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Role of the Laterodorsal Tegmental Nucleus in Scopolamine- and Amphetamine-Induced Locomotion and Stereotypy

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LAVIOLETTE, S. R., R. P. M. PRIEBE AND J. S. YEOMANS. *Role of the laterodorsal tegmental nucleus in scopolamine- and amphetamine-induced locomotion and stereotypy*. PHARMACOL BIOCHEM BEHAV **65**(1) 163–174, 2000.—Scopolamine (1.5 mg/kg; IP) or amphetamine (3 mg/kg; IP) increases locomotion and stereotyped behavior patterns in rats. Previous studies suggest that scopolamine acts via muscarinic receptors near the midbrain–pons border. In this study, unilateral microinjections in *N*-methyl-scopolamine (2.5–10 μg) into the laterodorsal tegmental nucleus (LDT) increased locomotion. Bilateral ibotenate lesions of the LDT attenuated scopolamine-induced locomotion by 68% 7 days postlesion, and by 35% 28 days postlesion. LDT lesions reduced scopolamine-induced stereotypy less than locomotion. The sensitization to amphetamine observed on repeated tests was attenuated by LDT lesions for stereotypy, but not for locomotion. These findings suggest that scopolamine induces locomotion largely, but not exclusively, by blocking muscarinic receptors in LDT. © 1999 Elsevier Science Inc.

Cholinergic Muscarinic Mesopontine Ibotenate Dopaminergic

MESOPONTINE cholinergic neurons are found in two large cell groups. The laterodorsal tegmental nucleus (LDT) contains a densely clustered group of cholinergic neurons (Ch6) intermingled with noncholinergic neurons. The more widely diffused Ch5 cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) are found lateral and rostral to the LDT neurons (38,58). Both cell groups are adjacent to the mesencephalic locomotion region of the caudal midbrain (19,39,48). They both connect directly with mesencephalic and medullary structures involved in patterned limb movements, and with midbrain dopamine neurons that activate locomotor activity (7,21,40).

Systemic injections of antimuscarinics increase locomotion and stereotypic behaviors in rats (1,25,36,37,50). Previous studies indicate that locomotion induced by systemic scopolamine occurs in large part via muscarinic receptors located near the PPT (37). For example, scopolamine injected near the PPT induces locomotion. Unilateral injections of carbachol, a muscarinic/nicotinic agonist, into the PPT decreases spontaneous, scopolamine- or NMDA-induced locomotor activity (7,20,37,44). The agents used in these studies (carbachol and scopolamine) are highly mobile, however, so that it is difficult to determine how locally the drugs act in the midbrain and pons.

Muscarinic agonists inhibit both cholinergic and noncholinergic cells in PPT and LDT (30,34,47). Atropine, a muscarinic blocker, excites LDT cells in slice (31), presumably by removing a tonic muscarinic inhibition. Therefore, it has been proposed that locomotion induced by scopolamine results from disinhibition of mesopontine cholinergic neurons (37).

Mesopontine cholinergic neurons monosynaptically activate dopamine neurons of the midbrain (6,11,18,29,58,59). Scopolamine, injected systemically or into the PPT at doses that increase locomotion and stereotypy, increases striatal dopamine efflux as measured by electrochemistry (12). The increase in dopamine efflux induced by systemic scopolamine was blocked by pretreatment with carbachol in the PPT (12). Accordingly, it was proposed that disinhibition of mesopontine cholinergic cells excites dopamine cells that induce locomotion. In support of this idea, haloperidol blocks locomotion

