Interactions between aging and cortical cholinergic deafferentation on attention☆

Joshua A. Burk, Christopher D. Herzog, M. Christine Porter, Martin Sarter*

Departments of Psychology and Neuroscience, The Ohio State University, Columbus, OH 43210, USA

Abstract

Pre-existing trauma to basal forebrain corticopetal cholinergic neurons has been hypothesized to render this system vulnerable to age-related processes. The present longitudinal study assessed the interactions between the effects of partial cortical cholinergic deafferentation and aging on sustained attention performance. After pre-surgical training, animals were given sham-surgery or bilateral infusions of the immunotoxin 192 IgG-saporin into the basal forebrain. The lesion was intended to yield a limited loss of cortical cholinergic inputs and thus to produce minor immediate effects on sustained attention performance. All animals were tested continuously until age 36 months. The attentional performance of lesioned and sham-lesioned animals did not dissociate until age 31 months, when the lesioned animals exhibited an impairment in overall sustained attention performance. Importantly, this impairment interacted with the effects of time-on-task, and thus reflected a specific impairment in attentional processes. These results support the notion that pre-existing damage to the basal forebrain corticopetal cholinergic neurons yields age-related impairments in the attentional capabilities that depend on the integrity of this neuronal system. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Among the numerous neuronal systems affected by aging and dementia, loss of, or decreases in the integrity of, basal forebrain corticopetal cholinergic neurons are most closely correlated with the decline in cognitive functions, and in fact are hypothesized to initiate such decline [6,8,12,13,18,23,24,27,45,50,52]. In rodents, the study of the functional consequences of age-related alterations in the basal forebrain corticopetal cholinergic system has therefore received much attention. Although some morphological differences have been observed in cholinergic neurons of aged animals [1,26], the evidence concerning the direct effects of age on the functional capacity of basal forebrain corticopetal cholinergic projection system has remained inconclusive [40,41,56]. For example, several studies have failed to find robust age-related differences in cortical choline acetyltransferase (ChAT) activity [4,31,43,59,60,70]. Furthermore, high affinity choline uptake, the rate-limiting step in the synthesis of acetylcholine (ACh), and thus possibly a more sensitive measure of cholinergic activation than ChAT activity, does not differ between young and aged animals [29,40,61]. Likewise, basal cortical acetylcholine release is unaffected by age in rats [10]. However, age-related differences in the regulation of cortical ACh efflux were demonstrated using pharmacological stimuli [19,40,41].

Although the available evidence in support of the notion that normal aging in rodents affects the function of cortical cholinergic inputs is complex, several experiments suggested the possibility that age can act as an intervening variable that may limit the capacity of residual corticopetal cholinergic basal forebrain neurons to respond to pharmacological or neurotoxic challenges [56]. Fadel et al. [10] demonstrated that, in aged rats with partial lesions of the basal forebrain cholinergic system, combined behavioral and pharmacological stimulation of residual cholinergic neurons remained ineffective. In contrast, in young rats with similar lesions, the responsivity of the residual cholinergic system remained normal (relative to their baseline). These data, similar to several other studies on the age-related effects of excitotoxin-induced lesions of the basal forebrain [44,63,66,67,71,72,74] support the general idea that aging acts

* Corresponding author. Tel.: +1-614-292-1751; fax: +1-614-688-4733.

E-mail address: sarter.2@osu.edu (M. Sarter).

to reveal the vulnerability of a damaged or abnormally developed basal forebrain cortical cholinergic input system (for discussion see [56,73]).

The present study was designed to test further the hypothesis that the effects of aging and pre-existing damage to the cholinergic system interact detrimentally. Based on an extensive literature that supports the role of cortical cholinergic inputs in attentional functions (for review see [9,55, 57]), including studies demonstrating strong correlations between the loss of cortical cholinergic inputs and impairments in sustained attention performance [35], or sustained attentional performance-induced increases in cortical acetylcholine (ACh) release [20,21], sustained attentional performance was utilized as a marker for the integrity of the cortical cholinergic input system (see also [57]). Furthermore, and in contrast to the majority of the previous studies in this field, the present experiment employed a longitudinal experimental design. Such a design has obvious heuristic advantages over studies comparing independent groups of differentially aged subjects, specifically the fact that each subject serves as its own control [54]. Furthermore, longitudinal designs limit the likelihood of invalid results that are due to comparing relatively homogeneous data from a young or adult population with the typically more heterogeneous data from aged subjects [54]. On the other hand, life-span studies of behavioral or cognitive abilities have been rarely conducted for obvious reasons, including their costly nature, and because of experimental problems such as data analyses across groups with decreasing numbers of subjects.

Widespread and nearly complete cortical cholinergic deafferentation (>80% decrease in the density of acetylcholinesterase (AChE)-positive fibers) has been demonstrated to produce substantial yet selective impairments in the sustained attentional performance of rats [35]. In the present study, cortical cholinergic deafferentation was intended to remain limited to a decrease in the density of cortical AChE-positive fibers by about 50%, and thus to produce only modest immediate effects on sustained attention performance [35,39]. If aging interacts with such a pre-existing cortical cholinergic deafferentation, performance of lesioned and sham-lesioned animals would be expected to dissociate as the animals age. Based on the data by Fadel et al. [10] that suggested that in aged animals, the excitability of residual cholinergic neurons is attenuated, the attentional performance of lesioned animals was expected to decline during aging, possibly reflecting the limited degree to which residual cholinergic neurons in aging animals can be recruited for the mediation of attentional performance.

