Maze Learning and Morphology of Frontal Cortex in Adult and Aged Basal Forebrain-Lesioned Rats

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Maze performance and morphology of frontal cortex were assessed in young adult, middle-aged, and aged rats with and without lesions of the nucleus basalis magnocellularis. Although maze performance did not vary with age, neuron number and the thickness of superficial laminae were reduced in aged rats. Lamina II-III neurons were hypertrophied in middle-aged rats relative to both younger and older groups. At all ages, lesions significantly impaired maze performance. In young adult rats, lesions moderately reduced the size of lamina II-III neurons. This effect was more pronounced in middle-aged rats. Lesions in aged rats did not affect neuron size. The neuronal changes seen in middle-aged rats may reflect a compensatory response to the expression of other age-related neural changes, which may affect the ability of cortical neurons to respond to lesion-induced loss of cholinergic input.

The majority of acetylcholine found in the neocortex is extrinsic in origin, and the bulk of this extrinsic supply arises in the nucleus basalis magnocellularis (NBM; e.g., Divac, 1975; Johnston, McKinney, & Coyle, 1981; Mesulam & Van Hoesen, 1976). Frontoparietal cortical areas receive specific and direct projections from the NBM (Bigl, Woolf, & Butcher, 1982; Johnston et al., 1981). Furthermore, the proportion of cholinergic innervation supplied by the NBM varies within frontoparietal cortex: Whereas frontal cortex receives essentially all of its cholinergic innervation from the NBM, parietal cortex receives substantial cholinergic projections from the lateral hypothalamus and lateral preoptic area as well (Bigl et al., 1982; Ichikawa & Hirata, 1986; Luiten, Spencer, Traber, & Gaykema, 1985; Woolf, Eckenstein, & Butcher, 1983; Woolf, Hermit, & Butcher, 1986). Acetylcholine supplied by the NBM modulates neocortical function (Kuroswa, Sato, & Sato, 1989; Rasmusson & Dykes, 1988; Tremblay, Warren, & Dykes, 1990a, 1990b; Webster et al., 1991), and changes in spontaneous NBM activity are correlated with changes in cortical electroencephalogram (EEG) and behavior (Buzsaki et al., 1988; Detari & Vanderwolf, 1987; Ray & Jackson, 1991; Steward, MacFabe, & Vanderwolf, 1984).

Consistent with the fundamental role that the NBM and its cholinergic projections appear to play in cortical function, lesions of the nucleus result in marked changes in a variety of behavioral variables. For instance, lesions of the NBM temporarily disrupt spontaneous behaviors such as locomotion, exploration, feeding, and hoarding (Dubois, Mayo, Agid, Le Moal, & Simon, 1985; Whishaw, O'Connor, & Dunnett, 1985). In addition, NBM lesions result in impaired performance in learning and memory tasks. Lesions of rat NBM impaired learning and retention of active and passive avoidance responses (Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Miyamoto, Shintani, Nagaoka, & Nagawa, 1985), acquisition of spatial learning tasks (e.g., Kesner, Crutch, & Measom, 1986; Markowska, Wenk, & Olton, 1990; Wozniak, Stewart, Finger, & Olney, 1989), and memory for the order of spatial locations (Kesner et al., 1986). Lesions of the NBM have also produced deficits in a variety of attentional tasks, including multiple-choice serial reaction-time tasks, two-choice reaction-time tasks, and divided-attention tasks (e.g., Muir, Dunnett, Robbins, & Everitt, 1992; Olton, Wenk, Church, & Meck, 1988; Pang, Williams, Egeth, & Olton, 1993; Robbins et al., 1989). Thus, the behavioral deficits induced by NBM lesions may be mediated by attention. Indeed, frontal cortex, the primary projection target of the NBM, plays a major role in attention (e.g., Kolb, 1984; Olton et al., 1988). Thus, NBM lesions may exert their behavioral effect through frontal cortex.

Lesions of the NBM produce morphological changes in both frontal and parietal cortex. In neonatal mice, NBM lesions disrupted morphogenesis of frontoparietal cortex, delayed differentiation of cortical laminae, and temporarily reduced cortical cholinergic markers (Hohmann, Brooks, & Coyle, 1988). Sixteen to 18 months after lesion of the NBM, remaining acetylcholinesterase (AChE)- and choline acetyltransferase (ChAT)-containing cortical fibers in aged rats showed abnormalities, including hypertrophied terminals and clusters of swollen varicosities (Gaykema, Gaal, Traber, Hersh, & Luiten, 1991). Lesions of the NBM altered dendritic arborization of pyramidal cells in frontoparietal cortex of both neonatal (Stearn, Mervis, Dvorak, & Arendash, 1989) and aged rats (Mervis, Bedo-Wierdl, Dvorak, & Arendash, 1989). Previously, Wellman and Sengelaub (1991) assessed cellular changes in the frontoparietal cortex of rats that exhibited deficits on a go/no-go task following lesion of the basal forebrain. These behavioral deficits were accompanied by gross morphological changes in neurons in frontoparietal cortex, including reduced soma size in particular cortical laminae, which resulted in decreased laminar thickness. These effects were present in all
the cortical areas examined and most pronounced in lamina II–III.

Normal aging results in a variety of functional and morphological changes in the frontal cortex and NBM of rats, including decreased conduction velocity in NBM fibers projecting to frontal cortex (Aston-Jones, Rogers, Shaver, Dinan, & Moss, 1985) and reductions in NBM neuron numbers and size (Fischer, Chen, Gage, & Bjorklund, 1991; Fischer, Gage, & Bjorklund, 1989). In addition, acetylcholine release is substantially reduced in frontal cortex of aged rats (Wu, Bertorelli, Sacconi, Pepeu, & Consolo, 1988). Recent studies have demonstrated age-related changes in frontal cortical EEG (Riekkinen, Riekkinen, Sirvio, Miettinen, & Riekkinen, 1992) and glial density (Peinado, Martinez, Pedrosa, Quesada, & Peinado, 1993).

