Differential effects of nucleus basalis lesions in young adult and aging rats

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Abstract

To characterize age-related changes in frontal cortical plasticity, we assessed maze learning and frontal cortical pharmacology in young adult, middle-aged, and aged rats. Rats received either ibotenic acid or sham lesions of the nucleus basalis magnocellularis (NBM) and were then trained on a radial maze task. After training, we assessed \[^{3}H\]desmethylimipramine (DMI), \[^{3}H\]muscimol, \[^{3}H\]AMPA, and \[^{3}H\]QNB binding using quantitative autoradiography. Both middle-aged and aged rats were impaired on the radial maze task. DMI binding was increased in both middle-aged and aged rats, while QNB binding was decreased in aged rats. While lesions impaired maze performance at all ages, middle-aged and aged rats showed more profound lesion-induced deficits. Lesions increased GABA\textsubscript{A} and AMPA receptor binding in young adult rats only. These lesion-induced changes may reflect a compensatory response that is lost with advancing age. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

Normal aging in rats is accompanied by deficits on a variety of cognitive tasks, including performance of the Morris water maze and radial arm maze, operant delayed-response tasks, and passive avoidance. Extensive work from multiple investigators (e.g., [19,21,22,42,56]) has consistently demonstrated that aged rats require more trials to learn the position of a hidden escape platform in the Morris water maze; this deficit appears to be due to age-related impairment in the use of a place-learning strategy (see [6] for review). Likewise, several studies have demonstrated impaired performance on the radial arm maze, a task that young adult rats learn readily (e.g., [4,28,72]). Others have demonstrated age-related deficits on complex maze tasks (e.g. [80], and reviewed in [29]), which have been interpreted as impaired memory for both specific information related to maze-learning and information related to more general rule-learning [80]. Furthermore, aged rats are impaired on an operant delayed matching to position task, suggesting short-term memory deficits in the absence of increased sensitivity to proactive interference [16,17]. Finally, aged rats typically are impaired in the acquisition of a passive avoidance response (e.g. [60]), which is considered to be a fairly nonspecific measure of cognitive impairment.

While aging alters the structure and function of a variety of brain regions (e.g. [22,42,53]), cognitive changes associated with aging may be mediated in part by changes in frontal and prefrontal cortex, which play a major role in aspects of information processing such as attention, sequencing and inhibiting responses, habituation, and place learning. For example, young adult rats with lesions of frontal cortex were unable to simultaneously process two temporal stimuli, although processing of individual stimuli was intact. This pattern of deficits suggests impaired selective attention [50]. Furthermore, lesions of medial prefrontal cortex increased perseveration in a delayed spatial alternation task (e.g. [18]), while observation of species-specific behaviors such as nest building, food hoarding, and maternal behaviors after medial prefrontal lesions have also suggested difficulties in inhibiting previous responses and sequencing behaviors [38]. Lesions of medial prefrontal...
cortex have been shown to disrupt habituation assessed in a variety of settings, including open field tests and investigative nosepoking (e.g. [35]); and habituation has been shown to eliminate the increased acetylcholine release in frontal cortex elicited by presentation of a novel stimulus [1]. Finally, lesions of frontal and prefrontal cortex impair performance on the radial arm maze (e.g. [6,40,37]) and the Morris water maze [37,36], suggesting a role for frontal cortex in spatial navigation and place learning. Thus, frontal and prefrontal cortex are involved in a variety of behaviors, many of which change with aging, and potential age-related changes in frontal and prefrontal cortex may underlie some of these cognitive deficits.

Consistent with this hypothesis, normal aging results in a variety of functional and morphological changes in the frontal cortex of rats, including changes in frontal cortical EEG [25], increases in glial density [52], and decreases in synaptic density [64]. Previously, we demonstrated age-related changes in frontal cortical morphology in rats. For instance, the thickness of superficial laminae was significantly reduced in aged rats. This reduction paralleled, and may reflect, a significant decrease in total neuron number in the aged rats. Interestingly, age-related changes in the size of neuronal somata were nonlinear: neuronal somata of middle-aged rats were hypertrophied relative to those of either young adult or aged rats [75]. These physiological and morphological changes are accompanied by neurochemical changes, including downregulation of neocortical nicotinic and muscarinic receptors [3], alterations in GABAergic receptor binding in prefrontal cortex [63], and changes in monoamnergic release and receptors in frontal cortex (e.g. [24,48]). Thus, a growing body of evidence demonstrates structural, functional, and neurochemical changes in frontal and prefrontal cortex in aging rats.