2. Materials and methods

2.1. Subjects

Subjects included 20 Fischer/Brown Norway male rats (Harlan Sprague-Dawley, Indianapolis, IN), aged 3 months

at the beginning of the experiment. Animals were housed individually in a temperature- and humidity-controlled vivarium with a 12-h light/dark cycle (lights on at 06:30). Rats received ad libitum access to water and were food restricted to 75–85% of free feeding body weight for this strain [62]. Animals were regularly inspected by the attending veterinarian (Dr. V. Bergdall, ULAR, OSU) to ensure that potential age-related conditions did not confound the data generated by aging animals. Subjects were treated in accordance with the guidelines of the Institutional Laboratory Animal Care and Use Committee (ILACUC) at The Ohio State University in facilities accredited by the American Association of Accreditation of Laboratory Animal Care (AAALAC).

2.2. General behavioral procedures

2.2.1. Apparatus

Behavioral training occurred in twelve operant chambers (Med Associates, East Fairfield, VT). Each chamber was equipped with two retractable levers and three panel lights on one side of the chamber. A food hopper was located on the opposite side of the chamber. Operant chambers were enclosed within a sound-attenuating box that was equipped with a fan that provided ventilation and background noise. A houselight was attached to the sound-attenuating chamber above the side of the chamber with the panel lights and levers. All data collection and the execution of programs were controlled by an IBM clone computer using MED-PC software (V1.10; Med Associates).

2.2.2. Presurgical operant training

Training occurred 5 days/week between 13:30 and 16:30. After being trained to lever press for reinforcement (45 mg food pellets; P.J. Noyes Co., Inc., Lancaster, NH), animals were shaped to perform the sustained attention task. In the initial stage of training, trials were initiated by a signal (1 sec illumination of the central panel light) or a non-signal (no illumination of the central panel light) event followed two seconds later by extension of the levers into the chamber. After a signal, a left lever press was rewarded and scored as a hit and a right lever press was not rewarded and scored as a miss for half of the animals. For the remaining animals, the rules were reversed (i.e. a right lever press was correct and scored as a hit and a left lever press was incorrect and scored as a miss). After a non-signal, a right lever press was rewarded and recorded as a correct rejection and a left lever press was not rewarded and recorded as a false alarm. Again, for half of the animals the rules were reversed with a left lever press being correct (i.e. a correct rejection) after a non-signal and a right lever press being incorrect (i.e. a false alarm). Thus, hits and misses reflected accurate and inaccurate responses in signal trials, and correct rejections and false alarms reflected accurate and inaccurate responses in nonsignal trials. At this stage of training, incorrect responses were followed by a correction trial that was identical to the previous trial in order to avoid the

development of a bias for pressing a particular lever. A forced trial was introduced if rats committed three consecutive errors on correction trials. During a forced trial, after illumination of the central panel light (if the error was committed during a signal trial) or no illumination (if the error was committed during a non-signal trial), only the correct lever was extended into the chamber until it was pressed or 90 s elapsed. The intertrial interval (ITI) was 12 ± 3 s and the houselight was not illuminated during this stage of training. Criterion performance was three consecutive days of 70% accuracy during signal and non-signal trials.

After reaching criterion, the task parameters were changed in two ways. First, signals of shorter and variable duration (500, 50, or 25 msec) were presented semi-randomly within a session. Second, correction trials and forced trials were eliminated. Trials were counted as omissions if animals failed to respond within four seconds following extension of the levers. Each session lasted for 162 trials, 27 trials with each of the three signal durations and 81 non-signal trials. Rats were required to reach a criterion of at least 70% accuracy during the 500 msec signal and non-signal trials and omit fewer than 25% of the trials for three consecutive days before training on the final task. The final task was the same as the previous stage except that the houselight was illuminated throughout each session and the ITI was decreased to 9 ± 3 s. Criterion performance during this stage of training was three consecutive days of at least 60% accuracy during the 500 msec signal and non-signal trials and fewer than 25% of trials were omitted during each session. After reaching criterion, rats were pseudo-randomly assigned to 192 IgG-saporin (192-SAP)-induced lesion or sham-lesion groups, with half the animals in each group using one set of rules (e.g. reinforcement for pressing the left lever after a signal) and half the animals using the reversed set of rules.

2.2.3. Post-surgical behavioral training

After seven days of post-surgical recovery, animals were retrained on the sustained attention task. Animals were trained five days/week until they reached stable performance, defined as three consecutive sessions with hits to the 500 msec signal varying by less than 15%, correct rejections varying by less than 10%, and omissions per session varying by less than 15. After reaching stable performance, training was reduced to three days/week (Monday, Wednesday, Friday). Preliminary data indicated that in well-trained animals, this training regimen did not affect performance. This step was taken to reduce workload for this longitudinal study. Animals continued training on the sustained attention task until they reached the age of 36 months.

2.2.4. Behavioral measures

The total number of hits (h), correct rejections (cr), misses (m), false alarms (fa), and omissions were calculated for the entire session and for each block of 54 trials. Based on these values, the relative number of hits $[h = h/(h + m)]$

and of correct rejections $[cr = cr/(cr + fa)]$ was calculated. In addition, a vigilance index (VI), an overall measure of sustained attention performance, was calculated based on the relative number of hits and false alarms using the formula: $VI = (h - fa)/2 \times (h + fa) - (h + fa)^2$. This index differs from the sensitivity index derived by Frey and Colliver [11] in that omitted trials are excluded from the analysis. Values for VI can vary from $+1.0$ to -1.0, with $+1.0$ indicating no errors (i.e. all responses were scored as hits or correct rejections) and 0 indicating an inability to discriminate between signal and non-signal events.

2.3. Surgical procedures

Animals were anesthetized with ketamine (90.0 mg/kg, i.p.) and xylazine (6.0 mg/kg, i.p) and then placed in a stereotaxic instrument (Kopf, Tujunga, CA). All surgical procedures were conducted under aseptic conditions. Animals received bilateral infusions into the basal forebrain using the following coordinates (AP and ML relative to bregma, DV relative to dura): AP -0.80 mm, ML $\pm$ 2.60 mm, DV -7.20 mm (based on the atlas by Paxinos & Watson [49]). Rats in the lesion group received 0.5 μl bolus infusions of 192-SAP in Dulbecco's saline (Advanced Targeting Systems, San Diego, CA; 0.15 μg/μl; lot # 4–1). Rats in the sham group received 0.5 μl bolus infusions of Dulbecco's saline. Animals received ad libitum access to food and water for seven days before food deprivation and behavioral testing were resumed.