These age-related changes in the NBM and frontal cortex may be related to cognitive deficits that occur in aged rats (e.g., Winocur & Moscovich, 1990). For instance, regressive changes in the basal forebrain of aged rats were significantly correlated with performance in the Morris water-maze task (Fischer et al., 1989; Fischer, Chen, et al., 1991). Furthermore, in aged rats with impaired retention of a passive avoidance response, the number of ChAT-positive NBM neurons was significantly reduced relative to both unimpaired aged and young adult rats (Riekkinen, Miettinen, Sirvio, Aaltonen, & Riekkinen, 1990). This age-related neuronal loss was significantly correlated with passive avoidance performance (Riekkinen et al., 1992).

Regressive changes in the NBM that occur with aging and with lesion may exert their effect on behavior through the frontal cortex, its major efferent projection. We hypothesized that age-related morphological changes in frontal cortex are related to deficits in learning and memory that occur in aged rats, as are NBM lesion-induced cortical changes, and that aging and NBM lesions in combination produce unique behavioral and morphological changes. To test these hypotheses, we examined the relationship between morphology of frontal cortex and changes in radial maze performance associated with normal aging, as well as the interactive effects of aging and lesions of the NBM on these variables.

The behavioral task used in the present study has previously been shown to be sensitive to alterations in frontal cortical function (e.g., Kolb, Pittman, Sutherland, & Whishaw, 1982; Kolb, Sutherland, & Whishaw, 1983). In this task, four arms of an eight-arm radial maze are baited. The rat’s task is to collect the food in the baited arms. Unimpaired young adult rats will quickly learn to visit the baited arms first and not return to arms previously visited within a trial (Kolb et al., 1982). Using this paradigm, one can assess both reference and working memory; whereas their ability to remember within a trial which arms they have visited and to visit each arm only once reflects short-term, or working, memory.

Despite comparable behavioral impairment, cholinergic denervation produced differential neuronal changes in frontal cortex across ages, with middle-aged rats being most profoundly affected. The neuronal changes seen in middle-aged rats may reflect a compensatory response to expression of other age-related neural changes, which may affect the ability of cortical neurons to respond to lesion-induced loss of cholinergic input.

Method

Behavioral Analysis

Subjects

Behavior was assessed in young adult, middle-aged, and aged male albino rats (Fischer 344; Harlan Laboratories, Indianapolis, IN) that received either excitotoxic or sham lesions of the NBM. Rats were 6 (n = 16), 13 (n = 20), and 21 (n = 21) months old at the beginning of the experiment. These ages were chosen to bracket a large portion of the adult life span of the Fischer 344 rat and represent ages both below and approaching the median mortality of this strain (Chesky & Rockstein, 1975); as the rats aged during the course of the experiment, the groups represented ages below and at median mortality. We will refer to these three age groups as 9-, 16-, and 24-month-old rats, which were their ages at the conclusion of the study.

To reduce the impact of possible cohort effects (see Coleman & Flood, 1987), rats were lesioned, tested, and submitted to morphological analyses in cohorts of 6–12 rats, with approximately equal numbers in each experimental condition, over a period of 12 to 18 months. Throughout the experiment, rats were housed in cages equipped with filter tops.

Surgery

Rats were anesthetized with chloropent, with the dose varying across ages (9-month-old rats, 0.3 ml/100 g ip; 13-month-old rats, 0.255 ml/100 g ip; 24-month-old rats, 0.225 ml/100 g ip). Pilot studies showed that these doses adequately anesthetized rats while decreasing mortality in older rats due to anesthetic overdose. Half of the rats in each age group received bilateral excitotoxic acid lesions of the NBM. They were placed in a stereotaxic instrument (Kopf); the incisor bar was set so that bregma and lambda were in the same horizontal plane. The scalp was incised and retracted, holes were drilled, and bilateral excitotoxic lesions were 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, 1982). A cannula attached to a Hamilton microsyringe was lowered to the appropriate stereotaxic coordinates and left in place for 2 min prior to injection. Ibotenic acid (0.6 µl, 5 µg/µl) was then pressure-injected in 0.1-µl steps at 1-min intervals, and the cannula was slowly withdrawn 5 min after the final injection. The other half of the rats served as controls. They received the surgical procedures described above but did not receive the ibotenic acid injections. Instead, the cannula was lowered to the appropriate stereotaxic coordinates and withdrawn after 5 min. Following surgery, rats that received ibotenic acid lesions were fed a liquid tube-feeding formula (Isocal; Mead-Johnson Nutritional, Evansville, IN) by gavage three times per day for 72 hr. In addition, they received injections of penicillin (0.1 ml im) once a day during this same period.

Behavioral Testing

To assess the effects of aging and NBM lesions on learning and memory, rats were trained in a radial maze task that previously has been shown to be sensitive to alterations in frontal cortical function (e.g., Kolb et al., 1982).

Apparatus. The maze consisted of a central platform (33 cm in diameter) with eight radial arms (each 39 x 12 cm), each of which was equipped with texturally unique flooring (e.g., rubber matting, indoor-
outdoor carpet, tile, linoleum) to provide distinct intramaze stimuli (see Bartus et al., 1985). Furthermore, extramaze stimuli such as a chalkboard and large posterboards were present in the room. Peanuts were used as bait and placed in small metal cups at the end of each arm.

Procedure. To motivate animals on the task, the 9-month-old rats' weight was gradually reduced to 85% of their free-feeding weight, whereas the 16- and 24-month-old rats' weight was reduced to 80% of their free-feeding weight (see Goodrick, 1972; Van Der Staay, van Nies. & Raaijmakers, 1990). After reaching this weight, all rats received three peanuts in their cages on two consecutive days to familiarize them with the bait.

