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Lesions of the Nucleus Basalis Magnocellularis in Immature Rats: Short- and Long-Term Biochemical and Behavioral Changes

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ŽUPAN, G., F. CASAMENTI, C. SCALI AND G. PEPEU. *Lesions of the nucleus basalis magnocellularis in immature rats: Short- and long-term biochemical and behavioral changes.* PHARMACOL BIOCHEM BEHAV 45(1) 19–25, 1993.—Short- and long-term effects of unilateral lesions of the nucleus basalis magnocellularis (NBM) on cortical choline acetyltransferase (ChAT) activity and passive avoidance conditioned responses were examined in immature rats. The lesions were made by stereotaxic injection of quisqualic acid on postnatal days 14 (P14), 17 (P17), and 21 (P21). A marked loss of ChAT activity was found 7 days after surgery in all age groups of lesioned rats. Unoperated P14 rats were unable to perform the passive avoidance conditioned responses. Acquisition began on P17. Lesions made on P17 and P21 strongly impaired the acquisition and retention of the task, evaluated 7 days postoperation. No biochemical but a partial behavioral recovery was observed 3 months after surgery in rats lesioned on P14. On the contrary, despite a persistent decrease in cortical ChAT activity, rats lesioned on P21 were able to acquire and retain the passive avoidance conditioned response. These results indicate that destruction of NBM cholinergic neurons shortly after birth is not compensated for by the developmental plasticity of the residual neurons but results in permanent cholinergic hypofunction. They also demonstrate that cholinergic NBM neurons play an important role in the acquisition and retention of a passive avoidance task; nevertheless, a behavioral recovery may take place 3 months after the lesion, even in the presence of a persistent cholinergic hypofunction.

Immature rats Lesions of the nucleus basalis magnocellularis Choline acetyltransferase
Passive avoidance conditioned response Postnatal recovery

THE nucleus basalis magnocellularis (NBM) provides the major cholinergic innervation to neocortical regions and amygdaloid complex (2,6,20-23,27,30). In adult primates, including man, this nucleus attains the highest level of development, and it has been possible to recognize the subdivisions (21) of the NBM according to its corticopetal projections. In the rat, the neurons of the NBM do not form a discrete nucleus as they do in primates, but instead exhibit a more diffuse distribution within the basal forebrain (22).

In rodents, the morphology of NBM neurons and the activity of central cholinergic systems undergo extensive modifications during postnatal development (4,10,11,13,14,16,17,28). The size of the cholinergic neurons of the NBM increases progressively from postnatal day (P) 7 (30) or P10 (10), reaching a peak value at P14 (30) or P18 (10,11). Thereafter, the size decreases and reaches adult values by P46 (29). The changes in cell body size are correlated with morphological changes of the dendrites (10).

In the rat, at birth all brain cholinergic markers are poorly developed. Choline acetyltransferase (ChAT) activity in the whole rat brain is between 3.5% (4) and 7% (17) of adult values. The level of ChAT activity remains low until the end of the first postnatal week (4,17) and thereafter increases linearly during the subsequent 3 weeks (4,17). The increase in high-affinity choline uptake (HACU) closely parallels the rise in ChAT activity (4). Acetylcholine (ACh) release from cortical slices is 50% and brain ACh concentrations are 27% of adult levels at birth; both reach adult values by 4 weeks postpartum (4,25). At P1, the density of muscarinic receptor sites in the whole rat brain is low but increases linearly during the first month postpartum, at the end of which it reaches adult levels (4).

In adult rats, the anterograde degeneration of cholinergic neurons of the NBM, induced by unilateral electrolytic or neurotoxic lesions of the NBM, is followed by a large decrease in ChAT activity, HACU rate, ACh release and turnover in the cortical areas ipsilateral to the lesion, and impairment in the performance of passive and active avoidance responses (7,18,24,26). Both the biochemical and behavioral deficits undergo spontaneous reduction and within 6 months no signifi-

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cant differences can be detected between lesioned and sham-operated rats (3,32).

In this study, we investigated the consequences of a unilateral lesion of the NBM placed during the postnatal period of rapid development of the brain cholinergic system. We measured neocortical ChAT activity, passive avoidance conditioned responses, and reflex behavior 1 week and 3 months after neurotoxic lesions of the NBM made on P14, P17, and P21.