2.4. Histological procedures and quantification of AChE-positive fibers

Rats were perfused transcardially with cold saline followed by 4% cold paraformaldehyde. Brains were stored in 4% paraformaldehyde overnight, cryoprotected in 30% sucrose for 2–3 days, and then sectioned in a cryostat at 40 μM. A modified protocol by Tago et al. [68] was used for AChE staining. Lesioned tissue was always processed together with sham-lesioned tissue. After rinsing in 0.1 M phosphate buffer (pH = 7.4), sections were incubated in 0.1% H_2O_2 for 30 min. Sections were rinsed in 0.1 M maleate buffer (pH = 6.0) and then immersed in a solution containing: 0.0025 g potassium ferricyanide, 0.0112 g copper sulfate, 0.0221 g sodium citrate, 0.005 g of acetylthiocholine iodide, and 1.18 ml of 0.173% tetraisopropylphoramide (iso-OMPA; Sigma, St. Louis, MO) to block nonspecific butylcholinesterase staining, in 200 ml of 0.1 M maleate buffer (pH = 6.0). After rinsing with 50.0 mM Tris Buffer (pH = 7.6), staining was visualized using a solution of 0.050 g of diaminobenzidine (DAB) and enhanced with 0.375 g nickel ammonium sulfate in 125.0 ml of 50.0 mM Tris Buffer (pH = 6.2). After 10 min, eight drops of 0.1% H_2O_2 were added and sections continued to incubate until cortical layering could be detected in the sham-lesioned tissue.

A quantitative estimate of AChE-positive fiber density was used to indicate the degree of the loss of cortical cholinergic fibers following lesions. The methods used to quantify AChE-positive fiber density were similar to those in previous studies on the behavioral and histochemical effects of 192-SAP [14,35,38,39]. As delineated in the atlas by Lysakowski et al. [32], cortical areas 3B (layers II/III and V), 4 (layer V), and 41 (layers II/III and V) were sampled. A modified grid counting method, initially described by Stichel and Singer [65] was used to quantify AChE-positive fibers [22,35]. At a magnification of 25X, the focusing magnifier of a VANOX Olympus Research Microscope (Olympus America, Melville, NY) was inserted such that four orthogonal double cross lines were superimposed over the cortical region of interest. Each fiber that crossed the double lines was counted. For each cortical area, counts were made in each hemisphere for three different sections.

2.5. Statistical analyses

Data were compared from the last five sessions prior to surgery (age 16 months) and immediately after reaching stable postsurgical performance (see above for criteria for stable postsurgical performance). The effects of aging on the attentional performance of lesioned and sham-lesioned animals were assessed by comparing data from the last five sessions of the month at ages 21, 26, and 31 months. Due to the relatively small number of animals remaining in the experiment and the high number of trials omitted at age 36 months (see below), these data are reported separately and descriptively. Furthermore, the animals included in the longitudinal analyses needed to be limited to the animals for which data from the 31st month of age were available in order to treat 'age' as a repeated within-subject factor. The relative number of hits and correct rejections were angularly transformed [35,75] and then tested with mixed factor ANOVAs that included lesion, signal length (where appropriate), block of trials, and age (21, 26, and 31 months of age). The Logrank Test was used to test whether the mortality rate differed between the lesioned and sham-lesioned animals [30]. The p-values reported for within-subject main effects and interactions were corrected with the Huynh-Feldt procedure [34]. Data analyses were conducted with SPSS 10.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Body weights and mortality rates

As illustrated in Fig. 1, body weights of lesioned and sham-lesioned animals did not differ throughout the experiment (top panel). One-way ANOVAs comparing the weights of 192-SAP- and sham-lesioned groups at ages 21, 26, 31, and 36 months confirmed this observation (all F's < 1). For both groups, body weight decreased following sur-
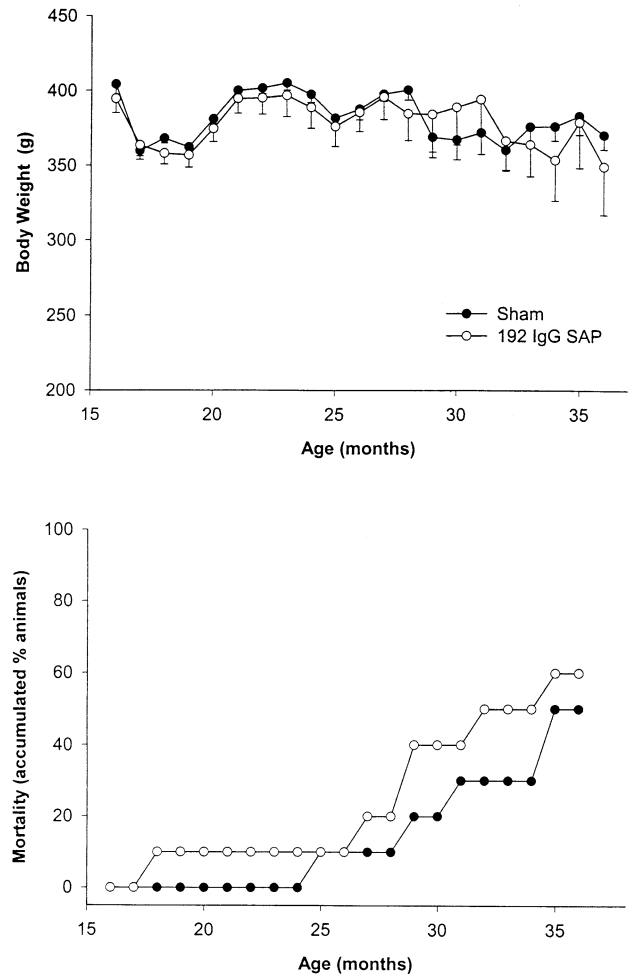

Fig. 1. Body weight (top) and mortality (bottom) of sham-lesioned and 192-SAP-lesioned animals. Body weight and rate of mortality did not differ significantly between the groups throughout the experiment (see Results section for details). Generally, body weight decreased following surgery (16 months of age) and then recovered and remained fairly stable throughout the experiment.

gery and then recovered and remained fairly stable throughout the experiment.

