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Neonatal 192 IgG-Saporin Lesions of Basal Forebrain Cholinergic Neurons Selectively Impair Response to Spatial Novelty in Adult Rats

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The role of the developing cholinergic basal forebrain system on cognitive behaviors was examined in 7 day-old rats by giving lesions with intraventricular injections of 192 IgG-saporin or saline. Rats were subjected to passive avoidance on postnatal days (PND) 22–23, water maze testing on PND 50–60, and a open-field test (in which reactions to spatial and object novelty were measured) on PND 54. Behavioral effects of the lesions were evident only in the open-field test with 5 objects. Unlike controls, the lesioned rats did not detect a spatial change after a displacement of 2 of the 5 objects. Control and lesioned rats, however, showed comparable novelty responses to an unfamiliar object. Lesion effectiveness was confirmed by 75% and 84% decreases in choline acetyltransferase activity in cortex and hippocampus. These results suggest that the developing cholinergic system may be involved in spatial information processing or attention to spatial modifications.

A considerable amount of data amassed during the past two decades has suggested an important role of the central cholinergic system in learning and memory in adult rodents and humans (for reviews, see Coyle, Price, & DeLong, 1983; Fibiger, 1991). Lesions of basal forebrain (BF) cholinergic neurons, or of their projections, produce severe deficits in behavioral tests assessing learning and memory in rodents (Dunnett, Everitt, & Robbins, 1991). Interpretation of these studies, however, has been hampered by a lack of specificity in the lesion techniques used in them. Recently, a specific cholinergic toxin has become available, thus providing new opportunities to study the cognitive role of the central cholinergic system (Everitt & Robbins, 1997; Wenk, 1997). This toxin consists of a monoclonal antibody to the low affinity nerve growth factor receptor (NGFr), 192 IgG, that is coupled with the ribosome-inactivating protein saporin. The immunotoxin exploits the fact that most cholinergic BF neurons express high levels of the NGFr relative to other cholinergic and noncholinergic neurons in nearby regions (Woolf, Gould, & Butcher, 1989; Yan & Johnson, 1988). When injected intracerebroventricularly in vivo, 192 IgG-saporin (192 IgG-sap) selectively destroys neurons bearing NGFr (Book, Wiley, & Schweitzer, 1994; Wiley, 1992). In adult rats, intracerebroventricular administration of 192 IgG-sap results in learning impairments on several behavioral tasks, including water maze, passive avoidance (PA), radial maze, and delayed non-match-to-sample (Berger-Sweeney et al., 1994; Leanza, Nilsson, Wiley, & Björklund, 1995; Steckler, Keith, Wiley, & Sahgal, 1995; Waite, Wardlow, Wiley, Lappi, & Thal, 1995; Walsh et al., 1995; Zhang, Berbos, Wrenn, & Wiley, 1996). After more focused intraparenchymal BF lesions, however, learning impairments are considerably less severe or nonexistent (Berger-Sweeney et al., 1994; Torres et al., 1994; Baxter & Gallagher, 1996; Dornan et al., 1997; Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996). The discrepancies in the magnitude of saporin-induced cognitive deficits between intracerebroventricular and intraparenchymal immunotoxin administration have been primarily ascribed to the saporin-induced loss of cerebellar Purkinje cells, a neuronal population that is also NGFr positive in the adult rat brain. Damage to these cells can affect motor skills and therefore confounds the observation of saporin-induced cognitive changes on many tasks.

Few studies, however, have used 192 IgG-sap to investigate the role of the developing cholinergic system in regulating the development of the neocortex, hippocampus, and cognitive functions. This information is particularly important for two reasons. First, developmental effects of neonatal selective cholinergic lesions can clarify the role played by BF on the onset of cognitive capabilities, a role that not is necessarily equal to that played by the same nuclei at adulthood. Indeed, our previous studies in mice suggest that neonatal damage to the BF produces long-lasting impairments in spatial navigation acquisition and retention, impairments more severe than those reported as a result of

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comparable adult lesions (Arters, Hohmann, Mills, Olaghere, & Berger-Sweeney, 1998; Bachman, Berger-Sweeney, Coyle, & Hohmann, 1994; Berger-Sweeney & Hohmann, 1997). Furthermore, neonatal BF damage produces synaptic reorganization that has not been reported after adult lesions (Hohmann & Berger-Sweeney, 1998). Second, neonatal lesions of the cholinergic BF can be considered an experimental model of clinical relevance. Indeed, cholinergic abnormalities have been documented in several developmental disorders in humans, including Down and Rett syndromes as well as after perinatal exposure to environmental contaminants (Marin-Padilla, 1976; Kaufmann, Taylor, Hohmann, Sanwal, & Naidu, 1997; Takashima, Becker, Armstrong, & Chan, 1981; Wenk, Naidu, Casanova, Kitt, & Moser, 1991; Winneke, Lilienthal, & Kramer, 1996).

The few studies available on the effects of neonatal 192 IgG-sap have documented that this treatment also successfully targets NGFr cholinergic BF neurons during development, inducing a marked and long-lasting selective loss of cholinergic markers in both the cortex and hippocampus (Leanza, Nilsson, Nikkhah, Wiley, & Bjšrklund, 1996; Pappas et al., 1996; Ricceri, Calamandrei, & Berger-Sweeney, 1997; Robertson et al., 1998; Sherren, Pappas, & Fortin, 1999). Moreover, intracerebroventricular 192 IgG-sap injections given on postnatal days (PND) 1 and 3 have resulted in abnormalities of the cortical cytoarchitecture after neonatal BF immunolesion, thus confirming a morphogenetic role for the cholinergic afferents in cortical development (Robertson et al., 1998).

It is important to note that all these developmental studies used intracerebroventricular rather than intraparenchymal 192 IgG-sap administration. Previous researchers have chosen this route of administration because, in contrast with the extensive Purkinje cell loss seen after intracerebroventricular 192 IgG-sap administration in adult rats, no Purkinje cell loss has been reported after neonatal treatments (Leanza et al., 1996). This difference is crucial: It renders the neonatal intracerebroventricular immunolesion procedure a powerful tool to evaluate cholinergic-regulated behavioral and neurochemical functions without confounding interferences that are due to cerebellar damage.

We therefore used the neonatal intracerebroventricular 192 IgG-sap lesion paradigm to verify two different hypotheses. The first one concerns the nature (transient or permanent) of learning deficits previously found in 15-day-old rats after neonatal cholinergic lesions on PND 7 (Ricceri et al., 1997). We hypothesised that the PA deficits in acquisition rate previously found in 15-day-old rats lesioned on PND 7 would be still detectable on PND 22, that is, postweaning. We initially chose the PA task because it is one of the few learning tasks that can be performed by 15-day-old rats and because cholinergic control of PA performance has been documented extensively during development (Dumery, Derer, & Blozovski, 1988; Calamandrei, Ricceri, & Valanzano, 1996). We did not assess the effects of neonatal 192 IgG-sap treatments at adulthood because adult rats acquire the PA task very rapidly (only one trial is usually needed), therefore, decrements in acquisitions rates are difficult to detect in adults.

The second hypothesis concerns the involvement of the cholinergic system in spatial acquisition during development. We hypothesized that after neonatal cholinergic lesions long-term deficits in rat spatial behavior would be apparent, despite the occurrence of synaptic reorganizing phenomena. This hypothesis is in contrast to previously published data. To date, three studies have examined spatial learning after developmental cholinergic BF lesions, and none of these studies reported dramatic long-term spatial memory deficits in lesioned rats, and only one reported longer latencies in finding the hidden platform in the initial phase of training in lesioned rats (Leanza et al., 1996; Pappas et al., 1996; Sherren et al., 1999). All three studies, however, used only the Morris water maze to evaluate spatial learning. This task may not be the best one for examining the contributions of the cholinergic system to spatial memory. The relatively high degree of stress associated with the task may mask subtle spatial deficits that are due to alteration of the BF cholinergic function (Everitt & Robbins, 1997; McMahan, Sobel, & Baxter, 1997). We therefore evaluated spatial performances of neonatally lesioned rats in a second spatial task, the spatial open-field. This is a nonaversive task in which spontaneous responses to a spatial rearrangement of familiar objects, as well as to a novel unfamiliar object, are measured (Poucet, 1989; Thinus-Blanc, Save, Rossi-Arnaud, Tozzi, & Ammassari-Teule, 1996; Usiello et al., 1998). Responses to spatial novelty are altered in this open-field task after medial septal lesions and pharmacological cholinergic blockade (Buhot, Soffie, & Poucet, 1989; Poucet, 1989), which suggests that performance in this task may be sensitive to removal of cholinergic input to the hippocampus and neocortex.

