Research report

Cholinergic and serotonergic neocortical projection lesions given singly or in combination cause only mild impairments on tests of skilled movement in rats: evaluation of a model of dementia

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Abstract

The cholinergic (ACh) projections of the nucleus basalis and the serotonergic (5-HT) projections of the raphe nuclei to the neocortex are required for the normal function of the neocortex. Nevertheless, damage to either system alone has little effect on the behavior of rats, but conjoint damage to both systems is reported to produce dementia to the point that animals are described as being unable to engage in intelligent behavior. Because rats with bilateral damage to both systems are so severely impaired, they are not useful for chronic studies. The objective of the present research was to determine whether unilateral depletions produce a functional impairment. Rats received unilateral neurotoxic lesions to either the nucleus basalis (quisqualic acid), or the medial forebrain bundle (5,7-dihydroxytryptamine), or both, which reduced neocortical levels of ACh (55%) and 5-HT (63%). The rats then received a battery of tests sensitive to unilateral neocortical injury. The 5-HT lesion produced no quantitative or qualitative deficits on reaching for food, walking across a horizontal ladder, forelimb placement in a cylinder, sensory detection of adhesive paper applied to the wrists, or forelimb inhibition during swimming. The ACh lesion produced mild qualitative deficits in reaching. Combined lesions produced mild deficits in skilled reaching, ladder walking, and sensory detection. In contrast to the mild impairments produced by the lesions, pharmacological blockade of either ACh with atropine or 5-HT with methiothepin mesylate systemically blocked skilled motor behavior as assessed by skilled reaching. The results are discussed in relation to the problems associated with the development of a unilateral model of dementia.

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1. Introduction

Cholinergic (ACh) and serotonergic (5-HT) afferents densely innervate the neocortex and have been implicated in a variety of behavioral functions. The nucleus basalis magnocellularis, a group of ACh neurons in the ventromedial corner of the globus pallidus of the rat, is the primary source of cholinergic afferents to the neocortex [34,48]. The principal 5-HT fibers to the neocortex arise from the median and dorsal raphe nuclei located in the brain stem, and they ascend to the forebrain via the medial forebrain bundle [25,27]. The most rostral of these projections terminate in the frontal lobes and sensorimotor cortex. There is evidence that the cholinergic projection facilitates the learning of conditioned motor responses [30], enhances plastic processes [15] such as those involved in compensatory responses to brain damage [21,24,33], is central to cortical map reorganization [19] and activates the cortical vasculature in order to enhance cerebral blood flow [6,36,40]. The serotonergic projection is also instrumental in certain aspects of brain plasticity, such as compensatory plastic responses after injury [28], developmental cortical
plasticity [16] and behaviors dependent on motor control [43]. In addition, cholinergic and serotonergic projections to the neocortex are associated with producing a low voltage fast activity (LVFA) pattern of the neocortical electroencephalogram (EEG) [10,44,46,47]. The ACh-related LVFA is associated with alert immobility, while the 5-HT-related LVFA is associated with overt movement of the body and limbs. Surprisingly, damage to either system has little, if any, effect on many learned behaviors. Conjoint damage to both, however, produces such severe impairments in learning and memory [4,7,11,20,26,31,39,42] to the point that such animals have been described as displaying “no intelligent behavior” [42]. Consequently, the combined blockade preparation has been proposed as a model of dementia.

In addition to predicting global dementia the ‘two transmitter’ theory predicts that unilateral depletion will produce impairments in those behaviors under the specific control of each hemisphere. Unilateral lesions have been used to study the effects of conditions such as stroke, pyramidal tract injury, and Parkinson’s disease in which combined lesions are also incapacitating [23,32,50,52]. Animals with unilateral damage have sensory and motor impairments mainly to the contralateral side of the body. Their ipsilateral side of the body, controlled by the intact hemisphere, is sufficient for self-maintenance. Surprisingly, there has been no previous investigation of the role of these ascending systems on sensory/motor behavior in the rat as studies have been limited to learning/memory. There also has been no previous examination of the effects of unilateral lesions of the two systems. Thus, the objective of the present research was to assess the effects of single unilateral lesion and conjoint unilateral depletion of the neocortical ACh and 5-HT projections on sensorimotor behavior. A demonstration of impairments following unilateral lesions has the additional advantage in providing an animal model in which the animals are able to care for themselves using their intact hemisphere.