Such widespread changes in structure, function, and neurochemistry may either alter or reflect changes in cortical plasticity. To explore age-related alterations in the plasticity of frontal cortex, we previously assessed morphology of frontal cortex after lesion of the nucleus basalis magnocellularis (NBM) in young adult, middle-aged, and aged rats. Frontal cortex receives specific and direct projections from the NBM [8,30], and acetylcholine supplied by the NBM plays a fundamental role in modulating neocortical structure [27,65,76] and function (e.g. [10,13,41,44,57,58,66,69,70]). We found that the effects of lesions of the NBM on frontal cortical morphology varied with age: lesions produced more profound deficits in cortical morphology in middle-aged and aged rats relative to young adults [75,77].

The present study assessed the neurochemical and behavioral correlates of these structural changes. To examine whether NBM lesions differentially affect maze performance at different ages, we assessed acquisition of a radial maze task in young adult, middle-aged, and aged rats with either ibotenic acid or sham lesions of the NBM. The radial arm maze task is sensitive to frontal cortical function: rats with prefrontal and frontal lesions are markedly impaired on this task (e.g. [6,40,37]), and lesion of NBM afferents to frontal cortex also results in profound deficits on this task (e.g. [20]).

Using quantitative autoradiography, we then assessed potential alterations in the noradrenergic, GABAergic, cholinergic, and glutamatergic systems in frontal cortex. These neurotransmitter systems play a major role in cortical functioning (e.g. [2,7,31,45,54]). In addition, these systems have extensive interactions in neocortex (e.g. [33,46]) and appear to play a role in cortical plasticity [5,11,25,32,39,74].

In this study, we have demonstrated age- and NBM lesion-related deficits in maze performance and concomitant alterations in frontal cortical pharmacology. These changes are consistent with the hypothesis that plasticity of frontal cortex is altered in the aging rat.

2. Methods

2.1. Animals

Radial maze performance was assessed in young adult, middle-aged, and aged male Long-Evans rats (Harlan Laboratories, Indianapolis, IN) that received either excitotoxic or sham lesions of the NBM. Rats were 3 (N = 25), 14 (N = 30), and 22 (N = 15) months old at the beginning of the experiment. These ages bracket a large portion of the adult life span of the Long-Evans rat and represent ages both below and approaching the median mortality of this strain: as the rats aged during the course of the experiment, the groups represented ages below and at median mortality. Throughout the experiment, rats were individually housed in cages equipped with filter tops.

2.2. Surgery

Rats were anesthetized with chlorapent, with the dose varying across ages (young adult rats, 0.3 mL/100 g ip; middle-aged rats, 0.255 mL/100 g ip; aged rats, 0.255 mL/100 g ip; see [75]). Approximately half of the rats in each age group received bilateral ibotenic acid lesions of the NBM. This relatively nonspecific excitotoxin was chosen to allow comparison with our previous studies [75,77], as well as to maximize potential behavioral and frontal cortical effects [43,15,71,79]. 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 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 [51]. A cannula attached to a Hamilton microsyringe was lowered into the appropriate stereotaxic coordinates and left in place for 2 min prior to injection. Ibotenic acid (0.8 µL per side, 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 sham-operated controls. They received the surgical procedures described above, but did not receive the ibotenic acid injections. To minimize the post-surgical aphagia that typically accompanies basal forebrain lesions, both sham and ibotenic acid lesions in each hemisphere were performed during separate surgeries three days apart.

2.3. Behavioral testing

To assess the effects of aging and NBM lesion on learning and memory, rats were trained in a radial maze task that previously has been shown to be sensitive to both age-related behavioral changes (e.g., [36,78]) and lesions of the NBM (e.g. [20]).

2.3.1. Apparatus

The maze consisted of a central platform (33 cm in diameter) with eight identical radial arms (each 39 × 12 cm). Peanuts were used as bait and placed in small metal cups at the end of each arm.