At the conclusion of behavioral testing, we measured choline acetyltransferase (ChAT) activity in three regions (cortex, hippocampus, and striatum) in eighteen 60-day-old brains to quantify the extent of the lesion-induced cholinergic loss in BF targets. In other brains, we performed ChAT immunostaining to assess lesion-induced cholinergic damage in the BF.

Method

Subjects and Toxin Administration

We purchased Wistar rats from Charles River Italia (Calco, Italy) and kept them in an air-conditioned room at 21 ± 1 °C and 60% ± 10% relative humidity, with a white–red light cycle (white light on from 8:30 a.m. to 8:30 p.m.). Males and multiparous females were housed in couples in 42 × 27 × 14 cm Plexiglas cages with metal tops and sawdust bedding. Pellet food (enriched standard diet purchased from Mucedola, Settimo, Milanese, Italy) and tap water were continuously available. Two weeks after the rats' arrival, we grouped them into 16 breeding pairs and housed these pairs together. After 10 days, the females were individually housed and inspected daily at 9:30 a.m. for delivery (PND 1).

Ten litters were culled at birth to 6 males and 2 females; both sexes were left in the litter to avoid maternal behavioral biases, as well as long-term effects on the offspring, induced by the presence of pups of one sex only (for a review see Laviola & Terranova, 1998). However, only male pups were used in the following experiments. The male pups in each litter were randomly assigned

to either the vehicle (phosphate-buffered saline [PBS] 0.1M; $n = 3$ in each litter) or the neurotoxin (IgG 192-sap, Chemicon; $n = 3$ in each litter) treatment condition. On PND 7, between 9 a.m. and 10 a.m., one group of pups received an intracerebroventricular injection of 0.84 μg of 192 IgG-sap dissolved in PBS (injection volume 2 μl); this dose was effective in previous neonatal lesion studies (Ricceri et al., 1997). Control littermates received a similar injection of saline. We injected pups under hypothermic anesthesia by a direct transcutaneous, 2.2-mm-deep puncture using a glass needle (gauge 0.3 mm) placed approximately 1.2 mm anterior to the interaural line along the sagittal suture. Dye control injections performed on 7-day-old rats showed that this procedure was an effective means of administering liquid into the third ventricle and that the liquid was then distributed throughout the ventricular system.

Passive Avoidance Testing (PND 22–23)

The avoidance apparatus, originally designed for mice (Ugo Basile, Comerio, Italy), consisted of a tilting-floor Plexiglas cage divided into two compartments (the start and the escape compartments, 18 × 9.5 × 16 cm each) by a sliding partition door. The start compartment was white and illuminated by a white light located on the top, whereas the escape compartment was black and stayed dark. The metallic grid floor (bars of 0.3 cm in diameter spaced 5 mm apart) was connected to a scrambling shocker set at 0.4 mA. We performed avoidance tests between 9:30 a.m. and 12:30 p.m., that is, during the initial portion of the light period. The procedure consisted of two phases, acquisition and retention, which took place on two consecutive days.

Acquisition phase (PND 22). Two control and 2 192 IgG-sap pups from each litter were randomly assigned to one of the two testing conditions (conditioned or nonreinforced group). We placed conditioned pups individually into the start compartment facing away from the doorway. The sliding door between the compartments was raised, and the pup was allowed to cross into the dark chamber. When the pup crossed (4-paw criterion), lowering the tilting floor, the door was shut and a 3-s, 0.4 mA footshock was delivered to the grid floor. The trial ended when the rat performed the step-through response or remained in the start compartment for 120 s, whichever event occurred first. At the end of each trial, we removed pups from the apparatus and left them undisturbed for a 45-s intertrial interval. The acquisition phase ended either when the subject had remained in the start compartment for two consecutive trials (learning criterion), or after 10 trials ended by stepping-through. If the learning criterion was achieved before the 10th trial, then all remaining trials (to a total of 10) were considered as 120-s latencies. Rats assigned to the nonreinforced control group were subjected to a similar multitrial session in the same apparatus, but step-through responses were not punished by footshock. We used the number of trials needed to achieve the learning criterion and the latency to step-through as measures of learning.

Retention phase (PND 23). The procedure, which was identical for conditioned and nonreinforced pups, consisted of a single trial not punished by footshock. The retention trial ended when the pup either gave the step-through response or remained in the start compartment for 120 s. Latency to step-through was used as a measure of retention.

Water Maze (PND 50–60)

In this phase of the experiment we used 1 control and 1 192 IgG-sap pup from each litter (from the nonreinforced groups of the PA experiment). The pool used for spatial navigation trials was 150 cm in diameter and constructed of black acrylic. The pool was located in a room surrounded by cues external to the maze, such as geometric paintings that could be used by the rat to guide its navigation in the pool. The pool was filled with 24 °C water, and a round acrylic platform (15 cm in diameter) was placed 15 cm from the edge on the pool, in the northeast quadrant. For the spatial navigation trials, we submerged the platform 4 cm below the surface of the water. For the cued navigation trials, we placed the platform 4 cm above the surface of the water. We recorded the rat's movement in the pool by using the computer-based video tracking system Ethovision (Noldus Information Technology, 1995), which provided path length and speed data. Water maze tests were performed between 9:30 a.m. and 12:30 p.m.

Spatial navigation (invisible platform and probe trial; PND 50–54). We used four equally spaced points around the edge of the pool as starting positions; we arbitrarily designated these points as north, south, east, and west. Each rat was given four trials per day, one from each of the start positions. Each rat was placed into the water facing the edge of the pool, but not touching it. The rat then had a maximum of 120 s to find and escape onto the platform. If the rat did not find the platform in the maximum time allotted, then the experimenter placed it on the platform. Latency, path length, and percentage of time in each quadrant of the pool were monitored. At the end of the four trials, the rat was removed from the pool, dried, and returned to its home cage. The spatial task was conducted for 5 consecutive days. On the 5th day, the spatial trials were followed by a single probe trial. During the probe trial, the platform was removed from the pool. Each rat was started from the north position, and its movement was tracked for 30 s.

Reversal learning (PND 55–57). The reversal-learning testing protocol was similar to that used during the previous test, except that the platform was located in the southwest quadrant. Each rat was placed in the pool, facing the wall, and was given a maximum of 120 s to find the platform. Each rat was given four trials per day, one from each of the start positions. We conducted the reversal task for 3 consecutive days.

Cued navigation (visible platform with platform relocation; PND 58–60). Two days after the completion of reversal testing, we began the cued navigation task. The testing protocol was similar to that used during the previous test, except that the platform was made visible above the surface of the water. On the 1st day of the cued task, the platform was located in the same quadrant that was used in the reversal learning procedure. On the subsequent 4 days, the platform was relocated in different quadrants of the pool, with a new position each day. Each rat was placed in the pool, facing the wall, and was given a maximum of 120 s to find the platform. We conducted four trials each day, one from each starting position, for 4 consecutive days.

Open Field With Objects (PND 54)

We used 1 control and 1 192 IgG-sap pup from each litter in the open-field task. The apparatus (Figure 1) was a black circular open-field arena, 120 cm in diameter with a 30-cm-high plastic wall. We placed the arena into a sound-proof cubicle and surrounded it with a visually uniform environment except for a striped pattern (20 × 10 cm) on the wall of the field. The apparatus was illuminated by a red light (80 W) located on the ceiling. A video camera above the field was connected to a video recorder.

Five different objects were simultaneously present in the open field: Object A, a gray plastic cylinder with a metal grid wall (height 15 cm; diameter 13 cm); Object B, a black plastic cylinder with a metal grid wall (height 12 cm; diameter 8 cm); Object C, a blue plastic parallelepiped (4 cm high × 13 cm wide × 9 cm long) with holes regularly distributed on the top; Object D, a transparent

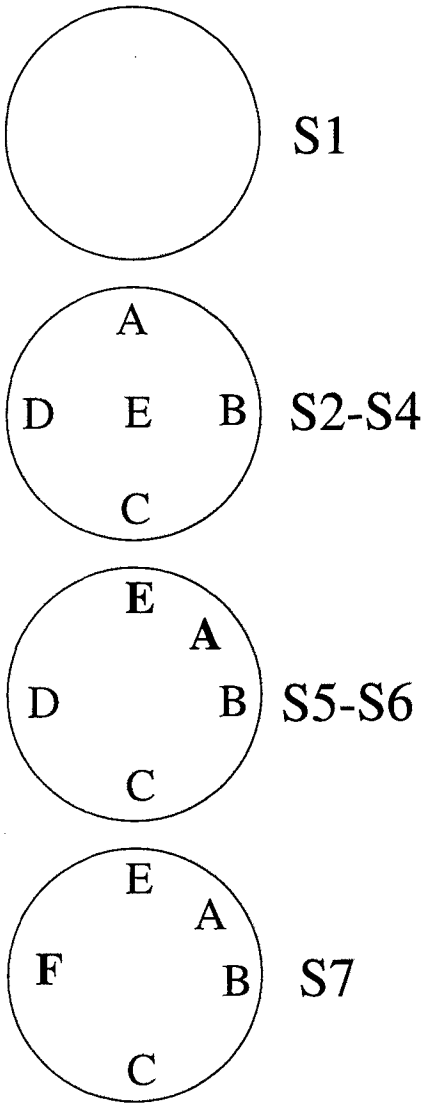

Figure 1. Schematic diagram of the apparatus and object positions throughout the seven sessions (S) of the open-field test. The arena was empty in Session 1. From Session 2 to Session 4, five objects (A, B, C, D, E) were placed in the arena. In Sessions 5 and 6, two objects were displaced (A and E). In Session 7, one of the objects (D) was replaced by an unfamiliar one (F).