METHOD

Animals

Male and female Wistar rat pups, Charles River strain, weighing 20–30 g at the beginning of the experiments were used. They were born and raised in our laboratory and kept with their mothers and litter mates except during the surgical procedure and behavioral testing, when they were removed from their home cages. Three age groups were selected: 14, 17, and 21 days. Animals that were not sacrificed during the first 4 postnatal weeks were weaned on P28, housed individually in stainless steel cages, and provided ad lib access to food and water until the day of sacrifice. Animals were maintained on a normal day and night cycle at a temperature of 22–24°C.

Surgery

Under 30 mg/kg IP ketamine (Ketalar, Parke Davis, Detroit, MI) anesthesia, each immature rat was placed in a stereotaxic apparatus (Stoelting Co. Stellar) with the incisor bars on the same plane as the ear bars. A small hole was drilled into the right parietal bone at a point corresponding to the stereotaxic coordinates for the NBM lesions. A unilateral lesion of the NBM was made by stereotaxic injection of 0.5 μl 0.12 M quisqualic acid (Sigma Chemical Co., St. Louis, MO) dissolved in sodium phosphate buffer (pH 7.4). The infusion volume was delivered over a period of 3 min with a 10μl Hamilton syringe (Hamilton, Reno, NV) furnished with a 26 S-ga needle. The needle was left in place for 5 min after the infusion. The following stereotaxic coordinates, taken from the Sherwood and Timiras (29) atlas, were used for all rats: 4.9 mm anterior, 1.9 mm lateral, and 2.7 mm dorsal with the intraauricular line and midline as 0. The NBM lesions were made in three different age groups: at P14, P17, and P21. Immediately after operation, all animals were injected with benzyl-penicillin (100,000 U) IM. All operated animals showed a slight loss (2–3 g) in body weight during the first 2 days after surgery, but no loss was detected 1 week after surgery. In sham-operated rats, the syringe needle was lowered into the cortex and no quisqualic acid was injected. At the end of the experiments, rats were killed by decapitation and their brains removed and fixed on 8% sodium phosphate-buffered formaldehyde. After inclusion in paraffin, brains were cut by use of a microtome and the placement and size of the lesions were checked by histological examination on 10-μm thick coronal slices stained with cresyl violet according to the Nissl method.

Behavioral Tests

Six days or 3 months after NBM lesions, rats were trained in a two-compartment step-through passive avoidance apparatus according to a previously reported procedure (18). The apparatus consisted of two compartments with grid floors that could be electrified separately. The first compartment was an illuminated chamber of 24 × 21 × 20 cm for 3-month-old rats. A smaller chamber (17 × 15 × 20 cm) was used for immature rats. The size was selected after observing the range of exploration of immature rats during the orientation period. A guillotine door connected the first with the second dark compartment, whose walls were painted black. In the training trial, each rat was placed in the illuminated compartment, facing the guillotine door and, after a 30-s orientation period, the door was manually raised. As soon as animals placed all four paws in the dark compartment, the guillotine door was lowered, the time elapsing before entering in this chamber was recorded, and a 0.8- or 1.0-mA scrambled shock (5 Hz, 20 ms) for immature and 3-month-old rats, respectively, was delivered to the grid floor for 5 s. The two shock intensities were selected so as to obtain comparable jumping and vocalization responses after establishing shock sensitivity threshold in immature and 3-month-old rats. Immediately after receiving the shock, the rat was removed from the dark chamber, returned to its home cage, and the chambers were cleaned from feces and urine.

Retesting was performed 30 min and 24 h posttraining. The latency between the door opening and the entrance in the dark compartment was measured. Better performance was indicated by longer retest latencies (up to a maximum time of 120 s). Comparison between unoperated adult rats retested twice, 30 min and 24 h posttraining, or once 24 h posttraining did not show significant latency differences at the 24-h retest.

Psychomotor Task

One hour after completing the passive avoidance test, rats were suspended by their forepaws from 1-m taut horizontal wire (3 mm in diameter, rubber coated) extending between two 50-cm high platforms. All rats would grasp the wire with their forepaws when made lightly to touch it. Three subsequent trials were given in a single session and the latencies (seconds) to fall were recorded for each rat.

