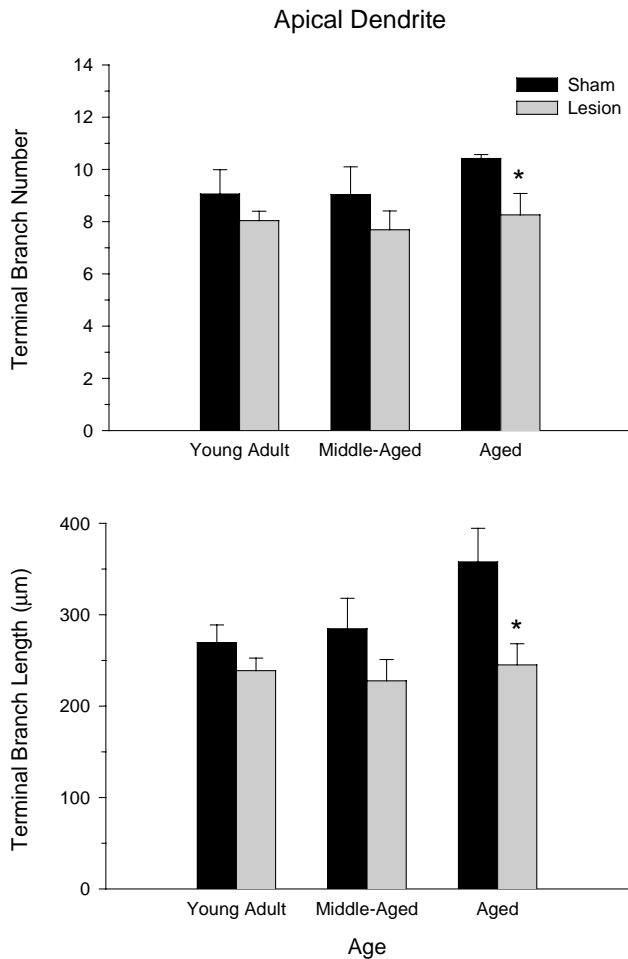


Fig. 5. Mean number (top) and length (bottom) of apical terminal branches ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Vertical bars represent S.E.M. values; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

($F(2, 28) = 17.90, P < 0.05$), and this effect varied with both age and distance from the soma (interaction of age and treatment: $F(2, 28) = 3.89$; interaction of treatment with distance from soma: $F(8, 224) = 6.48$; interaction of age with treatment and distance from soma: $F(16, 224) = 1.83$, all $P_s < 0.05$). Planned comparisons indicated that, as for apical dendrites, while lesions significantly decreased basilar dendritic material at all ages, the pattern and extent of this effect differed dramatically with age (Fig. 7). In young adult rats, lesion effects were restricted to the proximal portion of the basilar arbor, producing a 20% reduction in dendritic material within 20 μm of the soma ($F(1, 10) = 14.35, P < 0.05$; all other $F_s(1, 10) = 0.60$, ns). For middle-aged rats, the effect of lesion was somewhat more widely distributed, with a significant lesion-induced reduction in dendritic material of 28% within 20 μm of the soma ($F(1, 9) = 10.29, P < 0.05$) and reductions of 21 and 33% at 30–40 and 50–60 μm approaching significance ($F_s(1, 9) \leq 4.70, P_s < 0.075$; all other $F_s(1, 9) \leq 2.17$,