Twenty rats were given surgery ($N = 10$/group) when they were 16 months old. Fig. 1 (bottom panel) illustrates the mortality rate of lesioned and sham-lesioned animals. The most frequent cause of death in aging animals was kidney failure (V. Bergdall, personal communication). Importantly, the mortality rate did not differ between lesioned and sham-lesioned animals (L(1) = 0.38, $P = 0.537$). Behavioral data from animals aged 31 months were obtained from 6 lesioned and 7 sham-lesioned animals.

3.2. Pre-surgical and immediate post-surgical attentional performance

The sustained attention performance of animals assigned to receive 192-SAP lesions and sham-surgery did not differ prior to surgery. One lesioned animal that died shortly after

surgery (Fig. 1), and the perfusion of one lesioned animal yielded inadequate AChE-positive fiber staining; these two animals were not included in the analysis of presurgical performance. The absence of significant differences between the two groups of animals' ability to detect signals [$F(1,18) = 0.001$, $P = 0.973$] and to reject non-signal events [$F(1,18) = 0.067$, $P = 0.796$] indicated that the groups were well-matched prior to surgery.

As was intended by producing an only moderate loss of cortical cholinergic denervation (see histologic analyses further below), the lesioned animals' immediate post-surgery attentional performance was not robustly impaired. To assess the immediate effects of the lesion on post-surgical performance (see Methods for the criteria for stable post-surgery performance) a Time (pre- versus immediate post-surgery) X Lesion X Block X Signal Length ANOVA was conducted for VI. The first of three consecutive post-surgery sessions meeting the criteria for this analysis was session number 27.4 ± 2.7 for sham-lesioned animals and session number 32.5 ± 3.0 for lesioned animals. The overall vigilance performance, as indexed by VI, was not affected by the lesion [Lesion X Time: $F(1,16) = 1.40$, $P = 0.25$]. Furthermore, the effects of the lesion did not interact with Signal Length [$F(2,32) = 0.20$, $P = 0.82$] or with Block [$F[2,32] = 0.72$, $P = 0.50$], and there were no significant 3-way or 4-way interactions [Lesion X Time X Signal Length: $F(2,32) = 1.05$, $P = 0.36$; Lesion X Time X Block: $F(2,32) = 1.22$, $P = 0.31$; Lesion X Time X Block X Signal Length: $F(4,64) = 0.74$, $P = 0.57$]. However, lesions resulted in an increase in omissions [Lesion x Time: $F(1,16) = 4.93$, $P = 0.025$]. Inspection of the means indicated that lesioned animals continued to omit relatively few trials (20.1 ± 3.3; i.e. less than 15% of total trials omitted) but a larger number than sham-lesioned animals (9.50 ± 2.3) after reaching stable post-surgical performance.

3.3. Attentional performance through age 31 months

As described above, immediately after the 192-SAP-induced limited loss of cortical cholinergic inputs (histological analysis below), lesioned animals' performance, with the exception of a moderate increase in omissions, was not impaired. However, in the course of aging, lesioned animals' attentional performance dissociated from that of sham-lesioned animals. Specifically, the analysis of the overall attentional performance, as indexed by VI, at 21, 26, and 31 months of age, indicated a significant interaction between the effects of age, lesion and block of trials [$F(4,44) = 3.25$, $P = 0.020$; see Table 1 for all results from this analysis]. To further identify the nature of this interaction, the effects of lesion were analyzed for each age and block. This analysis did not reveal an effect of the lesion on the performance at the age of 21 and 26 months (all $P > 0.3$). However, the performance of the lesioned animals at 31 months of age, and in the third block of trials, differed

Table 1
Results of the statistical analysis of the effects of age (21, 26, and 31 months), lesion (sham vs. 192-SAP), block of trials per session (3 blocks) and signal length (3 durations) on attentional performance (indexed by the overall performance measure VI)

Age: $F(2,22) = 0.99$, $p = 0.383$
Lesion: $F(1,11) = 1.22$, $p = 0.293$
Block: $F(2,22) = 6.29$, $p = 0.007$
Signal Length: $F(2,22) = 126.3$, $p < .001$
Age × Lesion: $F(2,22) = 1.65$, $p = 0.217$
Age × Block: $F(4,44) = 1.45$, $p = 0.235$
Age × Signal Length: $F(4,44) = 2.59$, $p = 0.053$
Lesion × Block: $F(2,22) = 1.19$, $p = 0.323$
Lesion × Signal Length: $F(2,22) = 0.85$, $p = 0.440$
Block × Signal Length: $F(4,44) = 3.80$, $p = 0.010$
Age × Lesion × Block: $F(4,44) = 3.25$, $p = 0.020$
Age × Lesion × Signal Length: $F(4,44) = 1.82$, $p = 0.146$
Age × Block × Signal Length: $F(8,88) = 1.36$, $p = 0.225$
Lesion × Block × Signal Length: $F(4,44) = 0.82$, $p = 0.517$
Age × Lesion × Block × Signal Length: $F(8,88) = 0.65$, $p = 0.738$

significantly from sham-lesioned controls [$F(1,11) = 6.13$, $P = 0.03$; block 1: $P = 0.24$; block 2: $P = 0.52$].

