Pharmacology Biochemistry & Behavior, Vol. 18, pp. 973–981, 1983. Ankho International. Printed in the U.S.A.

Behavioral and Neurochemical Effects Following Neurotoxic Lesions of a Major Cholinergic Input to the Cerebral Cortex in the Rat[1]

CHARLES FLICKER,† REGINALD L. DEAN,* DOROTHY L. WATKINS,*
STEPHEN K. FISHER* AND RAYMOND T. BARTUS*†

**Department of Central Nervous System Research, Medical Research Division American Cyanamid Company, Lederle Laboratories, Pearl River, NY 10965 and †New York University, Department of Psychiatry, 550 First Avenue, New York, NY 10016*

Received 17 March 1983

FLICKER, C., R. L. DEAN, D. L. WATKINS, S. K. FISHER AND R. T. BARTUS. *Behavioral and neurochemical effects following neurotoxic lesions of a major cholinergic input to the cerebral cortex in the rat.* PHARMACOL BIOCHEM BEHAV **18**(6) 973–981, 1983.—The nucleus basalis magnocellularis (NBM) is the name given to a group of cholinesterase-reactive neurons in the ventromedial corner of the globus pallidus of the rat. This cell group appears to be the major extrinsic source of cortical acetylcholine and is believed to be homologous to the nucleus basalis of Meynert in primates. The excitotoxin ibotenic acid (2.4 μg/0.4 μl) was infused bilaterally into the ventromedial globus pallidus. These lesions depleted frontal cortical choline acetyltransferase (CAT) by a third. Neurotoxic lesions of the dorsolateral globus pallidus did not affect cortical CAT activity. Neither lesion affected the rats' performance on a battery of psychomotor tasks or on tests of shock sensitivity. Rats with NBM lesions were mildly impaired in the acquisition of a one-way active avoidance response, but did not differ from the other groups on extinction of the task. The NBM lesioned rats exhibited a severe deficit in the retention of a passive avoidance response. This effect was visible both 24 hours and one hour after training. Experimental controls suggested that the poor performance of the NBM lesioned rats involves a deficit in learning and/or memory of the training trial. Lesions of the dorsolateral globus pallidus also produced an impairment of passive avoidance retention, but this impairment was not as severe as that following NBM lesions. These results are discussed as they relate to the behavioral role of cholinergic innervation of the cortex, and the development of animal models for disorders involving cortical cholinergic deficiencies, including senile dementia of the Alzheimer's type.

Nucleus basalis Globus pallidus Magnocellular forebrain nuclei Acetylcholine Choline acetyltransferase Cholinergic denervation Animal models, senile dementia Animal models, Alzheimer's disease Avoidance learning Ibotenic acid Memory Psychomotor behavior Neurotoxin lesions

IN THE ventromedial corner of the rat globus pallidus is a group of large, cholinesterase-reactive neurons, referred to as the nucleus basalis or nucleus basalis magnocellularis (NBM), and believed to be homologous to the nucleus basalis of Meynert in human and nonhuman primates [16, 26, 27, 33]. A considerable body of evidence indicates that these cells are a major source of the extrinsic cholinergic input to the neocortex. An acetylcholinesterase-reactive fiber pathway originating in this region and terminating in cerebral cortex was first described by Shute and Lewis [48]. Since that time cells in the NBM staining positively for acetylcholinesterase (AChE) have been shown to be labelled by retrograde transport of cortically injected horseradish peroxidase [33,39]. Cortical ablation likewise produces retrograde degeneration of this neuronal population [8,33]. Furthermore, electrolytic or neurotoxic lesions of the NBM reduce cortical choline acetyltransferase (CAT) activity, AChE activity, ACh release, and choline uptake [26, 27, 28, 33, 36, 42, 50]. Finally, neurons in the rat sensorimotor cortex exhibit responsivity to acetylcholine (ACh) iontophoresis and NBM stimulation, partial blockade of responses by cholinergic antagonists, and supersensitivity to ACh after NBM lesions [15,32]. In summary, although there are almost certainly other externally and internally derived contributions, the NBM appears to be the major source of neocortical ACh [27].

The diffuseness of the projection from the NBM, the relatively compact distribution of the cells of origin, and the

[1]Requests for reprints should be addressed to Dr. Raymond T. Bartus, Director, Geriatric Research Program, Department of CNS Research, American Cyanamid Company, Pearl River, NY 10965. These data were initially presented by R.T.B. as part of an invited presentation at the Cold Spring Harbor Symposium on "Biological Aspects of Alzheimer's Disease," October 25, 1982, Cold Spring Harbor, NY.

apparent utilization of ACh as a neurotransmitter by these cells provide the opportunity to study the relationship between brain ACh and behavior with a new research strategy. That is, destruction of this neuronal population makes it possible to examine the role in behavior played by these cholinergic neurons and their cortical innervation. Furthermore, recent observations that cells in the NBM in man may be selectively degenerated in patients with senile dementia of the Alzheimer's type [51,52] has sparked further interest in the functional role of this brain region. However, in spite of the numerous biochemical investigations and the potential clinical relevance of this manipulation, only one study of the behavioral effects of lesions relatively specific to this area has been previously published [36]. The present study was conducted therefore to help characterize the behavioral consequences of destroying this major cholinergic input to the neocortex.

A variety of behavioral tests and observations were done in order to assess the animal's sensorimotor function and other, more complex cognitive capabilities. The rats were administered a battery of psychomotor tasks designed to evaluate reflexes, balance, and muscle strength. Learning and memory abilities were studied in two separate avoidance paradigms. Pain sensitivity was also measured. The results from this wide array of behavioral tests should provide a foundation for the construction of a behavioral profile of the NBM lesioned rat, and provide some insight into the neurobehavioral role of these cells and their projections.

METHOD

Subjects

The subjects used in this study were 38 male Fischer 344 rats weighing 350–400 grams (6–7 months of age). After surgery, the rats were housed individually and maintained on a 12-hour light/dark cycle. In addition to ad lib access to their pre-operative dietary regimen (Fisher Lab Chow), the animal's diet was supplemented with cheese, peanuts, chocolate and vitamins (Polyvisol®, Mead Johnson, 1.33 ml/l of drinking water).

Surgery

The rats were anesthetized with Nembutal, 50 mg/kg IP, and placed in a stereotaxic apparatus. The infusion apparatus consisted of a 30 gauge stainless steel infusion needle connected to a 10 μl Hamilton microsyringe via PE-10 polyetheylene tubing (Intramedic). The infusate consisted of ibotenic acid (Sigma) dissolved in phosphate-buffered saline (pH 7.4) at a concentration of 6 mg/ml. Ibotenic acid is a neurotoxic glutamate analogue that, like kainic acid, causes selective dendrosomatic degeneration, preferentially destroying dendrites and cell bodies, while sparing axonal processes [45]. Ibotenic acid was chosen because of evidence that it does not produce the distant damage which is associated with the epileptogenic effects of kainic acid injections [22,30].