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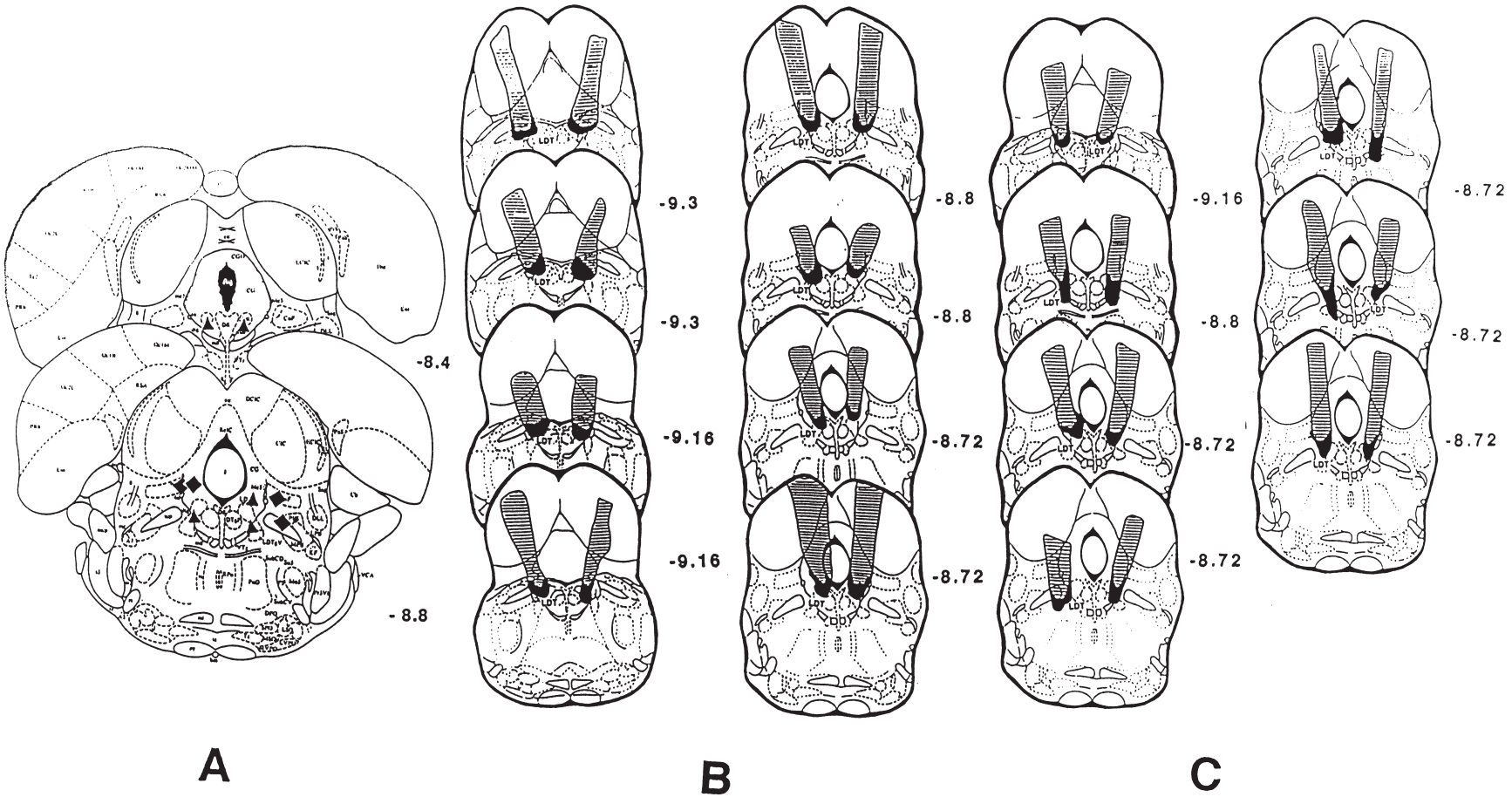
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and stereotypy induced by scopolamine (37). Alpha-methyltyrosine, a DA synthesis inhibitor, attenuates locomotion induced by antimuscarinics, such as scopolamine, benztropine, and atropine(50).

The most critical neurons for stimulant-induced locomotion are mesolimbic dopamine neurons (27). Therefore, the direct projections of LDT neurons to ventral tegmental area dopamine neurons may be important. LDT lesions block anticholinesterase-induced activation of mesolimbic dopamine systems, but PPT lesions have little effect (5). Injections of 6-hydroxydopamine into the nucleus accumbens, however, failed to block scopolamine-induced locomotion (25).