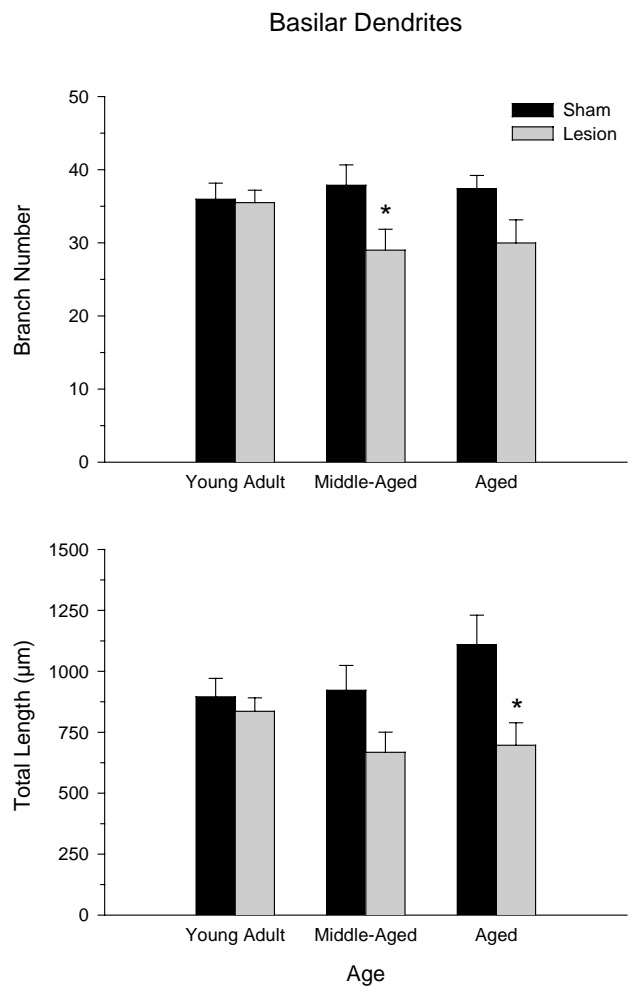


Fig. 6. Mean basilar branch number (top) and length (bottom) ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Vertical bars represent S.E.M. values; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

ns). On the other hand, in aged rats, lesions resulted in a marked reduction in dendritic material, ranging from 22 to 68%, throughout much of the basilar arbor (for radii $\leq 100 \mu\text{m}$, all $F_s(1, 9) \geq 6.69, P_s < 0.05$); at 110–120 μm , a lesion-induced decrease of 76% approached significance ($F(1, 9) = 4.69, P < 0.06$; all other $F_s(1, 9) \leq 3.33$, ns).

The number and length of basilar branch terminals did not vary significantly with age ($F_s(2, 28) = 0.01$ and 1.51, respectively, ns). However, lesions significantly altered both number and length ($F_s(2, 28) = 8.27$ and 12.70, respectively, $P_s < 0.05$), and planned comparisons indicated that this effect varied across ages (Fig. 8). Lesions failed to significantly alter basilar branch number or length in young adult rats ($F_s(1, 10) = 0.46$ and 0.88, respectively, ns). However, in middle-aged rats, lesions significantly reduced the number of basilar terminal branches by 25% ($F(1, 9) = 7.44, P < 0.05$), while a 27% reduction in basilar terminal branch length approached significance ($F(1, 9) = 4.06, P < 0.075$). In aged rats, lesions reduced basilar branch length