Subsequently, rats were habituated to the maze. The maze was cleaned with a deodorizer, and ¼ peanut was placed in each bait cup and at the juncture of each arm with the central platform. Each rat was then placed on the maze and allowed to move freely for 12 min. Each rat performed at least two habituation trials, and habituation trials continued until the rat made at least eight choices within a trial.

After rats reached criterion for habituation, learning trials began. For each rat, four randomly chosen arms were baited with ½ peanut. The location of the four baited arms remained constant throughout training. Each rat was placed on the maze and permitted to choose among the arms until either it completed a trial (by entering eight arms) or 10 min had elapsed. All rats were given one trial per day until four of their first five visits were to the baited arms on four of five consecutive trials. The length of each trial and arms visited during the trial were recorded for each rat. These measures were used to compute average run time (time between first and last visit divided by the number of visits), reference memory errors (number of visits to unbaited arms), working memory errors (number of visits to previously chosen arms), and trials to criterion. Each of these measures was compared across the six groups with a two-way analysis of variance (ANOVA; Age x Treatment). In addition, working and reference memory errors were compared across trials with three-way repeated-measures ANOVAs (Age x Treatment x Trials). All measures are reported as means with standard errors.

Morphometric Analysis

Subjects

Following behavioral testing, AChE staining and cortical morphology were examined. Rats were killed 3 months after initiation of the experiment.

Histology

Animals were overdosed with urethane and then transcardially perfused with saline and 10% buffered formalin solution. Brains were removed, postfixed, and then cryoprotected with a solution of 30% sucrose (wt/vol) in 10% formalin (vol/vol). Frozen sections were taken consecutively beginning at either 20 or 40 μm, and every second section was collected starting just anterior to the genu of the corpus callosum through the superior colliculus. Two series of sections were mounted and either stained with cresylecht violet or processed for AChE staining according to a modification of the Karnovsky–Roots method (Hedreen, Bacon, & Price, 1985).

To estimate the efficacy of NBM lesions, intensity of AChE staining in frontal cortex was measured with a computerized image analysis system (MCID; Imaging Research Inc., St. Catherines, Ontario). Relative optical density was measured in 5 sections spaced 80 μm apart per rat beginning just anterior to the genu of the corpus callosum and ending at the medial septum. Frontal cortex was identified according to the characteristic staining and laminar organization of this area in adjacent cresylecht violet-stained sections, and relative optical density averaged across all of the Frl area (nomenclature of Zilles & Wree, 1985; see the next section) contained within each section was measured. To control for possible artifactual differences in staining across rats, for each rat, optical density in frontal cortex was expressed relative to that in the superior colliculus. The AChE staining of the superior colliculus is quite intense and, because it neither directly innervates nor receives direct projections from the NBM, is unaffected by lesions of this structure (Johnston et al., 1981). Furthermore, preliminary analyses indicated that the intensity of AChE staining in superior colliculus did not differ across the three age groups. F(2, 12) = 2.02, ns. Average intensity was then compared across the six groups using a two-way ANOVA (Age x Treatment).

Measures of Cortical Morphology

Frl (nomenclature of Zilles & Wree, 1985), or primary motor cortex, was chosen for quantitative morphological analysis. This area was chosen because it receives projections from the NBM (e.g., Divac, 1975), shows reduced cholinergic activity following basal forebrain lesions that include NBM and ventral globus pallidus (Johnston et al., 1981; Mesulam & Van Hoesen, 1976), and because its borders and laminae are readily identifiable in Nissl-stained material. In addition, a subset of morphological measures was gathered from Oc1M, or the medial portion of primary visual cortex. Occipital cortex receives minimal cholinergic projections from the NBM (Bigl et al., 1982; Johnston et al., 1981) and thus served as an internal control to discriminate between neocortical effects specific to loss of cholinergic projections and other, nonspecific effects of lesion and aging. As in previous studies, identification of these areas and their laminar organization followed that of Zilles (1985). All measures of cortical morphology were obtained from cresylecht violet-stained sections.

Cortical and laminar thickness. For each rat, Frl was identified using its characteristic laminar structure and staining. Nine sections matched for their positions along the anterior–posterior axis of the brain were chosen, and the cortical area and laminar boundaries drawn with a projection microscope at a final magnification of 32×. Laminar boundaries were identified with standard morphological criteria of neuronal cell type and packing density (see Zilles, 1985; Zilles & Wree, 1985). For instance, although Frl can be distinguished from somatosensory cortex by the relative absence of a prominent, densely packed granular layer IV, a small, less densely packed lamina IV can nevertheless be identified in this area. The thickness of each lamina and total cortical thickness normal to the surface of the brain were measured from the drawings with a digitizing tablet and computer-morphometry system (Sigmascan; Jandel, San Rafael, CA). Cortical thickness was compared in young adult, middle-aged, and aged lesioned and sham-lesioned rats with a two-way ANOVA (Age x Treatment). To determine whether changes in cortical thickness were restricted to certain laminae, average laminar thickness was compared in young adult, middle-aged, and aged lesioned and sham rats using a three-way ANOVA (Age x Treatment x Lamina).