A post-hoc Age X Signal Length X Block ANOVA was conducted in order to compare the immediate postsurgical performance (VI) of sham-lesioned rats with their performance at age 31 months. This analysis did not yield a main effect of age [$F(1,6) = 1.99$, $P = 0.21$] and age did not interact with any other factors (all p's > 0.13), suggesting that age alone did not affect performance. In order to provide a broad illustration of the performance of the aging lesioned and sham-lesioned animals, Fig. 2 depicts their performance by presenting data by signal length (although signal length did not statistically interact with the effects of lesion and age; Table 1). Fig. 3 illustrates the main significant finding of this analysis, showing that in animals aged 31 months, the lesioned animals' performance in the third block of trials was significantly lower than in sham-lesioned animals. Note that the data shown in Fig. 3 are averaged across signals. As will be discussed later, the finding that the effects of the lesion emerge as a significant variable in aging animals only in interaction with the effects of block represents the main basis for an interpretation of this interaction specifically in terms of impairments in attentional performance.

In the analysis of the relative number of hits, the effects of the lesion interacted with the effects of block [$F(2,22) = 4.38$; $P = 0.04$] but did not change in the course of aging, as indicated by the lack of a 3-way interaction [$F(4,44) = 2.05$; $P = 0.12$]. One-way ANOVAs on the effects of the lesion on block did not yield significant results but indicated a trend for a significantly lower hit rate in lesioned animals in the third block of trials [$F(1,11) = 3.86$; $P = 0.07$; Fig. 4]. The lesion did not produce any other significant effects or interactions in the analysis of hits (all $P > 0.12$). The relative number of correct rejections remained unaffected by any factor (all $P > 0.50$).

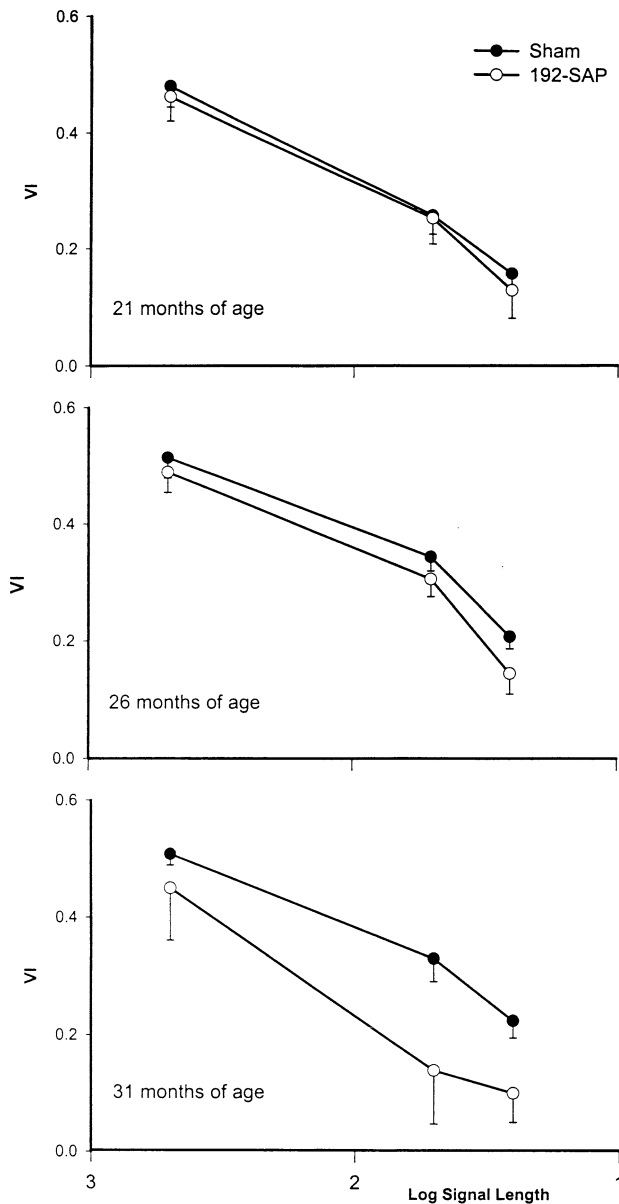Finally, age [$F(2,22) = 7.22$; $P = 0.004$] and block

Fig. 2. Sustained attention performance (as indexed by VI; see methods for details regarding calculation of VI) across log signal length of sham-lesioned and 192-SAP-lesioned animals at age 21 months (top), 26 months (middle), and 31 months (bottom). The attentional performance of lesioned animals differed from sham-lesioned animals at 31 months of age (see Table 1 and Fig. 3 for Age X Lesion X Block interaction).
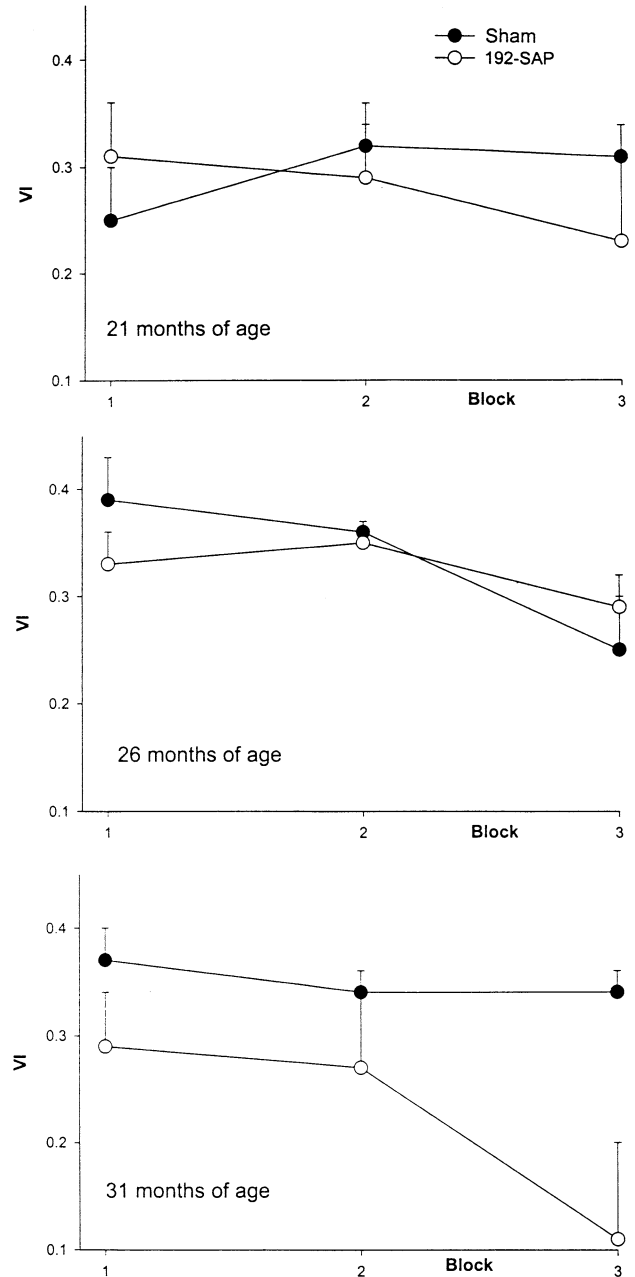

