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Effects of NBM Lesions With Two Neurotoxins on Spatial Memory and Autoshaping

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STECKLER, T., J. S. ANDREWS, P. MARTEN AND J. D. TURNER. *Effects of NBM lesions with two neurotoxins on spatial memory and autoshaping.* PHARMACOL BIOCHEM BEHAV 44(4) 877–889, 1993.—Four groups of Wistar rats received either vehicle, quisqualate, or one of two different ibotenic acid infusions into the basal forebrain. Following recovery from surgery, all rats were tested in three distinct behavioral paradigms: the Bättig radial arm maze, the Barnes circular platform, and autoshaping in an operant chamber. The results showed that the size and site of the ibotenic acid lesion had a profound effect on acquisition performance in some, but not all, procedures. Performance in the Bättig maze and acquisition of a food-rewarded lever press were in particular disrupted by ibotenic acid lesions. The severity of the reduction in cortical choline acetyltransferase (ChAT) did not correlate with performance in the tests. Quisqualate produced the largest reduction in ChAT levels but had no significant effect on performance in any of the three procedures used. Anatomic analysis revealed severe nonspecific damage to the striatum following ibotenic acid that was more pronounced in the group receiving a highly concentrated solution of ibotenic acid as compared to rats infused with a greater volume but less concentrated solution of the neurotoxin. Striatal damage was much less severe following quisqualic acid infusions. However, both types of neurotoxins produced equivalent nonspecific degeneration of the reticular thalamic nucleus. These data confirm reports that nonspecific damage appears to define the severity of ibotenic acid lesions on subsequent behavioral performance.

Autoshaping Autoradiography Circular platform Ibotenic acid Learning Lesion Memory Parvalbumin immunohistochemistry Quisqualic acid Radial arm tunnel maze Rats

ONE of the main projection sites of the cholinergic neurons situated in the basal forebrain is the frontal cortex (11). On the basis of this connection, and the marked decrease in cortical acetylcholine in Alzheimer's patients (14,47), it has been postulated that lesions of this structure in animals may produce mnemonic deficits analogous to those observed in man and thus serve as a relevant animal model (35). The lack of a specific neurotoxin for cholinergic neurones has led to a series of experiments using excitotoxins thought capable of destroying cell bodies in the area of infusion without affecting fibres of passage.

Initially, several reports suggested that ibotenic acid (Ibo) infusions reduced cholinergic activity in the frontal cortex and produced behavioral impairments in a number of animal models of cognition. However, there is an increasing body of evidence that the behavioral effects elicited by lesions of the basal forebrain are dependent not only upon the site of the lesion but also upon the neurotoxin used, that is, ibotenic or quisqualic acid (Quis) (21–24,52). In most studies, behaviorally disruptive effects of ibotenic acid lesions of the basal forebrain have been clearly demonstrated. In contrast, quisqualate lesions in a wide range of procedures have not always produced performance deficits, for example, passive avoidance acquisition and the Morris water maze (23), delayed matching (22,24,38) and nonmatching to position (22,38), delayed alternation tasks (24), or visual discrimination (52). Interestingly, this discrepancy between the effects of the two excitotoxins appears to be unrelated to the type of cognitive task used: It has been reported for both spatial and nonspatial procedures. Moreover, a common finding was that quisqualic acid reduced the cortical choline acetyltransferase (ChAT) activity to the same extent or even more than ibotenic acid. On the other hand, ibotenic acid-induced basal forebrain lesions produced a greater reduction of cortical cytochrome oxidase activity than quisqualic acid, which may be indicative for destruction of a noncholinergic pathway by ibotenic acid (54). This indicates that ibotenic acid may produce more widespread nonspecific damage to subcortical structures than quisqualate and,

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further, that the behavioral deficits are dependent upon the amount of nonspecific damage. Indeed, nonspecific damage at the site of injection following infusion of ibotenic acid into the basal forebrain has been clearly demonstrated (49,52,69). In particular, ibotenic acid destroys magnocellular as well as parvocellular basal forebrain neurons, whereas quisqualic acid leaves parvocellular neurons unaffected (69), and it may be the GABAergic neurons within the basal forebrain that are more vulnerable to ibotenic acid than to quisqualic acid (54).

Dissociative effects have also been demonstrated by comparing ibotenic acid- and AMPA-induced basal forebrain lesions, suggesting that ibotenic acid-induced noncholinergic lesions will result in behavioral deficits (44). Therefore, the earlier hopes that the behavioral deficits seen following basal forebrain damage would be unambiguously related to the loss of cholinergic cells situated within this region appears unlikely [for review, see (21)].

To investigate the behavioral and neuroanatomic distinctions between ibotenate- and quisqualate-induced lesions, a series of experiments involving different lesion sites and behavioral paradigms were planned. Pilot experiments indicated that reductions in ChAT activity in discrete brain regions differed depending upon the injection site and concentration of neurotoxin injected. In this pilot study, ibotenic acid was the more potent neurotoxin, as shown by the more pronounced decrease in ChAT activity induced when compared to the effects of equivalent concentrations of quisqualic acid [see (23) for similar results].

Following the results of the pilot experiments, four groups of rats were assigned to receive either quisqualic acid, ibotenic acid, or sham lesions. The lesions were chosen on the basis of the degree of reduction of the ChAT activity in the frontal cortex observed in the pilot experiments. The behavioral paradigms chosen (the Bättig tunnel maze, the Barnes platform, and autoshaping of a food-rewarded lever press) were designed to take into account different aspects of learning and memory, as well as basic motor effects.

Animals were initially tested for acquisition and long-term retention in a six-arm radial tunnel maze. This type of maze has no extramaze cues and a paucity of intramaze cues. Thus, in the absence of fixed auditory or visual cues animals are required to explore the maze by means of simple taxon strategies (61). This type of maze has previously been shown to be sensitive to basal forebrain lesions: Rats with ibotenic acid lesions of this region have difficulties exploring this maze optimally (55).

Rats were then tested on the circular platform task originally developed by Barnes (5). In contrast to the first task, the animal has to orientate solely on the basis of extramaze cues. The platform is illuminated by bright light, which the animal can avoid by escaping through a hole into a dark chamber. The escape hole has a stable position relative to the extramaze cues within the testing room.

Third, animals were assigned to a nonspatial autoshaping task to assess acquisition of food-rewarded lever pressing. In this task, the appearance and disappearance of a lever is matched with the delivery of food (3,4). After several pairings, the rat learns to associate the lever with food and begins to press.

For information concerning lesion selectivity and differences between the two different neurotoxins or the two different infusion sites, brains were assessed for histological and biochemical analysis at the end of the experiment. Thus, brain slices were stained for AChE histochemistry and parvalbumin immunohistochemistry, which stains a subpopulation of GABAergic interneurons in the striatum and the GABAergic projection neurons in the globus pallidus and reticular nucleus of the thalamus (13,29,36). In addition, autoradiographic studies with ligands that label selectively different cellular populations or elements were employed to assess damage to cholinergic systems, striatal neurons, and striatal and pallidal neurons and afferent terminals. Ligands employed were [^{3}H]hemicholinium-3, which labels choline uptake sites on cholinergic terminals (42), [^{3}H]SCH23390, which labels D_1 receptors on intrinsic striatal neurons (17,65), and [^{3}H]5-hydroxytryptamine (5-HT), which labels serotonin 1 and 2 receptors on intrinsic striatal and pallidal neurons and also the terminals of striatopallidal projection neurons (46). Specific cholinergic damage was quantified by cortical and hippocampal ChAT activity.

