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Serotonin Influences the Behavioral Recovery of Rats Following Nucleus Basalis Lesions

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MARKOWSKA, A. L. AND G. L. WENK. *Serotonin influences the behavioral recovery of rats following nucleus basalis lesions.* PHARMACOL BIOCHEM BEHAV **38**(4) 731–737, 1991.—The present study investigated the effects of serotonergic depletion upon the performance of rats with lesions of the nucleus basalis magnocellularis (NBM) in a nonspatial memory task. NBM lesions were made by injections of ibotenic acid. Serotonin was depleted by systemic injections of p-chloroamphetamine (PCA). After four weeks of testing, the choice accuracy of PCA rats was not different from that of control rats (CON), while the choice accuracy of NBM rats and rats with combined treatment (NBM + PCA) was significantly lower than CON rats, but not different from each other. After prolonged testing, performance improved in NBM rats, but not in NBM + PCA rats indicating that simultaneous loss of both cholinergic and serotonergic neurotransmission produced a significantly longer lasting behavioral deficit than the loss of cholinergic neurotransmission alone.

Acetylcholine Serotonin Learning and memory Rats Nucleus basalis magnocellularis

ACETYLCHOLINERGIC neurons that project to the cerebral cortex have cell bodies in the basal forebrain (4,28) adjacent to the globus pallidus extending from the level of the olfactory tubercle to the level of the lateral geniculate, referred to as the nucleus basalis magnocellularis (NBM). The terminals of these cholinergic cells are loosely distributed throughout all layers of the neocortical neuropil in the rat brain (4, 6, 28). The serotonergic innervation of all layers of the neocortex originates in the midbrain raphe (RA) nuclei (12,23). The pattern of overlapping cortical innervation provides ample opportunity for functional interactions. For example, cholinergic and serotonergic neural systems provide joint controls of neocortical electrical activity in relation to specific behaviors. Low voltage fast activity (LVFA) in the cortex is related to cholinergic inputs from the NBM and serotonergic inputs from the midbrain RA. These inputs are responsible for an atropine-sensitive and atropine-resistant LVFA, and each has distinctive behavioral correlates (25).

Serotonergic inputs into the cortex may directly modulate the influence of cholinergic inputs from the NBM (1). For example, RA lesions increased acetylcholine turnover in the cortex (19). Thus serotonergic RA cells may tonically inhibit the action of acetylcholine in the neocortex (21).

Recent studies support significant functional interactions between the cholinergic and serotonergic systems in the control of behavior. The serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) had no effect on performance of the spatial memory task in the water maze. Partial reduction of the cholinergic transmission using a low dose of atropine had no effect on the performance of such a task either. When rats were given both PCPA and atropine, performance was significantly impaired (18). Combined reduction in the cholinergic and the serotonergic transmission also impaired performance in a nonspatial memory task (cue task) in the water maze (24).

The acute effects of a combined cholinergic and serotonergic reduction of transmission greatly potentiated the learning impairment. It is not known whether this impairment remains for a long period of time and after extended training. The behavioral impairments due to cholinergic denervation alone usually recovered with continued testing; the rate of recovery was related to the nature of the lesions, the age of the rat, and difficulty of the behavioral task (2, 5, 10). Combined cholinergic and serotonergic denervation of the rat forebrain impaired spatial learning in the Morris water maze for at least 6 months (15). Unfortunately, no groups with lesions of only one system were included in this study as a control. The comparison of the effects produced by combined lesions with effects of lesions of only one system are not possible, and jeopardize the interpretation of above results.

The present study was designed to investigate the acute and long-term effects of combined cholinergic and serotonergic denervation, in comparison to the effects of either lesion alone. Partial cholinergic denervation was produced by bilateral lesions to NBM. The systemic injection of p-chloroamphetamine (PCA) was used to produce the serotonergic lesions. Pharmacological challenges to the cholinergic (scopolamine, physostigmine) and

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serotonergic (methysergide) systems were designed to further investigate the potential interactions between the two systems.

METHOD

Following surgery, the rats were trained in a cued delayed nonmatch-to-sample task. The acquisition of the task (no-injection trials) was followed by testing the rats under pharmacological treatment (IP injections of either saline or drug). At the end of behavioral testing, all rats were retested with no-injection trials.