For the present experiments, groups of rats received unilateral neurotoxic damage to the nucleus basalis, or medial forebrain bundle, or both. The animals were given a series of sensorimotor tests, all of which have been demonstrated to be sensitive to sensory/motor cortex integrity. The assessment included tests of limb placing while traversing a horizontal ladder with variably spaced rungs (the rung walking test), forelimb support (cylinder test), forelimb inhibition during swimming, responsiveness to sensory contact (adhesive dot removal test), and limb use in reaching for food (skilled reaching). The performance of the rats was videotaped and behavior scored frame-by-frame to assess the quantitative and qualitative performance of the animals. In addition to being compared to a control group, performance related to the limbs ipsilateral to the lesion was compared to performance of the limbs contralateral to the lesion with the expectation that contralateral limbs should be more affected, as typical-ly occurs following frank cortical injury. To evaluate the effects of bilateral blockade, the ACh muscarinic blocker atropine sulphate and the 5-HT blocker methiothepin mesylate were separately administered to control rats prior to one of the tests, skilled reaching.

2. Materials and methods

2.1. Subjects

Experiments were conducted according to standards set by the Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee. The subjects were 44 female Long-Evans hooded rats, 120 days old and weighing 250–300 g. They were raised in the University of Lethbridge vivarium. The animals were assigned to three different groups, control (n = 17), nucleus basalis lesion (n = 6), medial forebrain bundle lesion (n = 6), and combined lesion (n = 15). The animals were housed in groups of three or four individuals in hanging wire mesh cages. The colony room was maintained on a 12/12 h light/dark cycle (08:00–20:00 h).

2.2. Feeding

Three weeks prior to surgery, the rats were gradually food deprived to 85% of their original body weight by providing once a day feeding of Purina rat chow. Rats remained on food deprivation for the rest of the experiment.

2.3. Surgery

2.3.1. Nucleus basalis lesion

Animals received an injection of atropine nitrate (0.1 mg/kg, i.p.) (Sigma–Aldrich, St. Louis, MO, USA) to facilitate respiration throughout surgery. Under sodium pentobarbital anesthesia (65 mg/kg, i.p.), each rat received stereotaxic infusions of quisqualic acid (0.5 μg/μl, Sigma–Aldrich) via a 30-gauge cannula connected to a micro drive pump by a polythene tube [13]. Two 0.5 μl infusions were made unilaterally in the nucleus basalis. Each infusion was delivered over 3 min and an additional 5 min allowed for diffusion before the cannula was retracted. Stereotaxic coordinates [anterior (A), lateral (L), and ventral (V)] were A = 0.2 mm, L = 3.4 mm, V = 7.0 mm (below dura) and A = 1.0 mm, L = 2.6 mm, V = 7.3 mm (below dura), with the incisor bar set 5.0 mm above the interaural line. Animals were allowed 2 weeks to recover before behavioral testing.

2.3.2. Medial forebrain bundle lesion

Animals received an injection of desipramine HCl (25 mg/kg, i.p.) (Sigma–Aldrich), a norepinephrine reuptake inhibitor, 30 min prior to neurotoxin infusions. Animals
then received an injection of atropine nitrate (0.1 mg/kg, i.p.) (Sigma–Aldrich) to facilitate respiration throughout surgery. Under sodium pentobarbital anaesthesia (65 mg/kg, i.p.) (Sigma–Aldrich), each rat received stereotaxic infusions of 5,7-dihydroxytryptamine (5 μg/4 μl, Sigma–Aldrich) via a 30-gauge cannula connected to a micro drive pump by a polythene tube [13]. Two 2.0 μl infusions were made unilaterally in the medial forebrain bundle. Each infusion was delivered over 5 min and an additional 5 min allowed for diffusion before the cannula was retracted. Stereotaxic coordinates were: A = −2.0 mm, L = 1.5 mm, V = 9.5 mm (below skull) and A = −1.5 mm, L = 1.5 mm, V = 9.5 mm (below skull). The incisor bars were positioned such that the skull was flat. The lesion group was allowed 2 weeks to recover before behavioral testing.

2.3.3. Combined lesion

This lesion was produced using one of two methods. (1) Two-stage lesion: subjects received either a unilateral nucleus basalis lesion (quissacilic acid, 0.5 μg/μl) or a unilateral medial forebrain bundle lesion (5,7-dihydroxytryptamine, 5 μg/4 μl). After 8 weeks, they received an additional unilateral lesion with the other neurotoxin to produce a combined nucleus basalis and medial forebrain bundle lesion in the same hemisphere. (2) One-stage lesion: a separate group of rats received both nucleus basalis and medial forebrain bundle lesions in the same hemisphere and in one operation.