Together, deficits concerning spatial and nonspatial learning and memory can be differentiated and compared with lesion size and placement.

METHODS

Subjects

Subjects were 40 male Wistar rats (271–300 g, Dept. Tierzucht und Haltung, Schering AG, Berlin), housed individually under a 12 L : 12 D cycle, initially with food and water freely available. These animals were assigned to four groups (n = 10). With the introduction of the autoshaping procedure, animals were food restricted to 15 g of food pellets per day.

Surgery

Rats were lesioned under pentobarbital anesthesia (60 mg/kg). Ibotenic acid or quisqualic acid (Sigma Chemical Co., St. Louis, MO) were dissolved in phosphate buffer (0.1 M, pH 7.4) at the following doses: ibotenic acid 0.06 and 0.12 M and quisqualic acid 0.12 M.

The neurotoxins were injected bilaterally using the following stereotaxic coordinates (45), concentrations, and volumes: quisqualic acid (0.12 M), 0.2 mm anterior to bregma, 3.4 mm lateral to the midline, 7.0 mm ventral to the dura (0.5 μl), and 1.0 mm anterior to bregma, 2.6 mm lateral to the midline, and 7.2 mm ventral to the dura (0.5 μl) (group Quis; coordinates A). The two 0.5-μl injections were made using a 10-μl Hamilton syringe (Hamilton, Reno, NV). Each infusion was delivered over 2.5 min and the needle left in place for a further 2 min after injection to aid diffusion. Ibotenic acid (0.06 M) was infused using the same volumes and coordinates (group Ibo 2; coordinates A) as for the quisqualate lesions. In addition, 0.12 M ibotenic acid were injected bilaterally using the following coordinates: 0.8 mm anterior to bregma, 3.0 mm lateral to the midline, and 7.2 mm ventral to the dura (0.4 μl) (group Ibo 1; coordinates B). This one 0.4-μl infusion was delivered over 2 min. Rats in the control group were anesthetized, the skull drilled, but no infusion was given before suturing the skin.

Rats were allowed to recover for 3 weeks prior to testing. During that time they were handled daily.

Apparatus

The tunnel maze and the radial configuration used in this experiment has been described extensively elsewhere (53,55, 56). To minimize extramaze cues, the maze stands alone in its own room. It has a diagonal extension of 1.4 m, and each tunnel is 8 cm wide and 15 cm high (black Plexiglas). The

ceilings and walls are fitted together to form a unit that can be lifted from the floor for removing the animal and cleaning the maze after each run. Forty-two infrared photo cell units are distributed throughout the tunnels and interfaced to an IBM XT computer equipped with the PCI-2000 system (Burr-Brown Inc., Tucson, AZ).

The circular platform used was a modified version of the one originally developed by Barnes [(5); for illustration, see Fig. 4 in (6)]. The platform stands in a separate room that allows a stable arrangement of extramaze cues. Further, four distinct extramaze cues were available for orientation, consisting of a large solid white circle, three white triangles, white horizontal stripes, all on a black background (35 × 50 cm), or a black board alone. The cues were positioned near the dark goal box and then 90°, 180°, and 270° relative to the first and approximately 50 cm from the edge of the platform. Five floodlights were aimed at the platform to minimize shadows and force the animal to avoid the bright light. The platform has a diagonal extension of 1.22 m with 18 circular holes, 9.5 cm in diameter and evenly spaced around the circumference, only one of which allows access to the dark escape box. The rotatable surface stood 0.91 m above the floor. Animals were initially placed in the centre in a metal cylinder (30 cm in diameter) that could be quickly and quietly removed at the beginning of each trial.

Eight Coulbourn operant chambers (Coulbourn Instruments, Inc.) fitted with two levers, a pellet dispenser, houselights, and cue lights over each lever and centrally placed were connected to, and controlled by, an IBM XT and MedLab interface (MedLab Associates, Inc., East Fairfield, VT). Programs were written and data collected using the OPN programming system (60).

Testing Procedure

Following surgery, rats were allowed to recover for a period of 3 weeks. During this period, body weights were measured every day during the first week after surgery and thereafter every second or third day. The testing procedure is illustrated in Fig. 1.

Tunnel maze. At the beginning of the third week after surgery, animals were handled every day and put into the closed center of the maze [see (55) for illustration] with the entrances to the arms blocked for a 2-min daily session for 7 days. Animals were preadapted in the darkened room for 30 min before testing. Testing was performed between 0800 and 1300 h. Animals were introduced through a ceiling door at the center. The door was closed and testing began. Animals explored the maze on the basis of spontaneous exploration, that is, no reward was provided. Following seven 5-min acquisition sessions, a 7-day break was introduced, at the end of which animals were retested for three additional sessions.

To prevent interference between the different tasks used, testing was halted at the completion of the tunnel maze experiment for 12 days before testing began on the Barnes platform. During this stage, rats were handled daily.

Barnes' platform. Animals were tested between 0800 and 1300 h. Animals were adapted to the testing room for 30 min