ChAT Determination

After performing the behavioral tests, 7 days or 3 months after the lesion, lesioned and control rats were killed by decapitation, their brains removed, and the frontal and parietal neocortex dissected. The samples were homogenized in 20 vol 10 mM EDTA buffer (pH 7.4) and 0.2% (v/v) Triton X-100. ChAT activity was determined by measuring the conversion of 1-[^{14}C]acetyl-coenzyme A (Radiochemical Centre, Amersham Corp., Arlington Heights, IL, specific activity 60 mCi/mmol) to [^{14}C]acetylcholine according to the method of Fonnum (9). Incubation time was 15 min at 37°C. ChAT activity was expressed as μmol/h/100 mg protein. Protein content in the homogenates was determined by the method of Lowry et al. (19).

Statistical Analysis

Statistical significance of the changes in ChAT activity was calculated by Student's two-tailed *t*-test and in passive avoidance conditioned responses by two-way analysis of variance (ANOVA) and Kruskal–Wallis followed by a posthoc multiple comparison *Z*-values test; the differences between lesioned and sham-operated rats were evaluated with the Mann–Whitney *U*-test.

RESULTS

Lesions of the NBM

Nissl-stained coronal brain sections from P14, P17, and P21 quisqualic acid-lesioned rats revealed focal gliosis and a