2.4. Drugs

2.4.1. Atropine sulphate

Three doses of atropine sulphate (Sigma–Aldrich), 5, 10, and 25 mg/kg, were prepared in 0.9% sterile saline solution [41]. A single dose was administered (i.p.) to 10 subjects in the control group 20–30 min prior to testing in the single pellet reaching task, each dose was only used once. The drug doses were administered starting with the lowest dose to minimize tolerance effects. After each rat was injected with the drug, it was returned to its home cage until it was due for testing on the reaching task.

2.4.2. Methiothepin mesylate

Five doses of methiothepin mesylate (Sigma–Aldrich), 0.1, 0.15, 0.2, 0.25, and 0.3 mg/kg, were prepared in 0.9% sterile saline solution [11]. Six subjects in the control group were injected (i.p.) with the drug 20–30 min prior to testing in the single pellet reaching task. The drug doses were administered in systematic increments starting with the lowest dose to minimize tolerance effects. After each rat was injected with the drug, it was returned to its home cage until it was due for testing.

2.5. Behavioral training and test analysis

All subjects were tested on the rung walking, cylinder, swimming, and adhesive dot removal tests once a week for 4 weeks after surgery.

2.5.1. Rung walking

The runway consisted of a straight section 1 m in length with walls 19 cm high and a square goal box at one end in which food was located [22]. The width of the alley was adjusted to the size of the animal allowing 1 cm on either side of the animal to prevent it from turning around. The floor of the runway was made of a readily changeable arrangement of horizontal steel rods 3 mm in diameter. An irregular but unchanged rung pattern was maintained throughout all trials, gap sizes varied from 1 to 5 cm. Each animal received three trials during each testing session. A video camera was positioned at a slight ventral angle, so that the positions of all four limbs could be filmed simultaneously from a ventral view.

A foot-fault scoring system was used to assess forelimb and hind limb placement [22]. Each step was rated on a five-point scale: if the foot placement appeared normal where the midportion of the palm was placed on the rung, it was given a score of ‘0’; if placement on the rung was done using the wrist or digits of the forelimb or the heel or toes of the hind limb, it was given a score of ‘1’; if a limb was placed on a rung and slipped off during weight shifting without disturbing balance, it was given a score of ‘2’; if a limb was placed on a rung and slipped off during weight shifting causing a fall, it was given a score of ‘3’; and if a limb missed the targeted rung completely and fell through the gap compromising body posture and balance, it was given a score of ‘4’. Animals received three trials during each testing day. The asymmetry score, a ratio between foot faults committed by limbs on one side of the body and foot faults committed by limbs on the other side of the body, was calculated for each group (i.e., contralateral limb faults/ipsilateral limb faults).

2.5.2. Cylinder test

Forelimb use for weight support during explorative activity was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm high for 4 min [37]. A mirror was placed underneath the cylinder at an angle to allow the experimenter to videotape the animal’s activity from a ventral view. The cylindrical shape encouraged vertical exploration of the walls with the forelimbs, but the walls were high enough so that animals could not reach the top. Forelimb use was measured during vertical exploration during rearing. Each forepaw contact with the cylinder wall was counted. The animals were individually placed in the cylinder for 4 min during each testing session. The asymmetry score of forelimb use in wall exploration was calculated for each group (i.e., contralateral forelimb wall contact/ipsilateral forelimb wall contact).

2.5.3. Swimming test

Video recordings were made of rats swimming in a large
rectangular aquarium (120×43×50 cm) [51]. Water was high enough to prevent animals from touching the bottom of the aquarium but at the same time low enough to prevent them from escalating to the edge of the pool; temperature was maintained at 21 °C. At one end of the pool was an escape wire mesh platform onto which the animals could climb. The platform was visible to the animals at all times. During the training phase, animals were released close to the platform; after they learned to swim and climb onto the platform, they were released at progressively longer distances until they swam directly from the opposite end of the tank. Initially, most animals used all four limbs to stroke, rapidly changed direction, and sometimes swam aimlessly. Once animals learned to swim directly to the platform and were more familiar with the task, they held their forelimbs immobile under their chins and only used their hind limbs to propel through the water. Each animal performed four trials during which they had to swim directly to the platform. Animals were dried and returned to their home cages after completing four trials. Disruption to the normal swim pattern was quantified by counting the number of strokes by each forelimb. The asymmetry score of forelimb inhibition was calculated for each group (i.e., contralateral forelimb strokes/ipsilateral forelimb wall strokes).