2.3.2. Procedure

To motivate animals on the task, rats’ weight was gradually reduced to 80% of their free-feeding weight. 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. For habituation trials, only one of eight arms of the radial maze was attached to the central platform. The platform and arm were cleaned with a deodorizer, and four pieces of peanut were scattered along the length of the arm. Each rat was placed on the central platform and permitted to make up to 16 choices during the trial. Daily habituation trials continued until either it traveled the length of the arm at least once and ate all the bait or 10 min had elapsed. Each rat was given a maximum of 45 trials. After a rat reached criterion, maze training was discontinued; for the purposes of data analysis, subsequent trials were assigned the error score that rat received on its last trial. The length of each trial and arms visited during the trial were recorded for each rat. These measures were used to compute several performance- and acquisition-related variables, including average latency to first choice, average run time (time between first and last visit divided by the number of visits), and working memory errors (within-trials errors; number of visits to previously chosen arms on a given trial). In addition, a measure of perseveration was calculated for each rat. Perseverative errors were defined as more than two visits to an arm within a trial; these errors were summed for each trial. Thus, according to this definition, a rat that visited Arm A three times (one perseverative error), Arm B four times (two perseverative errors), and all other arms once each, made a total of three perseverative errors.

2.4. Autoradiographic analysis

After behavioral testing (four months after surgery; at 7, 18, and 26 months of age), rats were decapitated and their brains rapidly removed and placed on ice. The anterior portion of each brain (from the olfactory bulb to approximately the level of the medial septum) was dissected, frozen in isopentane, and stored at −70°C for use in quantitative autoradiography. The remainder of the brain was immersed in 10% neutral buffered formalin for histology.

2.4.1. Histology

After immersion in formalin, the portions of the brains containing the lesion sites were cryoprotected and frozen sections (40 μm) 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 cresyl echt violet or processed for AChE staining using a modification of the Karnovsky-Roots method [26].

2.4.2. Quantitative autoradiography

Brains were cut at 16 μm in a cryostat, and coronal sections thaw-mounted onto chrome-alum gelatin-coated slides. For each ligand, eight equally spaced sections from the anterior portion of the claustrum to the genu of the corpus callosum were saved. Total binding was assessed in four of these sections, while nonspecific binding was determined in four anatomically adjacent sections. All tritiated ligands were supplied by NEN (Boston, MA); unlabeled quinuclidinyl benzylate was supplied by RBI (Natick, MA); all other unlabeled competitors were supplied by Sigma (St. Louis, MO).

To label muscarinic receptors, sections were rinsed three minutes at 4°C in .05 M phosphate buffer (pH 7.4). To assess total binding, sections were incubated one hour at 4°C in buffer plus 1.5 nM [3H]quinuclidinyl benzylate (QNB, 43.5 Ci/mmol). Nonspecific binding was assessed by competing [3H]QNB against 20 μM atropine, and non-M1 receptor binding was assessed by competing [3H]QNB against 2.0 μM pirenzepine. Nonspecific binding of [3H]QNB averaged 2 ± 0.1 percent, which is consistent with previous reports (e.g. [47]). After two three-minute rinses at 4°C in buffer, slides were dipped in cold distilled H2O (see [47]).

To assess potential alterations in noradrenergic activity, noradrenergic reuptake sites were labeled with [3H]des-
methylimidipramine hydrochloride (DMI, 73 Ci/mmol) using the procedure described by Rapp [55]. Sections were incubated in dim light one hour at 4°C in 50 mM Tris-HCl (pH 7.4) plus 300 mM NaCl and 2.0 nM [3H]DMI. Nonspecific binding was assessed by competing against 100 µM desipramine, and averaged 51 ± 0.6%, which is consistent with previous studies (e.g. [55,44]). After three 20-minute rinses at 4°C in buffer, slides were dipped in cold distilled H2O.

GABA_A receptors were labeled with [3H]muscimol using a procedure similar to that of Xia and Haddad [83]. Sections were rinsed 30 min at room temperature in 50 mM Tris/Citrate (pH 7.0). To assess total binding, sections were incubated 45 min at 4°C in buffer plus 50 nM [3H]muscimol (17.5 Ci/mmol); nonspecific binding was assessed by incubation with the tritiated ligand plus 100 µM unlabeled GABA. Consistent with previous reports [9], nonspecific binding was minimal, averaging 12 ± 0.3%. After five 2-sec rinses in ice-cold buffer, sections were dipped once in ice-cold distilled H2O.