Plexiglas cube with holes irregularly distributed on the sides (height = 10 cm); Object E, a black-and-white-striped plastic cylinder (height = 13 cm; diameter = 6 cm). The initial arrangement was a square with a central object (E), as illustrated in Figure 1. A sixth object (F), which consisted of two red plastic squares (10 × 10 cm, regularly pierced) forming a 90° angle, was used to examine reaction to nonspatial change.

Behavioral procedure. We performed open-field tests between 9:30 a.m. and 2:30 p.m. Rats were individually submitted to seven successive 6-min sessions, separated by 3-min delays during which the subjects were returned to their home cage. During Session 1 we placed each rat into the empty open field to allow it to become familiar with the apparatus and to record a baseline level of locomotor activity. During Sessions 2–4 the objects were placed as shown in Figure 1. In Session 5, the spatial test session, the configuration was changed by moving two objects: Object E replaced Object A, which was itself displaced at the periphery of the apparatus so that the initial square arrangement was changed to a polygonal arrangement. In Session 6 (S6), we left the configuration of the objects unchanged to let the rats habituate to the new arrangement of the objects. In the last session one of the familiar, nondisplaced objects (D) was replaced by a new object (F) at the same location.

Data collection. Data collection was performed by using a video recorder and The Observer, a software system for the collection and analysis of behavioral data (Noldus Information Technology, 1993). During the first session, we measured the frequency of the following responses: rearing (standing on hind legs), wall rearing (standing on hind legs and placing forelimbs on the wall of the arena), and crossing (crossing the annulus in which the floor of the arena was subdivided on the monitor). From Sessions 2 to 7, object exploration was measured as time spent by the rat in contact with an object. A contact was defined as subject's snout or forelimbs actually touching an object. Habituation to the objects was assessed by averaging the duration of contacts with the objects during Sessions 2–4 in each group. In Session 5, the spatial arrangement of the objects was modified and response to spatial change was assessed by comparing the mean time spent in contact with displaced objects and nondisplaced objects in Session 5 minus the mean time spent in contact with the same object category in Session 4. Finally, in Session 7, the response to the nonspatial novelty was assessed by comparing mean time in contact with the substituted object and familiar, nonsubstituted objects in Session 7 minus the mean time spent with objects located in the corresponding position in Session 6. Data collection was performed by an investigator unaware of the treatment.

ChAT Activity (PND 60)

Eighteen rats were guillotined and the brains rapidly removed onto an ice-cooled metal plate. Fronto-parietal cortex was dissected bilaterally, followed by the hippocampus and striatum. Dissected samples were immediately frozen on dry ice and stored at −70 °C until the time of neurochemical assay; all assays were performed in triplicate. All reagents were obtained from Sigma Chemical (St. Louis, MO), except where indicated. ChAT activity measurements were based on the method of Fonnum (1975). Briefly, tissue samples (50 mg) were homogenized by sonication in 50 mM Tris buffer (pH 7.4) containing 0.2% Triton X-100 (dilution 1:100 wt/vol). Homogenates were spun at 10,000 × g for 10 min. Aliquots of the supernatants were incubated for 20 min at 37 °C in a mixture containing (final concentrations): 0.02 μCi 14C-acetyl coenzyme A (4 × 105 cpm, 55.9 μCi/mole; New England Nuclear, Boston, MA), 225 μM acetyl coenzyme A, 8 mM choline bromide, 100 μM physostigmine, 10 mM ethylenediamine tetraacetic acid, 0.05% bovine serum albumin, 0.3 M NaCl, and 50 mM Na-phosphate (pH 7.4). The acetylcholine product was separated from the acetyl CoA substrate by means of an organic–inorganic separation using Kalignost solution (5g Na-tetraphenylboron in 850 ml toluene and 150 ml acetonitrile). The supernatant was then transferred to a scintillation cocktail and radioactivity was measured. Protein content was determined according to the method of Bradford (1976), and ChAT activity was calculated as nmol ACh/hr/mg protein.

ChAT Immunocytochemistry (PND 60)

Four rats (2 controls and 2 192 IgG-sap-treated rats) were given an overdose of Nembutal before being perfused transcardially with

200 ml cold PBS 0.1 M, followed by 400 ml cold 4% (wt/vol) paraformaldehyde in PBS 0.1 M. The brains were removed and placed in fresh paraformaldehyde for 6 hr at 4 °C. The brains were then transferred to a 30% (wt/vol) sucrose solution in 0.1 M PBS and left overnight at 4 °C. Coronal sections (30 μm) of the forebrain were cut on a freezing microtome. The free-floating sections were rinsed in PBS plus 0.1% Triton X-100 (PBS-T), treated with 5% normal rabbit serum, and then incubated at 4% overnight in an anti-ChAT monoclonal antibody (Boheringer, Mannheim, Germany; 1:3.5 dilution in PBS-T). After rinsing with PBS-T, sections were incubated for 1 hr in a rabbit anti-rat IgG and then in avidin-biotin-horseradish peroxidase (Vectastine Elite Kit, Vector Laboratories, Burlingame, CA). After rinsing in PBS-T, sections were incubated at room temperature in 0.05% (wt/vol) diaminobenzidine (Sigma), and then in the same solution containing 0.005% (vol/vol) hydrogen peroxide in the dark. Following additional rinsing, sections were mounted on gelatin-coated slides, dried, dehydrated in ascending concentrations of ethanol, cleared in hystosol, and coverslipped. An observer unaware of the treatment examined microscopically the BF region in the slides.

Statistical Analysis

We used a mixed-model analysis of variance (ANOVA) with repeated measures to analyze passive avoidance, open-field, water maze, and ChAT activity data, always considering the litter as a random variable. Litters are therefore considered statistical units, and data from control and treated littermates are "repeated trials" of the litter unit (Zorilla, 1997). We performed post hoc comparisons by using Tukey's honestly significant difference (HSD) test, which can be used in the absence of significant ANOVA results (Wilcox, 1987).

Results

Passive Avoidance Testing (PND 22–23)

Passive avoidance data are shown in Figure 2. Saline- and 192 IgG-sap-treated pups did not differ significantly in the number of trials needed to reach learning criterion during the acquisition phase. Retention of the passive avoidance task was also not significantly different between the conditioned saline- and the 192 IgG-sap-treated pups. As expected, however, all of the shock-conditioned pups had a significantly longer latency to step-through than did the nonreinforced pups, $F(1, 10) = 146.48, p < .01$.

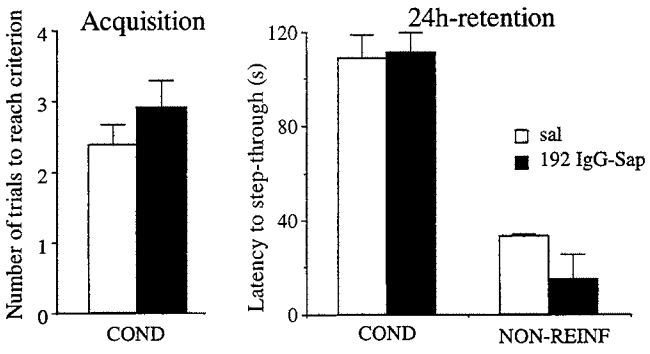

Figure 2. Passive avoidance acquisition and retention on Postnatal Day (PND) 22–23 in rats treated with saline (sal) or the immunotoxin 192 IgG-saporin (192 IgG-Sap) on PND 7. Each column represents the mean number of trials (±*SEM*) to reach learning criterion in conditioned rats (COND) on PND 22 (left panel), and latency to enter the dark compartment during retest on PND 23 in conditioned and nonreinforced rats (NON-REINF, right panel). $n = 11$ in each group.