2.5.4. Adhesive dot removal

The task required rats to detect and remove pieces of adhesive paper from their wrists [38]. Animals were removed from their home cages, and their forelimbs were washed with 50% ethanol solution, then wiped with cotton gauze and allowed to dry. Two parallel creases were formed in adhesive paper stimuli (113 mm², manufactured by Avery International) to facilitate wrapping them around the forelimb. The stimuli were attached to the distal–radial aspect of both forelimbs. The experimenter then firmly touched both forelimbs simultaneously and placed the animal in a clear Plexiglas tub (45×26×20 cm) without bedding for ease of recording. A stainless steel lid was used to cover the tub and contain the rat. Subjects first contacted the adhesive paper and then attempted to remove it. The latency to remove the stimulus from each forelimb on four trials was recorded. Each trial was ended after both pieces of adhesive paper were removed or after 3 min. The asymmetry score of latency of dot removal was calculated for each group (i.e., contralateral forelimb strokes/ipsilateral forelimb latency).

2.5.5. Single pellet reaching boxes and training

All animals were pre-trained to reach through a slot for single pieces of food for 2 weeks prior to surgery or drug administration [49]. Reaching boxes were made of clear Plexiglas, with the dimensions 45×14×35 cm. In the center of each front wall was a 1 cm wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm wide shelf. Two indentations on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit where rats could reach. Food pellets (45 mg Rodent Chow food pellets, Bioserve) were placed in the indentation contralateral to the limb with which the rat reached [53]. Following each reach, a short pause preceded the presentation of the next pellet, and an additional pellet could be dropped in the back of the box. This encouraged animals to return to the back of the box after each reach and so forced them to reposition themselves and prepare for the next reach. The animals were trained for 10 min each day for the first week and were presented with 20 pellets each day for the second week. Reaching performance was assessed on two measures: ‘reaching success’=number of pellets retrieved and ‘reaches/pellet retrieved’=number of reaching attempts/successful retrieval. After the recovery period following surgery, the animals were tested every day for 2 weeks. They were presented with 20 pellets in each testing session.

For a qualitative analysis of reaching, a reach was subdivided into 10 components [54]. (1) Limb lift: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) Digits close: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (3) Aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the addition of the elbow. (4) Advance: the head is lifted and the limb is advanced directly forward above and beyond the food pellet. (5) Digits open: as the limb is advanced the digits are extended and opened. (6) Pronate: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food. Full pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) Grasp: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn. (8) Supination I: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90° by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) Supination II: as the rat sits back with food held in the paw, the paw is further supinated by 90° and ventroflexed to present the food to the mouth. (10) Release: the digits are opened and the food transferred to the mouth.

Five successful reaches were analyzed frame-by-frame on the video tapes. Each movement was rated on a three-point scale. If the movement appeared normal, it was given a score of ‘0’; if it appeared slightly abnormal but
recognizable it was given a score of ‘1’; and a score of ‘2’ was assigned if the movement was absent or completely unrecognizable.

2.5.6. Tray reaching boxes and training

This task was only used after surgery with the one-stage lesion group and some subjects from the control group. The training phase involved placing each animal individually in a reaching box 26 cm high, 28 cm deep, and 19 cm wide, for 30–40 min each day for 14 days [52]. The fronts of the boxes were constructed of 2 mm bars separated from each other by a 9 mm gap. Clear Plexiglass tops allowed access to the inside of the box. A 4 cm wide and 0.5 cm deep tray was mounted in front of the bars. The tray contained food fragments weighing approximately 30 mg each. Animals had to reach between the bars, grasp the food and retract it where they were able to freely eat. The animals were free to reach with either of their forelimbs. Following this training period, animals were tested for 5 min on three consecutive days. Each time the rat placed its forelimb towards the tray, a ‘reach’ was scored. If the animal was successful in obtaining food, a ‘hit’ was scored. The percentage of reaches and hits was calculated and regarded as an indication of reaching accuracy. Following this testing phase, a bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew, Lachine, Quebec, Canada) was wrapped around the preferred paw of animals in each group to restrict its use for reaching [52]. The bracelet prevented the animal from inserting its paw through the gap to reach the food, thus forcing it to reach with its other paw. The bracelets did not impede the subject’s movement and could be easily slipped off by the experimenter without damaging the animal’s forelimb or ripping hair off. Once habituated to the bracelet, the animals ignored it and did not attempt to remove it; the rats learned to use the other limb and could do so even without the bracelet.