Soma area. For each lamina, the somata of 25–35 randomly selected neurons were measured in sections matched for position along the anterior–posterior axis of the brain. Cells were identified as neurons using the standard morphological criteria of nonhomogeneous staining, multipolar cell body, and the presence of Nissl substance and a well-defined nucleus. Small, oval cells with granular staining and lacking Nissl substance were considered glia and therefore were excluded. All somata were drawn at a magnification of 750× with a camera lucida. Cross-sectional areas of somata were then measured from these drawings with a digitizing tablet and computer-morphometry system (Sigmascan; Jandel, San Rafael, CA). Average soma area was computed for each lamina and compared with a three-way ANOVA (Age x Treatment x Lamina).
Neuron number. Changes in laminar and cortical thickness may result from changes in neuron number. An unbiased stereological technique (e.g., Coggshall, 1992; West, 1993; West & Gundersen, 1990) was used to obtain estimates of total neuron numbers in each lamina of frontal cortex. The numerical densities of neurons in each lamina were obtained with an optical dissector procedure similar to that of Srivastava et al. (Srivastava, Brouillet, Beal, Storey, & Hyman, 1993). Shrinkage of sections due to histological procedures was approximately 3.7%, and thus the length of the dissector was either 19.3 μm (in 20-μm sections) or 38.5 μm (in 40-μm sections). Both lengths were adequate for visualizing neurons in multiple focal planes. Six sections per rat were sampled in a systematically random fashion. That is, the location along the anterior–posterior axis of the brain of the initial section was randomly selected, and neuronal cell bodies in this section and every second subsequent section were then counted. Counts were made in each lamina in each section with an 85 × 85 μm grid and an unbiased counting frame (i.e., neuronal somata touching the upper and right edge of the grid were not counted). All counts were made at a final magnification of 937.5× with a custom computer-assisted cell counting system that provides both quantitative and spatial data collection. Cells were identified as neurons by the standard morphological criteria described above. Neurons in the first focal plane (i.e., “tops”) were not counted. A total of 60–120 neurons in each lamina of each animal were sampled. Average sampling error was 10% ± 0.38.

The volume of each lamina was estimated with a digitizing tablet and computer-based morphometry system (SigmaScan; Jandel, San Rafael, CA). Individual laminae were drawn with a projection microscope (32× magnification). The area of each lamina was measured in either 11 sections spaced at 400-μm intervals (for sections cut at 20 μm) or 16 sections spaced at 320-μm intervals (for sections cut at 40 μm). Volumes were estimated by multiplying the sums of these areas by the thickness of the sections and correcting for the sampling ratio. Finally, estimates of total neuron numbers in each lamina were obtained by multiplying the volume of each lamina by the numerical density of neurons in that lamina. These estimates were compared across groups with a three-way ANOVA (Age × Treatment × Lamina).

Results

Behavioral Analysis

Mortality due to surgery was 29%. In addition, rats that did not reach habituation criterion (n = 8) were excluded from behavioral testing. Four additional rats were excluded from analysis because of improper lesion placement (see below for details on histological verification of lesions). Thus, 5 sham and 7 lesioned young adult rats, 6 sham and 6 lesioned middle-aged rats, and 6 sham and 5 lesioned aged rats were included in the behavioral analyses.

Age Effects

To assess whether performance variables changed with age, the average number of arms visited per trial and average run time were compared in young adult, middle-aged, and aged rats. Average choices per trial were similar across groups: young adult sham-lesioned rats = 7.75 ± 0.11, middle-aged sham-lesioned rats = 7.45 ± 0.27, and aged sham-lesioned rats = 7.84 ± 0.15; F(2, 29) = 0.908, ns. Likewise, average run time did not vary significantly with age, F(2, 29) = 0.505, ns. On average, within a given trial, young adult rats spent 0.74 ± 0.13 min on each arm, middle-aged rats spent 0.85 ± 0.21 min on each arm, and aged rats spent 0.62 ± 0.13 min on each arm. Thus, performance variables were comparable across age groups.

To assess possible age-related changes in acquisition, average trials to criterion, reference-memory errors, and working-memory errors were compared in the sham-lesioned animals. Results of a two-way ANOVA revealed no significant difference across ages in the number of trials required to reach criterion, F(2, 29) = 0.355, ns. Young adult rats required 12.80 ± 2.29 trials to criterion; middle-aged rats required 14.33 ± 2.33 trials to criterion; aged rats required 11.67 ± 1.63 trials to criterion. Similarly, average reference-memory errors did not vary significantly with age: Across trials, young adult rats made about 13% of total possible reference-memory errors, whereas middle-aged and aged rats made about 15% and 12% of total possible errors, respectively, F(2, 29) = 0.242, ns. Finally, working-memory errors were also comparable across ages, with young adult, middle-aged and aged sham-lesioned rats making 20–22% of total possible working-memory errors, F(2, 29) = 0.237, ns. Thus, at all ages, sham-lesioned rats rapidly learned the location of the baited arms and tended to revisit only one or two previously visited arms within a trial.

Lesion Effects

NBM lesions significantly impaired radial maze performance at all ages tested. However, lesions did not affect the performance variables assessed, suggesting that deficits were due to impaired acquisition. Two-way ANOVAs revealed no significant difference between lesioned and sham-lesioned rats for either number of choices per trial, F(1, 29) = 1.05, ns, or average run time, F(1, 29) = 1.85, ns. Furthermore, no significant interaction between lesion and age was present; for average choices, F(2, 29) = 2.07, ns; for average run time, F(2, 29) = 0.88, ns.

Whereas NBM lesions did not affect performance variables, they did affect acquisition-related variables (see Figure 1). A two-way ANOVA revealed a significant lesion effect on trials to criterion, F(1, 29) = 10.20, p < .05, which was consistent across ages; the interaction of age and treatment, F(2, 29) = 1.05, was not significant. Thus, at each age, lesioned rats required approximately 55% more trials to reach criterion than did sham-lesioned rats (M = 12.94 ± 1.17 for sham-lesioned rats vs. M = 20.11 ± 1.70 for lesioned rats). This impairment was apparently due to an increase in reference-memory errors. A two-way repeated-measures ANOVA on this variable indicated a significant difference between lesioned and sham-lesioned rats, F(1, 29) = 12.07, p < .05. Again, this effect did not vary with age, F(2, 29) = 0.48, ns. Across trials, errors for young adult sham-lesioned rats averaged 13%, versus 27% for lesioned rats; for middle-aged rats, the errors averaged 15% versus 24%, respectively; and for aged rats, the errors averaged 12% versus 21%, respectively. On the other hand, working-memory errors were unaffected by lesions, F(2, 29) = 1.97, ns, with all groups making an average of 20% to 23% of total possible working-memory errors.
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Figure 1. Mean trials to criterion, reference-memory errors, and working-memory errors in 9-, 16-, and 24-month-old sham- and ibotenic-acid-lesioned rats. Vertical bars represent SEMs. Asterisk indicates significantly different from sham (p < .05).