Water Maze (PND 50–60)

Spatial navigation task. Mean latency to locate the platform (see Figure 3A) path length, and speed on successive days of the spatial navigation task are shown in Table 1. All rats showed a marked reduction in latency over the 5 days, $F(4, 32) = 3.81, p = .01$; no effect of the treatment on path length, latency, or speed was evident.

Probe trial. Analysis of the probe trial (Figure 3B) revealed that both control and 192 IgG-sap-treated rats spent most of their time in the quadrant where the platform had been during training, $F(1, 7) = 8.83, p < .05$. In addition, 192 IgG-sap-treated rats swam faster than did controls (saline = 23.7 cm/s; 192 IgG-sap = 31.2 cm/s), $F(1, 7) = 19.69, p < .01$.

Reversal task. All rats showed a marked reduction in latency over the 3 days, $F(2, 16) = 20.14, p < .01$. Although no effect of the treatment was evident on either latency or path-length measures, 192 IgG-sap-treated rats had faster mean swim speeds than did controls, $F(1, 8) = 7.15$, $p < .05$.

Cued task. As expected, when the platform was made visible, no effect of the treatment was evident on the latency, path length, or swim speed, thus confirming that there were no general sensorimotor or motivational deficits in 192 IgG-sap-treated rats.

Open Field With Objects (PND 54)

Locomotor activity (Session 1). Number of crossings, frequency of rearing, and wall rearing during the first session of the open-field test (no objects) are shown in Table 2. No effect of the treatment was evident in this phase of the task.

Object exploration (Sessions 2–4). Object-exploration scores during Sessions 2–4 are shown in Figure 4A. All rats showed a marked reduction in the time spent in contact with the five objects over the three sessions, main effect of the repeated trial factor, $F(2, 18) = 15.84, p < .01$. The ANOVA also revealed a significant interaction between intracerebroventricular treatment and the repeated trial factor, $F(2, 18) = 4.34, p < .05$. Post hoc comparisons performed on this interaction showed that 192 IgG-sap-treated rats spent more time in contact with the objects than did the control rats during Session 4 ($p < .05$).

Spatial change responses (Session 5). Exploration of displaced and nondisplaced objects is shown in panel B of Figure 4. The ANOVA revealed a significant Treatment × Displacement interaction, $F(1, 9) = 19.22, p < .01$. Post hoc comparisons performed on this interaction showed that control rats spent more time exploring the displaced objects than did the nondisplaced rats ($p < .01$), whereas 192 IgG-sap-treated rats did not show any reaction to the spatial changes.

Novel object responses (Session 7). Reactivity to object novelty is shown in Figure 4C. The ANOVA revealed a

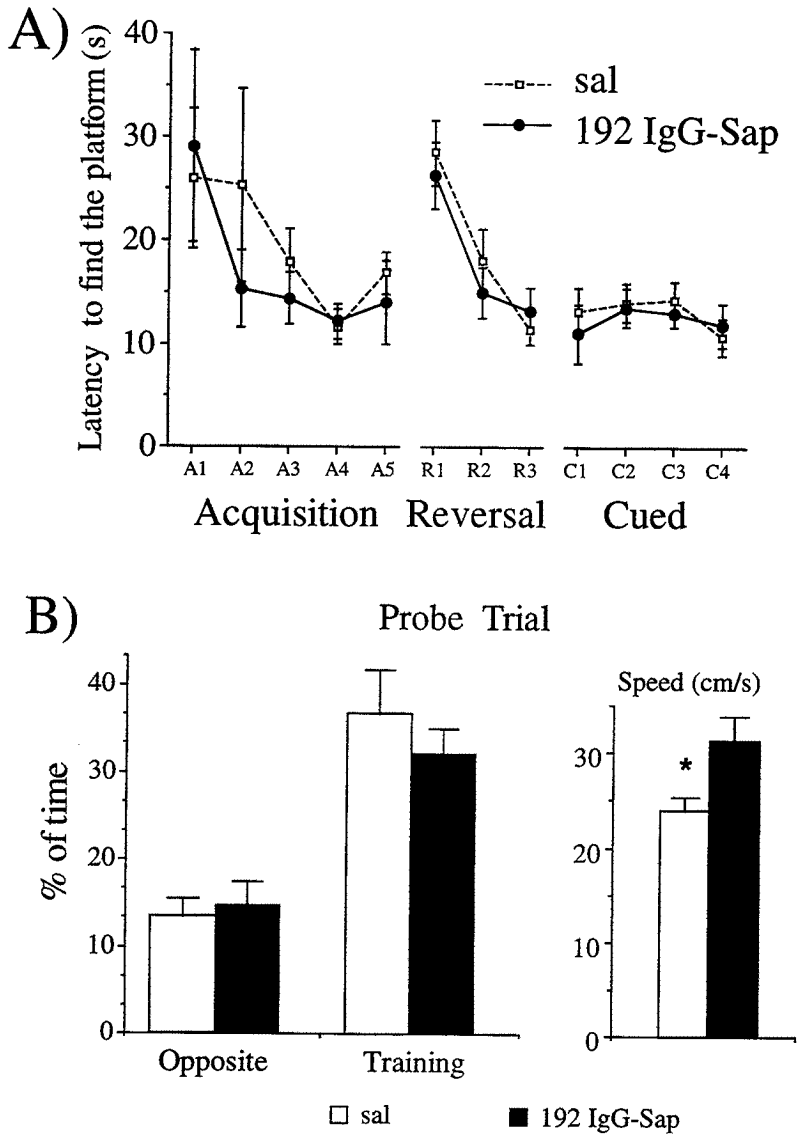

Figure 3. Water-maze performance on Postnatal Day (PND) 50–60 in rats treated with saline (sal) or the immunotoxin 192 IgG-saporin (192 IgG-Sap) on PND 7. A: Latency to find the platform during acquisition (hidden platform in northeast quadrant), reversal (hidden platform in southwest quadrant), and cued sessions (visible platform). Each point represents the mean value (±*SEM*) for the four trials in each session. B: Probe trial. Left panel shows the percentage of time spent in training (northeast) and opposite (southwest) quadrant during the 30-s probe trial; right panel shows average swimming speed during the probe trial. Each column represents the group mean (±*SEM*). $n = 8$ in each group. *significantly different at $p < .05$.

Table 1
Path Length and Speed During the Water-Maze Test

Swim parameters	Acquisition phase		Reversal phase		Cued phase	
	Saline	192 IgG-sap	Saline	192 IgG-sap	Saline	192 IgG-sap
Path length (cm)	480 ± 76	475 ± 60	484 ± 42	523 ± 49	319 ± 20	330 ± 18
Speed (cm/s)	15.0 ± 0.9	16.2 ± 0.9	15.2 ± 0.8	16.9 ± 0.9*	13.4 ± 0.3	14.3 ± 0.4

Note. Data are means ± *SEM*. $n = 9$ in each group. 192 IgG-sap = 192 IgG-saporin.
*$p < .05$, main effect of the treatment.

Table 2
Behavioral Items Recorded During the First Session of the Modified Open-Field Test on PND 54

Behavioral measures (frequencies)	Saline	192 IgG-sap
Crossings	158.4 ± 8.4	158.5 ± 10.1
Rearing	7.5 ± 1.3	7.9 ± 2.2
Wall rearing	17.6 ± 3.0	19.9 ± 3.3

Note. Data are mean number of episodes during 6 min ± *SEM*. $n = 10$ in each group. 192 IgG-sap = 192 IgG-saporin.

significant effect of the object replacement (the substituted object–nonsubstituted object ratio), $F(1, 9) = 29.73$, $p < .01$. In other words, the rats spent more time in contact with the novel objects than with the familiar objects. No effect of the treatment was evident, indicating that both control and 192 IgG-sap-treated rats reacted similarly to object novelty.

ChAT Immunocytochemistry (PND 60)

Examples of ChAT immunostaining in both the medial septal area and nucleus basalis magnocellularis region are shown in Figure 5. A dramatic decrease of ChAT immunopositive staining was evident in both regions. No enlargement of ventricles, gliosis, or disrupted cellular architecture was seen in the 192 IgG-sap-treated brains. Because of the limited sample size available ($n = 2$; the rest of the brains were used for biochemical analysis), no cell counting was performed.