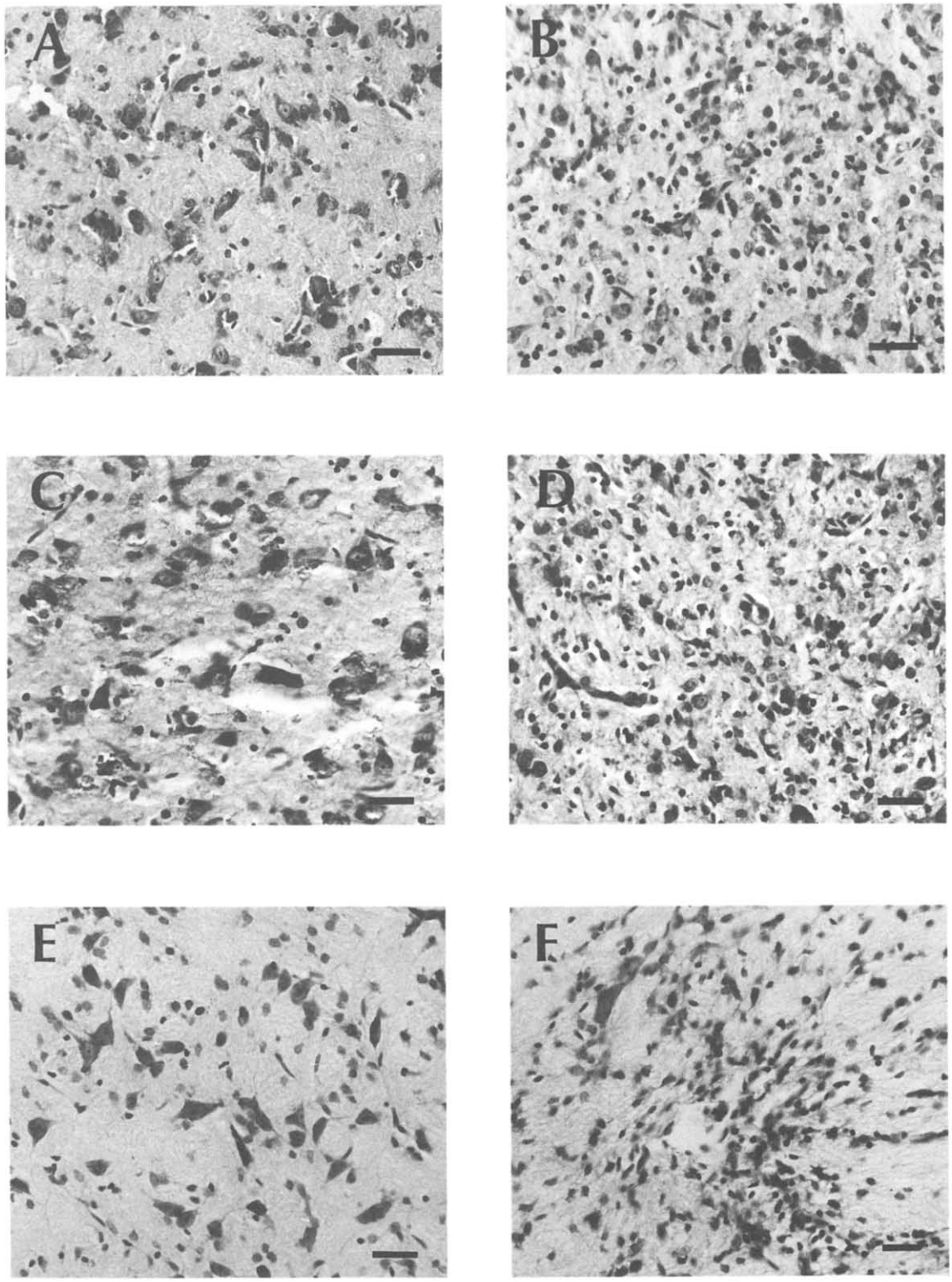

FIG. 1. Photomicrographs of cresyl violet-stained sections through the nucleus basalis magnocellularis (NBM) of P14 (A, B), P17 (C, D), and P21 (E, F) quisqualate-lesioned rats 7 days after surgery. Note the presence of glial cells and loss of magnocellular neurons in the lesioned side (B, D, F) as compared to the respective contralateral unlesioned side (A, C, E). Magnification, × 260. Scale bars, 30 μm.

TABLE 1

CHOLINE ACETYLTRANSFERASE ACTIVITY (μmol/h/100 mg PROTEIN ± SEM) IN THE CEREBRAL CORTEX OF IMMATURE RATS 1 WEEK AFTER UNILATERAL LESIONS OF THE NBM

Condition	Age at Operation (days)	Frontal Cortex		Parietal Cortex	
		Lesioned	Unlesioned	Lesioned	Unlesioned
Sham-operated (6)	14	2.23 ± 0.23	2.30 ± 0.23	2.54 ± 0.38	2.58 ± 0.36
Lesioned (9)	14	1.16 ± 0.22* (−48%)	2.61 ± 0.38 (+13%)	1.48 ± 0.16† (−42%)	3.31 ± 0.33 (+28%)
Sham-operated (5)	17	2.00 ± 0.08	2.06 ± 0.09	2.88 ± 0.28	2.92 ± 0.03
Lesioned (10)	17	1.10 ± 0.12‡ (−45%)	2.01 ± 0.15 (−3%)	1.60 ± 0.16‡ (−45%)	2.62 ± 0.27 (−10%)
Sham-operated (5)	21	2.36 ± 0.20	2.40 ± 0.20	2.83 ± 0.22	2.85 ± 0.20
Lesioned (14)	21	1.40 ± 0.19§ (−41%)	2.40 ± 0.21 (−)	2.10 ± 0.20*§ (−36%)	2.88 ± 0.32 (+1%)

The percent changes calculated vs. age-matched sham-operated rats and the number of rats are given in parentheses. Statistically significant differences vs. age matched sham-operated rats: ‡$p < 0.001$; *$p < 0.005$; †$p < 0.01$; §$p < 0.05$ (Student's t-test, two-tail probability).

loss of magnocellular cholinergic cells into the region of the NBM (Fig. 1). The NBM lesions extended anterior-posteriorly for approximately 1 mm [from A: 5.0 to 4.1-3.8, according to the atlas of Sherwood and Timiras (29)] and no differences in either extension or intensity of damage were seen between the age groups. In some animals, the cell loss extended slightly beyond the borders of the dorsal and ventral globus pallidus. Magnocellular neurons of both limbs of the diagonal band of Broca were in general spared.

ChAT Determination 7 Days After Lesioning

Table 1 shows ChAT activity levels in the frontal and parietal cortex of immature rats with unilateral destruction of the NBM made on P14, P17, and P21. Seven days postoperation, a large, statistically significant, decrease in ChAT activity in the frontal and parietal cortex ipsilateral to the lesion was observed in each group of lesioned rats when compared to age-matched sham-operated controls. ChAT activity in the unlesioned hemisphere of immature rats did not differ from that in sham-operated rats. A slight increase in ChAT activity was observed in the frontal and parietal cortices contralateral to the lesion in rats lesioned on P14.