Subjects

Male Long-Evans hooded rats (300 g) were housed in hanging cages with water available ad lib in a colony room with a 12:12 light/dark cycle. Each rat was assigned to one of four groups and given the appropriate surgery: NBM group—NBM lesions produced by injections of IBO (n = 11); PCA—PCA treatment (n = 11); NBM + PCA—NBM lesions in combination with serotonergic depletion produced by injections of PCA (n = 9); CON—sham operations (n = 11).

After recovery from surgery, the rats were maintained on a restricted feeding schedule designed to maintain body weight at 85% of the free feeding level, and adjusted for normal growth. All behavioral testing occurred during the light portion of the light/dark cycle.

Surgical Procedure

Each rat was given 0.3 ml of atropine methylbromide (5 mg/ml, IP), anesthetized with pentobarbital (50 mg/kg, IP), placed in a stereotaxic instrument with the incisor bar set 2 mm below the earbars, and the scalp was incised and retracted. Coordinates for the NBM lesions were 0.4 and 0.8 mm posterior to Bregma, 2.6 mm lateral from the midline, and 6.8 mm below the dorsal surface of the neocortex. The neurotoxin ibotenic acid (10 μg/μl, pH 7.7 in phosphate-buffered saline) was injected bilaterally into the NBM (0.4 μl/site). The CON group had cannulae lowered to the same anterior-posterior and medial-lateral coordinates used for the NBM lesions, but to a point 5.6 mm dorsal in order to avoid damage to the target area.

Five additional rats were given either NBM lesions or PCA treatment or sham operation, and sacrificed two weeks later to confirm the effectiveness of the lesions.

PCA Treatment

The serotonergic system (PCA group) was destroyed by the administration of four injections of the neurotoxin dl-p-chloroamphetamine HCl (PCA, 10 mg/kg, IP, Sigma) given over two consecutive days (two injections/day).

For the NBM + PCA group, the order in which the treatment was given, either NBM lesion or PCA treatment, was counterbalanced, so that the half of the rats received the NBM lesion prior to PCA, and the other half received the PCA treatment first.

Behavioral Testing

Apparatus. The apparatus consisted of a 37 × 10 cm runway with walls 25 cm high that expanded into a goal platform, 32 cm wide by 38 cm long. Two moveable goal boxes (each 14 × 23 cm with 3 walls 19 cm high) were placed side by side on the goal platform. One goal box was painted with white and black stripes and had strips of carpeting glued on the floor and on the walls. The other goal box had white spots on a black background and had smooth walls and floor. On the far end of each goal box was a food cup. The food cup had a 0.5 cm edge that prevented the rat from seeing its contents from the choice point. A guillotine door separated the start area from the choice point and goal boxes. Additional food was placed beneath the entrance to each goal box so that the rat could not use food odor as a cue to solve the task.

Procedure. Starting 10 days after surgery, each rat was placed on the apparatus for 5 min and allowed to explore and drink chocolate milk from the food cup in the goal boxes. During successive days, each rat was removed from apparatus just after it finished all of the milk from the food cup.

Each trial consisted of a force run and a choice run. For the force run, the entrance to one goal box was blocked and a few drops of chocolate milk were placed in the cup of the available goal box. The rat was placed in the start box and then allowed to run to the available goal box and drink the milk. For the choice run, the barrier was removed and milk was available only in the goal box not entered during the force run (irrespective of the location of the goal box). The correct (baited) goal box was either in the same location as during the force run, or it was moved to the opposite side. The rat was placed in the start box and allowed to choose between the two goal boxes. The delay between the force run and the choice run was about 5 seconds and the intertrial interval was about 3 minutes. Rats were trained 15 trials/day, 5–6 days a week.

When a rat made the correct choice (i.e., entered the goal box opposite to that entered during the force run), it was allowed to drink the milk and was returned to its home cage.

When a rat made an incorrect choice (i.e., entered the same goal box that it entered during the force run), two correction procedures were introduced. 1) For the within-trial correction procedure, the rat was returned to the start, given a second choice run, and then returned to its home cage whether or not it made an error. 2) For the between-trial correction procedure, the same trial was repeated for the next trial, i.e., the goal boxes were located in the same place for the force and choice runs. The between-trial correction procedure was used for a maximum of 4 consecutive trials.

When these correction procedures were not being used, the location of the two goal boxes was changed in a pseudorandom fashion and balanced across the test sessions.