Again, no significant interaction was present, $F(2, 29) = 0.03$, $ns$. Thus, at all ages examined, NBM lesions significantly impaired acquisition of this radial maze task due to a reference-memory deficit, but did not affect working memory.

Morphometric Analysis

All rats with adequate lesions, including those that did not reach habituation criterion in Experiment 1, were used in the morphological analyses. Thus, morphological and histochemical measures were assessed in 9- (7 sham lesioned and 7 lesioned), 16- (7 sham lesioned and 7 lesioned), and 24-month-old rats (8 sham lesioned and 6 lesioned).

Lesion Reconstruction and AChE Staining

Histological examination of the lesioned rats revealed the presence of well-defined bilateral lesions in the basal forebrain in all but 4 rats, which were therefore excluded from all analyses. In all remaining cases ($n = 20$), lesions involved the NBM, the ventral portion of the globus pallidus, and portions of the ventral pallidum and substantia innominata. In four cases, the lesions extended ventromedially into portions of the horizontal limb of the diagonal band of Broca. In two cases the lesions also extended posteriorly into the anterior aspect of the ventrolateral thalamic nucleus (see Figure 2). In some cases, a small number of magnocellular neurons remained in either the extreme anterior or extreme posterior portion of the NBM region. Additionally, collections of "translucent spherical bod-

Figure 2. Reconstruction of bilateral ibotenic acid lesion of the nucleus basalis magnocellularis in a representative animal. Lesion is shown in black.
ies" were observed in the globus pallidus of NBM-lesioned rats. These are typically found in NBM-lesioned rats with long postsurgical survival times and have been shown to be a form of calcification (Wozniak et al., 1989).

Lesions significantly reduced AChE staining in frontal cortex by about 39%, $F(1,28) = 29.91, p < .05$ (see Figure 3), and this effect was uniform across the three age groups; for interaction of age and lesion, $F(2, 28) = 0.39, ns$. Planned comparisons indicated that the difference in staining intensity was significant at all ages, all $F$s(1, 36) $\geq$ 5.81, $p < .05$. Preliminary analyses of the correlation between ChAT activity and AChE staining in frontal cortex following NBM lesions indicate that this reduction in AChE staining corresponds to a 47% reduction in ChAT activity (Wellman & Pelleymounter, 1994). Thus, these lesions produced a loss of cholinergic innervation comparable to that seen in previous studies (e.g., Moret, Pastrie, & Briley, 1989; Watson, Vickroy, Fibiger, Roeseke, & Yamamura, 1985).

Cortical and Laminar Thickness

To test whether cortical thickness or thickness of individual laminae changed with either age or lesion, we compared the average total thickness as well as the average thickness of each lamina of frontal cortex in young adult, middle-aged, and aged sham and lesional rats.

Age effects. Although overall thickness of frontal cortex did not vary with age, $F(2, 36) = 0.07, ns$, the thickness of specific laminae varied significantly across ages, $F(8, 36) = 5.08, p < .05$; see Figure 4. The average thickness of lamina I in aged sham-lesioned rats was decreased about 22% relative to both 9- and 16-month-old sham-lesioned rats, $F$s(1, 36) $> 10.35, p < .05$, reflecting a decrease from $M =$ 161.44 ± 12.50 µm and $M =$ 167.41 ± 9.65 µm (9 and 16 months, respectively) to $M =$ 127.39 ± 12.68 µm (24 months). Likewise, average thickness of lamina II-III decreased 27% in 24-month-old rats relative to the two younger groups, $F$s(1, 36) $> 11.92, p < .05$. Lamina II-III decreased from $M =$ 325.00 ± 16.29 µm and $M =$ 345.72 ± 20.63 µm at 9 and 16 months to $M =$ 243.48 ± 16.40 µm at 24 months. Alternatively, both laminae V and VI exhibited a slight but significant increase in thickness in 24-month-old rats compared to 9- and 16-month-old rats. Lamina V was 6-7% thicker in 24-month-old rats, $F$s(1, 36) $> 4.23, p < .05$; $M =$ 518.90 ± 8.10 µm and $M =$ 510.67 ± 8.84 µm, 9 and 16 months, versus $M =$ 548.42 ± 14.35, 24 months. Similarly, lamina VI averaged 10% thicker in 24-month-old rats, $F$s(1, 36) $> 11.57, p < .05$; $M =$ 630.86 ± 17.54 µm and 645.29 ± 16.70 µm, 9 and 16 months, versus $M =$ 701.70 ± 19.60, 24 months. The thickness of lamina IV did not vary significantly across the three age groups, $F$s(1, 36) $< 1.35, ns$.

Thickness of occipital cortex also varied with age, $F(2, 36) = 3.83, p < .05$, and this effect was not specific to particular laminae; interaction of age and lamina, $F(8, 36) = 1.44, ns$. Whereas average cortical thickness did not differ significantly in 9- and 16-month-old sham-lesioned rats—for 9-month-old rats, $M =$ 1625.80 ± 84.06 µm; for 16-month-old rats, $M =$ 1787.95 ± 96.54 µm; $F(1, 36) = 0.82, ns$—average cortical thickness decreased 19% (to $M =$ 1440.87 ± 69.92 µm) from 16 to 24 months of age. Planned comparisons showed that this difference was significant, $F(1, 36) = 9.45, p < .05$.