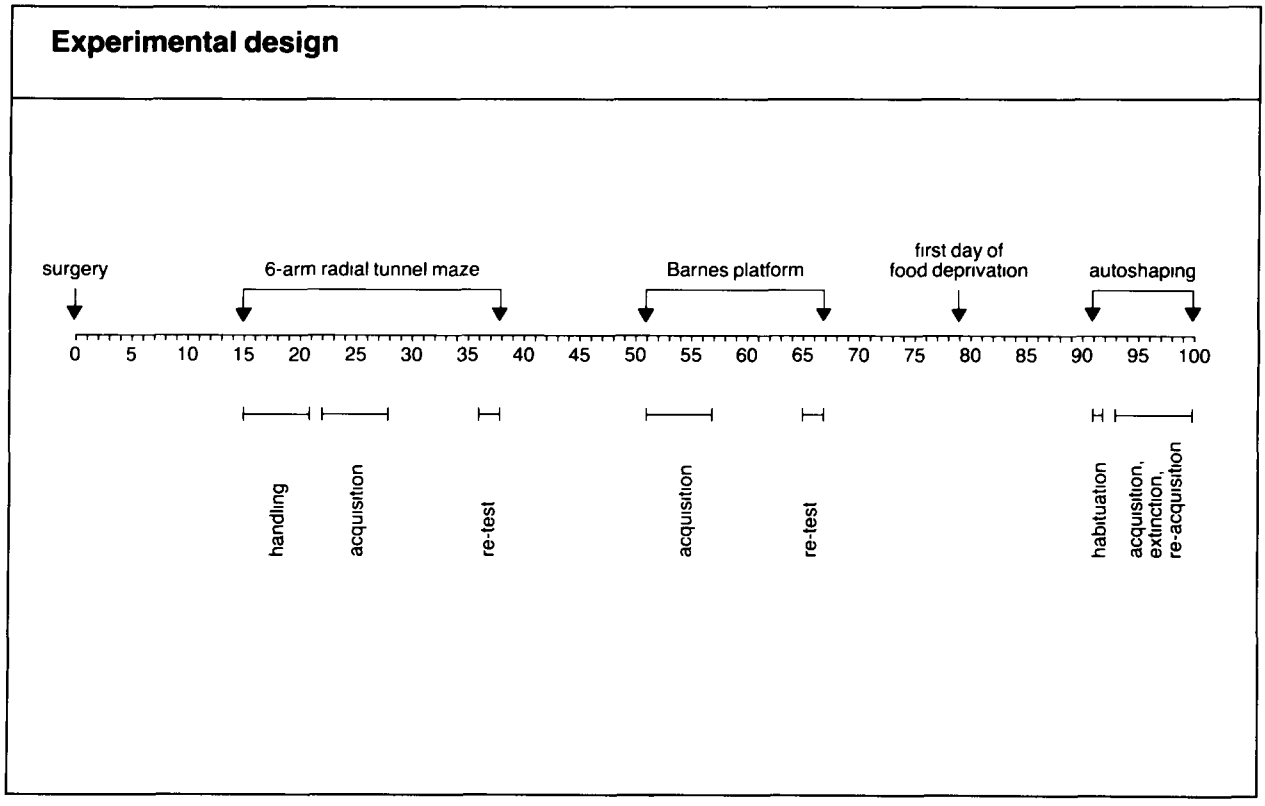

FIG. 1. Schematic illustration of the sequence of testing. Rats that completed autoshaping/acquisition were tested in extinction and re-acquisition; no significant effects were found.

before testing. During the first testing day, each rat was directly placed before the escape hole and left in place until it entered the dark box for an adaptation period of 5 min. Then, the animal was removed and returned to its home cage for a 1 min-period, followed by the first session. During the following testing days, animals were placed into the cylinder and following 1 min the cylinder was raised from the platform and the test session began. After each trial, the top of the platform was wiped clean and rotated; the goal box remained in the same position. This procedure was utilised to prevent the possible use of olfactory cues to find the entrance to the dark box. Rats were trained to learn the position of the dark box over sessions (days), and then following a 7-day break were retested for 3 sessions. As in the tunnel maze, each session was terminated after 5 min.

Following testing on the platform, an interval of 24 days was introduced before training in the autoshaping paradigm began. Twelve days before the beginning of the autoshaping task, animals were restricted to 15 g of food per day.

Autoshaping. During the first 2 days, rats were habituated to the boxes and the noise of the pellet dispenser. On the first day, each rat was placed in the box for 10 min with 20 pellets of food (Dustless pellets; Bio Serv, Holton Industries Comp.; 45 mg per pellet) available in the magazine tray with the magazine light on. On the following day, there was only one pellet in the magazine tray and all other pellets were randomly delivered into the magazine tray, on average once per minute for 10 min. The next day was the first acquisition day (session). The houselight was illuminated and the lever emerged (left or right at random) for 30 s. If an animal did not press the lever within that time, the lever was retracted and a food pellet delivered. If an animal responded on the lever, the lever was immediately retracted and a food pellet delivered. Fifty trials, separated by a 10- s intertrial interval, were scheduled for each session. Each animal was trained for maximally 150 trials, that is, during 3 acquisition sessions (days). Training stopped when five consecutive responses had occurred.

Data Recording

Tunnel maze. From each individual trial, the following behavioral measures were obtained:

Total locomotor activity: total number of photo beams interrupted.

Blind arm entries: the number of entries into the short blind arms. In the design used here, blind arm entries are considered to measure reference memory (2,27,34,67) but may also reflect the use of simple praxis strategies to explore the maze in the absence of adequate cues.

Time to visit all six maze arms.

Reentries: the number of repeated entries into previously visited arms until the animal visited all six different arms. Again, this parameter may represent a measure for reference memory based upon taxon strategies (55,61).

Choice stereotypy: the flexibility of choice behavior, defined as the most frequently chosen turn category based upon the arm entered after exiting a just visited arm, that is, arms at 0, 60, 120, and 180° etc., relative to the arm just visited, and calculated as $(x - c)/(1 - c) \times 100$ where x = relative frequency of the most frequent turn category and c = chance probability = 1/6 [see also (26,33,55)].

Incomplete entries: the percent frequency of visits not completed up to the arm endings in relation to the total number of arm entries.

Barnes' circular platform

Time needed to enter the dark box.

Number of hole-visits: total number of all visits to the holes until the animal had entered the dark chamber.

Autoshaping.

Trials to criterion: the number of trials required to press the lever five consecutive times during acquisition.

Biochemistry

Following the behavioral testing, half the animals were perfused transcardially for histological evaluation of the lesions (n = 5 per group). The remaining rats were decapitated and one (the left) hemisphere dissected according to the method of Heffner et al. (31); the frontal cortex, parietal cortex, and hippocampus were frozen in liquid nitrogen and stored at −80°C until analysis of the ChAT activity according to the method of Fonnum (28). The right hemisphere was used for autoradiographic analysis.

Histology

Horizontal cryostat sections from perfused brains (paraformaldehyde, 4%) were stained histochemically for acetylcholinesterase (AChE) according to Butcher (12).

Parallel sections were stained immunocytochemically for parvalbumin. Sections were preincubated in phosphate-buffered saline (PBS) (0.01 M, pH 7.2) buffer containing 0.2% Triton, 0.1% bovine serum albumin, and blocking serum (horse, 150 μl/10 ml buffer) for 2 h, rinsed, and then incubated overnight at room temperature with a monoclonal antibody directed against parvalbumin (Sigma Chemical Co., St. Louis, MO, 1:2,000, diluted in PBST 0.2%). Following three washes in PBST, sections were incubated for 2 h at room temperature in biotinylated antimouse immunoglobulin (Ig)G (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Sections were washed three times in PBS and then incubated for 1 h at room temperature in ABC (Vectastain kit). Afterward, sections were washed twice in PBS and incubated in diaminobenzidine HCl (DAB; 0.25%) + H_2O_2 (0.02 %) for 10 min, processed through a series of alcohols, and coverslipped.

Autoradiography

Right hemispheres of nonperfused animals were used to assess receptor distributions. Twenty-micrometer thick horizontal cryostat sections were mounted on glass slides and stored at −80°C until use. [^{3}H]Hemicholinium-3 (126 Ci/mmol) was purchased from New England Nuclear (Boston, MA); [^{3}H]SCH23390 (72.5 Ci/mmol) and [^{3}H]5-HT (12.5 Ci/mmol) were purchased from Amersham (Berks, UK). For [^{3}H]hemicholinium binding, sections were preincubated for 10 min at 4°C in 50 mM Tris-HCl, pH 7.4, containing 300 mM NaCl, washed twice, and were incubated in the buffer containing 8 nM of the titrated ligand for 60 min at 4°C. For determination of nonspecific binding, unlabelled hemicholinium-3 (10 μM) was included in the buffer. For dopamine D_1 receptor binding sites, sections were preincubated for 10 min in 50 mM Tris-HCl, pH 7.7, + 10 mM $MgCl_2$ + 1 mM EDTA at room temperature, washed twice, and incubated with 1 nM [^{3}H]SCH23390 in the same buffer for 60 min at room temperature. Nonspecific binding was defined in the presence of 10 μM unlabeled SCH23390. For [^{3}H]5-HT autoradiography, sections were preincubated for 30 min at room temperature in