Pharmacological Studies

The pharmacological challenges to the cholinergic and serotonergic neurotransmitter systems began after performance had stabilized (7 weeks after acquisition began and 9 weeks after surgery). Each rat was injected intraperitoneally (IP) 20 min or 5 min prior to the start of each behavioral test session (Table 1). Rats were injected with drug twice a week, with a minimum of two saline treatment days separating each drug administration. Data from no-injection trials (weeks 3–9 of acquisition and week 32 of retest) and from saline control trials were collected on days when the rats were given no drugs. Drug doses, time of injection and order of drug injections are presented in Table 1.

Three replications of testing were completed, with the order of drug × dose randomly determined. A different sequence was used for each replication. All drugs were freshly prepared in saline prior to each session. Drug concentrations were varied to permit the injection of a constant volume (1 ml/kg) to each rat. Each drug container was coded so that the experimenter did not know the specific drug or dose being given.

TABLE 1

PHARMACOLOGICAL TREATMENTS

Drug (weeks of treatment)	Dose (mg/kg)	Time of Injections Before Testing (min)
Scopolamine (weeks 10–19)	0.06	20
	0.12	20
	0.25	20
	0.50	20
	1.00	20
	2.00	20
N-Methylscopolamine (weeks 11 and 19)	2.00	20
Physostigmine (weeks 21–27)	0.05	20
	0.10	20
	0.17	5
	0.17	20
	0.25	20
Neostigmine (weeks 22 and 27)	0.10	20
Methysergide (weeks 20, 28–31)	5	20
	10	20
	15	20

Neurochemistry

After behavioral testing was completed, each rat was sacrificed by decapitation, and the brain was rapidly removed and dissected on ice. Tissue samples were removed bilaterally from each hemisphere, including the frontolateral/somatosensory and parietal/occipital cortex, and from the hippocampus and dorsal caudate nucleus, and stored frozen (−80°C) until assayed. All assays were performed in triplicate. The effectiveness of the NBM lesion was determined by a decrease in neocortical levels of choline acetyltransferase (ChAT) activity. The effectiveness of the PCA treatment was determined by a decrease in regional brain levels of serotonin.

Choline acetyltransferase. Tissue samples were homogenized in 0.4% Triton X-100 containing 10 mM EDTA (pH 7.4). ChAT activity was measured by formation of [^{14}C] Ach formed from [1-^{14}C] acetyl-coenzyme A and choline according to the method of Fonnum (7). Protein content of the homogenate was assayed by the method of Lowry et al. (11) with bovine serum albumin as a protein standard.

Endogenous serotonin. Fresh tissue samples (25 mg) were homogenized in 1500 μl of ice-cold mobile phase containing 0.01% sodium metabisulfite, 0.01% Na_2EDTA and 100 ng of 3,4-dihydroxy-benzylamine (as internal standard). A 200 μl aliquot of the homogenate was removed for protein analysis (11). Homogenate (1.0 ml) was centrifuged at 30,000 × g (4°C) for 15 minutes. An aliquot of supernatant from each sample was filtered (pore size, 0.45 μm) and injected into a high performance liquid chromatography system with an electrochemical detector (26). The level of serotonin was quantitated by comparison to an injected standard.

Histology

The remainder of the brain after dissection was immersion-fixed in a 10% formalin/30% sucrose solution. The tissue was frozen and sectioned coronally at 30 μm with a cryostat. Every fifth section through the NBM was mounted on a glass slide and stained with cresyl violet to determine areas of cell loss.

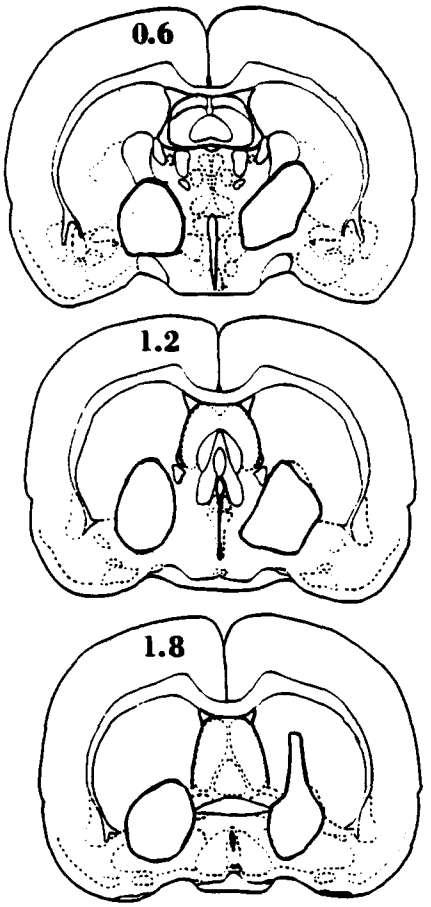

FIG. 1. Schematic representation of estimated maximal extent of gliosis in typical lesion. Numbers with each section refer to the distance (in mm) anterior to bregma.