For the NBM lesion, the tip of the infusion needle was placed 1.6 mm caudal to bregma, 3.1 mm lateral to the midline, and 7.6 mm ventral to the surface of the skull. The incisor bar of the stereotaxic apparatus was set 2.4 mm below the interaural line. As a control for the non-specific destruction of non-cholinergic cells in the globus pallidus, neurotoxic lesions were also placed in the dorsolateral globus pallidus remote from the NBM. Coordinates for animals receiving lesions of the dorsolateral globus pallidus were 1.4 mm caudal to bregma, 4.1 mm lateral to the midline, and 5.6 mm ventral to the surface of the skull. The infusion volume was 0.4 μl, which was delivered over a period of two minutes. The infusion needle was removed five minutes after the infusion. All placements were bilateral. In sham animals the needle was lowered to 2 mm dorsal to the NBM infusion site and left there for five minutes without any infusion. The rats were injected with 0.1 ml bicillin, IM, immediately post-operatively. Three of the 18 NBM lesioned rats died during the three week interim before testing was begun.

BEHAVIORAL PROCEDURES

Psychomotor Tasks

The rats were run on a battery of four psychomotor tasks designed to assess lesion effects upon reflexes, balance, strength, endurance, coordination and other motor functions.

The wire hanging task is a measure of the animal's prehensile reflex [49]. This capacity is evaluated by testing the rat's ability to grasp a taut, horizontal wire with its forepaws and to remain suspended without dropping off. The amount of time spent hanging was recorded for each animal. The test is sensitive to changes in forelimb muscle strength.

The inclined screen task is another measure of muscle tone [1]. On this test, the animal is placed on a 22 inch high wire mesh screen which is inclined at a 60° angle. The amount of time the animal remains on the screen was recorded (up to a 15-minute maximum cutoff).

The rod walking and plank walking tasks were used to assess motor coordination and the integrity of the animal's vestibular system [9,53]. For the rod walking test, each rat was placed on the center of a stationary horizontal wooden rod (28.5 mm in diameter). On the plank walking test, each animal was placed on three successively narrower planks (38, 25 and 13 mm in width). For both tasks, the amount of time the animal maintained its balance, as well as whether or not he traversed the rod/plank was recorded.

Passive Avoidance Task

The single trial passive avoidance procedure is intended to measure the animal's ability to learn and remember to avoid a brief footshock by inhibiting a response which normally occurs with a high probability [9]. A two-chambered step-through passive avoidance apparatus was used.

On the training trial, each rat was placed in the front, illuminated chamber and, after a three-second orientation period, the guillotine door separating the two chambers was raised. As soon as the animal placed all four paws in the rear, dark chamber, the door was lowered and a 1 mA scrambled shock (Coulbourn Instruments) was delivered to the floor grids for three seconds. The rat was removed from the rear chamber five seconds later and returned to its home cage. The latency to enter the rear chamber was recorded. Passive avoidance responding was tested either 1 or 24 hours post-training, by placing the rat in the front chamber and raising the door three seconds later, as on the training trial. The latency to re-enter the rear chamber was recorded (up to a maximum cutoff time of 10 minutes).

Active Avoidance Task

Active avoidance testing was conducted in an automated one-way shuttle box (Lafayette model 85200) equipped with a metal ledge and a retractable wall. The animals were con-

ditioned to climb up onto the metal shelf to avoid a pulsed shock delivered to the stainless steel grid floor. The conditioned stimulus (CS) consisted of a light and a tone.

During training, the rats were placed on the grid floor. Simultaneous with the onset of the CS, the metal wall pulled back to permit access to the shelf. Ten seconds later, a 0.7 mA pulsed shock was applied to the floor grids (0.5 second duration, 1.5 second interpulse interval).

After twenty seconds of this unconditioned stimulus (UCS) both the CS and UCS were terminated and the metal wall pushed forward, blocking access to the shelf. This was followed by a 10 second inter-trial interval. If at any point during the 30 second CS-UCS interval the animal performed a shelf-jump response, a microswitch was activated and the light, buzzer, and shock (if on) were turned off. Ten seconds later the metal wall was moved forward, forcing the animal off the shelf and initiating the inter-trial interval.

The animals received 50 training trials per day, with acquisition of the task operationally defined as a minimum of avoidances on 90% of the trials within a 25-trial block. Upon achieving this criterion, each animal received an additional 50 "overtraining" trials. On the day following overtraining, the extinction procedure was begun.

During extinction the same procedure as during training was followed with two exceptions: (1) on the first trial the rat was placed on the shelf for 10 seconds before the metal wall advanced forward (pushing the rat off) and the CS was turned on, and (2) no UCS (shock) was presented. Each subject received 50 extinction trials per day for up to three days or until the rat failed to exhibit the shelf-jump response on 90% of the trials in a 25-trial block.

Shock Sensitivity Measurement

The rats were evaluated for differences in shock sensitivity using the same shock grid and source as had been used in the passive avoidance task. The scrambled shock was delivered at seven different intensity levels between 0.05 and 1 mA, applied in ascending order with a 30 second inter-shock interval. Vocalization (any audible response to the shock) or a jump response (the lifting of at least two paws from the grid) were recorded for each subject at each shock level.

Biochemistry

Approximately ten weeks after surgery, the rats were sacrificed by decapitation and their brains were removed and dissected. Brain regions assayed for CAT activity included frontal cortex, temporo-parietal cortex at the level of the lesion, occipital cortex, and rostrol-dorsal hippocampus. Bilateral tissue slices from these regions were frozen at −20°C. CAT activity was determined by the method of Fonnum [17]. Protein determinations were conducted in accordance with Lowry's method as modified by Geiger and Bessman [18].

Histology

A block of tissue including the needle track was fixed in 10% formaldehyde. Frozen coronal sections 40 μ thick were mounted on slides and stained with cresyl violet. Under microscopic examination, the area of neuronal degeneration was defined by the total absence of cells with visible nucleoli, in areas where neurons were normally present. Elsewhere the lesion area was defined by the presence of gliotic activity. At least three coronal sections through each lesion were schematically illustrated.