To assess potential changes in AMPA receptor binding, sections were labeled with [3H]α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), using a procedure similar to those of Olsen et al. [49] and Tocco et al. [68]. Slides were rinsed 30 min at 35°C in 100 mM Tris-Acetate (pH 7.2–7.4) containing 100 mM potassium thiocyanate and 100 µM EGTA before incubation 45 min at 4°C in this buffer plus 34 nM [3H]AMPA (53.0 Ci/mmol). Nonspecific binding was assessed by competing the tritiated ligand against 250 µM quisqualate. As in previous studies [49], nonspecific binding averaged 12 ± 0.4%. After incubation, all slides were rinsed twice (10 sec) in ice-cold buffer, followed by one 5-sec rinse in ice-cold buffer diluted 1:1 with distilled H2O and three dips in ice-cold distilled H2O.

All slides were dried in a stream of cold air, stored under vacuum with desiccant overnight and then placed in autoradiographic cassettes (Hypercassettes; Amersham, Cleveland, OH), opposed to film (3H Hyperfilm; Amersham) along with standardized autoradiographic microscales (Amersham), and stored at 4°C for either five weeks (for muscimol autoradiography, four weeks (for QNB and DMI), or two weeks (for AMPA). The films were then developed (Kodak D-19), fixed (Kodak Fixer), and air dried. Slides were exposed to paraformaldehyde vapors and stained with cresylecht violet.

Density of binding in the Fr1, Fr2, and Fr3 areas [nomenclature of Zilles and Wree (84)] of the resulting autoradiographic images were quantified using a computer-based image analysis system (MCID; Imaging Research Inc., St. Catharines, Ontario). These cytoarchitectural areas correspond to primary motor and portions of prefrontal cortex. Histological slides were placed on a light box (Northern Light; Imaging Research, Inc.) and digitized; the corresponding autoradiograms were then placed on the light box, digitized, and the computerized images aligned with the histological section. Regions of interest were then defined on the histological sections and samples taken from the corresponding areas of the autoradiograms. For each ligand, average optical density was measured in the superficial and deep layers of Fr1, Fr2, and Fr3 in each hemisphere of each section. Thus, six total and six nonspecific samples per section, or a total of 24 total and 24 nonspecific samples per animal, were obtained. Measures were standardized against the microscales included on each film and expressed in fmol/mg wet tissue weight. Specific binding was calculated by subtracting nonspecific binding from total binding for each pair of sections. For each ligand, specific binding measures for superficial and deep layers were then averaged across the three frontal cortical areas for each animal.

2.5. Statistical analyses

To eliminate the possibility of spurious age effects due to differential effects of lesions across ages, age effects were assessed by comparing young adult, middle-aged, and aged sham-lesioned rats. Average latency to first choice, average run time and trials to criterion were compared across the three groups using one-way ANOVAs. Additionally, working memory and perseverative errors were averaged across blocks of three trials and compared across blocks using two-way repeated measures ANOVAs (Age X Block). Specific binding of each ligand in the superficial and deep layers was compared in young adult, middle-aged, and aged sham-lesioned rats using two-way ANOVAs (Age X Layer).

Lesion effects were assessed by comparing young adult, middle-aged, and aged sham- and ibotenic-acid lesioned rats. Average latency to first choice, average run time and trials to criterion were compared across the six groups using two-way ANOVAs (Age X Treatment). Additionally, working memory and perseverative errors were averaged across blocks of three trials and compared across blocks using three-way repeated measures ANOVAs (Age X Treatment X Block). Specific binding of each ligand in the superficial and deep layers was compared across groups using three-way ANOVAs (Age X Treatment X Layer).

3. Results

3.1. Age effects

3.1.1. Behavioral testing

To assess whether performance variables changed with age, the average latency to first choice and average run time were compared in young adult, middle-aged, and aged sham-lesioned rats. One-way ANOVAs indicated that neither latency to enter the first arm nor average run time varied across ages [F(2, 32) = 0.88 and 0.53, respectively; ns]. Average latency for young adult rats was 5.79 ± 2.16 s; for middle-aged rats, 3.06 ± 0.83 s; and for aged rats, 3.52 ± 1.31 s. Average run time for young adult rats was...
18.64 ± 1.27 s; for middle-aged rats, 18.02 ± 0.79 s; and for aged rats, 19.95 ± 1.75 s. Thus, these performance variables did not change with age.