ChAT Determination 3 Months After Lesioning

The results of ChAT activity determinations in the frontal and parietal cortices 3 months after the NBM unilateral lesion made 14 and 21 days after birth are shown in Table 2. Three months after lesioning, both groups of rats showed a statistically significant decrease in cortical ChAT activity, ipsilateral to the lesion. ChAT activity in the unlesioned contralateral cortical areas was not significantly different from that of sham-operated rats. No difference in ChAT activity was found between P14 and P21 sham-operated rats, so the two groups were pooled.

Psychomotor Task

The wire hanging task is sensitive to changes in forelimb muscle strength and is used to measure the animal's prehensile reflex (8). The NBM lesions made on P14, P17, or P21 days did not impair the performance in this type of psychomotor task when measured either 7 days or 3 months postoperatively. Seven days and 3 months postoperation, in sham-operated and lesioned rats the latencies to fall ranged between 89 and 75 s and between 38 and 28 s, respectively.

TABLE 2

CHOLINE ACETYLTRANSFERASE ACTIVITY (μmol/h/100 mg PROTEIN ± SEM) IN THE CEREBRAL CORTEX 3 MONTHS AFTER UNILATERAL LESION OF THE NBM

Condition	Age at Operation (days)	Frontal Cortex		Parietal Cortex	
		Lesioned	Unlesioned	Lesioned	Unlesioned
Sham-operated (8)	14 and 21	2.70 ± 0.19	2.68 ± 0.23	2.96 ± 0.20	2.89 ± 0.16
Lesioned (6)	14	1.78 ± 0.17* (−34%)	2.35 ± 0.18 (−12%)	1.90 ± 0.19† (−36%)	2.48 ± 0.19 (−14%)
Lesioned (10)	21	1.62 ± 0.13‡ (−40%)	2.29 ± 0.13 (−15%)	1.91 ± 0.12‡ (−35%)	2.50 ± 0.08 (−14%)

The percent changes calculated vs. sham-operated rats and the number of rats are in parentheses. Statistically significant differences from sham-operated rats: *$p < 0.01$; †$p < 0.005$; ‡$p < 0.001$ (Student's t-test, two-tail probability).

TABLE 3

PASSIVE AVOIDANCE CONDITIONED RESPONSE OF SHAM-OPERATED RATS DURING DEVELOPMENT

Age	Retest latency at 30 min (s ± SEM)	Retest latency at 24 h (s ± SEM)
P14 (8)	3.50 ± 0.73*†	3.00 ± 0.69*†
P17 (8)	20.80 ± 6.96*	10.20 ± 4.29*
P21 (10)	61.25 ± 18.9*	19.70 ± 3.90*
3 months (10)	108.20 ± 9.70	88.43 ± 18.0

The number of rats are given in parentheses. Statistical analysis: Kruskal–Wallis ($p < 0.001$) followed by a post-hoc multiple comparison Z-value test: *$p < 0.05$ vs. 3 months and †$p < 0.05$ vs. P21.

Passive Avoidance Conditioned Responses

Table 3 shows that sham-operated P14 rats were unable to negotiate the passive avoidance conditioned response task; learning and retention began on P17 and improved on P21, gradually reaching adult levels. In P21 rats, the reentry latencies carried out 30 min after the learning trial were much longer than at the 24-h test–retest interval. It appears, therefore, that at this age acquisition is already well developed while retention ability is still immature.