RESULTS

Histology

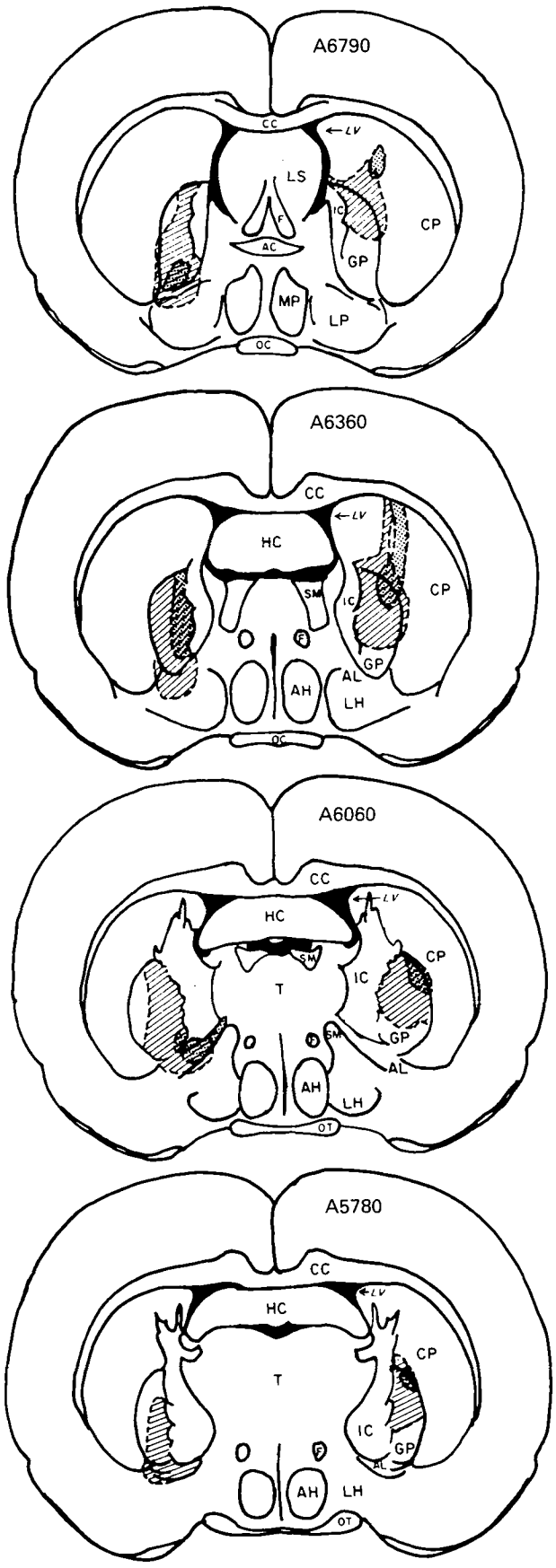
Examination of frontal sections through the lesion site revealed that in the NBM lesioned group the infusion site was typically located in, slightly medial, or slightly ventral to the ventromedial corner of the globus pallidus at approximately A6060 of König and Klippel. From this point, spread of the infusate appeared to be biased rostrally and dorsally, along the axis of the infusion needle.

Neuronal degeneration normally extended over the ventromedial one-half to two-thirds of the globus pallidus at its maximal extent (see Fig. 1). The lesion drifted laterally in more rostral sections through the globus pallidus, where the structure lies farther medial. The lesion area had an estimated average diameter of 1.5 mm, although it was longer dorso-ventrally and shorter rostro-caudally, being elliptical in shape. Aside from the needle tracks, degeneration was rarely detectable in the internal capsule because of the paucity of nerve cells in that fiber bundle. Ventral to the globus pallidus, however, gliosis was almost always discernible over the ansa lenticularis. More medially, neurotoxic effects were occasionally visible to the border of the stria medullaris. In most animals there was some degeneration of the dorsal-most neuronal elements of the lateral hypothalamus and/or preoptic area. There was virtually no destruction of cells in the striatum and the edge of the lesion commonly skirted along the ventrolateral border between the globus pallidus and the caudate-putamen. This finding is attributable to several possible causes: (1) some diffusion barrier at the border between the two structures, (2) relative insensitivity of striatal neurons to the neurotoxic effects of ibotenic acid, or (3) the very high relative density of caudate nucleus neurons augmenting and accelerating the uptake and intracellular metabolism of the neurotoxin.

The animals receiving ibotenic acid infusions into the dorsolateral globus pallidus had elliptically-shaped lesions approximately 1.5 mm in diameter, similar to those in ventromedial globus pallidus (see Fig. 1). At their maximal extent, between A6000 and A6500 of König and Klippel, the lesions typically covered the dorsal half of the globus pallidus with the medial edge of the structure normally included in the area of destruction. In no case did neuronal degeneration extend as far as the ventral border of the globus pallidus. Again, there was no detectable damage to the internal capsule. In more caudal sections, the lesions were restricted to the globus pallidus. Rostrally, however, where the globus pallidus sits farther medially, there was usually damage to the striatum beyond that produced by the infusion needle. This region of neuronal degeneration was primarily lateral and often dorsal to the globus pallidus, and in a few cases was also visible anterior to the pallidum.

Biochemistry

Neurochemical determination of CAT activity revealed that the NBM lesions decreased CAT activity in the frontal cortex by about a third, relative to the other groups (see Table 1). CAT activity in the temporo-parietal cortex was decreased by about 16% relative to the sham and dorsolateral globus pallidus lesioned rats and by 23% relative to the naive rats. Both of these effects were statistically significant, $F(3,76)=64.94$, 10.88, $p<0.001$. Specific comparisons on both sets of data showed significant differences between the NBM lesioned rats and each of the other three groups, with



no significant differences among the controls. The NBM lesions nonsignificantly decreased CAT activity levels in the occipital cortex by approximately 7%, $F(3,36)=1.15$, N.S. In the hippocampus, CAT activity was significantly lower in the sham group than in the other groups, $F(3,36)=6.74$, $p<0.05$. Specific comparisons revealed no significant differences among the naive, NBM lesioned, and dorsolateral globus pallidus lesioned rats.

Viewed with respect to the biochemical data, no striking topographical relationship between lesion site and cortical CAT depletions emerged from the histological data. Lesions that extended farthest ventral to the globus pallidus did not differ markedly from more dorsally situated lesions in their corresponding cortical CAT activity levels. The more caudal placements appeared to produce optimal CAT depletions in both frontal and temporo-parietal cortex. One animal from the NBM lesion group was discarded from this study because its bilateral lesion sites were rostral to A6360 of König and Klippel and its CAT activity in the temporo-parietal cortex was higher than most of the sham-operated controls.