To assess potential age-related differences in acquisition, average trials to criterion, working-memory errors, and perseverative errors were compared in the sham-lesioned animals. Statistical analyses demonstrated that age significantly impaired maze performance, reflected in a variety of measures. One-way ANOVA revealed a significant effect of age on trials to criterion \( F(2, 32) = 5.37, p < .05 \); Fig. 1a. Subsequent contrasts revealed that middle-aged rats required 60% more trials to reach criterion than did young adult rats \( F(1,32) = 10.60, p < .05 \). Although aged rats required 40% more trials to reach criterion than did young adults \( F(2,32) = 3.07, p = .09 \), this effect did not reach significance. Middle-aged and aged rats did not differ significantly \( F(1,32) = 0.81, \) ns.

The age-related impairment of acquisition was apparently due to an increase in both within-trials and perseverative errors. Two-way repeated-measures ANOVAs indicated a significant effect of age on within-trials errors \( F(1,32) = 6.92, p < .05 \); Fig. 1b. This effect did not vary over trials [interaction of age and block, \( F(2,28) = 0.75, \) ns]. Subsequent contrasts indicated that both middle-aged and aged rats made significantly more within-trials errors overall than did young adult rats \( F(1,32) = 7.71 \) and 6.41, respectively, \( p < .05 \). Mean within-trials errors for middle-aged and aged rats were not significantly different \( F(1,32) = 0.01, \) ns.

In addition, two-way repeated-measures ANOVA demonstrated that perseverative errors also varied significantly with age \( F(1,32) = 5.36, p < .05 \); Fig. 1c. Again, this effect did not vary significantly over trials [interaction of age and block, \( F(2,28) = 0.95, \) ns]. Contrasts indicated that both middle-aged and aged rats made significantly more perseverative errors than did young adults \( F(1,32) = 10.03 \) and 4.48, respectively, \( p < .05 \); mean perseverative errors for middle-aged and aged rats were not significantly different \( F(1,32) = 0.21, \) ns.

3.1.2. Autoradiography

The distribution of binding in frontal and prefrontal cortex of all ligands examined was similar to that reported in previous studies (Fig. 2; e.g. [47,14,83,9,49]). Two-way ANOVAs demonstrated that neither AMPA nor muscimol binding was significantly altered in aging rats \( F(2,64) = 0.44 \) and 2.27, respectively, both ns; data not shown. However, two-way ANOVAs demonstrated a significant effect of age on both DMI and QNB binding \( F(2,64) = 33.75 \) and 11.83, respectively, both \( p < .05 \); Figs. 3 and 4. Contrasts indicated that binding of DMI to noradrenergic reuptake sites was significantly increased in both superficial and deep layers in middle-aged and aged rats relative to young adults [43% overall; all \( F(1,64) \geq 12.48, p < .05 \)]. DMI binding in middle-aged and aged rats did not differ significantly [all \( F(1,64) \leq 0.94, \) ns]. In contrast, QNB binding to muscarinic receptors in aged rats was significantly decreased in the superficial and deep layers relative to both middle-aged and young adult rats [31% overall; all \( F(1,64) \geq 5.96 p < .05 \)], while middle-aged rats did not differ significantly from young adults [all \( F(1,64) \leq 0.30 \),

![Fig. 1. (a) Average trials to criterion for young adult, middle-aged, and aged sham-lesioned rats. Asterisk indicates significant difference relative to young adults. (b) Average within-trials errors over blocks of 3 trials for young adult, middle-aged, and aged sham-lesioned rats. (c) Average perseverative errors over blocks of 3 trials for young adult, middle-aged, and aged sham-lesioned rats. For all graphs, vertical bars represent SEMs.](attachment:image.png)
ns]. The decrease in QNB binding in the aged rats was apparently due to decreased M₁ receptor binding: aged rats showed a significant decrease in M₁ but not non-M₁ receptor binding relative to young adult and middle-aged rats [for M₁ binding, all \( F_{(1,64)} \geq 5.74, p < .05 \); for non-M₁ binding, all \( F_{(1,64)} \leq 0.81, \text{ns} \); Fig. 4]. Again, middle-aged rats did not differ significantly from young adults [all \( F_{(1,64)} \leq 1.99, \text{ns} \].