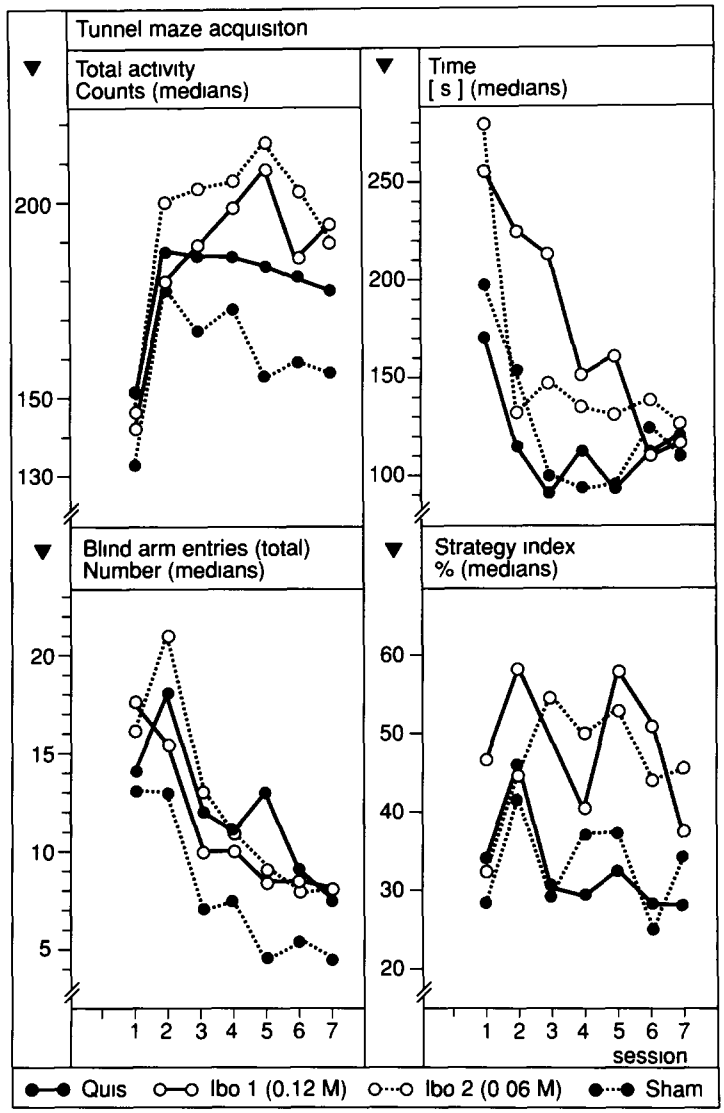

FIG. 2. Tunnel maze performance during acquisition. Data show medians. Note there is no significant difference between the Quis- and sham-lesioned groups. However, there was a trend for the Quis group to exhibit an increased number of blind arm entries.

TABLE 1

SUMMARY OF THE RESULTS OF THE STATISTICAL TESTS USED TO ANALYSE THE TUNNEL MAZE

Total Activity	Blind	Time	Reentries	Strategy	Incomplete Entries
Acquisition (level)					
$H' = 11.096$	$H' = 9.251$	$H' = 17.700$	$H' = 8.041$	$H' = 10.707$	$H' = 10.339$
$p = 0.0072$*	$p = 0.0207$*	$p = 0.0001$*	$p = 0.0396$	$p = 0.0091$*	$p = 0.0112$*
$I_2 > S$	$I_1 > S$	$I_1 > Q,S$		$I_1, I_2 > S$	$I_1 > S$
Retest (1st session)					
$H' = 6.491$	$H' = 3.175$	$H' = 3.020$	$H' = 4.680$	$H' = 13.354$	$H' = 1.522$
$p = 0.0863$	$p = 0.3760$	$p = 0.03996$	$p = 0.1999$	$p = 0.0016$*	$p = 0.6879$
				$I_1,I_2 > S$	

*$p < 0.05$ for each test.
For details of analysis see text under Methods.

50 mM Tris-HCl, pH 7.7, containing 10 mM $MgCl_2$ and 1 mM EDTA. Sections were washed twice and incubated for 60 min with 1 nM [^{3}H]5-HT dissolved in the Tris-HCl buffer as described above at room temperature. Unlabelled 5-HT (10 μM) was included for definition of nonspecific binding. After two buffer washes and a brief wash in distilled water, sections were dried and exposed to Ultrofilm (LKB, Sweden) with [^{3}H]methacrylate standards. In sections incubated with [^{3}H]hemicholinium-3, optical densities of the frontal cortex were measured using a Loats image analysis system (Amersham) and transformed into tissue radioactivity values. Films from sections incubated with [^{3}H]SCH23390 or [^{3}H]5-HT were used for qualitative analysis of lesion-induced nonspecific damage to the striatum. These films were not quantified.

Statistical Analyses

Results obtained from the tunnel and Barnes' mazes were analyzed by means of the Kruskal-Wallis test. Comparisons were computed from the medians of the value of the behavioral measures from all acquisition sessions (except the first session because of the variability of the animal's behavior on initial exposure to the tunnel or Barnes' maze) and from the values of the first retest session (α = 0.05 for each univariate test). In case of a significant difference, multiple comparisons were carried out using the Bonferroni criterion (method of Dunn; $\alpha' = \alpha/6 = 0.0083$). The interdependence of the behavioral measures was not taken into account.

Data obtained from the autoshaping schedule were cast into a contingency table for analysis with the χ^2 test. Three categories were used: animals that reached the criterion in less than 75 trials, between 76 and 150 trials, and more than 150 trials, that is, animals that failed to learn the lever press. Due to the small cell sizes, multiple comparisons between the groups were performed by means of the Fisher exact probability test; again, the Bonferroni criterion was used to correct the α level to 0.0083.

Biochemical data and [^{3}H]hemicholinium-3 autoradiographic data were analyzed by the use of the Kruskal-Wallis test as described for the behavioral data. Further, the reduction of the ChAT activity in the different regions of the brains, frontal cortical, and [^{3}H]hemicholinium-3 binding were compared to tunnel maze activity, blind arm entry and repetition acquisition data, tunnel maze repetition retest data, circular platform acquisition data, and the number of trials needed to reach criterion during the acquisition schedule of the autoshaping procedure by the use of the Spearman rank correlation statistic.

RESULTS

One animal from the second Ibo group died following surgery. As has been previously reported, the lesions produced a loss of body weights compared to the control group. Rats treated with Quis showed a recovery of body weights to control levels during the 3-week recovery period. The two groups that received ibotenic acid infusions did not attain the same weight as controls and remained approximately 10% lighter during the course of the experiment. Food restriction resulted in an equivalent decrease of body weights in all groups.

Tunnel Maze

Total locomotor activity. During acquisition, group Ibo 2 was significantly more active than controls (see Fig. 2; the results of the statistical tests are summarized in Table 1).

Blind arm entries. Ibotenic acid lesions resulted in more entries into the blind arms during acquisition (group Ibo 1 made more total blind arm entries than the control group).

Time to enter six different arms. Ibo 1 took more time to reach criterion than the control and Quis groups.

Reentries. No differences were observed.

Choice stereotypy. Both Ibo groups explored the maze in a less optimal manner than control, that is, there was less flexibility in exploration strategies (see Fig. 2).