Behavioral Assessment

The three groups failed to show significant differences on any of the four psychomotor tasks (see Figs. 2, 3). Moreover, there were no discernible trends in the data and no signs of consistently poorer performance by one of the groups across tasks. There were no significant differences among the three groups on either the wire hanging task, $F(2,30)=0.84$, N.S., or the inclined screen, $F(2,31)=0.19$, N.S., the two tests most sensitive to muscular strength and prehensile reflexes. On the plank walking task (see Fig. 3) with the 3.8 cm board width (the least demanding of the tests of motor equilibrium and coordination), the sham group exhibited an apparent increase in mean time on the plank. However, analysis of variance revealed no statistically reliable differences among the three groups, $F(2,30)=2.95$, N.S. The high variability in these data seemed to be attributable to several animals that failed to fall before the cutoff latency. There was no sign of such an effect on the more difficult plank walking tasks, $F(2,30)=0.04$, 0.52, N.S., and the order of the means was in fact reversed on the rod walking task (see Fig. 3), $F(2,30)=1.97$, N.S.

The lesions were found to produce significant effects upon passive avoidance retention. On the training day there were no reliable group differences in the latency to enter the compartment of the chamber in which the shock was received (see Fig. 4) Kruskal Wallis, $H(2)=5.6$, N.S. Twenty-four hours later both groups of lesioned animals entered the shock compartment significantly sooner than the sham

FIG. 1. Schematic representation of neuronal degeneration produced by infusions of ibotenic acid (2.4 μg/0.4 μl) into the NBM (left side) and dorsolateral globus pallidus (right side). Four coronal sections modified from König and Klippel, are depicted, with numbers in upper right hand corner referring to distance (microns) anterior to the interaural line. The lesion illustrated at each level was the one that produced the maximal (hatched area) or minimal (stippled area) damage to ventromedial globus pallidus (left side) or dorsolateral globus pallidus (right side). Abbreviations: AC, anterior commissure; AH, anterior hypothalamus; AL, ansa lenticularis; CC, corpus callosum; CP, caudate-putamen; F, fornix; GP, globus pallidus; HC, hippocampal commissure; IC, internal capsule; LH, lateral hypothalamus; LP, lateral preoptic area; LS, lateral septal nucleus; LV, lateral ventricle; MP, medial preoptic area; OC, optic chiasm; OT, optic tract; SM, stria medullaris; T, thalamus.

TABLE 1

REGIONAL BRAIN CAT ACTIVITY FOLLOWING IBOTENIC ACID LESIONS OF NBM

	Naive	Sham	NBM Lesioned	Dorsolateral GP Lesioned
Frontal Cortex	0.92 ± 0.02 (12)	0.89 ± 0.02 (20)	0.61 ± 0.02* (28)	0.88 ± 0.02 (20)
Temporo-Parietal Cortex	0.71 ± 0.02 (12)	0.66 ± 0.02 (20)	0.55 ± 0.02* (28)	0.65 ± 0.02 (20)
Occipital Cortex	0.74 ± 0.02 (6)	0.75 ± 0.04 (10)	0.69 ± 0.01 (14)	0.74 ± 0.02 (10)
Hippocampus	0.88 ± 0.02 (6)	0.77 ± 0.02* (10)	0.87 ± 0.03 (14)	0.92 ± 0.02 (10)

CAT activity (nmol ACh synthesized/mg protein/min) in four brain regions in naive, sham-operated, NBM lesioned, and dorsolateral globus pallidus lesioned rats. For tissues derived from either frontal cortex or temporo-parietal cortex, left and right sides were assayed separately. For occipital cortex, left and right sides were combined. For hippocampus, CAT activity was determined on one side only (left). Numbers in parentheses refer to the number of tissue samples assayed.

*Significantly different from the other three groups.

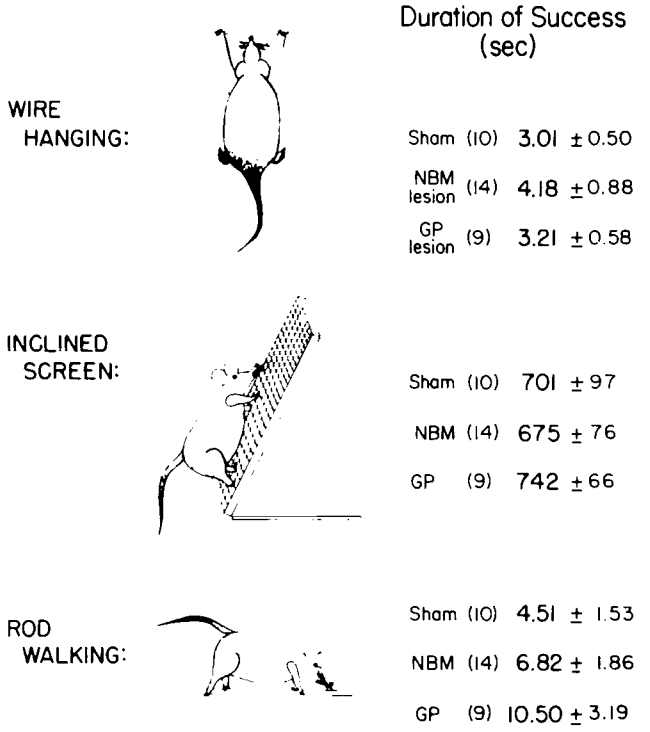


FIG. 2. Mean duration of successful performance (±S.E.M.) on three of the psychomotor tasks for sham, NBM lesioned, and dorsolateral globus pallidus lesioned rats. Dependent variables are the time spent suspended from or balanced upon the wire, screen or rod. There were no significant differences among the three groups on any of the tests.

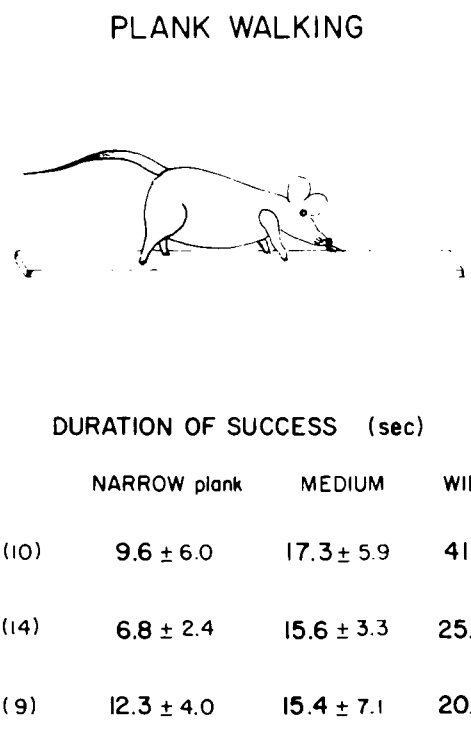


FIG. 3. Mean duration of successful performance (±S.E.M.) on the plank walking task for sham, NBM lesioned, and dorsolateral globus pallidus lesioned rats. Dependent variable is the time spent balanced upon the plank. Narrow, medium, and wide plank widths were 1.3, 2.5, and 3.8 cm respectively. There were no significant differences among the three groups on any of the tests.

operated controls (see Fig. 4) Kruskal Wallis, $H(2)=14.5$, $p<0.001$. Latencies for the NBM lesioned rats were significantly lower than those of the dorsolateral globus pallidus lesioned rats (Mann-Whitney $U=14$, $p<0.05$). Five additional animals with NBM lesions were tested one hour after the training trial. Even at this shorter test-retest interval the NBM lesioned rats exhibited deficient passive avoidance behavior, with latency scores statistically indistinguishable from those of the NBM lesioned rats tested a day later (Mann-Whitney $U=20.5$, N.S.). Since the performance of these rats was impaired as early as one hour after training, it is not clear whether the lesion disrupted the acquisition or the retention of the passive avoidance response.