2.6. Histological procedures

After 6 weeks of behavioral testing, all animals were sacrificed using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed, post-fixed and cryoprotected in a solution of 30% sucrose in 4% paraformaldehyde solution for 3 days at 4 °C. All brains were then sectioned into 40 μm using a cryostat (2800 Frigocut, Reichert-Jung). Sections from the nucleus basalis lesion and combined lesion groups were mounted onto glass slides and stained for acetylcholinesterase using a procedure modified from Karnovsky and Roots [18]. Sections from the medial forebrain bundle lesion and combined lesion groups were stored in 0.1 M PBS solution. The following day, free-floating sections were incubated for 15 min in a quench solution, 20 ml of 3% H2O2 in 180 ml 0.1 M PBS, to reduce background staining. The sections were then washed three times with 0.1 M PBS. The tissue was then incubated in a primary antibody solution, 15 ml 0.1 M PBS, three drops goat serum, 100 μl of 3% Triton-X, 20 μl of serotonin antibody (donated by Dr. Richard Dyck); bovine serum albumin 150 mg was added to reduce background staining [29]. Six sections per centrifuge tube were rotated at 40 rpm while refrigerated at 4 °C for 20 h. All sections were washed three times with 0.1 M PBS and incubated in a secondary antibody solution, 10 ml 0.1 M PBS, three drops goat serum and one drop anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA). The sections were rotated at 40 rpm at 4 °C for 1 h. The tissue was washed three times with 0.1 M PBS and then incubated in an AB complex solution (Vector Laboratories), 5 ml 0.1 M PBS, two drops solution A, two drops solution B and centrifuged at 40 rpm at 4 °C for 30 min. The tissue was washed three times with 0.1 M PBS and then dipped into a solution containing: 5 ml distilled H2O, two drops 7.5 M PBS, four drops d-aminobenzodine (DAB), two drops H2O2, and two drops Ni2+ (Vector Laboratories). All sections were finally rinsed three times with 0.1 M PBS, mounted onto slides, and had cover slips placed on top.

2.7. Density measurements

Six coronal brain sections were selected from the lesion groups at [anterior to bregma (A)] A = +4.70, +2.70, +1.2, +0.2, −0.8, −1.8 for density analysis. Digital images of the stained tissue were captured using a Kodak digital camera DCS 410. The camera was mounted 5.5 cm above a Northern Light Precision Illuminator Model 890 manufactured by Imaging Research and run using a Macintosh PowerPC G4 computer. The aperture was set to f/3.6 and the shutter speed to 1/500th of a second. The images captured were opened using NIH image V1.62 (written by W. Rasband at the United States National Institutes of Health and available from the Internet at http://rsb.info.nih.gov/nih-image/). The cortex of the lesion and control hemispheres were separately outlined by making boundary drawings around each and density measurements expressed in pixles/square pixles were recorded. Statistical significance of the density measurements between the lesion and control hemispheres was assessed using a paired-sample t-test.

3. Results

3.1. Histological results

Fig. 1 presents coronal hemisections from animals with combined nucleus basalis and medial forebrain bundle lesions, stained with acetylcholinesterase (left) and immunohistochemistry for serotonin (right). The lesions were selective and did not damage other structures in the basal
forebrain. The tissue stains revealed significant ($P < 0.0001$) reduction of acetylcholinesterase, 55% loss in the ipsilateral hemisphere and significant ($P < 0.0001$) reduction of serotonin, 63% loss in the ipsilateral hemisphere.

3.2. Behavioral analysis

The performance of the ACh lesion, 5-HT lesion, combined lesion, and control groups was compared on rung walking, cylinder, swimming, adhesive dot removal, and single pellet reaching tests using a simple ANOVA. Data collected from the one-stage and two-stage lesion groups were pooled and will be referred to as the combined lesion group (Table 1).