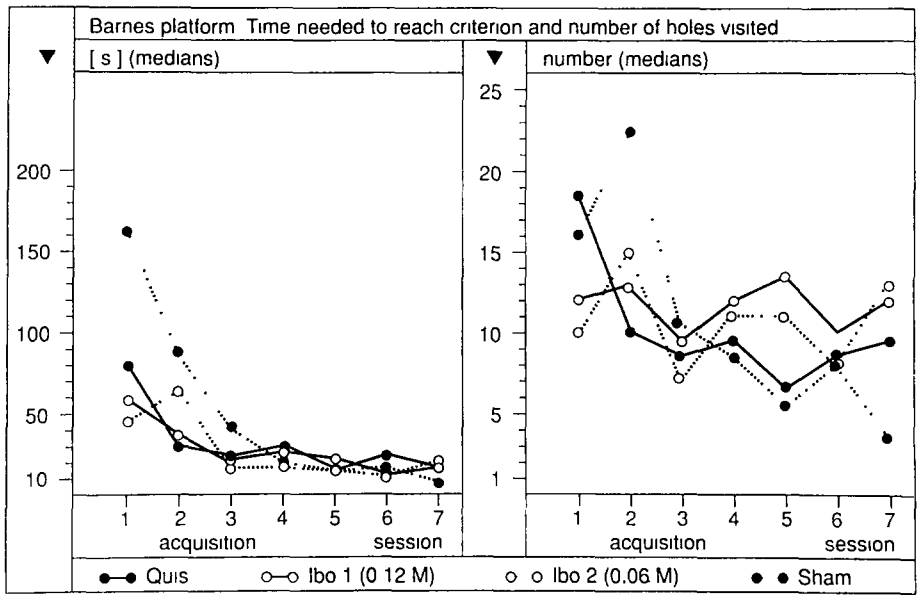

FIG. 3. Exploration of the circular platform. Data show median time and number of holes visited in the Barnes platform before entering the dark box over 7 test days.

Incomplete entries. During the acquisition stage, Ibo 1 made fewer incomplete arm entries than controls.

Barnes' Platform

Inspection of the raw data suggested that lesioned rats were more active than sham controls on the initial test day and showed no apparent learning, that is, no improvement or decline in performance over time. However, no significant differences were observed between the groups on any one test day (see Fig. 3).

Autoshaping

As indicated in Fig. 4, Ibo 2 needed more trials to reach criterion than Quis and Sham groups on the initial acquisition (Quis vs. Ibo 2 $p < 0.0037$; Sham vs. Ibo 2 $p < 0.004$).

ChAT activity

All lesions reduced ChAT activity in cortical regions by approximately 30%. The largest decrease in frontal cortex ChAT activity compared to the control group was seen in the Quis group ($H' = 9.078$, $p = 0.0148$). No differences were found in the hippocampus (Fig. 5).

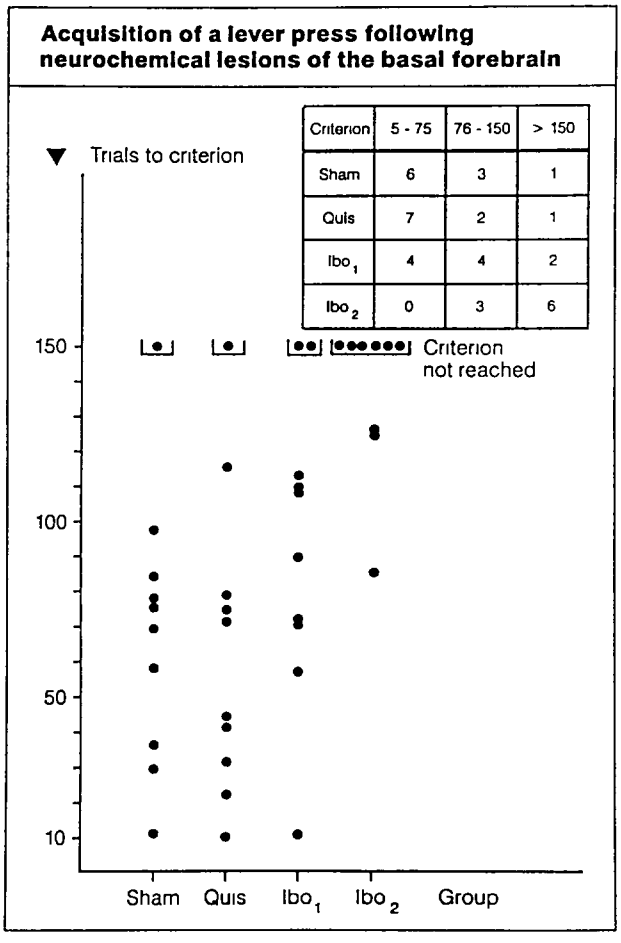

FIG. 4. Trials to criterion in the autoshaping acquisition. Note that only three of nine animals of the Ibo 2 group reached the criterion within 150 trials.

[^{3}H]hemicholinium-3 Autoradiography

[^{3}H]Hemicholinium-3 binding to frontal cortex did not differ between groups ($H' = 3.254$, $p = 0.381$; Table 2).

Correlations

Neither the ChAT activity nor the [^{3}H]hemicholinium binding did correlate in any of the regions measured with the behavioral data.

Histology, [^{3}H]SCH23390, and [^{3}H]5-HT binding

As indicated by autoradiographic as well as by parvalbumin immunohistochemical and AChE histochemical analyses, both types of excitotoxins caused striatal damage. However, this nonspecific damage was much more extensive in ibotenic acid-lesioned rats when compared to quisqualic acid-lesioned animals. Figure 6 illustrates the autoradiographic results in four representative animals (one animal from each group): Although nonspecific lesion size varied within the groups, group Ibo 1 exhibited by far the greatest nonspecific destruction to the striatum. Further, striatal degeneration in group Ibo 1 was even more pronounced than in group Ibo 2. The distinction between striatal neurotoxic effects of quisqualic acid and ibotenic acid was also observed in slices stained for AChE and parvalbumin (Fig. 7). In contrast to the relative sparing of the striatum by quisqualic acid, all treatments damaged the reticular nucleus of the thalamus to the same extent (Fig. 7).

DISCUSSION

The effects of various lesions of the basal forebrain of the rat were compared in several paradigms thought to test differing aspects of learning and memory: one nonspatial autoshaping task and two spatial tasks, further differentiated into one based upon taxon strategies (tunnel maze) and one related to spatial mapping strategies (Barnes platform). The lesions differed in their position, concentration, or type of excitotoxin used. Despite the fact that quisqualate-induced lesions caused the greatest reductions in ChAT levels, quisqualate-lesioned animals showed no significant deficit in exploration of the Bättig tunnel maze or in the acquisition of a food-rewarded lever press. Ibotenic acid lesions caused significant deficits in these procedures depending upon the position of the lesion. In the third test, the Barnes circular platform, quisqualate- and ibotenic acid-lesioned rats showed no obvious learning curves in comparison to sham-lesioned controls, although the differences were not significant.