In the active avoidance paradigm, a significant difference in acquisition was observed (see Fig. 5; trials to 18/20 avoidances: sham 27.2±2.1, NBM 39.3±5.3, dorsolateral

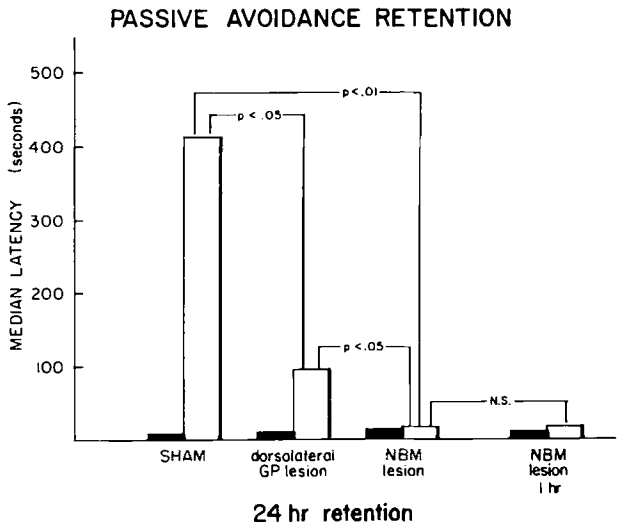
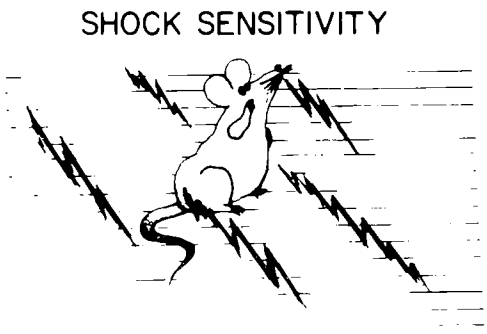


FIG. 4. Median latencies to enter the shock chamber of the passive avoidance apparatus for rats with NBM lesions (N=9), dorsolateral globus pallidus lesions (N=10), and sham-operated controls (N=10). On the training trial (left columns) the animals received a 3-second 1.0 mA footshock upon entering the rear chamber. All animals were tested for retention 24 hours later (right columns) except for the fourth group of NBM lesioned rats (N=5), which was tested one hour after training. Compared to the sham group, the lesioned animals exhibited significantly shorter latencies to enter the shock chamber on the retention trial (with no differences observed on the training trial). Between-group p values are presented, based upon Mann-Whitney U tests.



PERCENT RESPONDING

Current Intensity (mA)	VOCALIZATION RESPONSE			JUMP RESPONSE		
	sham	NBM lesion	GP lesion	sham	NBM lesion	GP lesion
0.05	20	0	10	0	0	0
0.10	10	0	40	10	36	10
0.20	60	71	80	60	93	80
0.40	100	93	100	100	93	100
0.60	100	100	100	100	100	100
0.80	100	100	100	100	100	100
1.00	100	100	100	100	100	100

FIG. 6. Percentage of rats in each group responding to footshock at each of seven intensity levels. Sham (N=10), NBM lesioned (N=14), and dorsolateral globus pallidus lesioned (N=10) rats received one-second footshocks in ascending order of intensity with an inter-shock interval of 30 seconds. A jump was defined as the lifting of at least two paws from the floor grid.

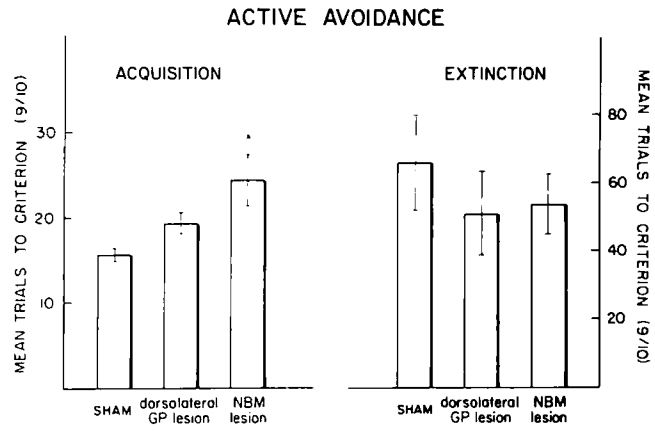


FIG. 5. Active avoidance acquisition and extinction. The acquisition criterion was 9 out of 10 successful footshock-motivated shelf-jump avoidances. The extinction criterion was 9 out of 10 failures to respond to the conditioned stimulus. See text for details. *Significantly different from sham-operated controls, $p<0.05$.

GP 30.7±2.4), $F(2,25)=3.47$, $p<0.05$. Specific comparisons revealed that this mild effect was due to the difference in trials to criterion between the sham operated rats and the NBM lesioned rats; the dorsolateral globus pallidus lesioned group did not significantly differ from either of the other two. The active avoidance acquisition deficit was thus greatest in the NBM lesioned group. Extinction of the active avoidance response was not affected by neurotoxic lesions of the NBM or dorsolateral globus pallidus (see Fig. 5; trials to 9/10 failures to escape: sham 66.0±14.2, NBM 53.7±8.8, dorsolateral GP 51.2±12.3), $F(2,25)=0.48$, N.S.

The lesions did not affect the animals' shock sensitivity. Vocalization and jump response thresholds were very similar in the three groups (see Fig. 6). Some of the sham and dorsolateral globus pallidus lesioned rats emitted vocalizations at the lowest shock intensities, while the NBM lesioned rats did not; however, the NBM lesioned group exhibited more jump responses than the other groups at the lower intensities. Most importantly, it was found that footshock intensities of 0.7 mA and 1.0 mA, used in the active avoidance and passive avoidance tasks respectively, were well above response threshold for every animal in the study.