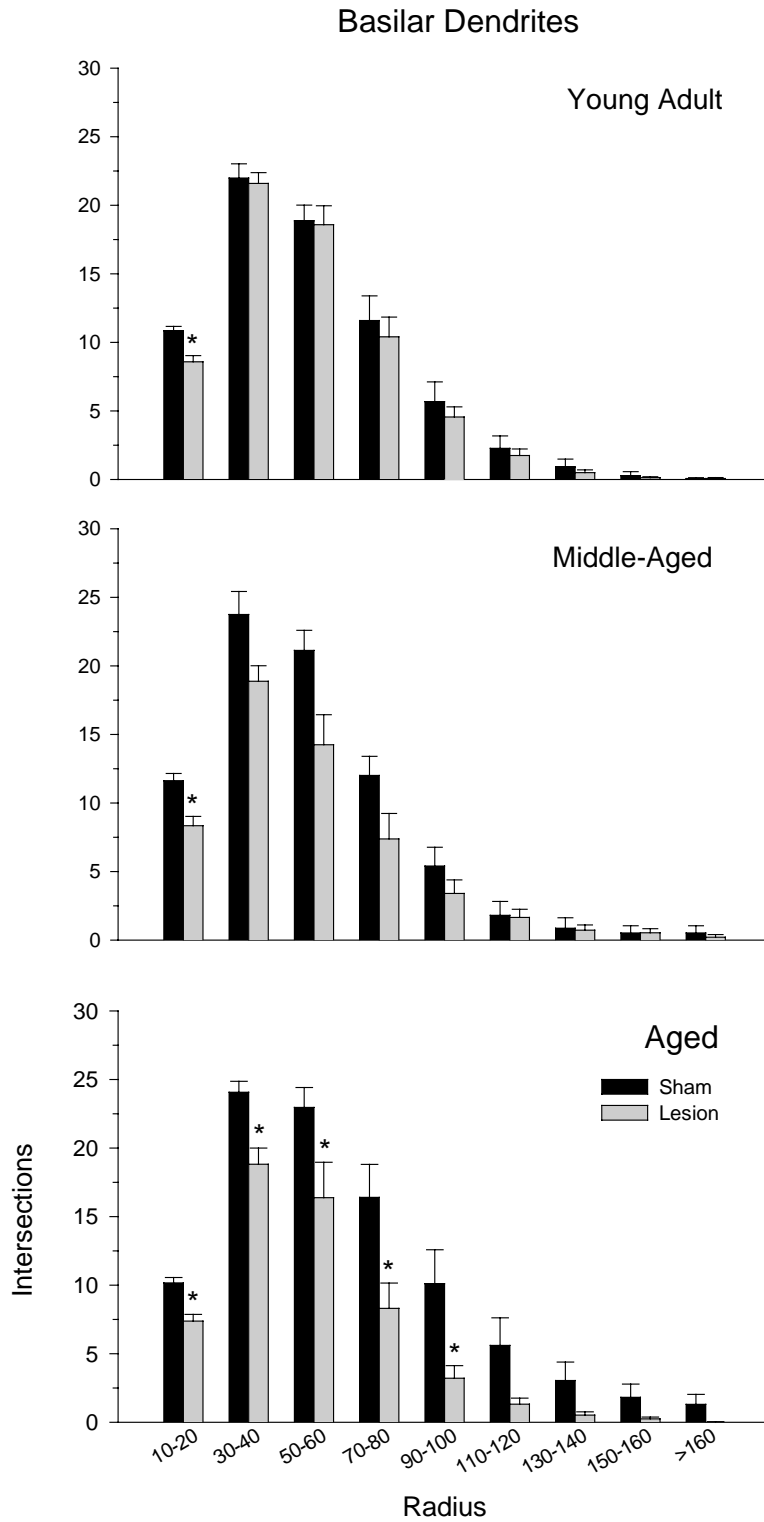


Fig. 7. Mean intersections of basilar dendrites with 10- μ m concentric spheres ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Data have been summed into seven bins. Vertical bars represent S.E.M. values; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

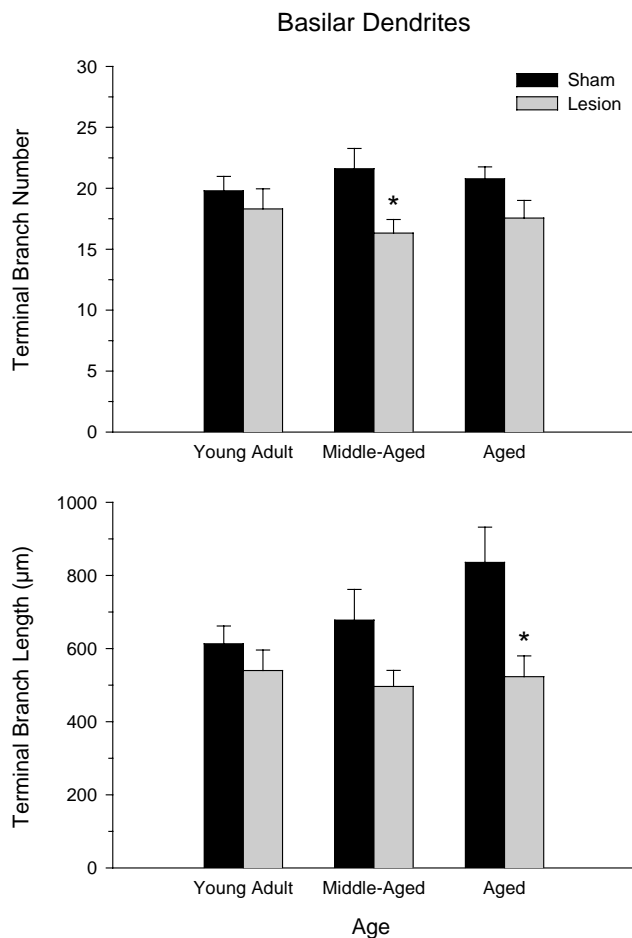


Fig. 8. Mean number (top) and length (bottom) of basilar terminal branches ipsilateral to sham and 192-IgG-saporin lesions of the NBM in young adult, middle-aged and aged rats. Vertical bars represent S.E.M. values; asterisks (*) indicate significant difference relative to sham-lesioned, age-matched rats.

by 37% ($F(1, 9) = 8.49, P < 0.05$), but did not significantly alter terminal branch number ($F(1, 9) = 3.12, ns$).