LDT and PPT cell groups may also affect amphetamine-induced locomotion. Carbachol or procaine injected into PPT attenuates amphetamine-induced locomotion (7,8). Scopolamine potentiates apomorphine-induced locomotion (9). However, recent studies have failed to find any effect of PPT lesions on amphetamine-induced locomotion (2,23) or apomorphine-induced locomotion (49), making the role of PPT neurons uncertain.

The LDT provides a convenient target for drug and lesion manipulations. It contains a small (1 cubic mm), tightly clustered group of cholinergic cells. The PPT, by contrast, contains a more diffuse distribution of cholinergic cells that are difficult to lesion or stimulate (41,58).

Here we study the role of the LDT in the mediation of scopolamine- and amphetamine induced locomotion and stereotypy. To localize the effects of antimuscarinics on locomotion, *N*-methyl-scopolamine (NMS), a muscarinic blocker with limited mobility in brain tissue (15,54), was injected into the LDT, and locomotion was measured. Bilateral ibotenate lesions of the LDT were made to measure the importance of LDT neurons to locomotion and stereotypy induced by scopolamine and amphetamine.

METHOD

Surgery

Fifty male Wistar rats weighing 300–400 g at the time of surgery were housed in individual Plexiglas cages with access to food and water ad lib, in a light-controlled room under a 12 L:12 D cycle.

Animals were anaesthetized with sodium pentobarbital (Somnotol, 60 mg/kg; MTC Pharmaceuticals) and placed in a stereotaxic apparatus. Stainless steel, 23-gauge guide cannulae (Plastic One Products, Roanoke, VA), were aimed for the LDT bilaterally using the following stereotaxic coordinates: 8.8 mm posterior to bregma; 2.13 mm lateral to bregma, and 7.12 mm ventral to the lambda–bregma line (45). All cannulae were angled medially by 10 degrees to avoid passing through the anterior fourth ventricle. Guide cannulae were fixed to the skull using jeweler's screws and self-curing dental acrylic (Dentsply Fast-cure; UK). Following surgery, rats were given at least 7 days to recover before testing began.

As anatomical controls for ibotenate lesions, six of these rats received bilateral cannula implants aimed for the region of the inferior colliculus/cuneiform nucleus, also at a 10-degree angle, using the following coordinates: 8.8 mm posterior to bregma; 2.75 mm lateral to bregma, and 5.5 mm ventral to the lambda–bregma line. Ibotenate lesions performed on these animals followed the same procedure as described below for LDT lesioned animals.

In addition, nine rats had unilateral cannulae aimed for the LDT at the coordinates specified above, for microinjection studies.

Drugs

Scopolamine hydrobromide (Sigma Chemicals) was dissolved in 0.9% isotonic saline at a concentration of 1.5 mg/ml. *d*-Amphetamine (RBI Chemicals) was dissolved in 0.9% isotonic saline for a concentration of 3 mg/ml. Isotonic saline served as the control for all injections.

For central injections, *N*-methyl-scopolamine (Sigma) was dissolved in isotonic saline. The concentrations were prepared so that a 0.5 μl injection would include 2.5, 6, or 10 μg NMS. Microinjections were performed with a 1-μl Hamilton syringe attached to a 28-gauge internal injector (Plastic Products) via microinjection tubing. The injector cannula was inserted into the guide cannula, and drug or saline vehicle was infused slowly over a 60-s period.

Ibotenate Lesions

Ibotenate was prepared as a 2.5 μg/0.5 μl solution by dissolving 1 mg of ibotenic acid (RBI Chemicals) in 0.2 ml of 0.9% isotonic saline and 0.02 ml of titrated citric acid (0.1 M) to dissolve the ibotenic acid powder.

Animals were anesthetized with pentobarbital, and lesions were performed bilaterally by infusing the ibotenate solution through the implanted guide cannulae via microinjector tubing attached to a 1-μl Hamilton syringe at a rate of 0.1 μl/min. The microinjectors were left in place in the cannulae for 15 min following infusions to ensure adequate diffusion of the ibotenate from the injector tip.