DISCUSSION

The combined biochemical and histological data confirm previous reports [26, 27, 33] that neurotoxic lesions of the rat NBM reduce CAT activity in frontal, temporal, and parietal but not occipital cortex. The results are therefore in agreement with other evidence for a cholinergic pathway originating in the NBM and terminating in neocortex. It was also observed that the lesions did not affect CAT activity in the dorsal hippocampus. These results thus illustrate the relative lack of cholinergic input of the rat NBM to the hippocampus, and simultaneously demonstrate that our lesions did not invade the septal area, which contributes the major cholinergic input to the hippocampus.

Johnston *et al.* [27] found that unilateral injection of 10 μg of ibotenic acid in a volume of 1 μl reduced frontal cortical CAT activity by about half relative to the contralateral side. In the present study CAT activity was only diminished by a third, probably because of the lower dosage and infusion vol-

ume used (2.4 μg/0.4 μl). The former infusion parameters might also be expected to produce more extensive degeneration along the rostro-caudal axis of the NBM, as well as greater spread of the infusate to other cholinergic magnocellular forebrain nuclei situated ventral, slightly lateral, and mostly rostral to the NBM (see [12, 16, 42, 50]).

The battery of psychomotor tasks administered to the experimental animals failed to reveal any consistent sensorimotor disturbances caused by the lesions. Likewise no indications of any such disturbances were detected by gross observation. These negative results are somewhat surprising in light of previous reports of sensorimotor deficits, such as hypotonia, forelimb weakness, and postural abnormalities, after globus pallidus lesions [11,34]. These symptoms, however, were produced by lesions larger than those inflicted in the present study. Morgane [41] found no motor dysfunctions in rats with small, bilateral electrolytic lesions of the globus pallidus. Thus the amount of neuronal degeneration produced by our infusions may not have been of sufficient magnitude to cause detectable changes in these behaviors. An alternative explanation for our failure to replicate the sensorimotor deficits is that their appearance may be dependent upon the destruction of fibers of passage through the globus pallidus. Unlike the neurotoxic lesions used in the present study, the electrolytic lesions used in previously published studies would destroy thalamocortical, corticofugal, nigrostriatal, and other fiber pathways coursing through the globus pallidus. This discrepancy therefore suggests that current thinking about pallidal involvement in sensorimotor activity based upon the results of lesion and stimulation experiments should be re-evaluated, and an attempt made to differentiate between effects attributable to globus pallidus neurons as opposed to projection pathways in the area. Regardless of the explanation, however, it would appear that the cortically projecting cholinergic neurons of the globus pallidus do not play a critical role in the types of psychomotor behavior measured in this study.

Evaluations of shock responsivity revealed no changes in the lesioned animals. The lack of changes in shock sensitivity in the NBM lesioned group seems at odds with the extensive literature indicative of cholinergic involvement in the regulation of pain threshold. Cholinomimetics such as physostigmine, arecoline, and oxotremorine all decrease pain sensitivity [23,40], an effect blocked by the muscarinic antagonist atropine [25]. There are several possible explanations for the lack of effects on pain threshold observed in the present study. It could be that the above effects of systemic pharmacological agents are peripherally mediated. However, they are not blocked by quaternary muscarinic antagonists such as atropine methylbromide [25] and antinociception is also seen after intraventricular administration of oxotremorine or ACh [40,43]. Another possibility is that central cholinergic control of pain is subcortically localized to a cholinergic pathway distinct from the projection originating in the NBM. Lesions of the cholinergic septo-hippocampal pathway, for example, have been shown to produce increased footshock sensitivity [44]. On the other hand, in spite of their apparent cholinergic deficiencies, aged rodents exhibit no changes in shock sensitivity [9, 20, 35]. Certainly it seems probable that responses to different pain-provoking stimuli are effected via different neurochemical pathways. A consideration of differences in the nature of the stimuli used to elicit a pain response in different experiments is likely to account for many apparent discrepancies. In any event, it seems clear that the effects observed in the footshock-motivated avoidance tasks were not secondary to effects upon shock sensitivity.

The deficit in active avoidance acquisition produced by the lesions suggests that the cortical cholinergic input from the NBM may be important in the learning process. Cholinergic blockers such as scopolamine likewise disrupt the acquisition of an active avoidance response [21]. These results are also comparable to those of Lo Conte *et al.* [36], who found that unilateral electrolytic lesions destroying the NBM and adjacent magnocellular forebrain nuclei impaired two-way active avoidance conditioning. On the other hand, these effects might also be interpreted as the consequence of a more general impairment of avoidance responding. However, the concept of a learning deficit, unlike the concept of deficient avoidance behavior, receives corroboration from electrophysiological studies demonstrating that neurons in the primate NBM are responsive to food reinforcement [10].

The lack of effects upon the extinction of one-way active avoidance is of interest in comparison to the effects of other cholinergic manipulations. Cholinergic blockers and lesions of the septo-hippocampal pathway both produce resistance to extinction [6, 24, 37, 38, 46]. In contrast, we observed no reliable differences in extinction rate among our three groups, and the trend in the NBM lesioned animals was in the opposite direction from that reported for septal lesions and muscarinic antagonists. Thus, the behavioral syndrome of "response disinhibition" often attributed to animals with hippocampal cholinergic denervation or pharmacological cholinergic blockade [7,37] clearly is not manifested following loss of the cholinergic input to the cortex. This dissociation between the behavioral effects of NBM lesions and those of other cholinergic lesions underscores the apparent functional differences that exist between different cholinergic neural pathways. This contention is further supported by the deficit in two-way shuttle box avoidance learning observed by Lo Conte *et al.* [36] in their NBM lesioned rats, since lesions of the medial septal nucleus are known to result in an improvement in shuttle box avoidance acquisition [29].

The most robust effect of the NBM lesions was the deficit in passive avoidance retention. Based upon the overall behavioral profile of these animals, the deficit in passive avoidance responding seems to be attributable to impairments in learning and/or memory of the task. The various controls and other tests exclude alternative explanations such as hyperactivity, increased pain threshold, perseveration, or motor disequilibrium. The last three possibilities were ruled out by the results from the shock sensitivity, active avoidance, and psychomotor tests, respectively. Hyperactivity cannot easily account for the lesioned rats' more rapid entry into the rear of the chamber on the test day (day 2) because no differences in latency between groups was observed on the passive avoidance training trial (day 1).

Unilateral electrolytic lesions of the magnocellular forebrain nuclei likewise impair passive avoidance retention [36]. Also in agreement with these results are previous experiments showing that cholinergic blockers such as scopolamine produce passive avoidance deficits which can be antagonized by the AChE inhibitor physostigmine [5]. Aged rats exhibit a variety of deficiencies of cholinergic transmission [19, 35, 47], and likewise display a severe memory deficit when tested in the passive avoidance paradigm [20,35].