ChAT Activity (PND 60)

Cortical ChAT activity in the 192 IgG-sap group (9.7 ± 0.6 nmol/hr/mg) was decreased 75% relative to the control group (40.1 ± 2.7 nmol/hr/mg). Hippocampal ChAT activity in the 192 IgG-sap group (9.8 ± 1.5 nmol/hr/mg) was decreased 84% relative to the control group (58.9 ± 2.5). In contrast, striatal ChAT activity was similar in the two groups: 177 ± 6.6 nmol/hr/mg in the control group compared with 176 ± 7.3 nmol/hr/mg in the 192 IgG-sap group (see Figure 6). Post hoc comparisons performed on the Treatment × Brain Region ANOVA interaction, $F(2, 16) = 25.02$, $p < .01$, revealed significant differences between the control and 192 IgG-sap group in the cortex and hippocampus ($p < .01$). No differences in striatal ChAT activity were evident between control and 192 IgG-sap-treated rats.

Discussion

The present data demonstrate that neonatal (PND 7) intracerebroventricular administration of 192 IgG-sap has long-term behavioral and neurochemical effects. The lasting effectiveness of the neonatal treatment in inducing a cholinergic loss specifically in the BF is indicated by the marked reduction of ChAT activity in both the hippocampus and cortex, but not in the striatum (in agreement with the lower

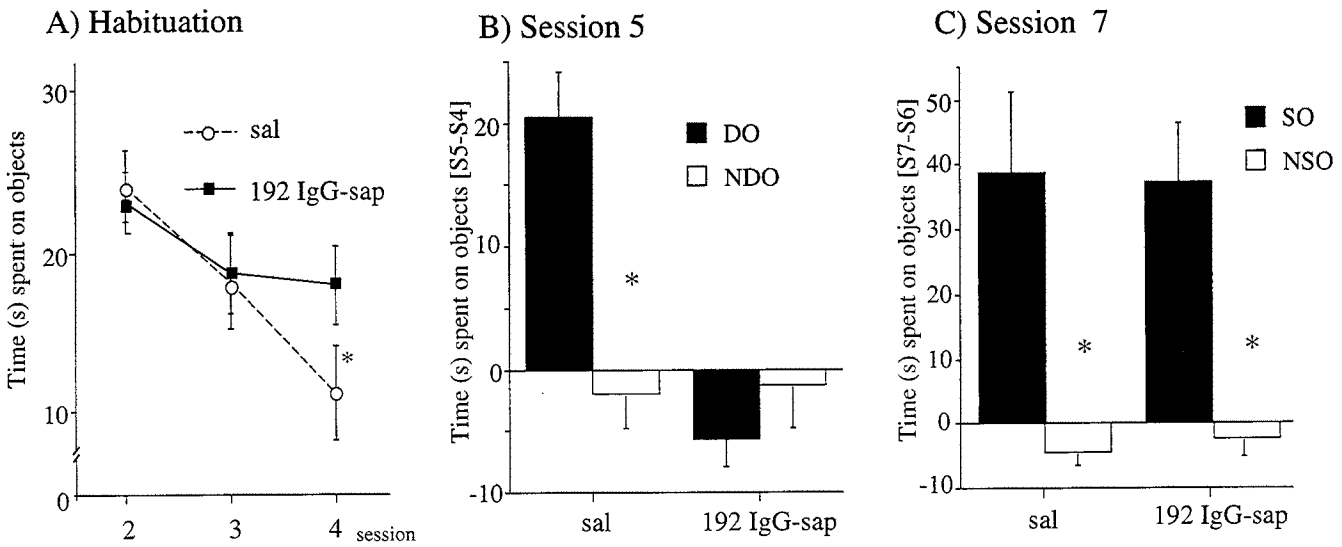

Figure 4. Spatial open field on Postnatal Day 54. $n = 9$ in each group. A: Object exploration in Sessions 2, 3, and 4. Each point represents the mean time (± *SEM*) spent in contact with the objects. *$p < .05$ after post hoc comparisons. B: Response to spatial change in Session 5. DO = mean time on displaced objects in Session 5 minus mean time on the same objects in Session 4; NDO = mean time on nondisplaced objects in Session 5 minus mean time on the same objects in Session 4. A positive value for DO indicates increased exploration toward displaced objects. *$p < .01$ of DO–NDO factor in the saline (sal) group. C: Response to object replacement in Session 7. SO = mean time on substituted object in Session 7 minus mean time on the object in the same position in Session 6; NSO = mean time on nonsubstituted objects in Session 7 minus mean time on the same objects in Session 6. A positive value for SO indicates increased exploration toward the new object. *$p < .01$ for the SO–NSO factor in both the sal and 192 IgG-saporin (192 IgG-sap) groups.

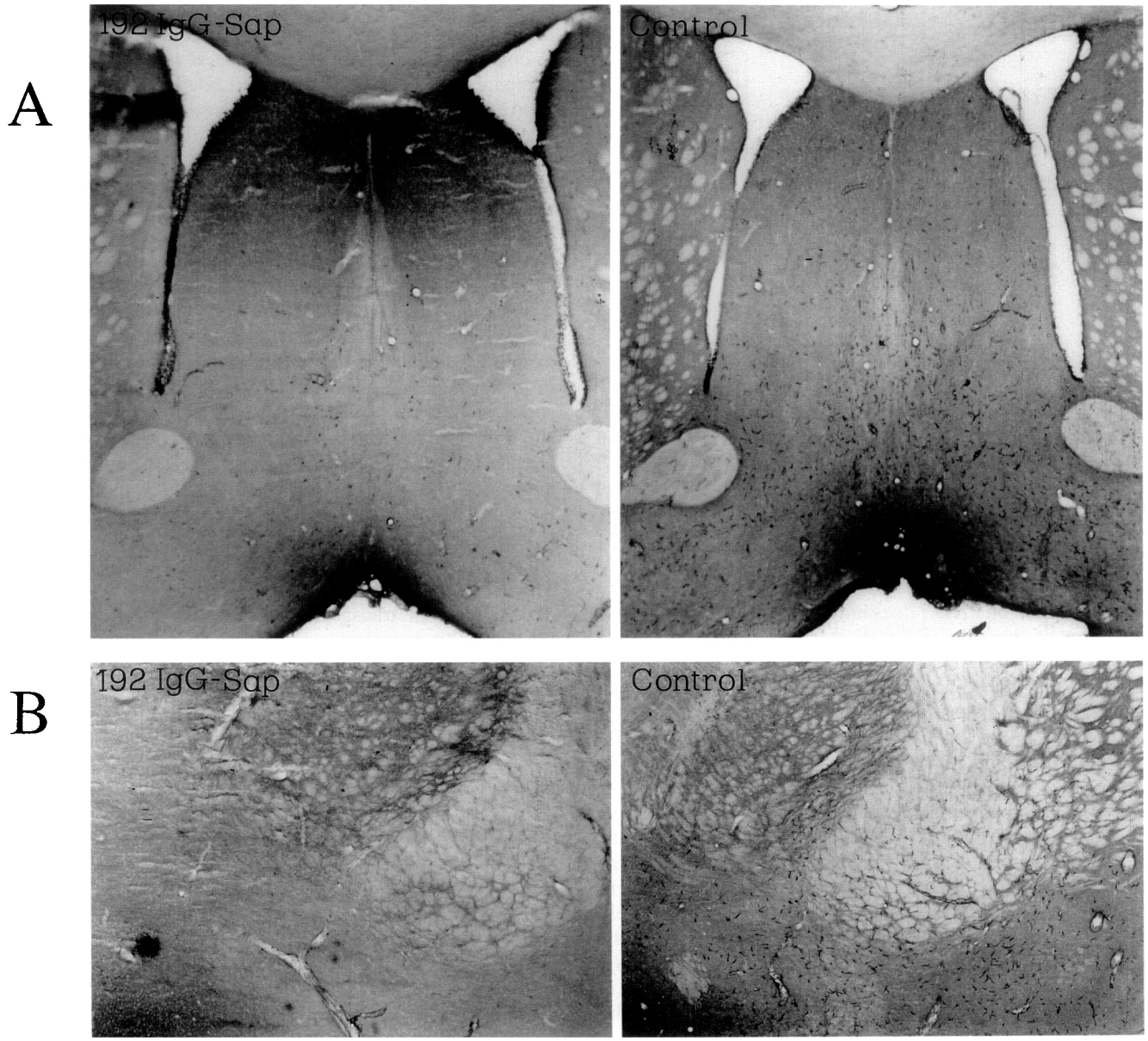

Figure 5. Extent of the lesion in the adult (Postnatal Day [PND] 60) rat brain induced by the 192 IgG-saporin (192 IgG-Sap) intracerebroventricular treatment on PND 7. Photomicrographs of coronal sections stained with antibodies against choline acetyltransferase in control (right) and 192 IgG-Sap-treated (left) brains. Panel A shows the medial septal area (2.5×); Panel B shows the nucleus basalis magnocellularis area (5×).

expression of NGFr in striatal neurons on PND 7; Mobley et al., 1989). The persistent cholinergic loss is also confirmed by the widespread decrease of ChAT immunoreactivity in BF cholinergic neurons. The removal of the cholinergic innervation to both neocortex and hippocampus on PND 7 also had long-term behavioral consequences. On PND 54, reactions of 192 IgG-sap-treated rats to the spatial rearrangement of familiar objects during the open-field test were clearly decreased relative to controls, suggesting a selective deficit for spatial information processing during exploration. However, the massive and persistent cholinergic depletion was not paralleled by deficits in performance of PA and water-maze learning tasks, which were performed on PND 22–23 (i.e., post-weaning) and on PND 50–60, respectively.