3.2. Lesion effects

3.2.1. Behavioral testing

To assess whether NBM lesions altered performance variables, the average latency to first choice and average run time were compared in young adult, middle-aged, and aged rats with either sham or ibotenic acid lesions of the NBM. Although latency to first choice was unaffected by lesion at any age [effect of treatment, \( F_{(1,52)} = 0.33, \text{ns} \); interaction of age and treatment, \( F_{(2,52)} = 0.004, \text{ns} \)], lesions significantly altered average run time \([F_{(1,52)} = 15.58, p < .05]\). Furthermore, this effect varied across ages \([F_{(2,52)} = 4.50, p < .05]\; data not shown). Subsequent contrasts indicated that lesions in young adult and middle-aged rats did not significantly affect average run time [in young adults, \( M = 18.64 \pm 1.27 \) s and \( 20.34 \pm 2.05 \) s for sham and lesioned rats, respectively, \( F_{(1,52)} = 0.27, \text{ns} \); in middle-aged rats, \( M = 18.02 \pm 0.79 \) s and \( 23.47 \pm 1.97 \) s, respectively, \( F_{(1,52)} = 2.82, \text{ns} \)]. However, lesions significantly increased average run time in aged rats \([M = 19.95 \pm 1.75\) and \( 36.48 \pm 6.32, F_{(1,52)} = 18.35, p < .05]\). Thus, lesions differentially increased the amount of time aged rats spent on each arm.

Lesions also significantly altered acquisition-related variables. Two-way ANOVA yielded a significant effect of treatment on number of trials necessary to reach criterion \([F_{(1,52)} = 10.79, p < .05]\; data not shown). Overall, lesioned rats required approximately 31% more trials to reach criterion than did sham-lesioned rats, and this effect did not vary significantly across ages \([F_{(2,52)} = 1.44, \text{ns} \].

Results of a three-way repeated measures ANOVA indicated that lesions significantly increased within-trials errors for all ages [effect of treatment, \( F_{(1,52)} = 12.13, p < .05 \); interaction of age and treatment, \( F_{(2,52)} = 0.24, \text{ns} \); Fig. 5a]. Overall, lesions increased within-trials errors by 61%; however, a significant interaction of block and treat-
ment was present \([F(14,52) = 2.84, p < .05]\). While lesions did not significantly alter performance on the first six trials [for Blocks 1 and 2, \(F(1,52) = 0.81\) and \(1.69, ns\)], they significantly impaired performance on all subsequent trials [for Blocks 3–15, all \(F(1,52) \geq 4.52, p < .05\)]. Thus, sham-lesioned rats’ performance improved more rapidly than did ibotenic-acid lesioned rats’ performance.

Interestingly, despite the lack of an interactive effect of age and lesion on trials to criterion, within-trials or perseverative errors, lesions appeared to differentially affect acquisition of the task, as reflected in the proportion of lesioned animals at each age reaching criterion (Fig. 6). In young adult rats, lesions reduced the proportion achieving criterion from 100% to 75% \([\chi^2(1) = 3.59, ns]\); in middle-aged rats, 53% of sham-lesioned and 50% of lesioned rats reached criterion \([\chi^2(1) = 0.02, ns]\); and in aged rats, lesions reduced the proportion reaching criterion from 71% to 14% \([\chi^2(1) = 4.67, p < .05]\).

### 3.2.2. Histology

Examination of cresylecht violet-stained sections revealed the presence of well-defined bilateral lesions in the basal forebrain in all but two of the ibotenic acid-lesioned rats, which were therefore excluded from all analyses. In all remaining cases, lesions involved the NBM, the ventral portion of the globus pallidus, and portions of the ventral pallidum and substantia innominata. Additionally, collections of “translucent spherical bodies” were observed in the globus pallidus of the 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 \([81]\). Extent of lesions appeared to be equivalent across ages. Finally, in all lesioned rats used for analyses, examination of AChE-stained sections revealed a pronounced decrease in staining of cholinergic fibers in frontal and prefrontal cortex, which was equivalent across ages (Fig. 7).