3.2.1. Rung walking

The ability of the various groups to cross a horizontal ladder with randomly spaced bars was assessed by counting the number of foot faults. A simple ANOVA revealed a significant main effect of group, $F(3,40) = 5.210$, $P = 0.0039$. A follow up Fisher LSD post hoc ($P < 0.05$) analysis showed that both the 5-HT and combined lesion groups committed significantly more foot faults than the control group. A simple ANOVA on ratio of foot faults (by both forelimbs and hind limbs) between the contralateral and ipsilateral sides of the body revealed a significant main effect of treatment on foot fault asymmetry, $F(3,40) = 4.809$, $P = 0.0059$. A follow up Fisher LSD post hoc ($P < 0.05$) analysis showed that the combined lesion group
Table 1
Sensorimotor tasks: performance of nucleus basalis lesion (quisqualic acid), medial forebrain bundle lesion (5,7-dihydroxytryptamine), combined lesion, atropine sulphate, and methiothepin mesylate groups. See text for details of the individual tests. (0) No impairment; (–) impairment; (×) unable to perform task.

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<th>Test</th>
<th>Group</th>
<th>ACh  (n=6)</th>
<th>5-HT (n=6)</th>
<th>Combined lesion (n=15)</th>
<th>Atropine sulphate (n=10)</th>
<th>Methiothepin mesylate (n=6)</th>
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had a significantly higher magnitude of asymmetry than both the control and the nucleus basalis lesion groups (Fig. 2A).

3.2.2. Cylinder test
All groups actively explored the cylinder, reared and supported their body against the walls with their forelimbs.

Fig. 2. Magnitude of asymmetry score (mean and standard error) = contralateral limb score / ipsilateral limb score, for control and lesion groups on four behavioral measures: (A) rung walking; (B) cylinder test; (C) adhesive dot removal; (D) swimming. Note, there are no group differences on the cylinder and swimming tasks. *P<0.05, **P<0.01.
The total number of wall contact (contralateral + ipsilateral) was calculated for each group, and a simple ANOVA showed a significant main effect of treatment, $F(3,40) = 3.034, P = 0.0402$. A follow up Fisher LSD post hoc ($P < 0.05$) analysis showed that the medial forebrain bundle lesion groups contacted the walls of the cylinder significantly more than the control and combined lesion groups. A simple ANOVA on the ratio of contralateral forelimb contacts to ipsilateral forelimb contacts gave no significant main effect of treatment in forelimb use asymmetry, $F(3,40) = 1.557, P = 0.2148$ (Fig. 2B).

### 3.2.3. Swimming task

Rats in all groups swam directly to the platform and successfully climbed onto it. A simple ANOVA revealed no significant main effect of treatment on the overall (contralateral + ipsilateral) number of forepaw strokes, $F(3,40) = 2.482, P = 0.0748$. There was also no significant main effect of treatment on the ratio of ipsilateral to contralateral forelimb movements, $F(3,40) = 1.583, P = 0.2086$ (Fig. 2C).

### 3.2.4. Adhesive dot removal

All groups successfully removed the adhesive paper from both of their paws within the 3-min time limit. A simple ANOVA revealed a significant main effect of treatment on the overall latency (contralateral + ipsilateral) to remove both stimuli, $F(3,24) = 23.553, P = 0.001$. A follow up Fisher LSD post hoc ($P < 0.05$) analysis gave the following group differences, combined lesion group $> 5$-HT $= ACh = control$. A simple ANOVA revealed a significant main effect of ratio of time to remove the stimulus from the ipsilateral vs. the contralateral forelimb, $F(3,24) = 3.52, P = 0.03$. A follow up Fisher LSD post hoc ($P < 0.05$) analysis showed that the combined lesion group had a significantly larger magnitude of asymmetry than the other groups (Fig. 2D). Thus, this was the only group to show an impairment in responding to the stimulus on the limb contralateral to the lesion.

### 3.2.5. Single pellet reaching

A mean success score was calculated for all animals and compared across groups. A simple ANOVA on the mean success scores of each group revealed a significant main effect of group, $F(3,40) = 4.23, P = 0.01$. A follow up Fisher LSD post hoc analysis ($P < 0.05$) showed that the combined lesion group had a significantly lower success score than the other groups, which did not differ (Fig. 3A). A mean reaches/pellet retrieved score was calculated for all animals as well and compared across groups. A simple ANOVA on reaches/pellet retrieved revealed no significant main effect of treatment, $F(3,40) = 1.89, P = 0.14$ (Fig. 3B).

A repeated measures ANOVA on the qualitative measures of reaching revealed a significant effect of group, $F(3,40) = 84.8, P = 0.001$. A follow up Fisher LSD post...
hoc analysis \((P<0.05)\) gave the following comparisons: combined lesion group = ACh > 5-HT = control (Fig. 3C).