Passive Avoidance

We have shown previously that rats lesioned on PND 7 with 192 IgG-sap were slower than controls in the acquisition of a PA task performed on PND 15–16 (preweaning), although 24 hr retention of the task was not affected (Ricceri et al., 1997). The present findings indicate that immunotoxin

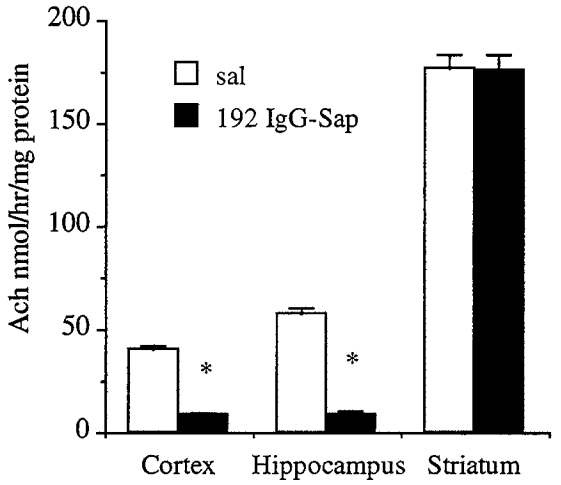

Figure 6. Activity of choline acetyltransferase in various brain regions on Postnatal Day (PND) 60 in rats treated with saline (sal) or 192 IgG-saporin (192 IgG-Sap) on PND 7. Each column represents the mean activity (±*SEM*). $n = 9$ in each group. The asterisk represents a significant difference between the sal and 192 IgG-Sap groups ($p < .01$).

administration on PND 7 does not affect either acquisition or retention of the same PA task in postweaning rats (PND 22–23). This age-related difference in performance of the 192 IgG-sap-treated pups suggests that neonatally lesioning the cholinergic neurons in the BF is accompanied by a transient effect on the acquisition rate of a step-through PA learning paradigm. It has been reported extensively in ontogenetic studies that, because of its requirement of withholding a punished response, PA is extremely sensitive to both motor and cognitive maturational influences in rats, and performance on this task usually improves with age (Ricceri, Alleva, Chiarotti, & Calamandrei, 1996; Schulenburg, Riccio, & Stikes, 1971; Stehouwer & Campbell, 1980). Such maturational influences appear to make the same PA task much easier to acquire at PND 22 than at PND 15 (the number of trials needed to learn the task in 22-day-old rats was less than half the number needed by 15-day-old control rats in Ricceri et al.'s 1997 experiment). This age effect may render undetectable the relatively subtle effects of neonatal cholinergic depletion on PND 22. We have suggested previously that the slower acquisition rate of the 192 IgG-sap-treated rats found on PND 15 could reflect an impairment of attentional rather than associative capabilities (Ricceri et al., 1997).

Comparisons between PA data from neonatally lesioned rats and adult lesioned rats should be considered with great caution for several reasons. First, the effects of developmental damage to the cholinergic BF can be quite different from those induced by adult BF lesions, because they affect a still-growing system and not an already stabilized and functional network. Second, the effects of adult 192 IgG-sap lesions on PA performance are unclear because conflicting results have been reported following these lesions. PA deficits have been mainly documented following intracerebroventricular 192 IgG-sap lesions (Leanza et al., 1995; Waite et al., 1995; Zhang et al., 1996), but possible cerebellar damage and the absence of nonreinforced groups to control for locomotor biases make these data difficult to compare with the present data; mild deficit in PA retention (Torres et al., 1994) or lack of effects have been reported after 192 IgG-sap lesions restricted to the nucleus basalis magnocellularis (nBM) area (Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994). Third, the same differences here revealed between 15- and 22-day-old rats strongly indicate that—because of different behavioral repertoires—the same test can represent different difficulty levels at different ages.

The fact that, in contrast with what we hypothesized, the saporin-induced PA deficit observed on PND 15 was no longer evident on PND 22 also highlights the importance of examining behavior at several points during development. From a methodological point of view, the possibility that some early learning deficits can disappear after weaning should not be neglected when studying behavior of rodents raised with neonatal cerebral lesions. This type of behavioral analysis could also be applied to the study of mice with deletions or overexpressions of selected genes relevant for learning and memory processes, a field that has received particular attention in recent years (Lipp & Wolfer, 1998). Thus far, few studies have assessed the neurobehavioral development of transgenic mice despite the fact that (a) missing or overexpressing genes might affect many developmental processes throughout ontogeny and (b) compensatory mechanisms might account for the absence of behavioral abnormalities at adulthood.

Water Maze

In agreement with previous findings (Leanza et al., 1996; Pappas et al., 1996), data from the present experiment indicate that neonatal saporin treatment did not affect spatial learning and memory in the water maze. Throughout the entire battery of water-maze tests (spatial learning, reversal, and cued sessions), the immunolesioned rats showed performance levels comparable with those of their control littermates.

This behavioral evidence is also in full agreement with the histological evidence that suggests that cerebellar damage does not occur following neonatal 192 IgG-sap lesions (Leanza et al., 1996). Indeed, the primary behavioral symptoms of cerebellar damage in rodents usually include ataxia, general motor impairments, slower swimming speed, and impaired spatial learning in the water maze (Berger-Sweeney et al., 1994; Goodlett, Hamre, & West, 1992; Waite et al., 1995). Furthermore, in a recent study with the OX7-saporin immunotoxin that selectively destroys cerebellar Purkinje cells without affecting cholinergic neurons, rats with cerebellar damage learned the spatial water-maze task very poorly (Waite, Wardlow, & Power, 1999). In the present study, no motoric impairments or water-maze deficits were seen in 192 IgG-sap-lesioned rats, thus suggesting that cerebellar Purkinje cells were spared.

Despite the absence of lesion effects on water-maze performance, long-term detrimental effects of the neonatal

192 IgG-sap treatment were clearly evident in a spatial open-field test that required gathering information about spatial relationships among familiar objects. As such, it appears that the lack of, or extremely mild, deficits of 192 IgG-sap lesions on water-maze tasks reflects the fact that the water-maze tasks were not sensitive to the type of spatial processing deficits induced by BF cholinergic loss either during development (Leanza et al., 1996; Pappas et al., 1996; the present results) or in adulthood after intraparenchymal administration of 192 IgG-sap (Baxter & Gallagher, 1996; Berger-Sweeney et al., 1994; Janis, Glasier, Fulop, & Stein, 1998; McMahan et al., 1997). The open-field task appears to be sensitive to mild impairments in spatial information processing, probably because, unlike water-maze tasks, it evaluates spontaneous exploratory activity without the involvement of any additional motivational stimulus. As for the water maze, it has been hypothesized that the stress induced by submersion in water could trigger neuromodulatory compensations in adult rats that could mask mild spatial working-memory deficits (McMahan et al., 1997; Everitt & Robbins, 1997). Such a neuromodulatory compensation appears to be even more likely after neonatal administration of 192 IgG-sap than after administration in adult rats because of the plasticity of the developing nervous system.

Spatial Open Field

The neonatal 192 IgG-sap treatment did not interfere with spontaneous locomotor activity in adulthood, as measured during the first session of the open-field test in which objects were not presented in the arena. From Session 2 to Session 4 in the open field, both control and 192 IgG-sap-treated rats progressively decreased their contact with objects, indicating that both control and treated groups showed a habituation profile that allowed for subsequent measurements of reactions to spatial change in the following sessions. However, a subtle difference in the habituation profile was evident, with 192 IgG-sap-treated rats showing a less dramatic decrease in interest toward the objects during Session 4 compared with controls.