In man, cholinergic and mnemonic dysfunction are similarly coupled. Pharmacological blockade of cholinergic transmission produces memory impairments [14], an effect antagonized by physostigmine [13]. Senile dementia of the

Alzheimer's type has been associated with decreased cortical CAT activity [4] and with neuronal degeneration in the NBM [51,52]. It has been demonstrated that the NBM lesioned rats in the present experiment share the above two neurophysiological correlates of Alzheimer's disease. Furthermore, it has been pointed out that the prominent deficit in one-trial passive avoidance retention seen in aged as well as NBM lesioned rodents shares many operational and conceptual similarities with the loss of recent memory seen with age-related cognitive disorders in man [3,9]. Taken together, the preceding considerations raise the possibility that neurotoxic lesions of the rat NBM may constitute a valid animal model for some of the major symptoms of Alzheimer's disease.

However, the results from the present study contain two important caveats with regard to these speculations. Unlike aged rats, the NBM lesioned rats showed deficient passive avoidance responding at a retention interval of only 60 minutes. The passive avoidance deficit might therefore be interpreted as the consequence of impaired learning rather than rapid forgetting. The former interpretation would also be consistent with the acquisition deficit seen in the active avoidance paradigm. It might be possible to differentiate the relative contribution of learning versus memory disorders in the passive avoidance deficit by employing shorter retention intervals, or possibly a multiple trial passive avoidance paradigm, or else by testing the lesioned rats on other more specific tasks of recent memory.

Another note of caution concerns the implicit causal relationship between loss of cortical CAT activity after NBM destruction and the passive avoidance deficit. It is possible that the effects observed were due to the degeneration of non-cortical projections of the NBM, for it has been noted that the NBM innervates other areas in addition to neocortex [16]. With respect to the question of cholinergic specificity, it must be noted that rats with lesions in the dorsolateral globus pallidus suffered passive avoidance deficits, even though they exhibited no loss of cortical CAT activity. These data therefore undermine the conclusion that degeneration of cholinergic neurons was necessarily responsible for the passive avoidance deficit in the NBM lesioned animals. However, it is well established that many drugs void of anticholinergic activity can significantly impair performance on passive avoidance tasks [2]. It would therefore not be surprising if one of the noncholinergic efferents from the globus pallidus were also somehow involved in mediating passive avoidance responding. The extent to which the cholinergic system plays an important role in the deficit might be clarified in future studies by examination of the interaction between lesion effects and the effects of systemic cholinergic agents. Such a strategy might also serve to more clearly differentiate the two lesion groups, as would further characterization of behavioral effects in a wider variety of tasks.

ACKNOWLEDGEMENTS

The authors appreciate the support of Drs. B. Beer, T. Crook, and S. Ferris during the course of this research and Drs. J. Ordy and J. Rogers for critical evaluations of the manuscript. The technical assistance of Kathy Schill, Stanley Jones, David Pawlyk and Dr. Margaret Parham, as well as help provided in the preparation of this manuscript by Mary Wilfred and Rhonda Sheppard are also greatly appreciated.

REFERENCES

1. Allmark, M. G. and W. M. Bachinski. A method of assay for curare using rats. *J Am Pharmacol Assoc* **38:** 43–45, 1949.
2. Bammer, G. Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neurosci Biobehav Rev* **6:** 247–296, 1982.
3. Bartus, R. T. The one-trial passive avoidance procedure as a possible marker of aging. In: *Biological Markers of Aging*, edited by M. E. Reff and E. L. Schneider. NIH Publication No. 82–2221, 1982.
4. Bartus, R. T., R. L. Dean, B. Beer and A. S. Lippa. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217:** 408–417, 1982.
5. Bohdanecky, Z. and M. E. Jarvik. Impairment of one-trial passive avoidance learning in mice by scopolamine, scopolamine methylbromide, and physostigmine. *Neuropharmacology* **6:** 217–222, 1967.
6. Carlton, P. L. Some behavioral effects of atropine and methylatropine. *Psychol Rep* **10:** 579–589, 1962.
7. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. *Psychol Rev* **70:** 19–39, 1963.
8. Das, G. D. Projection of the interstitial nerve cells surrounding the globus pallidus: A study of retrograde changes following cortical ablations in rabbits. *Z Anat EntwGesch* **133:** 135–160, 1971.
9. Dean, R. L., J. Scozzafava, J. A. Goas, B. Regan, B. Beer and R. T. Bartus. Age-related differences in behavior across the life span of the C57BL/6J mouse. *Exp Aging Res* **7:** 427–451, 1981.
10. DeLong, M. Activity of pallidal neurons during movement. *J Neurophysiol* **34:** 414–427, 1971.
11. Dickinson, S. L. and P. Slater. Effects of striatal and pallidal lesions and intrapallidal GABA and opiate drugs on tremorine-induced rigidity in the rat. *Neurosci Lett* **29:** 163–168, 1982.
12. Divac, I. Magnocellular nuclei of the basal forebrain project to neocortex, brain stem, and olfactory bulb. Review of some functional correlates. *Brain Res* **93:** 385–398, 1975.
13. Drachman, D. A. Memory and cognitive function in man: Does the cholinergic system have a specific role? *Neurology* **27:** 783–790, 1977.
14. Drachman, D. A. and J. Leavitt. Human memory and the cholinergic system: A relationship to aging? *Arch Neurol* **30:** 113–121, 1974.
15. Edstrom, J. P. and J. W. Phillis. A cholinergic projection from the globus pallidus to cerebral cortex. *Brain Res* **189:** 524–529, 1980.
16. Fibiger, H. C. The organization and some projections of cholinergic neurons of the mammalian forebrain. *Br Res Rev* **4:** 327–388, 1982.
17. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. *J Neurochem* **24:** 407–409, 1975.
18. Geiger, P. J. and S. P. Bessman. Protein determination by Lowry's method in the presence of sulphydryl reagents. *Anal Biochem* **49:** 467–473, 1972.
19. Gibson, G. E., C. Peterson and J. Sansone. Neurotransmitter and carbohydrate metabolism during aging and mild hypoxia. *Neurobiol Aging* **2:** 165–172, 1981.
20. Gold, P. E. and J. L. McGaugh. Changes in learning and memory during aging. In: *Neurobiology of Aging*, edited by J. M. Ordy and K. M. Brizzee. New York: Plenum Press, 1975, pp. 145–158.
21. Gruber, R. P., G. C. Stone and D. K. Reed. Scopolamine-induced anterograde amnesia. *Neuropharmacology* **6:** 186–190, 1967.
22. Guldin, W. O. and H. J. Markowitsch. Epidural kainate, but not ibotenate, produces lesions in local and distant regions of the brain: A comparison of the intracerebral actions of kainic acid and ibotenic acid. *J Neurosci Methods* **5:** 83–93, 1982.