4. Discussion

The present study describes an interactive effect of age and experimentally-induced cholinergic deafferentation on dendritic morphology of neurons in frontal cortex. Lesion of the NBM with the cholinergic immunotoxin 192-IgG-saporin altered dendritic morphology of layer II–III pyramidal neurons in frontal cortex; however, despite comparable depletion of cortical cholinergic innervation across the three age groups, lesions produced more profound dendritic atrophy in aging rats. Two weeks after lesion of the NBM, young adult rats showed only mild dendritic atrophy, which was restricted to the distal portion of the apical arbor and the proximal portion of the basilar arbor. In middle-aged rats, lesions essentially failed to significantly

alter apical dendrites, but produced a 23% reduction in the number of basilar dendritic branches. This reduction was restricted to the proximal portion of the basilar arbor, and was paralleled by decreases in both the number and length of terminal branches (25 and 27%, respectively). On the other hand, in aged rats, cholinergic lesions dramatically reduced both apical and basilar dendritic material. Aged lesioned rats showed a profound reduction in apical dendritic branch length and number (25 and 32%, respectively), which was distributed throughout the apical arbor and was apparently due to reductions in the number and length of terminal branches (21 and 32%, respectively). Similarly, in aged rats, lesions reduced overall basilar branch length by 37%. This reduction was again distributed throughout the arbor and was paralleled by a 32% reduction in the length of terminal branches. Thus, cholinergic lesions produced more pronounced atrophy of basilar dendrites of pyramidal cells in layers II–III of frontal cortex in both middle-aged and aged rats, and this atrophy was most profound in aged rats.

The present findings complement a growing body of evidence for age-related alterations in neuronal plasticity. For instance, previous studies have demonstrated that aged rats show slower behavioral recovery from traumatic brain injury [16], more pronounced changes in cortical EEG after administration of a noradrenergic neurotoxin [46], reduced axonal sprouting of hippocampal neurons following lesions of entorhinal cortex [52], and more pronounced attenuation of benzodiazepine-induced potentiation of acetylcholine release in frontoparietal cortex after NBM lesions [51]. The age-dependent effect of cholinergic deafferentation on dendritic morphology of frontal cortical neurons that we describe here provides further evidence of age-related changes in plasticity, and lends further support to Sarter and Bruno's [51] argument that an understanding of the interactive effects of aging and pathology may be critical to understanding age-related neurodegenerative disorders.

Interestingly, we found a marked increase in the length of apical dendrites of layers II–III pyramidal neurons in frontal cortex of aged, sham-lesioned rats. This finding is consistent with previous studies documenting age-related increases in dendritic arbors of cortical pyramidal neurons in rats (e.g. [11–13]). This dendritic proliferation may be a compensatory response to the death of other cortical neurons (see [10]), and suggests that cortical neurons retain some degree of plasticity in aged rats. Perhaps the proliferative response to normal age-related changes limits their ability to respond to further insult—in this case, loss of cholinergic input.

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; however, the design of the present study, in which we assessed dendritic changes at only one time point post-lesion, prevents an assessment of the precise nature of this alteration in plasticity. Previous studies have demonstrated that deafferentation results in significant alteration of dendritic morphology of target neurons in spinal cord [54], olfactory bulb [32], and hip-

pocampus [9]. Specifically, after denervation, the dendritic arbors of cortical and subcortical neurons undergo initial regression followed by proliferation [9,54,57]. For instance, whereas the dendritic arbors of hypoglossal neurons in rats were significantly reduced by 4 weeks after deafferentation, a re-expansion of the arbors occurred 5–10 weeks after deafferentation [57]. Similarly, after loss of afferents from entorhinal cortex, pyramidal neurons of the dentate gyrus in rats undergo an initial loss of distal branches, followed by recovery of these branches and a substantial remodeling of their dendritic arbors [9]. Our data are consistent with the hypothesis that neurons ipsilateral to lesions in young adult rats had already advanced to a proliferative phase, whereas neurons in the two older groups continued to demonstrate regression. This suggests that the older groups may have lost the proliferative phase. Alternatively, the time course of cortical neurons' response to cholinergic deafferentation may simply be slower in aging rats. The dendritic changes documented in the present study were assessed 2 weeks post-lesion. It is possible that at this time point, dendrites of cortical neurons in young adults had progressed from the initial reduction to subsequent proliferation, while older animals may have not yet reached the proliferative phase. 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 [1,18,19]. 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 [18]. This delay appears to be due to slower clearing of degenerative material rather than an impairment of synaptic growth [19]. Similarly, while not due to differential degeneration clearance, 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 [1]. Examination of the time course of changes in dendritic arbors of cortical neurons following NBM lesions in aged rats would determine whether neurons fail to respond via proliferation, or if the response is simply slowed.