Following habituation, the new spatial rearrangement of the objects in Session 5 elicited a renewal of exploration in control rats, selectively directed toward the displaced objects. This result is in agreement with previous studies in rats (Poucet, 1989). In contrast, 192 IgG-sap-treated rats did not show any selective interest toward the displaced objects, suggesting that they did not recognize the occurrence of a change in the spatial relationships among the objects. The 192 IgG-sap-treated rats, however, did show selective interest in a novel object, similar to control rats. Reinvestigation of the displaced objects implies that some internal representation of the topographical arrangement of the objects had been formed and compared with the new arrangement. The present findings suggest that neonatal BF cholinergic lesioning selectively affects the acquisition of relevant spatial stimuli (spatial mapping) during the open-field test while leaving acquisition of relevant object stimuli intact. Moreover, the subtle but consistent differences found in the habituation profile during the first part of the test (Sessions 2–4) may support this hypothesis. If the decreased interest toward an object over time reflects the time-course of acquiring spatial information, then the less pronounced habituation to the objects in Sessions 2–4 by the 192 IgG-sap-treated rats could be interpreted as a spatial information encoding or processing impairment that was later manifested as a spatial-mapping deficit when reactions to spatial novelty were measured in Session 5. Further experiments with additional sessions before the spatial change could clarify whether a longer exposure to the same object setting is able to facilitate spatial mapping in BF-lesioned rats. In support of this hypothesis, alterations in spatial mapping have also been reported following 192 IgG-sap lesion of the medial septum (MS) in adult rats (Janis et al., 1998). It is interesting that these authors reported that lesioned rats tested in both radial-maze and modified water-maze tasks abandoned allocentric (spatial-mapping-based) strategies in favor of egocentric (nonspatial) strategies that allowed them to locate the goal on the basis of internal response patterning rather than external landmarks.

There is, however, another possible interpretation that cannot be excluded on the basis of the present behavioral results. The lack of responses to spatial novelty in 192 IgG-sap rats may be due to an attentional deficit in encoding relevant spatial information from the environment rather than a deficit in spatial mapping. This type of deficit would prevent 192 IgG-sap-treated rats from making a clear representation of the object arrangement in the arena before object displacement. Our data suggest that this attentional deficit, however, would be limited to modifications of the spatial relationship among the objects. When the spatial modifications presented were of a greater magnitude and consist of a totally novel object, reactions of 192 IgG-sap-treated rats were indistinguishable from those of control rats.

Such an attentional-deficit interpretation is consistent with several other studies in which attentional capabilities in different paradigms were impaired following 192 IgG-sap lesions of the cholinergic input to both cortex (nBM lesions) and hippocampus (MS lesions) in adult rats (Baxter, Holland, & Gallagher, 1997; Chappell, McMahon, Chiba, & Gallagher, 1998; McCaughy, Kaiser, & Sarter, 1996; Stoehr et al., 1997). These studies provide evidence that the behavioral effects of 192 IgG-sap are evident primarily on tasks measuring specific aspects of attention, for example, stimulus discriminability, behavioral vigilance, or latent inhibition paradigms. Testing neonatally 192 IgG-sap-lesioned rats on attentional tasks similar to those used in previous studies after adult lesions may help determine whether the observed spatial-novelty deficit is due to an impairment in spatial mapping or in attention to relevant spatial stimuli.

We have reported previously the short-term effects of neonatal removal of BF cholinergic neurons on acquisition of a PA task in preweaning rats (PND 15) and on exploratory behavior in a standard open-field task on PND 19 (Ricceri et al., 1997). In the present study we showed, for the first time, that the behavioral effects of neonatal BF depletion are in fact long-lasting. Spatial information mapping and/or spatial

attentional processing deficits are detectable in adult rats (PND 54) who received neonatal BF lesions. Previous reports that suggest that the developing cholinergic BF system does not play a role in spatial-map processing are based primarily on the results of water-maze testing. However, this task does not appear to be sufficiently sensitive to detect the subtle spatial deficits induced by neonatal BF cholinergic depletion. The results of the present study, in conjunction with our previous studies (Ricceri et al., 1997) and those by Robertson et al. (1998), suggest strongly that the selective neurotoxin for the BF cholinergic neurons 192 IgG-sap can be successfully used to elucidate not only the function of the BF cholinergic populations in the adult brain, but also the long-term effects of the neonatal cholinergic depletions on brain morphology and behavior.

References

Arters, J., Hohmann, C. F., Mills, J., Olaghere, O., & Berger-Sweeney, J. (1998). Sexually dimorphic responses to neonatal basal forebrain lesions in mice: I. Behavior and neurochemistry. *Journal of Neurobiology, 37,* 582–594.

Bachman, E. S., Berger-Sweeney, J. E., Coyle, J. T., & Hohmann, C. F. (1994). Developmental regulation of adult cortical morphology and behavior: An animal model for mental retardation. *International Journal of Developmental Neurosciences, 12,* 239–253.

Baxter, M. G., & Gallagher, M. (1996). Intact spatial learning in both young and adult rats following selective removal of the hippocampal cholinergic input. *Behavioral Neuroscience, 110,* 1–8.

Baxter, M. G., Holland, P. C., & Gallagher, M. (1997). Disruption of decrements in conditioned stimulus processing by selective removal of hippocampal cholinergic input. *Journal of Neuroscience, 17,* 5230–5236.

Berger-Sweeney, J., Heckers, S., Mesulam, M. M., Wiley, R. G., Lappi, D. A., & Sharma, M. (1994). Differential effects on spatial navigation of immuno-toxin induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *Journal of Neuroscience, 14,* 4507–4519.

Berger-Sweeney, J., & Hohmann, C. (1997). Behavioral consequences of abnormal cortical development: Insights into developmental disabilities. *Behavioural Brain Research, 86,* 121–142.

Book, A. A., Wiley, R. G., & Schweitzer, J. B. (1994). 192 IgG-saporin: I. Specific lethality for cholinergic neurons in the basal forebrain of the rat. *Journal of Neuropathology and Experimental Neurology, 53,* 95–102.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72,* 248–254.

Buhot, M. C., Soffie, M., & Poucet, B. (1989). Scopolamine affects the cognitive processes involved in selective object exploration more than locomotor activity. *Psychobiology, 17,* 409–417.

Calamandrei, G., Ricceri, L., & Valanzano, A. (1996). Systemic administration of anti-NGF antibodies to neonatal mice impairs 24-h retention of an inhibitory avoidance task while increasing ChAT immunoreactivity in the medial septum. *Behavioural Brain Research, 78,* 81–91.

Chappell, J., McMahan, R., Chiba, A., & Gallagher, M. (1998). A re-examination of the role of basal forebrain cholinergic neurons in spatial working memory. *Neuropharmacology, 37,* 481–487.

Coyle, J. T., Price, D. L., & DeLong, M. R. (1983, March 11). Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science, 219,* 1184–1190.

Dornan, W. A., McCampbell, A. R., Tinkler, G. P., Hickman, L. J., Bannon, A. W., Decker, M. W., & Gunther, K. L. (1997). Comparison of site specific injections into the basal forebrain on water maze and radial arm maze performance in the male rat after immunolesioning with 192 IgG saporin. *Behavioural Brain Research, 86,* 181–189.

Dumery, V., Derer, P., & Blozovski, D. (1988). Enhancement of passive avoidance learning through small doses of intra-amygdaloid physostigmine in the young rat. *Developmental Psychobiology, 21,* 553–565.

Dunnett, S. B., Everitt, B. J., & Robbins, T. W. (1991). The basal forebrain–cortical cholinergic system: Interpreting the functional consequences of excitotoxic lesions. *Trends in the Neurosciences, 14,* 494–501.

Ethovision (Version 1.7) [Computer software]. (1995) Wageningen, The Netherlands: Noldus Information Technology.

Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic systems and cognition. *Annual Review of Psychology, 48,* 649–684.

Fibiger, H. (1991). Cholinergic mechanisms in learning, memory and dementia: A review of recent evidence. *Trends in the Neurosciences, 14,* 327–388.

Fonnum, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *Journal of Neurochemistry, 24,* 407–409.

Goodlett, C. R., Hamre, K. M., & West, J. R. (1992). Dissociation of spatial navigation and visual guidance performance in Purkinje cell degeneration (pcd) mutant mice. *Behavioural Brain Research, 47,* 129–141.

Hohmann, C. F., & Berger-Sweeney, J. (1998). Sexually dimorphic responses to neonatal basal forebrain lesions in mice: II. Cortical morphology. *Journal of Neurobiology, 37,* 595–606.

Janis, L. S., Glasier, M. M., Fulop, Z., & Stein, D. G. (1998). Intraseptal injections of 192 IgG saporin produce deficits for strategy selection in spatial memory tasks. *Behavioural Brain Research, 90,* 23–34.

Kaufmann, W. E., Taylor, C. V., Hohmann, C. F., Sanwal, I. B., & Naidu, S. (1997). Abnormalities in neuronal maturation in Rett syndrome neocortex: Preliminary molecular correlates. *European Child Adolescence Psychiatry, 6,* (Suppl.) 75–77.