3.2.6. Tray reaching (one-stage combined lesion)

The one-stage combined lesion group and a subset of the control group were tested on this task without any training prior to surgery. On initial training, all animals in the lesion group reached with their ipsilateral-to-lesion forelimb. Animals in the control group reached with a paw, which will be referred to as the contralateral forelimb. Following initial testing, the animals were trained to use their non-preferred forelimb. A simple ANOVA revealed no significant effect of treatment on success score following additional training, \(F(1,15) = 2.20, P=0.15\). Thus, both the lesion and the control groups performed equally as well with both their contralateral and ipsilateral forelimbs (Fig. 4A).

3.2.7. Single pellet reaching (one-stage combined lesion)

Both groups were trained and tested in this task after 4 weeks of training in the tray reaching task. All animals in the lesion group reached with their ipsilateral-to-lesion forelimbs. The control group used the same forelimb they last reached with in the tray task (i.e., the ipsilateral forelimb). A simple ANOVA on hit percent scores gave no significant main effect of treatment on success rate, \(F(1,15) = 0.01, P=0.91\). Elastoplast bracelets were then used to force both control and lesion groups to use their nonpreferred forelimbs. One rat from each group failed to reach when the bracelet was placed on its ipsilateral paw, and so was not included in the following analysis. An ANOVA on hit percent scores gave a significant group difference, \(F(1,13) = 12.8, P=0.003\). The control group was more successful in retrieving food pellets than the one-stage lesion group (Fig. 4B). A simple ANOVA on reaches per pellet revealed a significant group difference, \(F(1,13) = 16.3, P=0.001\). The one-stage lesion group made more reaches/pellet retrieved than the control group. A simple ANOVA on the ratio of number of reaches/pellet retrieved with the ipsilateral vs. the contralateral forelimb gave a significant group difference in that the lesion group made more reaches with the contralateral than with the ipsilateral forelimb, \(F(1,13) = 11.3, P=0.005\) (Fig. 4C).

3.2.8. Single pellet reaching (atropine sulphate)

A repeated measures ANOVA showed that the atropine sulphate treatment had a significant effect on the reaching success score, \(F(3,8) = 18.980, P=0.0001\) (Fig. 5A). A follow up Fisher post hoc analysis \((P<0.05)\) showed that the success rate at the highest dose was significantly lower than at the other doses. The lowest dose (5 mg/kg) had no effect, the medium dose (10 mg/kg) did not have a significant effect on hit percent, but it did cause a qualitative impairment, and most animals did not reach under the high dose (25 mg/kg). Movements of aiming and the advancing of the paw through the slot were most affected by the medium drug dose.

![Fig. 4. Skilled reaching scores (mean and standard error) for the one-stage combined lesion and control groups using both ipsilateral and contralateral forelimbs in (A) tray reaching, hit percentage; (B) single pellet, success, number of pellets retrieved out of 20; (C) single pellet, reaches/pellet, number of reaches performed for each successfully retrieved pellet. **\(P<0.01\).](image-url)
damage. Whereas slight impairments were obtained on skilled reaching following ACh and combined lesion and in rung walking following combined lesions, in general, neither single nor combined lesions had major disruptive effects on motor behavior.

A number of studies have demonstrated that combined, but not single ACh and 5-HT lesions, although allowing animals to walk, severely impair performance on mazes and simple motor tasks. The impairment is so severe that Vanderwolf [42] has described the animals as lacking all intelligent behavior. Accordingly, it has been proposed that animals with such lesions can serve as an animal model of Alzheimer’s disease or other dementias. Unfortunately, animals that are so severely impaired cannot be studied for long periods of time because they are unable to sustain themselves.

Since the afferent and efferent projections of the rat brain are organized within hemispheres, it might be expected that unilateral lesions to these two ascending projections to the neocortex should disrupt behaviors dependent upon the denervated hemisphere. For example, unilateral decortication disrupts a wide range of behavior dependent on the cortex [32]. In addition, focal lesions to the neocortex [52], unilateral depletion of dopamine [23], or damage to cortical efferents including the pyramidal tract [50] also disrupt behavior dependent on that cortex. Given that the integrity of the neocortex is required for the production of normal behavior, especially on the contralateral side of the body, it follows that the loss of ACh and 5-HT might result in contralateral deficits similar to those produced by frank unilateral lesions.