Fig. 3. Sustained attention performance (VI) across blocks of trials (abscissa), at age 21 months (top), 26 months (middle), and 31 months (bottom). At 31 months of age, and compared with sham-lesioned control animals, 192-SAP-lesioned animals exhibited a significant decrease in performance during the third block of trials within a session (see Table 1). Note that the calculation of VI, and thus the interaction between the effects of lesion, age and block, was not confounded by errors of omission. This interaction between the effects of lesion, age and block forms the basis for an interpretation of the effects of aging on lesioned animals' performance specifically in terms of an impairment in sustained attentional processing.

$[F(2,22) = 6.55; P = 0.007]$, but not lesion $[F(1,11) = 3.68) P = 0.08]$ produced main effects on omissions. With increasing age, animals omitted more trials (21 months: 12.8 ± 3.0 omissions; 26 months: 21.8 ± 4.7 omissions; 31 months: 42.7 ± 7.0 omissions). Generally, omissions increased in the course of a session (block 1: 6.0 ± 1.1 omissions; block 2: 7.9 ± 1.5 omissions; block 3: 11.8 ± 1.7 omissions). Furthermore, the effects of lesion and block interacted significantly $[F(2,22) = 5.62; P = 0.01]$, reflecting the lesion-induced augmentation of the number of omissions in blocks 1 and 2 [block 1: $F(1,11) = 7.86, P = 0.017$;

block 2: $F(1,11) = 5.23, P = 0.043$; block 3 $F(1,11) = 2.27, P = 0.160]$. Moreover, the effects of all three factors also interacted significantly $[F(4,44) = 3.58; P = 0.03]$, but post-hoc analysis failed to reveal further the nature of this interaction.
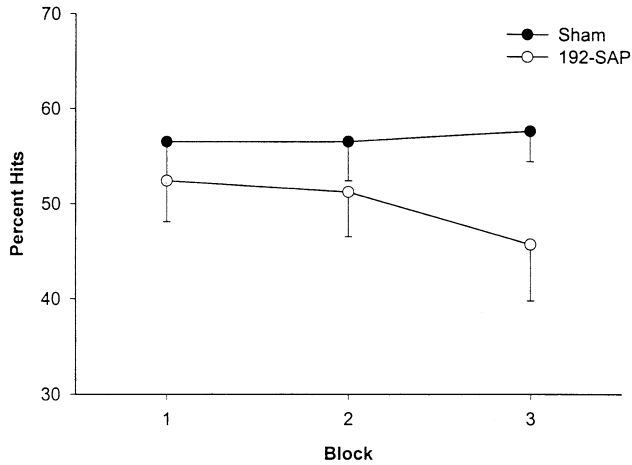
Fig. 4. Percentage of hits (ordinate) across the three task blocks (abscissa) in lesioned and sham-lesioned animals at 31 months of age. The block-dependent decline in VI in lesioned animals shown in Fig. 3 primarily reflected a decrease in the detection of signals (for details see Results section).

3.4. Sustained attention performance at age 36 months

Only descriptive analyses are presented for sustained attention performance at age 36 months due to the small number of animals remaining in the experiment and the high number of trials omitted by these animals. Four lesioned and four sham-lesioned rats continued to respond in the sustained attention task at 36 months of age. The number of omissions in either group was similar and relatively high (sham: 105.7 ± 18.0 omissions/session; 192-SAP: 99.17 ± 21.3 omissions/session). The percentage of hits for the 192-SAP group continued to be lower than for the sham group at 36 months (sham/192-SAP; 500 msec: $73.8 \pm 9.5\%/54.0 \pm 11.0\%$; 50 msec: $67.3 \pm 6.5\%/38.4 \pm 9.5\%$; 25 msec; $54.8 \pm 9.5\%/29.7 \pm 3.1\%$, 25 msec. Furthermore, the correct rejection rate of the sham-lesioned animals appeared lower ($51.6 \pm 7.2\%$) than in lesioned animals ($77.8 \pm 4.7\%$).

3.5. Histological analyses

Using procedures similar to those described in McGaughy et al. [35], an estimate of the number of cortical AChE fibers was attained to evaluate the extent of the 192-SAP-induced loss of cortical cholinergic inputs. The number of AChE-positive fibers could not be determined for one lesioned rat that died of natural causes at age 29 months, and thus the data from this animal were eliminated from any post-surgical statistical analyses. As intended, this dose of 192-SAP produced a relatively modest 40–60% decrease in cortical AChE-positive fibers (see Table 2 for details; Fig. 5).