Panels A and B of Fig. 2 show that the unilateral NBM lesion placed on P17 and P21 strongly impaired the ability of rats to acquire and retain the passive avoidance conditioned response when tested 1 week after surgery. Latency scores for NBM-lesioned rats of these two age groups were significantly lower than those of age-matched control animals both when the retest occurred 30 min and 24 h after the training. The impairment appears to involve both acquisition and retention. Two-way ANOVA conducted for both 30-min and 24-h test–retest intervals indicated reliable main effects of the independent variables (group, age) for both test–retest intervals, $F(1, 45)$ group 30 min = 16.632, $p < 0.001$, and $F(2, 44)$ age 30 min = 7.24, $p < 0.005$; $F(1, 45)$ group 24 h = 15.896, $p < 0.001$, and $F(2, 44)$ age 24 h = 7.343, $p < 0.005$. Such analysis also indicated the presence of the significant interaction between the groups and ages for both 30-min and 24-h test–retest intervals, $F(2, 44)$ 30 min = 5.680, $p < 0.01$; $F(2, 44)$ 24 h = 4.033, $p < 0.05$. No differences were found in the training trial latencies between sham-operated and lesioned rats and between different age groups. The latencies were 11.7 ± 2.4 s for immature rats and 17.5 ± 4.0 s for adult rats. Panels C and D of Fig. 2 show the passive avoidance conditioned responses in sham-operated and lesioned rats 3 months after operation. Two groups of rats were tested: In one, the lesion was placed on P14; in the second, on P21. There were no significant differences in body weight and gross behavior and training trial latencies between sham-operated and lesioned rats. The performance of lesioned rats of both age groups, retested 30 min after training, was indistinguishable from that of control rats (Fig. 2C). Similarly, no behavioral impairment was observed 3 months after surgery at a 24-h test–retest interval in rats lesioned on P21 (Fig. 2D). Twenty-four hours after training, rats lesioned on P14 showed marked impairment in the passive avoidance conditioned responses as indicated by a latency shorter than in sham-operated rats. A statistically significant difference was observed between the two groups. The Mann–Whitney U-test demonstrated a significant increase in the latency scores when the comparison was done 7 days and 3 months after surgery in age-matched lesioned rats (P14 at 30 min: $p < 0.005$; P21 at 30 min: $p < 0.001$; P14 at 24 h: $p < 0.01$; and P21 at 24 h: $p < 0.001$).

DISCUSSION

The aim of this work was to ascertain whether the destruction of the cholinergic neurons of the NBM shortly after birth, during a period of rapid development of the central cholinergic system (4,10,11,13,14,16), would result in permanent cholinergic hypofunction and, conversely, whether the developmental plasticity of the residual cholinergic neurons would bring about a neurochemical and behavioral recovery. Our results demonstrate that a unilateral lesion made by local injection of quisqualic acid in the NBM of immature rats is followed by a long-lasting decrease in ipsilateral cortical ChAT activity. Either a partial or complete recovery of cortical ChAT activity was found 3 (32) and 6 (3) months after a similar lesion was made with ibotenic acid in adult rats. Moreover, according to Hohmann et al. (12), 1 month after placing an NBM unilateral electrolytic lesion in 1-day-old mice ChAT activity in the lesioned hemisphere did not differ significantly from the contralateral side. Differences in species, procedure, and time of the lesion, which was placed at the beginning of cholinergic development, may explain the difference between these and our results. The latter indicate that a lesion of the NBM made in the early stages of postnatal life in the rat does not undergo a more rapid recovery than in adult life, and it persists for a long time. Although approximately 70% of cortical ChAT activity is contained in the nerve fibers originating from the NBM neurons (15,31), its decrease 7 days and 3 months after surgery in rats lesioned on P14, P17, and P21 ranged from 41–48%. A similar decrease in ChAT activity after neurotoxic lesions of the NBM has been observed in adult rats (3,5). The diffuse distribution of NBM cholinergic neurons within the basal forebrain in the rat makes it impossible to achieve their complete destruction. ChAT activity in developing rats has been previously measured in the whole brain (4,17). The activity levels we found in the cerebral cortex on P14 are similar to those reported by Ladinsky et al. (17) in the whole brain and only a small increase was found between P17 and P100–110. Fourteen-day-old rats were unable to acquire the passive avoidance conditioned response. Blozovski et al. (1) reported that under their experimental conditions 14-day-old Brown Norway rats needed nine trials for acquisition and adult rats only three trials. The ability to acquire the passive avoidance conditioned response increases markedly between P14 and P21 but the ability to retain the information is still poorly developed, as shown in our experiments, by the short latencies at the 24-h retest. In lesioned rats, the conditioned response is strongly impaired 7 days postoperation in both 30-min and 24-h retest trials. Because lesioned rats negotiated normally the psychomotor task, and their training trial latencies did not differ from those of sham-operated rats, the possibility that the poor performance could depend upon a motor deficit can be ruled out. By investigating the behavioral effects of NBM lesions made with different neurotoxins, Dunnett et al. (5) concluded that deficits in the passive avoid-