ACh depletions were produced using quisqualic acid. Based on previous work comparing various neurotoxin lesions of the nucleus basalis, bilateral infusion of quisqualic acid into the nucleus basalis produces a selective reduction of neocortical ACh by 70–75% with the fewest side effects [13]. Damage in the present experiment was restricted to the ipsilateral cortical cholinergic pathway as assessed by acetylcholinesterase reactivity in the neocortex and thus only produced a 55% loss. The lesion did not affect performance in rung walking, cylinder, swimming, adhesive dot removal, or success rate in single pellet reaching. Despite the unimpaired performance on these tests, qualitative deficits in single pellet reaching were observed in forelimb advancement and supination of the paw. Similar qualitative impairments are observed after unilateral motor cortex lesions, but such deficits are usually accompanied by reduced success scores [55]. Thus, the qualitative deficit without a quantitative effect suggests that the cortical cholinergic innervation has only a small, although interesting, effect on skilled reaching. The impairment in the qualitative features of skilled reaching may be related to abnormalities in the cortical representation or map of movement as other work has shown that ACh is necessary for other sensorimotor representations [17,19,35].
The present findings indicating little, if any, permanent movement deficits following nucleus basalis lesions are consistent with previous studies. Dunnett et al. [13] assessed animals with bilateral nucleus basalis lesions on a battery of sensorimotor tasks and reported initial impairments that disappeared with recovery time. Other studies confirmed that lesions restricted to the nucleus basalis do not affect learning and memory [2,3,5]. Abdulla et al. [1], on the other hand, described sensorimotor deficits following unilateral AMPA neurotoxic lesions of the nucleus basalis. Furthermore, Dubois et al. [12] used radiofrequency current and ibotenic acid to bilaterally damage the nucleus basalis and reported profound disturbances in spontaneous behaviors. Both findings of Abdulla et al. [1] and Dubois et al. [12] should be interpreted with caution because the lesions produced were not selective. Thus, with the exception of the qualitative impairments observed in reaching, the main result that motor behavior is spared by cholinergic depletion was confirmed.

To deplete serotonin in the neocortex, the neurotoxin 5,7-dihydroxytryptamine was unilaterally infused into the medial forebrain bundle. Based on Giambalvo and Snodgrass [14], this method produces chronic damage restricted to the ipsilateral ascending serotonergic projections. The lesion was assessed using immunohistochemical methods and revealed decreased levels of serotonergic innervation in the ipsilateral hemisphere several weeks after the surgery. The lesion did not affect performance on any of the behavioral measures. The present findings indicating no movement deficits following serotonergic depletion or receptor blockade are consistent with the findings of Dringenberg et al. [8]. They administered the serotonin synthesis inhibitor para-chlorophenylalanine (PCPA, 1000 mg/kg, i.p.), which depleted 90% of the serotonin in the rat’s brain as detected using biochemical assays, and they found no deficits on a battery of sensorimotor tests.

Depleting the neocortex of both ACh and 5-HT caused some mild deficits in the quantitative and qualitative measures of single pellet reaching and running, but generally behavior was intact. The impairments in the qualitative features of skilled reaching were similar to those produced to ACh lesions alone and thus are likely due to the ACh depletion. Thus, the only unique effect of the combined lesions was an impairment in running, and this impairment was relatively mild.

There are a number of potential explanations for why unilateral depletions did not produce the severe deficits reported to follow bilateral lesions. First, the ACh and 5-HT lesions are not total, and the remaining acetylcholine and serotonin in the neocortex may have been sufficient to maintain relatively normal behavior. Second, the impairments in behavior following bilateral lesions may be secondary to the impairments in the EEG. Possibly, slowing of the EEG due to unilateral lesions [9,45] may not have been large enough to produce behavioral impairments. Third, Vanderwolf’s [42] preparations, as well as those of others [4,11], used animals in which either ACh or 5-HT were inactivated pharmacologically. In the present study we confirmed that pharmacological blockade of either ACh or 5-HT could completely impede skilled motor behavior as exemplified by skilled reaching. It is, therefore, not surprising that subjects may be impaired on behavioral tests when either of these pharmacological treatments is used singly or in combination [11,42].

In conclusion, the present study is the first to describe the effects of single or conjoint unilateral lesions of cholinergic and serotonergic cortical input using a comprehensive battery of motor tests. The present findings demonstrate that a loss of ACh and 5-HT in the neocortex causes some mild motor impairments on some motor tests. The absence of more severe impairments of the kind produced by bilateral lesions/blockade of these two ascending systems suggests that it may not be possible to produce a similar unilateral model of dementia.

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References