4. Discussion

The present longitudinal study was designed to characterize the interactions between the attentional effects of partial cortical cholinergic deafferentation and aging. The lesions resulted in partial loss of cortical cholinergic inputs and, as intended, did not produce immediate significant effects on post-surgery performance. However, at 31 months of age, the lesioned animals' attentional performance in the last block of trials was significantly impaired when compared with sham-lesioned animals. At 36 months of age, age-related differences between the performance of sham-lesioned and lesioned animals were confounded by the increasing mortality rate and high numbers of errors of omissions. Below, the discussion will focus on (1) the attentional nature of the interactions between lesion and age, (2) the stable performance of sham-lesioned, aging animals and the possible role of long-term caloric restriction, (3) the potential mechanisms mediating the interactions between the effects of aging and lesion, and (4 the significance of the present data for the understanding of role of the basal forebrain cholinergic system in age- and dementia-associated decline in cognitive functions.

4.1. The attentional nature of the interaction between the effects of lesion and aging

A major core component of the psychological construct of sustained attention refers to the decline of performance over time-on-task. Such performance decrements hypothetically reflect the depletion of attentional capacities and, like no other measure of sustained attention performance, are devoid of potential non-attentional confounds [25,46,48]. For example, in rats, as in humans, such decrements can be provoked or augmented by increasing the frequency of signal and non-signal events [36]. Thus, the present finding

Table 2
Cortical AChE-positive fiber density: group data (mean $\pm$ S.E.M.)

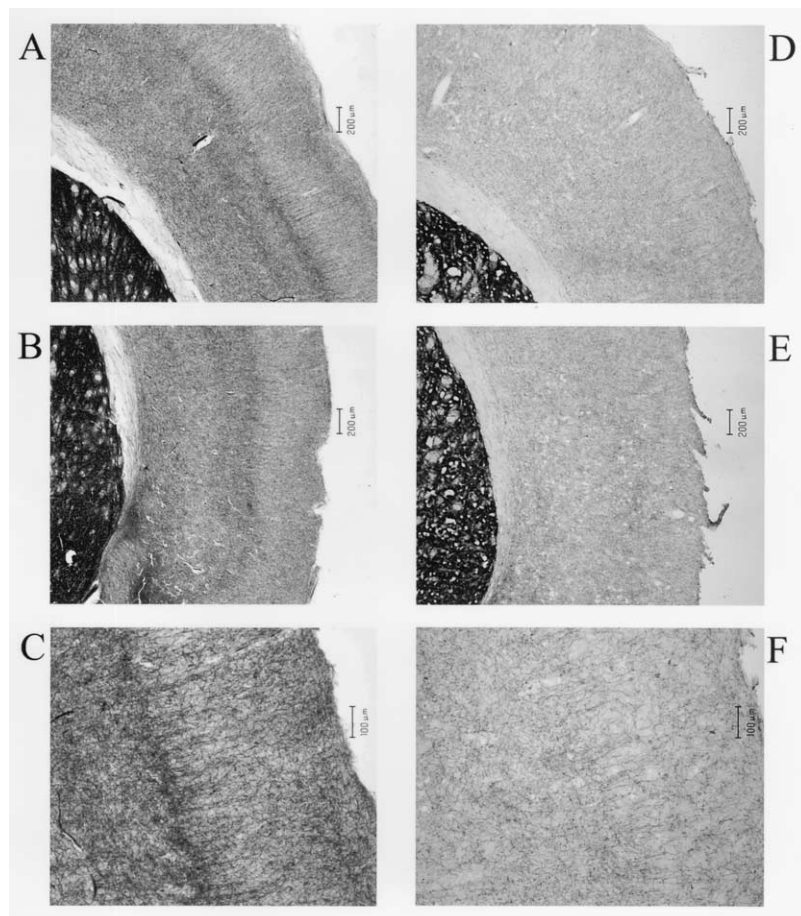| Cortical area:	4 (frontal cortex)	3b (somatosensory cortex)		41 (temporal cortex)	
Layer:	V	II/III	V	II/III	V
Sham-lesioned:	28.7 ± 1.1	29.8 ± 1.1	29.2 ± 1.1	27.4 ± 1.2	27.6 ± 1.3
192-SAP-lesioned:	10.9 ± 1.5	11.0 ± 1.8	11.8 ± 1.5	14.9 ± 1.8	15.9 ± 1.9
% Δ	-62.0	-63.1	-59.5	-45.7	-42.5

Fig. 5. Cortical AChE-positive fibers in sham-lesioned (left column) and 192-SAP-induced lesioned (right column) animals. The degree of AChE-positive fiber loss was fairly consistent (approximately 40–60%) across cortical regions, including the somatosensory cortex (A and D), temporal cortex (B and E; see Table 2 for details; 200 μm scale inserted in A, D, B, and E). C and F are higher magnifications (100 μm scale inserted) of the somatosensory cortex and clearly indicate the moderate loss of cortical cholinergic inputs produced by infusions of 192-SAP.

that the interaction between the effects of age and lesion manifested specifically as a decrease in performance in the third block of trials represents the strongest basis for an interpretation of the performance change in terms of an impairment in sustained attention performance. It should be reiterated that the behavioral measures underlying this interaction were not confounded by omissions (see Methods), and that therefore it is unlikely that this interaction may be explained alternatively by any trivial behavioral or cognitive mechanisms.

The size of the decline in the performance of lesioned, aging animals from blocks 1 and 2 to the last block of trials was substantial. In terms of differences in VI, this decline in fact was larger than the immediate and lasting effects of extensive lesions of the cortical cholinergic input system on attentional performance [35]. In other words, the within-session decline in the performance of aging animals with pre-existing moderate loss of cortical cholinergic inputs reached a level that was at least comparable to the immediate effects of lesions of over 80% of all cortical cholinergic inputs. Thus, the present data suggest that, in the course of aging, the initial absence of attentional impair-

ments in lesioned animals developed into a decline in within-session performance that mirrored the immediate consequences of a more complete loss of cortical cholinergic inputs.