In keeping with an earlier study (55), ibotenic acid lesions resulted in hyperactivity during the initial acquisition. The significant increase in locomotor activity observed in the Ibo 2 group may have been due either to the different amounts of neurotoxin used or the different sites of injection. This may also account for the conflicting reports indicating that locomotor activity may be either unchanged (25) or increased (20,32,68) after ibotenate lesions. It is important to note that quisqualic acid injected at the same sites as ibotenic acid in group Ibo 2 was without significant effect on locomotor activity and that a single high-dose ibotenic acid infusion had no significant effect on locomotor activity (Ibo 1), although nonspecific striatal damage was more severe in group Ibo 1. Interestingly, ibotenic acid-induced caudate lesions were without effects on locomotor activity during radial tunnel maze acquisition (63), suggesting that the striatal damage would not account for the hyperactivity elicited by ibotenic acid. Accord-

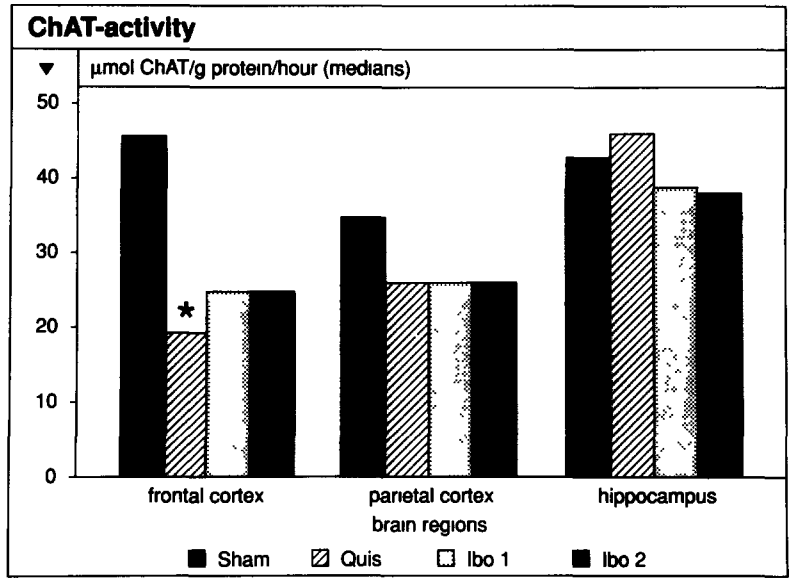

FIG. 5. ChAT activity of the left hemisphere of five randomly assigned animals from each group.

ingly, it has also been demonstrated that injections of both AMPA (which binds to the same glutamate receptor subtype as quisqualic acid) or NMDA (which is thought to act at the same receptor as ibotenic acid) into the lateral preoptic area are able to produce hyperactivity (57). Thus, the effect on locomotor activity appears to be more dependent upon the exact site of infusion as opposed to the neurotoxin used. Inconsistencies of the effects of basal forebrain lesions in many tasks may therefore reflect differences in locomotor activity per se rather than in specific cognitive deficits. In line with this view are contradicting results indicating that quisqualic acid-induced basal forebrain lesions were without effects on water maze acquisition (1,23,48,50) or disrupted water maze learning (49).

As indicated in Fig. 2, all three types of lesions tended to increase the number of blind arm entries during acquisition. However, as in the earlier study (55), ibotenic acid-lesioned rats (Ibo 1) entered the blind arm entries more frequently during acquisition than controls. As blind arm entries may reflect reference memory, different infusion sites may again account for discrepancies concerning basal forebrain lesions and effects on reference memory [this study, Ibo 1 vs. Ibo 2; see also (25)]. Interestingly, a recent study indicated that ibotenic- but not quisqualate-induced lesions disrupted reference memory (as measured by retention), even though quisqualate had a greater effect on ChAT activity (38).

Group Ibo 1 needed more time to reach the criterion during acquisition than the control and Quis groups. This may be related to the increased number of complete arm explorations made by this group.

During the first retest session, both groups lesioned with ibotenic acid used a more stereotyped strategy with which to explore the maze. This effect may not be related to a cognitive deficit, especially as no other differences appeared during this stage, but instead to a deafferentation of the cortex resulting in a disconnection of sensorimotor areas (55,68). However, this interpretation has been challenged by another study showing that the effects of basal forebrain lesions and somatosensory cortical ablation differ (70). It may be speculated that the increase in the number of repetitive entries typically observed during the first retest session (55,62) is related to deficits resulting from a faster decay of the memory trace formed under the lesion. Although there was a tendency for ibotenic acid-lesioned rats to make more reentries during the first retest session (data not shown), there were no significant differences to support this proposal.

The circular platform of Barnes has been shown to be sensitive to detecting deficits in aged rats (5,7,8) and manipula-

TABLE 2

[^{3}H]HEMICHOLINIUM BINDING IN FRONTAL CORTEX (MEAN AND STANDARD DEVIATION)

	Group			
	Sham	Quis	Ibo 1	Ibo 2
[^{3}H]HC-3 (fmol/mg protein)	11.3 ± 3.0	8.4 ± 3.1	9.4 ± 1.2	8.6 ± 2.8