Lesion effects. Overall, lesions did not affect cortical thickness at any age, $F(1, 36) = 1.49, ns$. However, lesions affected the thickness of certain laminae, $F(4, 36) = 5.69, p < .05$, and this effect varied across ages, $F(8, 36) = 4.61, p < .05$; see Figure 5. In young adult rats, lesions did not significantly affect the thickness of any lamina, $F$s(1, 36) $< 0.40, ns$. In contrast, in middle-aged rats, lesions produced a small but significant decrease (9%) in the thickness of lamina IV only; for lamina IV, $F(1, 36) = 5.22, p < .05$; all other $F$s(1, 36) $< 2.51, ns$. For sham-lesioned middle-aged rats, the average thickness of lamina IV was 231.08 ± 8.84 µm, versus 210.73 ± 7.96 µm for lesioned rats.

Aged rats exhibited a different pattern of changes after NBM lesions. Both laminae I and II-III were significantly thicker (30% and 27%, respectively) in lesioned aged rats compared to their sham-lesioned counterparts, $F$s(1, 36) $> 8.81$ and 11.61, respectively, $p < .05$. The average thickness of lamina I increased from $M =$ 125.97 ± 5.38 to $M =$ 163.67 ± 4.93, whereas the average thickness of lamina II-III increased from $M =$ 299.04 ± 10.15 µm to $M =$ 381.31 ± 15.39 µm. On the other hand, lamina VI was 12% thinner in lesioned aged rats relative to sham-lesioned rats, $F(1, 36) = 17.93, p < .05$; for sham-lesioned rats, $M =$ 701.70 ± 19.60 versus $M =$

![Figure 3 (opposite). Top: Photomicrographs of acetylcholinesterase (AChE)-stained fibers in frontoparietal cortex of a sham- (left) and an ibotenic-acid-lesioned (right) rat. Reduction in staining is apparent. Scale bar = 750 µm. Bottom: Mean intensity of AChE staining in 9-, 16-, and 24-month-old sham- and ibotenic-acid-lesioned rats. Vertical bars represent SEMs. Asterisks indicate significantly different from sham ($p < .05$).](image)

![Figure 4. Mean laminar thickness in frontal cortex of 9-, 16-, and 24-month-old sham-lesioned rats. Vertical bars represent SEMs. Asterisks indicate significantly different from 9- and 16-month-old rats ($p < .05$).](image)
628.43 ± 19.10 for lesioned rats. Laminae IV and V were unaffected by NBM lesions, F(1, 36) < 2.25, ns.

In occipital cortex, NBM lesion did not significantly alter laminar thickness at any age, F(1, 36) = 3.17, ns.

Soma Area

Changes in laminar thickness due to aging and lesion may have resulted from changes in the size of cortical neurons. To test this hypothesis, we compared the average cross-sectional area of neuronal somata in each lamina of frontal cortex in young adult, middle-aged, and aged sham-lesioned and lesioned rats (see Figure 6).

Age effects. A three-way ANOVA showed that cross-sectional soma area changed significantly with age, F(2, 36) = 6.73, p < .05, and that the effect was specific to certain laminae; interaction of age and lamina, F(6, 36) = 2.64, p < .05; see Figure 7. The cross-sectional area of lamina II–III somata increased about 24% from 9 to 16 months of age (from $M = 163.22 ± 8.34$ to $M = 202.01 ± 7.03$), followed by a 19% decrease from 16 to 24 months of age (from $M = 202.01 ± 7.03$ to $M = 163.70 ± 6.24$) for all F(1, 36) > 10.76, p < .05.

Although similar trends were present in occipital cortex, they were not significant; main effect of age, F(2, 23) = 2.47, ns; interaction of age and lamina, F(6, 23) = 1.22, ns. Overall, mean cross-sectional soma area was 152.48 ± 9.29 μm² for 9-month-old sham-lesioned rats, 161.24 ± 13.58 μm² for 16-month-old sham-lesioned rats, and 151.55 ± 5.69 μm² for 24-month-old sham-lesioned rats.

Lesion effects. A three-way ANOVA revealed a significant lesion effect, F(1, 36) = 22.32, p < .05. The magnitude of these effects varied across ages (see Figure 8). In 9-month-old rats, lesions reduced neuronal soma area in lamina II–III by 15% (for sham-lesioned rats, $M = 163.22 ± 8.34$; for lesioned rats, $M = 139.34 ± 11.87$) and in laminae IV and V by 19% (in lamina IV, $M = 235.41 ± 11.41$ and $M = 189.93 ± 7.34$ for sham and lesioned rats, respectively; in lamina V, $M = 135.17 ± 13.24$ and $M = 109.13 ± 4.39$ for sham and lesioned rats, respectively). Planned comparisons indicated these differences were significant, F(1, 36) > 4.08, p < .05. Likewise, in 16-month-old rats, lesions resulted in a 27% decrease in neuronal soma size in lamina II–III (for sham-lesioned rats, $M = 202.01 ± 7.03$; for lesioned rats, $M = 148.45 ± 9.98$), a 14% decrease in lamina IV (for sham-lesioned rats, $M = 129.74 ± 5.35$; for lesioned rats, $M = 112.09 ± 6.78$), and a 16% decrease in lamina V (for sham-lesioned rats, $M = 235.84 ± 10.68$; for lesioned rats, $M = 199.31 ± 12.51$) F(1, 36) > 4.46, p < .05. On the other hand, in 24-month-old rats, neuronal soma size was unaffected by lesion, all F(1, 36) < 1.16, ns.

In occipital cortex, NBM lesions failed to alter neuronal soma size across ages and laminae; main effect of treatment, F(1, 23) = 1.91, ns; interaction of treatment and age, F(2, 23) = 1.91, ns; interaction of treatment, age, and lamina, F(6, 23) = 0.25, ns.

Neuron Number

Age effects. A three-way ANOVA revealed a significant difference in neuron numbers across ages, F(2, 36) = 3.76, p <
6. Photomicrographs of cresylecht violet-stained cells in lamina II-III of frontal cortex in 16-month-old sham- (top) and ibotenic acid- (bottom) lesioned rats. Lesions markedly reduced soma size. Scale bar = 100 μm.