### 3.2.3. Autoradiography

Lesions significantly altered AMPA and GABA\(_A\) receptor binding [for AMPA, \(F(1,110) = 4.06, p < .05\); for GABA\(_A\), \(F(1,110) = 4.42, p < .05\); Fig. 8]. However, despite comparability of lesion placement and extent across ages, contrasts indicated that AMPA and GABA\(_A\) receptor binding were affected by lesions only in young adult rats. In young adults, lesions increased AMPA binding in the superficial layers by 19% [for superficial layers, \(F(1,110) = 11.65, p < .05\); interaction of age and treatment, \(F(1,52) = 0.11, ns\); Fig. 5b]. Overall, lesions increased perseverative errors by 129%, but again, a significant interaction of block and treatment was present \([F(14,52) = 2.84, p < .05]\). While lesions did not significantly alter performance on the first six trials [for Blocks 1 and 2, \(F(1,52) = 0.81\) and \(1.69, ns\)], they significantly impaired performance on all subsequent trials [for Blocks 3–15, all \(F(1,52) \geq 4.52, p < .05\)]. Thus, sham-lesioned rats’ performance improved more rapidly than did ibotenic-acid lesioned rats’ performance.

Fig. 4. Mean specific [\(^3\)H]QNB, and M\(_1\) and non-M\(_1\) receptor binding in superficial and deep layers of frontal cortex in young adult, middle-aged, and aged, sham-lesioned rats. Vertical bars represent SEMs. Asterisks indicate significant differences relative to young adults.
4. Discussion

In the present study, we have demonstrated dissociable age- and NBM-lesion-related deficits in maze performance and concomitant alterations in frontal cortical pharmacology. These changes are consistent with previous studies documenting neural and behavioral changes associated with
both aging and cholinergic deafferentation of frontal cortex. Furthermore, the changes we have found are consistent with the hypothesis that plasticity of frontal cortex is altered in the aging rat.

4.1. Age-related changes in frontal cortex

Aging rats were significantly impaired on a radial maze task. Overall, middle-aged and aged rats required more trials to reach criterion and made more within-trials and perseverative errors than did young adult rats. This is consistent with a variety of studies documenting deficits in maze performance in aged rats (e.g. [21,56,4,28,72]). Interestingly, we found that middle-aged rats were also impaired on the radial maze task. In fact, they appeared to show somewhat greater impairment than did the aged rats. For instance, middle-aged but not aged rats required significantly more trials to reach criterion, and a larger proportion of middle-aged rats (47% versus 29%) failed to reach criterion within 45 trials. This nonlinear pattern of results is in contrast to the linear pattern of age-related decline in per-
formance on the Morris water maze (e.g. [56,23,24]), a hippocampally mediated task. Thus, the nonlinear pattern of age-related changes in performance of our radial maze task may reflect age-related changes in nonhippocampal structures. For instance, previously, we found nonlinear age-related changes in the size of neuronal somata in the frontal cortex: middle-aged rats demonstrated hypertrophy of neuronal somata, while aged rats did not [75].

The age-related deficit in maze performance documented in this study paralleled changes in the noradrenergic and cholinergic systems. Binding to noradrenergic reuptake sites was markedly increased in both the superficial and deep layers of frontal cortex in middle-aged and aged rats. This change in noradrenergic reuptake is consistent with the other findings of age-related changes in norepinephrine levels in frontal cortex. For instance, norepinephrine levels in medial frontal cortex are increased overall in aged rats [67]. Others have found increased levels of frontal cortical norepinephrine specific to aged rats that are impaired on the Morris water maze [24].

In contrast, M₁ receptor binding was decreased in the superficial and deep layers only in aged rats. Age-related alterations in the cholinergic system are well-documented, including substantially reduced acetylcholine release [82] and choline acetyltransferase activity [3] in neocortex. In fact, others have demonstrated downregulation of M₁ and M₂ receptors in neocortical homogenates [3]. In this study, we found significant decreases in M₁ but not non-M₁ binding. However, we did not specifically assess binding to M₂ receptors; thus, significant changes in M₂ binding may have been obscured by binding to other muscarinic receptor subtypes. Nonetheless, our data complement a growing body of evidence for age-related cholinergic dysfunction in a variety of forebrain structures.