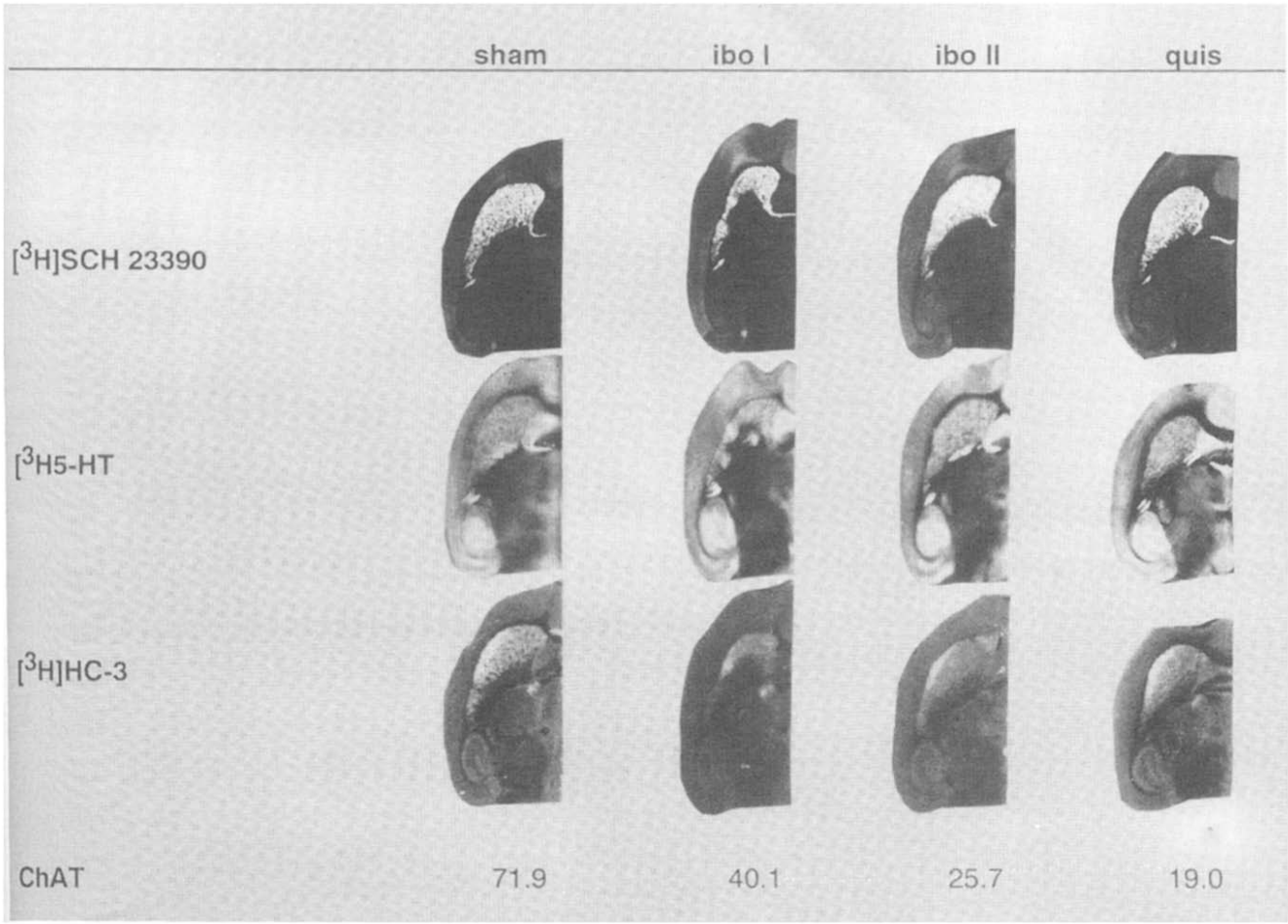

FIG. 6. Autoradiographic distribution of dopaminergic D_1 ([^{3}H]SCH23390), serotonergic ([^{3}H]5-HT), and hemicholinium ([^{3}H]HC-3) binding sites in four representative animals (horizontal plane, right hemispheres), shown as direct prints from autoradiograms. White represents highest binding; black represents no binding. Note that the rat taken from group Ibo I shows the most extensive nonspecific striatal damage, although the ChAT activity (expressed as μmol ChAT/g protein/h in the frontal cortex) was highest in the contralateral hemisphere when compared to the other two lesioned animals.

tions of the hippocampus (6,40). Whereas only taxon strategies have been considered to be necessary for exploration of the tunnel maze, animals use spatial mapping strategies to avoid the bright light in the platform test (5,8). Comparable data on the performance of rats with basal forebrain lesions in the circular platform are not available. However, data from circular water mazes, which are similar in principle, are available for comparison: To solve this type of maze, animals must also use extramaze cues to locate the hidden platform and escape from the water, although it can be argued that the strength of the negative reinforcer (bright light in one case, water in the other) may be more intense in the water maze than in the Barnes circular platform. Using a Morris water maze, deficits following basal forebrain lesions have been repeatedly (19,37) but not always demonstrated (30). Given the superficial similarity between the two mazes, and the fact that performance in both tasks is similarly disrupted by hippocampal lesions (18,40,41), it is unclear whether the lack of significant effects in the Barnes maze in the present experiment reflects a fundamental difference between the two tests or a similarly large variability in effect dependent upon the size of lesion, strain of rat, etc. Clarification of this effect awaits further experimentation. Behavioral recovery has been reported previously following basal forebrain lesions (9,10); however, in this experiment such an effect is not so apparent: Rats in the autoshaping schedule exhibited clear deficits in performance although this task was performed several weeks after the Barnes platform experiment. Thus, this study suggests long-lasting task-specific deficits following basal forebrain lesions. A recent study by Markowska et al. (38) also demonstrated long-lasting performance deficits following ibotenic- but not quisqualate-induced lesions. However, this latter study showed no difference in the effect of ibotenic acid lesions on separate cued and noncued spatial tasks. In addition, Riekkinen Jr. et al. (49) demonstrated acute as well as chronic effects of ibotenate- as well as quisqualate-induced lesions of the basal forebrain in water maze performance.

Lesions of the basal forebrain may result in attentional deficits that in turn are exhibited as poorer learning (16). Ten-