RESULTS

Histology

The borders of a typical lesion were determined as the area of detectable gliosis (Fig. 1). Ibotenic acid produced focal gliosis throughout the substantia innominata and a variable area of extensive cell loss adjacent to the injections in the vicinity of the NBM.

Behavior

For each rat and each block of 15 trials, the choice accuracy was defined as the percentage of the total responses that were correct (i.e., the total number of correct responses/total number of responses). Choice accuracy is reported as the mean and standard error of the mean (SEM).

The saline control data were collected on days on which the rats were not given any drug. Because an examination of post-treatment saline trials did not reveal any "carry-over" effect of

the drugs, which could possibly generate variance between saline trials, the saline data from one week (at least 3 saline injections) were pooled into a single value and analyzed across 22 weeks of the pharmacological studies.

The response accuracy of each rat in the drug trials was compared to its response accuracy in the respective saline trials. There were no systematic differences between the three repetitions at any of the drug × dose conditions, so these data were pooled to yield a single value for each rat per drug × dose. ANOVAs with repeated measures were performed for the behavioral data, and Student *t*-tests for the neurochemical data.

Acquisition. Immediately after surgery, choice accuracy was near chance level for all four groups (Fig. 2). CON and PCA rats steadily improved their choice accuracy, while NBM and NBM + PCA rats showed less improvement. During the last block of trials, choice accuracy was: CON; 81% (±3), PCA; 80% (±3), NBM; 68% (±4), and NBM + PCA; 69% (±4).

A two-way ANOVA (groups × blocks of trials) showed a main effect of group, $F(3,38) = 12.0$, $p<0.002$, a main effect of blocks of trials, $F(26,78) = 16.1$, $p<0.001$, and a nonsignificant interaction. Post hoc Duncan tests found that choice accuracy of NBM rats and NBM + PCA rats was significantly lower than choice accuracy of either CON rats and PCA rats ($0.005 < ps < 0.001$). The choice accuracy of NBM rats was not significantly different from that of NBM + PCA rats. There were no significant differences between CON rats and PCA rats.

Pharmacological Studies

Saline trials. The mean baseline choice accuracy of CON rats increased slightly from 81% (±3), during the first week of saline injections, to 84% (±3) at the end of testing (Fig. 3). PCA rats given saline showed an asymptotic performance during testing, with a minimum, 75% (±4), and maximum, 82% (±4), choice accuracy.

NBM rats improved their baseline choice accuracy from 63% (±5), during the first week of testing, to 73% (±4), during the last week.

NBM + PCA rats showed an asymptotic performance during the entire training under saline injections with the minimum choice accuracy of 58% (±5) and the maximum of 68% (±6).

An ANOVA performed on choice accuracy during 22 weeks of saline (control) trials (groups × blocks of trials) yielded an overall group effect, $F(3,38) = 8.2$, $p<0.003$, and a significant group × blocks of trials interaction, $F(63,798) = 1.39$, $p<0.03$, indicating the different changes in choice accuracy across groups with continued training. A post hoc Duncan test found a significant improvement in choice accuracy of NBM rats between week 10 (first week of saline treatment) and weeks 21 to 31 ($0.05 < ps < 0.01$). The CON rats showed a slight improvement in performance with training as compared to PCA rats, whose performance declined slightly between weeks 10–31. However, neither changes were statistically significant. NBM + PCA rats did not improve choice accuracy throughout testing.