Two separate experiments were performed on LDT-lesioned animals. In one experiment, eight animals were injected with scopolamine (1.5 mg/kg; IP) at three times: prior to, 7, and 28 days following LDT lesions. In each case, locomotion and stereotypy measures were taken for 60 min. One control group ($n = 6$) received sham lesions of the isotonic saline/citric acid vehicle for the ibotenate. The other control group received sham lesions of only isotonic saline ($n = 7$) to assess the effects of citric acid. Behavioral testing of control groups was identical to that described for the experimental groups.

In the second experiment, seven animals were given *d*-amphetamine (3 mg/kg; IP) and were tested for locomotion and stereotypical behavior before, and 7, and 28 days after ibotenate lesions. The control group consisted of seven animals given saline sham lesions.

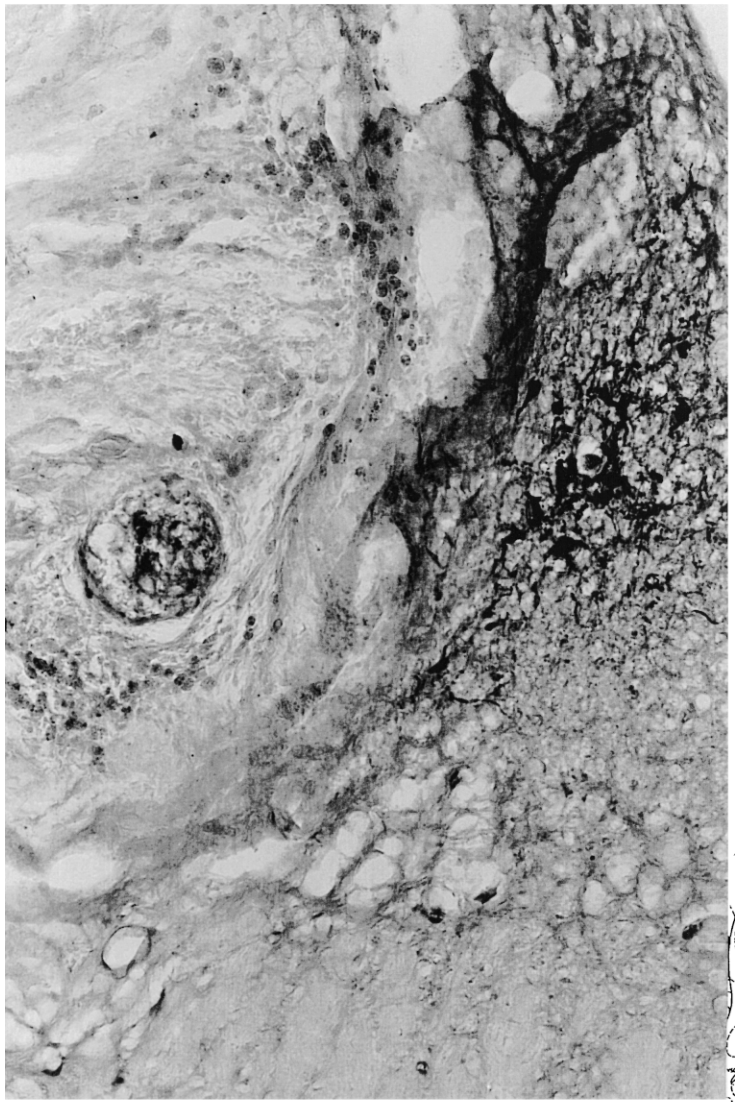
NMS Injections in LDT

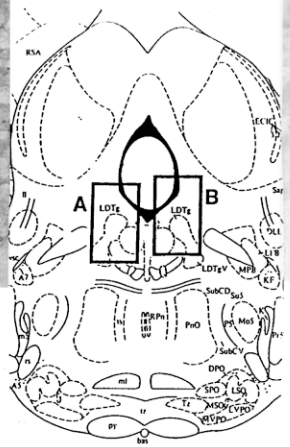
Each infusion was made over a 2-min period. Then the rat was placed in the locomotion boxes followed by a 60-min test