In addition, the present results are consistent with our previous study [64], in which we found that ibotenic acid 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 3 months after the lesions. The pattern of changes seen in middle-aged and aged rats is also similar to that resulting from ibotenic acid lesions. Previously, we demonstrated that ibotenic acid lesions in middle-aged rats produced the most extensive dendritic atrophy in the proximal portion of the basilar arbor, while lesions in aged rats produced more extensive atrophy in the distal portion of the arbor [64]. In the present study, we found that specific cholinergic lesions in middle-aged rats produced basilar dendritic atrophy relatively close to the soma, while lesion-induced atro-

phy in aged rats was present throughout most of the basilar arbor. Thus, the previously-documented age-dependent lesion-induced dendritic atrophy was likely due specifically to loss of cholinergic input from the NBM.

One observation in the present study is inconsistent with our previous results. Three months after ibotenic acid lesion of the NBM, middle-aged rats demonstrated dendritic atrophy that was more pronounced than that seen in either young adult or aged rats [64]; in the present study, 2 weeks after cholinergic lesions, middle-aged rats demonstrate dendritic atrophy that is intermediate to that seen in younger and older rats. One possible explanation for this discrepancy is time course: perhaps middle-aged rats show a continued atrophy over time, which is not seen in younger or older rats. Alternatively, given the nonspecific nature of ibotenic acid lesion versus 192-IgG-saporin lesion, the more pronounced changes seen previously in middle-aged rats may be due to loss of noncholinergic inputs. A study assessing dendritic changes with longer survival periods following 192-IgG-saporin lesions could discriminate between these possibilities.

Regardless of whether the middle-aged rats show a slower time course of dendritic changes or a unique response to loss of noncholinergic innervation, the possibility of a unique pattern of altered plasticity in middle-aged rats is consistent with previous work from our lab showing nonlinear age-related changes in frontal cortex of middle-aged rats. For instance, both middle-aged and aged rats showed increased binding of desmethylimipramine to noradrenergic reuptake sites in frontal cortex, whereas M₁ muscarinic receptor binding in frontal cortex was decreased in aged rats only. Thus, middle-aged rats appear to exhibit a transient imbalance in the noradrenergic and cholinergic systems in frontal cortex [62]. Given the important roles these systems play in cortical plasticity [4,23,27,36,60], an imbalance in middle-aged rats may result in a unique pattern of cortical plasticity.

Dendrites are a major site of synaptic connectivity, with adult cortical neurons receiving approximately 15,000 synaptic inputs [21]. In the present study, we assessed alterations in dendritic morphology, but did not directly examine lesion-induced changes in synaptic connectivity. Nonetheless, given that the geometry of the dendritic arbor (e.g. dendritic branching patterns, distribution, and overall shape) determines many functional properties of neurons (e.g. [15,25,30,31,44]), the more pronounced lesion-induced dendritic changes we have documented in older rats likely result in important functional changes in these neurons and therefore in frontal cortex. Indeed, this is consistent with data showing more pronounced NBM lesion-induced impairment in performance of radial maze [62] and attentional tasks [8], both of which are mediated by frontal and prefrontal cortex [5,26,28,34,38], in aged rats.

Finally, acetylcholine supplied by the NBM acts as a neuromodulator at glutamatergic synapses in the neocortex, increasing glutamate-evoked excitation when iontophoretically applied (e.g. [35,58]). The present results suggest

that acetylcholine from the NBM is involved in the maintenance of cortical dendritic arbor, perhaps through its modulatory effects on glutamate. Glutamate is the major excitatory neurotransmitter in neocortex [43], 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 [33,67]. Indeed, preliminary data from our laboratory suggests that in young adult rats, specific cholinergic lesions result in a large increase in expression of the GluR1 subunit of the AMPA receptor, and that this increase is absent in aging rats [24]. Thus, the age-related alteration we have found in dendritic plasticity of frontal cortical neurons may be mediated by changes in glutamatergic transmission at AMPA receptors.

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