Scopolamine. Scopolamine produced a dose-dependent decrease in the choice accuracy in all groups of rats, while the dose-response curve was different among the groups. An overall ANOVA group by treatment (drug vs. saline) by dose (0, 0.6, 0.12, 0.25, 0.5, 1, 2; methylscopolamine = dose 0) with repeated measures, yielded a significant main effect of group, $F(3,38) = 9.5$, $p<0.0001$, a significant main effect of treatment, $F(1,38) = 183.0$, $p<0.0001$, and a significant effect of dose, $F(6,228) = 23.1$, $p<0.0001$. A significant treatment × dose interaction, $F(6,228) = 26.8$, $p<0.0001$, indicated the various magnitudes of drug effect with the different doses. Other interactions were non-

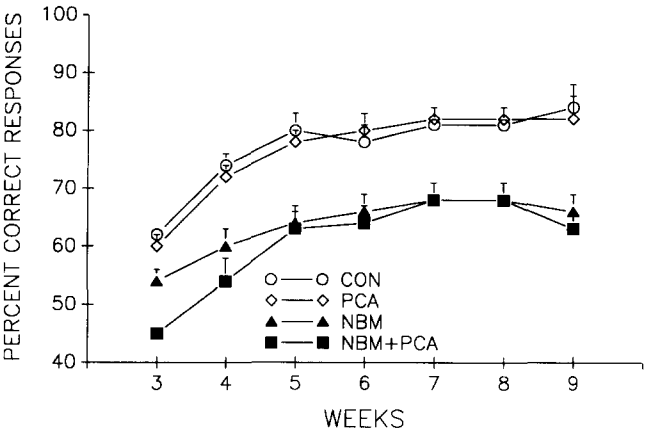

FIG. 2. The choice accuracy of four groups of rats during acquisition of the task (weeks 3–9 after surgery, no-injection trials).

significant. To further analyze significant main effects and interaction, separate two-way ANOVA's (group × treatment) and post hoc tests were conducted. The two lowest doses of scopolamine, 0.06 and 0.12 mg/kg, impaired the choice accuracy of NBM and NBM + PCA rats (post hoc Duncan tests, $p<0.005<ps<0.01$), but did not impair choice accuracy of CON and PCA rats. Higher doses of scopolamine (0.25, 0.5, 1, and 2 mg/kg) impaired choice accuracy of all groups of rats, $F(1,38)>50.0$, $p<0.0001$. Methylscopolamine had no significant effects on choice accuracy.

Physostigmine. Physostigmine injected 5 minutes (0.17 mg/kg) or 20 minutes prior to testing (0.05, 0.1, 0.17, 0.25 mg/kg), did not significantly alter choice accuracy (ANOVA). Neostigmine had no significant effects on choice accuracy.

Methysergide. All doses of methysergide impaired choice accuracy in all groups of rats. An overall three-way ANOVA (groups × treatment × dose) found a significant group effect, $F(3,38) = 7.5$, $p<0.0001$, and a significant treatment effect, $F(1,38) = 60.7$, $p<0.0001$. The dose effect and the interactions were nonsignificant. A two-way ANOVA performed for each dose separately yielded a significant main effect of drug [5 mg/kg: $F(1,38) = 18.8$, $p<0.002$; 10 mg/kg: $F(1,38) = 8.3$, $p<0.01$; 15 mg/kg: $F(1,38) = 26.4$, $p<0.0001$]. Methysergide at 10 mg/kg produced a significantly greater impairment in choice accuracy in the NBM + PCA group than in the other three groups [dose × drug interaction, $F(3,38) = 3.1$, $p<0.04$].

Retest Under No-Injection Conditions

During the last week of testing (week 32 in Fig. 3), the choice accuracy was: CON rats, 84% (±3); PCA rats, 75% (±4); NBM rats, 75% (±4); NBM + PCA rats, 67% (±4). A two-way ANOVA (groups × blocks of trials) revealed a main effect of group, $F(3,38) = 5.5$, $p<0.005$. Post hoc Duncan tests found that the choice accuracy of CON rats was significantly greater than that of all three lesioned groups: from PCA: $p<0.05$, NBM: $p<0.05$, and NBM + PCA: $p<0.005$. The choice accuracy of NBM rats and PCA rats was significantly greater than NBM + PCA rats ($p<0.05$). The choice accuracy of PCA rats did not differ from NBM rats.