.05, which was consistent across laminae, interaction $F(3, 36) = 0.61, ns$. Planned comparisons indicated that total neuron number in frontal cortex decreased from $M = 922,224 ± 67,506$ at 9 months of age to $M = 791,835 ± 72,943$ at 24 months of age, $F(1, 36) = 7.51, p < .05$. This 14% loss is apparently gradual, with no significant difference between either young adult and middle-aged, $F(1, 36) = 2.03, ns$, or middle-aged and aged rats, $F(1, 36) = 1.76, ns$. Moreover, although neuron number in individual laminae tended to decrease with age, this trend did not reach significance, $F_{s}(1, 36) < 2.79, ns$; see Table 1.

Lesion effects. A three-way ANOVA demonstrated no significant difference in neuron number as an effect of lesion, $F(1, 36) = 1.43, ns$, and rats of different ages were not differentially affected; interaction of age and treatment, $F(2, 36) = 0.101, ns$. Furthermore, no lesion effects were found for individual laminae; interaction of treatment and lamina, $F(3, 36) = 0.61, ns$; interaction of age, treatment, and lamina, $F(6, 36) = 0.40, ns$.

Discussion

In the present study, we have demonstrated age- and lesion-related morphological changes in frontal cortex, as well as lesion-induced deficits in radial maze performance. These observations complement previous studies documenting neural and behavioral changes associated with both aging and cholinergic deafferentation.
Age-Related Changes in the Basalocortical System

Radial Maze Performance

In contrast to previous studies, we found no significant age-related differences in performance of this radial maze task. This is surprising in that age-related behavioral deficits have been demonstrated on certain tasks (e.g., Fischer et al., 1989; Fischer, Chen, et al., 1991; Winocur & Moscovitch, 1990). One possible explanation for this discrepancy is that the present task was not sufficiently attentionally demanding and therefore was not sensitive to potential age-related deficits. For instance, aged rats trained on a more complex Hebb-Williams maze were significantly impaired relative to young adult rats (Winocur & Moscovitch, 1990). Studies have shown that damage to frontal and parietal cortex affects performance of radial maze tasks that are attentionally demanding (e.g., DiMattia & Kesner, 1988; Kolb et al., 1983), but not simpler tasks (Wozniak et al., 1989). The speed with which intact rats acquired the present task suggests that it is relatively simple. Thus, it may have been insensitive to age-related regressive changes in frontal cortex.

Neocortical Morphology

Despite the comparability of behavior across ages, quantitative analysis of frontal cortex revealed age-related changes in several morphological measures. The thickness of superficial laminae was significantly reduced in frontal cortex of 24-month-old rats, whereas the thickness of deeper laminae was slightly increased. Changes in the thickness of superficial laminae may partially reflect changes in neuronal size and number. In lamina II-III, neuronal somata of 16-month-old rats were hypertrophied relative to those of both 9- and 24-month-old rats. The decrease in average neuronal soma size from 16 to 24 months of age may have been due to loss of larger neurons: In 24-month-old rats, total neuron number was decreased by 14%, with a nonsignificant trend toward greater neuronal loss in the superficial laminae. Although less pronounced, similar morphological changes were present in occipital cortex, which receives minimal cholinergic projections from the NBM.

The pattern of age-related changes seen here is similar to
that found in previous studies. For instance, the 14% decrease in neuron number in frontal cortex is similar to that seen in occipital cortex of aged rats (Ordy, Brizzee, Kaack, & Hansche, 1978). Additionally, Wellman and Sengelaub (1990) previously demonstrated decreased neuron numbers in frontoparietal cortex of aged Sprague-Dawley rats. These changes were more pronounced than those documented in the present study, likely due to differences in counting techniques (in the present study we used an optical disector technique, which is considered to more accurately reflect actual neuron numbers than estimates based on counts corrected for neuronal soma size; see West, 1993). Others have reported subtle decreases in the thickness of frontal, parietal, and occipital cortices in aging rats (Diamond, Johnson, & Ingham, 1975), which could correspond to the decreases in the thickness of superficial laminae documented here. Given the similarity of age-related morphological changes in frontal and occipital cortex, both within the present study and across studies, these changes may reflect a general age-related regressive process that occurs in the neocortex.

Coleman and Flood (1986) have hypothesized that age-related increases in dendritic arbors of cortical neurons reflect a compensatory response to death of neighboring neurons. While the present study documents hypertrophy of lamina II–III neurons in 16-month-old rats, this does not reflect proliferation of dendritic arbors: Previous work in our lab has not revealed significant increases in the dendritic arbors of 16-month-old rats relative to 9-month-old rats (Wellman & Sengelaub, 1995). Furthermore, it is unlikely that it is a compensatory response to loss of neighboring neurons, as the present results reveal no significant neuronal loss at 16 months. However, hypertrophy of lamina II–III neuronal somata may reflect a compensatory response to the expression of other neural changes related to aging. Several studies have documented marked regressive changes in cholinergic neuron number and morphology in the NBM of aged rats (Altavista, Rossi, Bentivoglio, Crociani, & Albanese, 1990; Fischer et al., 1989; Fischer, Nilsson, & Bjorklund, 1991; Riekkinen et al., 1990). Additionally, extensive loss of neurons in the ventromedial and lateral hypothalamus, septum, corticoamygdaloid nucleus, substantia nigra, and the reticular formation of aged rats has been documented by Sabel and Stein (1981). However, most studies documenting these changes compare young adult rats with aged rats. Thus, the time course of these regressive changes is unknown. Hypertrophy of cortical neurons could be a dynamic compensatory response to the onset of changes in subcortical innervation. Such changes might in turn affect the ability of cortical neurons to respond to NBM lesion-induced loss of cholinergic input, reflected in the more pronounced decrease in neuronal size after lesion relative to both younger and older rats. If this is the case, the basolocortical system of middle-aged rats may prove particularly useful in modeling age-related changes in neuronal plasticity and function, and their relationships to behavior.