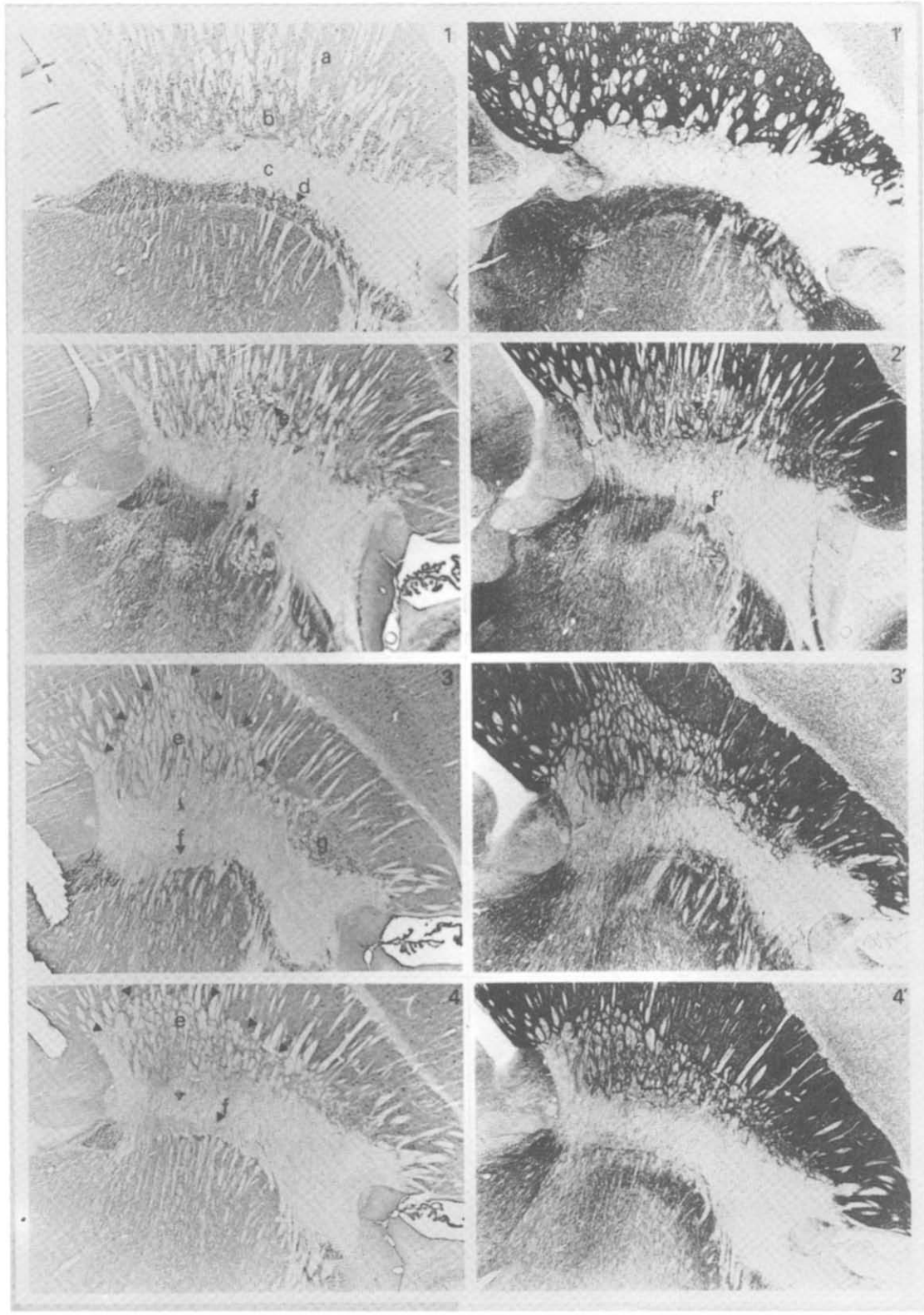

FIG. 7. Parvalbumin immunohistochemistry (left column) and acetylcholinesterase histochemistry (right column), horizontal plane, lateral to the left, medial to the right: (1 and 1′) sham-lesioned animal—Parvalbumin-like immunoreactive neurons are present in the caudate nucleus (a), globus pallidus (b), and reticular nucleus of the thalamus (d); (c) internal capsule. (2 and 2′) Animal with the largest lesion induced with quisqualic acid. There is only limited damage to the globus pallidus (e) but considerable destruction of the reticular nucleus (f and f′) and underlying thalamic structures. (3 and 3′) Sections from animals from group Ibo 1 and from group Ibo 2 (4 and 4′). In both groups lesioned with ibotenic acid, nonspecific damage to the striatum including the caudate nucleus (e) was more extensive than following quisqualic acid infusions. Damage to the reticular nucleus was comparable in all groups. Note that the parvalbumin-like immunoreactive neurons are destroyed within the lesion site in all lesion groups. (g) Remaining globus pallidus in the animal from group Ibo 1.

tative evidence to support this view can be found in the results of the autoshaping experiment. Autoshaping demands an efficient level of attention because the animal must learn to associate the lever with food reward (4). Further, attentional deficits may also account for the poorer performance of ibotenic acid-lesioned rats in the tunnel maze: As the maze is characterized by a paucity of cues, a relatively high attentional level may be required to explore the maze most efficiently. One way to test this hypothesis would be to consider the effects of basal forebrain lesions on attentionally demanding tasks such as conditioned blocking experiments (15). However, a purely attentional deficit is unlikely to be the sole cause of the behavioral effects of the lesion. Studies showing that already learned tasks are minimally disrupted by basal forebrain lesions, whereas new learning of the same task in rats lesioned prior to training is severely disrupted, despite intensive training suggests more than a simple attentional deficit (24,25). It may be that ibotenic acid induced a motivational deficit that was not seen following quisqualic acid lesions.

In agreement with Robbins et al. (52), histological and autoradiographic data clearly demonstrated more extensive nonspecific damage following ibotenic acid lesions of the striatum as shown by AChE histochemistry, [^{3}H]hemicholinium-3, [^{3}H]SCH23390, and [^{3}H]5-HT autoradiography, and parvalbumin immunohistochemistry, although specific damage was most severe following quisqualic acid (ChAT activity). Although nonspecific damage was extensive following both regimens of ibotenic acid lesions, damage tended to be more extensive following one high-dose infusion than following two low-dose infusions.

The parvalbumin immunohistochemical results are of special importance as it has been hypothesized that GABAergic neurons situated within the basal forebrain may be damaged by ibotenic acid but spared following quisqualic acid lesions (54). In contrast to this view, in vitro experiments demonstrated that cortical parvalbuminergic neurons, which represent a subpopulation of GABAergic neurons, are severely degenerated by AMPA but relatively spared by NMDA neurotoxicity (66). As quisqualate and AMPA bind to the same receptor subtype, and as ibotenic acid binds to the NMDA receptor, similar results may be expected following in vivo application. Our results do not confirm the hypothesis of Sarter and Dudchenko (54), and damage to the reticular nucleus of the thalamus, which is rich in neurons exhibiting parvalbumin-like immunoreactivity, was extensive in all lesioned groups, thus contradicting speculations that neurons containing parvalbumin are relatively resistant to overexcitation (58,59). However, parvalbumin-like immunoreactivity represents only a subpopulation of GABAergic neurons and therefore we cannot exclude the possibility that these GABAergic neurons that lack parvalbumin may be spared by quisqualic acid.

Examination of the reticular nucleus of the thalamus for nonspecific damage following basal forebrain lesions by looking at AChE histochemistry may be insufficient as this nucleus receives a dense input from the cholinergic basal forebrain that makes clear statements difficult. Further, it seems harder to separate this nucleus from other structures on the basis of AChE histochemistry only. Therefore, more selective staining procedures like parvalbumin immunohistochemistry are advantageous and important as it seems unlikely that all studies dealing with basal forebrain lesions, and that do not mention nonspecific damage to thalamic structures, show complete sparing of the reticular nucleus, which is also linked to mnemonic function (39).

It seems evident from the behavioral results that only animals receiving ibotenic acid lesions of the basal forebrain performed consistently and significantly worse than controls in novel learning situations. As quisqualic acid-lesioned rats had the most pronounced decrease of ChAT activity but showed only nonsignificant trends, this study supports the hypothesis that many of the deficits observed after basal forebrain lesions may not be related to specific damage of the cholinergic system but may also be due to effects on other neurotransmitter systems. It may be that other cholinergic structures such as the medial septum are more important for learning and memory, as double lesions of both the basal forebrain and medial septum with quisqualic acid result in the same deficits as following ibotenic acid lesions (64), an effect that may be mediated by septohippocampal dysfunction rather than by the disruption of the basal forebrain–cortical connection.

The lack of group differences concerning [^{3}H]hemicholinium-3 binding seems puzzling. However, recovery of hemicholinium-3 binding 3 months following ibotenic acid lesions has been demonstrated (33). Although this study showed also recovery of cortical ChAT activity, and an additional decrease 12 months following the lesion, it may be that both measures did not change in parallel. Further, autoradiographic data showed greater standard deviations, which may affect the significance of the results. Nevertheless, the discrepancy between cholinergic activity and behavioral effects is demonstrated in all three procedures reported here. The proposal that lesions of the basal forebrain may be a simple model for producing deficits similar to those seen in senile dementia of the Alzheimer type cannot therefore be supported [but, see (70) for contradictory conclusions]. Both recently published data and those presented here suggest that neurotoxic lesions of this region are by no means cholinergic specific and that the deficits seen in many tasks are based upon nonspecific damage. Considering the lack of a reliable and specific cholinergic toxin, it is apparent that quisqualic acid [or even AMPA (44)] should be the preferred neurotoxin for producing local lesions and minimizing nonspecific damage (21,23).

In addition, the site of infusion as well as the type of neurotoxin used appears to be an important factor for the behavioral consequences. Riekkinen Jr. et al. (51) have shown that rats with basal forebrain lesions (ibotenic acid lesions) and extensive nonspecific subcortical damage performed less accurately in a place navigation task than a second group with correspondingly less subcortical damage. In the present study, different concentrations and sites of injections of ibotenic acid also resulted in different behavioral effects.

In summary, results indicating distinctive effects of ibotenic acid and quisqualic acid lesions of the basal forebrain have been supported by experiments involving two different spatial tasks and one nonspatial task. Deficits are dependent both upon the neurotoxin used and the location of the lesion within the basal forebrain. This may account for some of the discrepancies reported in the literature in the behavioral consequences of basal forebrain lesions. Thus, as more evidence accumulates suggesting that some of the behavioral deficits observed following basal forebrain lesions are the result of nonspecific damage, initial hopes that this model could represent an animal analog of Alzheimer's disease must be treated with skepticism.

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