Laviola, G., & Terranova, M. L. (1998). The developmental psychobiology of behavioral plasticity in mice: Role of social experiences in the family unit. *Neuroscience & Biobehavioral Reviews, 23,* 197–213.

Leanza, G., Nilsson, O. G., Nikkhah, G., Wiley, R. G., & Björklund, A. (1996). Effects of neonatal lesions of the basal forebrain cholinergic system by 192 IgG-saporin: Biochemical, behavioural and morphological characterization. *Neuroscience, 74,* 119–141.

Leanza, G., Nilsson, O. G., Wiley, R. G., & Björklund, A. (1995). Selective lesioning of the basal forebrain cholinergic system by intraventricular 192 IgG-saporin: Behavioural, biochemical and stereological studies in the rat. *European Journal of Neuroscience, 7,* 329–343.

Lipp, H. P., & Wolfer, D. P. (1998). Genetically modified mice and cognition. *Current Opinion in Neurobiology, 8,* 272–280.

Marin-Padilla, M. (1976). Pyramidal cell abnormalities in the motor cortex of child with Down syndrome. *Journal of Comparative Neurology, 167,* 63–82.

McGaughy, J., Kaiser, K., & Sarter, M. (1996). Behavioral vigilance following infusions of 192 IgG saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behavioral Neuroscience, 110,* 247–265.

McMahan, R. W., Sobel, T. J., & Baxter, M. G. (1997). Selective immunolesions of cholinergic input fail to impair spatial working memory. *Hippocampus, 7,* 130–136.

Mobley, W. C., Woo, J. E., Edwards, R. H., Riopelle, R. J., Longo, F. M., Weskamp, G., Otten, U., Valletta, J. S., & Johnston, M. V. (1989). Developmental regulation of nerve growth factor and its receptor in the rat caudate-putamen. *Neuron, 3,* 655–664.

The Observer (Version 3.0) [Computer software]. (1993). Wageningen, The Netherlands: Noldus Information Technology.

Pappas, B. A., Davidson, C. M., Fortin, T., Nallathamby, S., Park, G. A. S., Mohr, E., & Wiley, R. G. (1996). 192 IgG-saporin lesion of basal forebrain cholinergic neurons in neonatal rats. *Developmental Brain Research, 96,* 52–61.

Poucet, B. (1989). Object exploration, habituation, and response to a spatial change in rats following septal or medial frontal cortical damage. *Behavioral Neuroscience, 103,* 1009–1016.

Ricceri, L., Alleva, E., Chiarotti, F., & Calamandrei, G. (1996). Nerve growth factor affects passive avoidance learning and retention in developing mice. *Brain Research Bulletin, 39,* 219–226.

Ricceri, L., Calamandrei, G., & Berger-Sweeney, J. (1997). Different effects of postnatal day 1 vs. 7 192 IgG saporin lesions on learning, exploratory behaviors and neurochemistry in juvenile rats. *Behavioral Neuroscience, 111,* 1292–1302.

Robertson, R. T., Gallardo, K. A., Claytor, J. K., Ha, D. H., Ku, K. H., Yu, B. P., Lauterborn, J. C., Wiley, R. G., Yu, Y., Gall, C. M., & Leslie, F. M. (1998). Neonatal treatment with 192 IgG-saporin produces long-term forebrain cholinergic deficits and reduces dendritic branching and spine density of neocortical pyramidal neurons. *Cerebral Cortex, 8,* 142–155.

Schulenburg, C. J., Riccio, D. C., & Stikes, E. R. (1971). Acquisition and retention of a passive avoidance response as a function of age in rats. *Journal of Comparative and Physiological Psychology, 74,* 75–83.

Sherren, N., Pappas, B. A., & Fortin, T. (1999). Neural and behavioral effects of intracranial 192 IgG-saporin in neonatal rats: Sexually dimorphic effects? *Developmental Brain Research, 114,* 49–62.

Steckler, T., Keith, A. B., Wiley, R. G., & Sahgal, A. (1995). Cholinergic lesions by 192 IgG-saporin and short-term recognition memory: Role of the septohippocampal projection. *Neuroscience, 66,* 101–114.

Stehouwer, D. J., & Campbell, B. A. (1980). Ontogeny of passive avoidance: Role of task demands and development of species typical behaviors. *Developmental Psychobiology, 13,* 385–398.

Stoehr, J. D., Mobley, S. L., Roice, D., Brooks, R., Baker, L. M., Wiley, R. G., & Wenk, G. L. (1997). The effects of selective cholinergic basal forebrain lesions and aging upon expectancy in the rat. *Neurobiology of Learning and Memory, 67,* 214–227.

Takashima, S., Becker, L. E., Armstrong, D. L., & Chan, F. (1981). Abnormal neural development in the visual cortex of the human fetus and infant with Down's syndrome: A quantitative and qualitative Golgi study. *Brain Research, 225,* 1–21.

Thinus-Blanc, C., Save, E., Rossi-Arnaud, C., Tozzi, A., & Ammassari-Teule, M. (1996). The differences shown by C57BL/6 and DBA/2 inbred mice in detecting spatial novelty are subserved by different hippocampal and parietal-cortex interplay. *Behavioural Brain Research, 80,* 33–40.

Torres, E. M., Perry, T. A., Blokland, A., Wilkinson, L. S., Wiley, R. G., Lappi, D. A., & Dunnett, S. B. (1994). Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience, 63,* 95–122.

Usiello, A., Sargolini, F., Roullet, P., Ammassari-Teule, M., Passino, E., Oliverio, A., & Mele, A. (1998). N-methyl-D-aspartate receptors in the nucleus accumbens are involved in detection of spatial novelty in mice. *Psychopharmacology, 137,* 175–183.

Waite, J. D., Wardlow, M. L., & Power, A. E. (1999). Deficit in selective and divided attention associated with cholinergic basal forebrain immunotoxic lesion produced by 192-saporin: Motoric/sensory deficits associated with Purkinje cell immunotoxic lesion produced by OX7-saporin. *Neurobiology of Learning and Memory, 71,* 325–352.

Waite, J. D., Wardlow, M. L., Wiley, R. G., Lappi, D. A., & Thal, L. J. (1995). 192 immunoglobulin G-saporin produces graded behavioral and biochemical changes accompanying the loss of cholinergic neurons of the basal forebrain and cerebellar Purkinje cells. *Neuroscience, 65,* 463–476.

Walsh, T. J., Herzog, C. D., Gandhi, C., Stackman, R. W., & Wiley, R. G. (1996). Injection of IgG 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Research, 736,* 69–79.

Walsh, T. J., Kelly, R. M., Dougherty, K. D., Stackman, R. W., Wiley, R. G., & Kutscher, C. L. (1995). Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: Cholinergic and non-cholinergic effects following icv injection. *Brain Research, 702,* 233–245.

Wenk, G. L. (1997). The nucleus basalis magnocellularis cholinergic system: One hundred years of progress. *Neurobiology of Learning and Memory, 67,* 85–95.

Wenk, G. L., Naidu, S., Casanova, M. F., Kitt, C. A., & Moser, H. (1991). Altered neurochemical markers in Rett's syndrome. *Neurology, 41,* 1753–1756.

Wenk, G. L., Stoehr, J. D., Quintana, G., Mobley, S., & Wiley, R. G. (1994). Behavioral, biochemical, histological, and electrophysiological effects of 192 IgG-saporin injections into the basal forebrain of rats. *Journal of Neurosciences, 14,* 5986–5995.

Wilcox, R. R. (1987). *New statistical procedures for the social sciences.* Hillsdale, NJ: Erlbaum.

Wiley, R. G. (1992). Neural lesioning with ribosome-inactivating proteins: Suicide transport and immunolesioning. *Trends in the Neurosciences, 15,* 285–290.

Winneke, G., Lilienthal, H., & Kramer, U. (1996). The neurobehavioural toxicology and teratology of lead. *Archives of Toxicology, 70,* (Suppl. 18), 57–70.

Woolf, N. J., Gould, E., & Butcher, L. L. (1989). Nerve growth factor receptor is associated with cholinergic neurons of the basal forebrain but not the pontomesencephalon. *Neuroscience, 30,* 143–152.

Yan, Q., & Johnson, E. J. (1988). An immunohistochemical study of the nerve growth factor receptor in developing rats. *Journal of Neuroscience, 8,* 3481–3498.

Zhang, Z. J., Berbos, T. G., Wrenn, C. C., & Wiley, R. G. (1996). Loss of nucleus basalis magnocellularis, but not septal, cholinergic neurons correlates with passive avoidance impairment in rats treated with 192-saporin. *Neuroscience Letters, 203,* 214–218.

Zorilla, E. P. (1997). Multiparous species present problems (and possibilities) to developmentalists. *Developmental Psychobiology, 30,* 141–150.

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