Interestingly, our data indicate that the timing of age-related changes differed in the noradrenergic and cholinergic systems in frontal cortex: while noradrenergic reuptake is altered in both middle-aged and aged rats, alterations in muscarinic receptor binding do not appear until after 18 months of age. The noradrenergic and cholinergic systems have extensive interactive effects on aspects of cognition mediated by frontal and prefrontal cortex (e.g. [12,59]). Thus, the poorer performance of middle-aged rats relative to aged rats may reflect a transient imbalance in these two systems.

Finally, the noradrenergic and cholinergic systems play
critical roles in cortical function and plasticity. For instance, norepinephrine is necessary for plasticity in developing neocortex (e.g. [45,5,39]). Iontophoretic application of norepinephrine modulates the firing rate of cortical neurons [31, 33,73], as does activation of noradrenergic neurons of the locus coeruleus [50]. Likewise, acetylcholine modulates neocortical activity [41,44,57,69], and acetylcholine is necessary for cortical reorganization following injury [32,74]. Thus, the age-related alterations in these systems may decrease plasticity of frontal cortex.

4.2. Lesion-induced changes in frontal cortex

Consistent with previous studies (e.g. [20,34,43,81]), NBM lesions impaired maze performance. However, this effect differed across ages. While NBM lesions produced comparable increases in within-trials and perseverative errors across ages, fewer aging lesioned rats reached criterion within 45 trials. Thus, aging rats appeared to be more affected by the lesions.

This effect was paralleled by lesion-induced changes in frontal cortical pharmacology. Lesions of the NBM increased AMPA and GABA\textsubscript{A} receptor binding in young adult rats. This effect replicates a previous study, in which NBM lesion-induced cholinergic, GABAergic, and glutamatergic receptor binding were assayed in cortical homogenates [62]. These authors found increases in both AMPA and GABA\textsubscript{A} receptor binding one week after lesioning, which they suggest are compensatory responses to cholinergic deafferentation. Indeed, alterations of both the glutamatergic and GABAergic systems are associated with cortical deafferentation (e.g. [11,25]).

Interestingly, in the present study, NBM lesions failed to significantly increase GABA\textsubscript{A} and AMPA receptor binding in middle-aged and aged rats. This differential age effect is consistent with the hypothesis that the lesion-induced changes in AMPA and GABA\textsubscript{A} receptor binding are a compensatory response that is lost with advancing age. Thus, the changes in the GABAergic and glutamatergic systems in frontal cortex of young adult rats seen after NBM lesion may be related to the less profound lesion-induced impairments in these animals.

Furthermore, the age-dependent effect of NBM lesions on frontal cortical pharmacology found in the present study is consistent with our previous data demonstrating differential effects of NBM lesions on frontal cortical morphology in aging rats. For instance, lesion-induced atrophy of frontal cortical neurons varied across ages, with middle-aged rats being more profoundly affected [75]. In addition, NBM lesion-induced alterations in dendritic morphology of frontal cortical neurons varied across ages: both middle-aged and aged rats exhibited profound regressive changes in cortical dendritic morphology following NBM lesions, whereas young adult rats were less affected. Interestingly, the pattern of dendritic reorganization was also different: in middle-aged rats, lesion-induced alterations were most pronounced proximal to the soma, whereas in aged rats, lesion-induced changes were most pronounced more distal to the soma [77]. Thus, the results of our previous studies combined with the present data suggest that the plasticity of frontal cortex is altered in aging rats, perhaps due to age-related changes in the noradrenergic and cholinergic systems.

Finally, we have demonstrated a clear dissociation between the age-related and lesion-induced changes in maze performance and pharmacology of frontal cortex. Normal aging resulted in impairment on a task requiring a win-shift strategy, with the aged rats making more errors that reflect inflexibility, a pattern consistent with frontal lesions (e.g. [18,38,6]). This behavioral alteration was accompanied by increased binding to noradrenergic reuptake sites and decreased binding to M\textsubscript{1} receptors. In contrast, the long-term effects of NBM lesions in young adults were overall impairment of radial maze performance, accompanied by increases in AMPA and GABA\textsubscript{A} receptor binding. This dissociation suggests that the age-related changes in frontal cortical pharmacology are not the result of the well-documented age-related neurodegeneration of basal forebrain nuclei (e.g. [19,61]). Thus, the age-related change in frontal cortical plasticity may reflect alterations in either the function of other subcortical nuclei (for instance, the locus coeruleus) or intrinsic cortical circuitry.

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References

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