The interaction between the effects of age and prior lesion was observed on all animals and not just in a sub-group of animals that was *ex post facto* classified as impaired. Many studies on the cognitive effects of aging suggested the need to differentiate aging populations by performance; this perspective supports the limited role of age as a main, independent variable (see the discussion in [5]). In contrast, however, the present data suggest that in terms of attentional performance, the consequences of aging for the performance of animals with pre-existing insult to the cholinergic system is mediated via common mechanisms of aging, at least for the particular strain of rats used in this experiment.

4.2. Absence of age-related changes in attentional impairments in sham-lesioned animals

Sham-lesioned animals maintained a consistent and high level of performance through the age of 31 months. This

observation is important because it allows the attribution of the age-related emergence of performance differences between the two groups exclusively to changes in the lesioned animals' performance. Furthermore, given the close relationship between the integrity of the cortical cholinergic input system and attentional performance [20,37–39], this finding corresponds with the limited degree to which age per se affects the ability of cortical cholinergic inputs to respond to activating stimuli [56].

Previous studies on the attentional capabilities of aging rats likewise did not demonstrate reliable effects of age. Using a precursor to the present task, we previously did not find differences between the performance of 12, 21, and 26 months old Fisher/BNNia rats [37,69]. Similarly, Grilly et al. [17] did not find effects of age on the performance of rats in a two-choice stimulus detection task (see also [66]). The study by Muir et al. [42] represents an exception in that a decrease in the performance of a five choice serial reaction time task was observed in 23–24 month-old female Sprague Dawley rats.

Similar to the discussion in Grilly et al. [17], it cannot be excluded that persistent caloric restriction was a factor contributing to the consistently high level of attentional performance in aged animals. However, the popular idea that caloric restriction generally attenuates the behavioral or cognitive effects of age has not been consistently supported [33]. Furthermore, the beneficial effects of caloric restriction on the mortality rate of rodents were typically observed in animals much more severely food deprived than was the case for the animals in the present study [7,15,62,64]. Thus, although data to exclude this possibility are not available (such data in fact would be very difficult to produce as some deprivation is required to motivate task performance), it seems unlikely that moderate caloric restriction as implemented in this study contributed significantly to the stable performance of aging rats.

4.3. Mechanisms mediating the interactions between age and partial cortical cholinergic deafferentation

The neuronal mechanisms underlying the interaction between aging and pre-existing loss of cortical cholinergic inputs to impair sustained attention performance are a matter of speculation. The data from previous studies assessing age-related changes in cortical ACh indicated that, rather than age affecting the functions of cholinergic neurons directly, age may affect the cortical regulation of ACh release by other cortical, non-cholinergic inputs to cholinergic terminals [41]. Recent studies in our laboratory substantiated the possibility that cortical GABAergic mechanisms may be facilitated during aging [19]. Although the status of cortical GABAergic transmission in aged rats remains to be determined by direct assessments, other studies showing decreases in cortical muscimol-binding [2] or increases in $GABA_A$ currents [16] appear to be in agreement with the possibility that during aging, increases in GABAergic trans-

mission emerge. In interaction with a reduced cortical cholinergic input system, such increases in cortical GABAergic transmission may manifest as a limited reactivity of cortical cholinergic inputs [10], or as impairments in cognitive functions that depend on the normal recruitment of the (residual) cortical cholinergic input system (present study). Such an age-related increase in the cortical inhibitory regulation of a residual cholinergic input system may have morphological correlates, as suggested by Wellman and Sengelaub ([72]; see also [71]).

It cannot be excluded that the neuronal mechanisms mediating the interactions between the effects of age and prior partial loss of cortical cholinergic inputs include alterations in other components of basal forebrain circuits, including changes in the afferent regulation of residual basal forebrain cholinergic neurons, or even alterations in neuronal systems entirely unrelated to the basal forebrain cholinergic system. The partially damaged basal forebrain cholinergic system may have exacerbated the onset of other age-related neuropathological changes. For example, intraventricular infusions of 192 IgG-saporin have been shown to increase cortical amyloid precursor protein expression [28] and activate microglia in the basal forebrain [53]. Such consequences of the lesions may have interacted with the neuronal effects of aging to impair the functioning of the telencephalic circuitry mediating attentional processing.

4.4. General significance for the understanding of the role of aging in the decline in cognitive functions

Persistent and escalating impairments of executive functions, specifically in the abilities to select, discriminate, and process significant stimuli and associations, and to allocate processing resources to competing cognitive activities, are hypothesized to contribute to the development of age-related cognitive impairments, including impairments in the acquisition of new materials and in the recall of memories [47,51,58]. Clearly, however, age-related cognitive impairments do not occur universally, and the chronological age of a subject per se is a poor predictor of such impairments. Thus, concerning cognitive abilities, age appears to act largely as an intervening variable, the neuronal consequences of which presumably require interactions with other neuropathological processes to yield age-related impairments in cognitive abilities. The nature of these neuropathological processes remains speculative. The possibility that developmental events that interfere with the maturation of cholinergic and/or other neuronal systems [3] suffice to initiate age-related decline in cognitive functions is intriguing. Likewise, the nature of the age-related neuronal mechanisms that reveal the existence of prior neuropathological processes is unclear. To the extent the manifestation of age-related cognitive impairments are a result of interactions between pre-existing neuropathological processes and aging, research on the neuronal mechanisms mediating age-related cognitive disorders need to consider the conse-

quences of neuronal events occurring across the entire life span, and thus to integrate approaches traditionally more typical for research in developmental neuroscience.

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