### Lesion-Induced Changes in the Basolocortical System

#### Radial Maze Performance

Lesions markedly impaired maze performance at all ages tested. Overall, lesioned rats required almost twice as many trials to reach criterion and made significantly more reference-memory errors. The pattern of lesion-induced behavioral deficits observed in the present study is similar to that seen after ablation of frontal cortex in rats. For instance, Kolb et al. (1982) trained rats on a radial arm maze task similar to that used in the present study. They found that rats with lesions of the medial prefrontal cortex (which included a large portion of the Frl area assessed in our study) were significantly impaired at learning the location of rewards compared with both intact controls and rats with lesions of the dorsomedial nucleus of the thalamus. Thus, lesions of prefrontal cortex and of its major source of cholinergic innervation result in similar behavioral deficits.

#### Neocortical Morphology

NBM lesions significantly decreased AChE staining in frontal cortex. This histological measure, which indirectly assesses the number of AChE-positive fibers in frontal cortex, does not provide information about absolute levels of cortical cholinergic activity. However, it does indicate that our lesions significantly decreased cholinergic innervation of frontal cortex. Furthermore, this decrease was equivalent across ages. However, despite similar depletion of cortical cholinergic innervation, the effect of NBM lesions varied across ages. In 9-month-old rats, lesions did not alter laminar thickness or neuron number and produced a moderate decrease in neuronal soma size in both superficial and deep laminae, with lamina II–III most affected. Alternatively, in 16-month-old rats, lesions did not affect neuron number but moderately reduced the thickness of lamina IV. Furthermore, lesions markedly reduced neuronal soma size in both superficial and deep laminae, again with lamina II–III most affected. NBM lesions produced much different effects in 24-month-old rats. Lesions did not alter neuron size or number; instead, lesions resulted in a pronounced increase in the thickness of superficial laminae.
laminae, possibly the result of gliosis, and a moderate decrease in the thickness of lamina VI. Finally, in occipital cortex, NBM lesions did not affect laminar thickness or neuronal soma size at any age, consistent with the minimal projections from the NBM to occipital cortex.

The NBM lesion-induced changes in cortical morphology in 9- and 16-month-old rats replicate previous findings (Wellman & Sengelaub, 1991). In the previous study, 7-month-old rats received NBM lesions, and quantitative morphological analyses were performed 6 months later (at 13 months of age). NBM lesions reduced soma size in particular cortical laminae, which resulted in decreased laminar thickness. These effects were present in frontal, forelimb, hindlimb, and parietal cortices, and most pronounced in lamina II–III. The overall pattern of results seen here is similar although somewhat less pronounced. The difference in magnitude may reflect continued degeneration over a longer postlesion interval in the original study. An investigation of the time course of degenerative changes after NBM lesions would clarify this point. In addition, we have examined dendritic morphology of lamina II–III frontal cortical neurons after unilateral NBM lesions in young adult, middle-aged, and aged rats. The changes in dendritic morphology seen in these groups parallel that seen in the present study. That is, middle-aged rats exhibit profound regressive changes in cortical dendritic morphology following NBM lesions, whereas young adult and aged rats are less affected (Wellman & Sengelaub, 1995).

Given the fundamental role played by acetylcholine in the modulation of cortical function, loss of the NBM projection likely results in a widespread decrease in frontal cortical activity. This decrease may be reflected in the morphological changes we have demonstrated. The absence of lesion effects in occipital cortex, which receives minimal input from the NBM, supports this hypothesis.

Nonetheless, it should be noted that these cortical changes could have been the result of loss of noncholinergic neurotransmitters. Several studies have localized noncholinergic transmitters, including somatostatin, neuropeptide Y, neurotensin, and galanin (e.g., Walker et al., 1989) in NBM neurons, and others have demonstrated that at least 10% of neocortical afferents from the NBM are noncholinergic (e.g., Rye, Wainer, Mesulam, Mufson, & Saper, 1984). Thus, we cannot rule out the possibility that noncholinergic transmitters play a role in the morphological changes induced by NBM lesions. Further studies assessing the relationships between cortical morphological changes and levels of these neurotransmitters after NBM lesion would clarify this issue.

The apparently minimal effect of NBM lesions at 24 months is somewhat counterintuitive. Given the regressive changes in laminar thickness and neuron number seen in the normal 24-month-old rats, one might hypothesize that NBM lesions would more greatly compromise frontal cortex in these rats compared to either 9- or 16-month-old rats. One possible explanation for the minimal changes observed in 24-month-old lesioned rats is that in these rats the lesions had a smaller functional impact. Although age-related morphological changes in basal forebrain nuclei are well-documented, these regressive changes are not accompanied by a decrease in cortical markers for acetylcholine (e.g., Fischer et al., 1991), suggesting that the system has functionally compensated for regressive changes in basal forebrain nuclei. This compensatory mechanism may operate to reduce the impact of further lesion-induced loss of NBM neurons. Thus, at 13 to 16 months of age, the system may be in the process of adjusting to changes in neural input, and this process could limit its ability to respond to further insult. Later, however, the system may have reached an equilibrium that enables it to function adequately despite the previous loss of subcortical input.

Alternatively, the 24-month-old rats could have been less affected by NBM lesions due to a selection bias. Lesions resulted in substantial mortality, which was not uniform across ages. Thus, while extent and placement of the lesions, reductions in cortical AChE, and behavioral deficits were equivalent across ages, it is possible that the 24-month-old NBM lesioned group is biased due to the differential death of more severely affected rats. However, other work in our lab demonstrates that unilateral NBM lesions produce a similar pattern of regressive changes in frontal cortex in middle-aged and aged rats, but no differential mortality (Wellman & Sengelaub, 1995). Thus, the differential patterns of changes seen in young adult, middle-aged, and aged NBM-lesioned rats likely reflects differential vulnerability to neural insult in the basalocortical system of middle-aged rats.

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