Neurochemistry

NBM lesions significantly decreased ChAT activity in the frontal somatosensory cortex while PCA treatment significantly

TABLE 2

ENDOGENOUS LEVELS OF CHAT ACTIVITY AND SEROTONIN 8 MONTHS AFTER LESION

Group	ChAT Activity (nanomoles/mg protein/hour) Somatosensory Cortex	Hippocampus
CON	25.7 ± 5.1	34.8 ± 7.2
NBM	18.0 ± 3.6* (70)	38.9 ± 8.6 (111)
NBM+ PCA	15.0 ± 3.2* (58)	31.4 ± 6.5 (90)
PCA	27.9 ± 5.2 (108)	37.7 ± 9.9 (108)
NBM†	15.7 ± 5.7* (61)	32.4 ± 1.5 (93)

Group	Serotonin (nanograms/mg protein) Occipital Cortex	Hippocampus	Caudate Nucleus
CON	2.13 ± 0.48	3.55 ± 0.12	3.64 ± 1.09
NBM	2.08 ± 0.48 (97)	3.71 ± 1.03(104)	4.45 ± 2.36(122)
NBM+ PCA	0.30 ± 0.11*(14)	0.69 ± 0.23*(19)	1.58 ± 1.12*(43)
PCA	0.49 ± 0.32*(23)	0.87 ± 0.55*(24)	2.59 ± 0.98*(71)
PCA	0.16 ± 0.02* (7)	0.38 ± 0.12*(10)	1.11 ± 0.34*(29)

*Indicates $p<0.01$ vs. CON levels by Student's t-test.
†Two weeks after lesion.
Numbers in parentheses show percent of control.

decreased serotonin levels in the occipital cortex, hippocampus and caudate nucleus (Table 2). Although the drop in ChAT activity and serotonin was greater two weeks after treatment than 33 weeks after treatment (at the end of behavioral testing), the differences were not statistically significant.

DISCUSSION

The results from this experiment showed that a serotonergic lesion, produced by intraperitoneal injection of PCA, severely impaired the choice accuracy of rats subjected to an additional lesion in the NBM, but had no effect on the choice accuracy of intact rats. Rats with NBM lesions were initially impaired, and gradually increased their choice accuracy with continued training. Rats with combined NBM + PCA treatment showed only a slight improvement in choice accuracy during acquisition of the task but no further improvement with prolonged training over the next 23 weeks. These data are consistent with the hypothesis that the serotonergic neural system is necessary for recovery of performance of rats with NBM lesions in a nonspatial working memory task.

The recovery of performance following NBM lesions has been reported by many others (2, 3, 10) and may depend upon the opportunity for continued testing or training. For example, when the rat was passively detained in its cage for 6 months after surgery, the performance deficit in a passive avoidance task persisted (3).

In the present study, the destruction of forebrain serotonergic neurons, following injection of the serotonergic neurotoxin PCA did not affect the acquisition of this nonspatial task. However, with prolonged training, CON rats improved their choice accuracy, while the PCA rats slightly declined their choice accuracy. These changes in performance may have been produced by the repeated drug injections or some alternative mechanism related to an interaction between the type of the lesion and the drug injections.

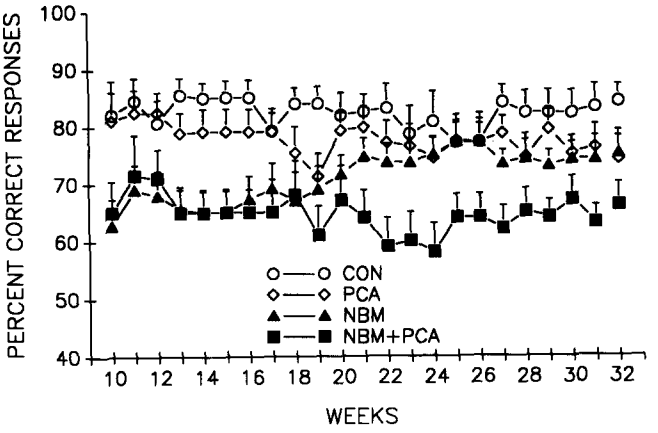

FIG. 3. The choice accuracy of four groups of rats during pharmacological treatment. Only data from saline trials (weeks 10–31 after surgery), and retest week (no-injection trials; week 32 after surgery) are included.