23. Harris, L. S., W. L. Dewey, J. F. Howes, J. S. Kennedy and H. Pars. Narcotic-antagonist analgesics: Interactions with cholinergic systems. *J Pharmacol Exp Ther* **169:** 17–22, 1969.
24. Herrnstein, R. J. Effects of scopolamine on a multiple schedule. *J Exp Anal Behav* **1:** 351–358, 1958.
25. Ireson, J. D. A comparison of the antinociceptive actions of cholinomimetic and morphine-like drugs. *Br J Pharmacol* **40:** 92–101, 1970.
26. Johnston, M. V., M. McKinney and J. T. Coyle. Evidence for a cholinergic projection from neurons in basal forebrain. *Proc Natl Acad Sci USA* **76:** 5392–5396, 1979.
27. Johnston, M. V., M. McKinney and J. T. Coyle. Neocortical cholinergic innervation: A description of extrinsic and intrinsic components in the rat. *Exp Br Res* **43:** 159–172, 1981.
28. Kelly, P. H. and K. E. Moore. Decrease of neocortical choline acetyltransferase after lesion of the globus pallidus in the rat. *Exp Neurol* **61:** 479–484, 1978.
29. King, F. A. Effects of septal and amygdaloid lesions on emotional behavior and conditioned avoidance responses in the rat. *J Nerv Ment Dis* **126:** 57–63, 1958.
30. Kohler, C., R. Schwarcz and K. Fuxe. Intrahippocampal injections of ibotenic acid provide histological evidence for a neurotoxic mechanism different from kainic acid. *Neurosci Lett* **15:** 223–228, 1979.
31. König, J. F. and R. Klippel. *The Rat Brain: A Stereotaxic Atlas*. Williams and Wilkins, Baltimore, 1963.
32. Lamour, Y., P. Dutar and A. Jobert. Spread of acetylcholine sensitivity in the neocortex following lesion of the nucleus basalis. *Brain Res* **252:** 377–381, 1982.
33. Lehmann, J., J. I. Nagy, S. Atmadja and H. C. Fibiger. The nucleus basalis magnocellularis: The origin of a cholinergic projection to the neocortex of the rat. *Neuroscience* **5:** 1161–1174, 1980.
34. Levine, M. S., N. Ferguson, C. J. Kreinick, J. W. Gustafson and J. S. Schwartzbaum. Sensorimotor dysfunctions and aphagia and adipsia following pallidal lesions in rats. *J Comp Physiol Psychol* **77:** 282–293, 1971.
35. Lippa, A., R. W. Pelham, B. Beer, D. J. Critchett, R. L. Dean and R. T. Bartus. Brain cholinergic dysfunction and memory in aged rats. *Neurobiol Aging* **1:** 13–19, 1980.
36. Lo Conte, G., L. Bartolini, F. Casamenti, I. Marconcini-Pepeu and G. Pepeu. Lesions of cholinergic forebrain nuclei: Changes in avoidance behavior and scopolamine actions. *Pharmacol Biochem Behav* **17:** 933–937, 1982.
37. McCleary, R. A. Response modulating functions of the limbic system: Initiation and suppression. In: *Progress in Physiological Psychology*, edited by E. Stellar and J. Sprague. New York: Academic Press, 1966, pp. 209–272.
38. McNew, J. J. and R. Thompson. Role of the limbic system in active and passive avoidance conditioning in the rat. *J Comp Physiol Psychol* **61:** 173–180, 1966.
39. Mesulam, M. and G. W. Van Hoesen. Acetylcholinesterase-rich projections from the basal forebrain of the rhesus monkey to neocortex. *Brain Res* **109:** 152–157, 1976.
40. Metys, J., N. Wagner, J. Metysova and A. Herz. Studies on the central antinociceptive action of cholinomimetic agents. *Int J Neuropharmacol* **8:** 413–425, 1969.
41. Morgane, P. J. Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. *Am J Physiol* **201:** 420–428, 1961.
42. Pedata, F., G. Lo Conte, S. Sorbi, I. Marconcini-Pepeu and G. Pepeu. Changes in high affinity choline uptake in rat cortex following lesions of the magnocellular forebrain nuclei. *Brain Res* **233:** 359–367, 1982.
43. Pedigo, N. W., W. L. Dewey and L. S. Harris. Determination and characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. *J Pharmacol Exp Ther* **193:** 845–852, 1975.
44. Poncy, M. P., P. S. Bernard and H. I. Chernov. Biochemical and behavioral modifications in septal and hypothalamically-lesioned rats. *Neuropharmacology* **11:** 39–44, 1972.
45. Schwarcz, R., T. Hokfelt, K. Fuxe, G. Jonsson, M. Goldstein and L. Terenius. Ibotenic acid-induced neuronal degeneration: A morphological and neurochemical study. *Exp Brain Res* **37:** 199–216, 1979.
46. Schwartzbaum, J. S., M. H. Kellicutt, T. M. Spieth and J. B. Thompson. Effects of septal lesions in rats on response inhibition associated with food-reinforced behavior. *J Comp Physiol Psychol* **58:** 217–224, 1964.
47. Sherman, K. A., J. E. Kuster, R. L. Dean, R. T. Bartus and E. Friedman. Presynaptic cholinergic mechanisms in brain of aged rats with memory impairments. *Neurobiol Aging* **2:** 99–104, 1981.
48. Shute, C. C. D. and P. R. Lewis. The ascending cholinergic reticular system: Neocortical, olfactory and subcortical projections. *Brain* **90:** 497–521, 1967.
49. Valzelli, L. *Psychopharmacology: An Introduction To Experimental and Clinical Principles*. New York: Spectrum, 1973.
50. Wenk, H., B. Volker and U. Meyer. Cholinergic projections from magnocellular nuclei of the basal forebrain to cortical areas in rats. *Brain Res Rev* **2:** 295–316, 1980.
51. Whitehouse, P. J., D. L. Price, A. W. Clark, J. T. Coyle and M. R. DeLong. Alzheimer's disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol* **10:** 122–126, 1981.
52. Whitehouse, P. J., D. L. Price, R. G. Struble, A. W. Clark, J. T. Coyle and M. R. DeLong. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* **215:** 1237–1239, 1982.
53. Zornetzer, S. F. and J. Rogers. Animal models for assessment of geriatric mnemonic and motor deficits. In: *Behavioral Assessment in Geriatric Pharmacology*, edited by T. Crook, S. Ferris and R. Bartus. New Canaan, CT: Powley Assoc., in press, 1983.