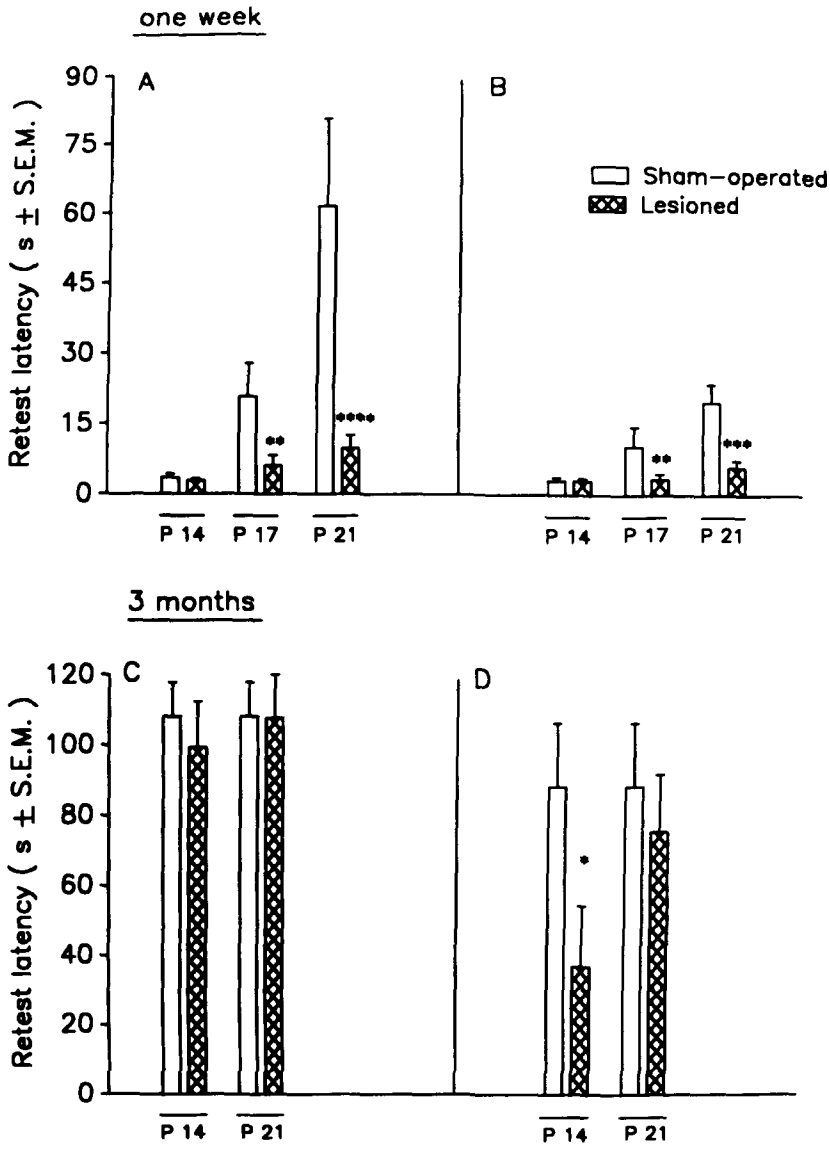

FIG. 2. Passive avoidance conditioned responses of sham-operated and nucleus basalis magnocellularis (NBM)-lesioned rats 7 days (upper panel) and 3 months (lower panel) after surgery. Lesions were made on P14, P17, and P21. Latency to reenter the dark chamber was tested at 30 min (A and C) and 24 h (B and D) after training. Columns represent means ± SEM of 8–15 rats. Statistical significance: $*p < 0.05$, $**p < 0.02$, $***p < 0.005$, and $****p < 0.001$ relative to age-matched sham-operated rats (Mann-Whitney *U*-test).

ance test reflect a hypofunction of the cortical cholinergic system. This finding is supported by our experiments because a marked cortical cholinergic hypofunction and an impairment in the passive avoidance conditioned response were found in sham-operated 14-day-old rats and in lesioned immature rats. However, 3 months after the lesion, despite the persistent ipsilateral decrease in cortical ChAT activity, lesioned rats are able to acquire the passive avoidance conditioned response. An impairment in retention was only detected in rats lesioned on P14. Several possibilities may be taken into consideration to explain this contradiction. The contralateral cortical cholinergic neurons and the intact septohippocampal cholinergic pathway may functionally replace the lost neurons, and other noncholinergic neurons may take over the function. On the other hand, because it has been shown in mice (12) that marked alterations in cortical layers result from NBM lesions placed at birth, it is possible that the delayed behavioral deficits may not directly depend upon persistent cholinergic hypofunction.

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