Combined manipulation of the cholinergic and serotonergic systems suggests that these two systems interact in the mediation of learning and memory processes (16, 24, 25). The behavioral impairment following combined cholinergic and serotonergic denervation did not recover after 6 months of testing, as compared to sham-operated rats (15). In addition, lesions in both systems produced a far more severe impairment in the spatial memory task than after a lesion in only one system (15). In the present study, the extent of initial acquisition impairment following simultaneous blockade of cholinergic and serotonergic function was similar to that seen after only an NBM lesion. During acquisition, prior to drug treatment, PCA + NBM rats appeared to recover as little as the NBM rats. However, after several months of testing, the combined NBM and serotonergic lesions produced a greater behavioral deficit than an NBM lesion or PCA treatment alone. In the study by Nilsson et al. (15), the nature of combined cholinergic and serotonergic manipulations was quite different from that used in the present study, i.e., the cholinergic projection to the hippocampus was destroyed by septal lesions, and the serotonergic denervation was produced by intraventricular injections of 5,7-DHT. The behavioral effects of septal lesions are different from the effects of NBM lesions [for review see (17,27)]. In addition, the effects of PCA are not directly comparable to the effects of 5,7-DHT. Therefore, the discrepancy between the results of the present study and others may be related to the different method and locations of the lesions or the different mnemonic processes required for each task, i.e., spatial reference memory in the Morris water maze versus nonspatial working memory in a T-maze.

Treatment with the muscarinic cholinergic antagonist scopolamine produced a dose-dependent impairment in performance of all rats. Rats with NBM lesions, with or without PCA treatment, were more sensitive to the effects of scopolamine than unlesioned rats. In contrast, the presence of a serotonergic deficit did not significantly interact with the ability of scopolamine to impair performance of rats with or without NBM lesions. This is in contrast to a previous study that found that treatment with both PCPA and atropine significantly increased the magnitude of impairment in the water maze task (18). However, this discrepancy may be

related to the different types of serotonergic deficit produced in both studies, i.e., PCA vs. PCPA, and to different antimuscarinic compounds used, i.e., scopolamine vs. atropine. In contrast to the present study, the degree of impairment produced by scopolamine was attenuated by pretreatment with PCA when tested in a nonmatch-to-sample task in an operant chamber (20). The different ways in which cholinergic and serotonergic systems interact may depend therefore upon the different mnemonic processes required for the accurate performance of each task.

Physostigmine did not affect the choice accuracy of any group at any dose tested when administered either 5 minutes (0.17 mg/kg) or 20 minutes (all doses) prior to testing, in contrast to previous reports where rats with NBM lesions showed improved spatial memory after physostigmine injections (13,14). The discrepancy between these studies may be attributed to the mnemonic demands of the task, i.e., spatial memory vs. nonspatial memory, the schedule of drug administration, or the doses of physostigmine given, i.e., injections of the drug every day for 1 week vs. injections every third day.

Interestingly, methysergide, a serotonergic antagonist, impaired choice accuracy of all groups at each dose, while the PCA treatment, which permanently destroyed the serotonergic neurons, did not impair performance. The lack of behavioral impairment after PCA treatment may be related to the partial depletion of forebrain serotonin, ranging from 77% in occipital cortex, and 75% in the hippocampus, to 29% in the caudate nucleus. Rats with combined NBM + PCA lesions were significantly more sensitive to a single dose of methysergide (10 mg/kg), as compared to the other groups, or to the other doses of methysergide administered. The importance of the effect of a single dose of methysergide is unknown, and may indicate that the effects of methysergide on choice accuracy in this task are represented by an inverted-U dose-response curve. Alternatively, the drug effect may not be the result of a central antiserotonergic action since the methysergide may not be selective antagonist of the central serotonergic system (8).

None of the pharmacological challenges revealed a significant interaction between the two neural systems. Rats with NBM lesions were clearly impaired in the performance of this task while rats with serotonergic depletion were unimpaired or impaired much less, suggesting that the disruption of cholinergic function can impair learning and memory and that acetylcholine plays a critical role in the performance of this task. However, a role for serotonergic function cannot be eliminated. Treatment with methysergide also impaired choice accuracy. The effects of methysergide may be task-dependent. Methysergide did not impair choice accuracy in a continuous nonmatching-to-sample working memory task in an operant chamber (20).

The results of this study may have significant implications for an understanding of the development of the dementia syndrome observed in patients with Alzheimer's disease (AD). AD patients have a significant loss of basal forebrain (particularly cholinergic) neurons and serotonergic receptors and midbrain RA neurons (9). The results are consistent with the hypothesis that the loss of basal forebrain cells may underlie the amnestic component associated with the AD, and that any potential for recovery may depend upon the integrity of the midbrain serotonergic RA system.

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