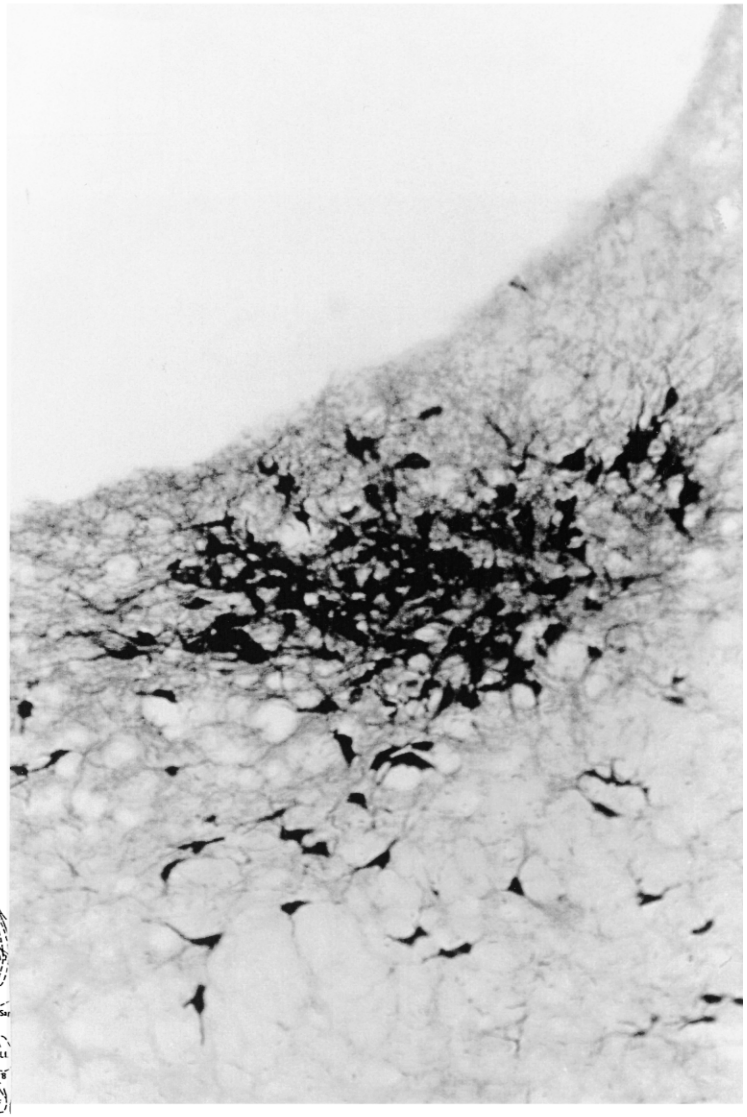
FIG. 1. (A) LDT sites for NMS injections in nine rats. Triangles show "excellent" sites, while diamonds show "adequate" sites more than 0.5 mm from LDT. (B) Tracings of maximal damage produced by ibotenate lesions of LDT, for eight rats given scopolamine, and (C) for seven rats given amphetamine. Ibotenate-induced gliosis is indicated by filled-in black areas. Cannula track-induced damage is indicated by line-shaded areas. For each rat, one section from the Paxinos and Watson (47) atlas was chosen that was closest to the center of the greatest damage near the LDT.

LDT (LESIONED)

LDT (INTACT)







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period in which activity was monitored electronically. For this study, total beam crosses were measured rather than crossovers. Each animal was habituated in the box for at least 12 h before testing. One test was performed every second day, beginning with a saline test, and then ascending through the three doses.

Behavioral Observations

Locomotor activity was measured in four locomotion boxes each 20 × 25 × 36 cm. Boxes were fully enclosed with the exception of the front of the box, which was constructed of wire mesh to allow for behavioral observations during test sessions. Each locomotion box contained two infrared photocell beams situated across the long axis of the box and placed 2 cm above the mesh floor. Total crossovers of the beams (i.e., crossing the rear beam followed by a crossing of the front beam, or vice versa) were recorded by computer for 12 5-min intervals per test session. Prior to testing, all animals were habituated for 20 h in the locomotion boxes.

During all test sessions, rats were observed for displays of stereotyped or other behaviors. Stereotypy was rated using a stereotypy scale similar to that described by Kelly et al. (28). Ratings ranged from 0–6 and were assigned according to the following criteria: 0—asleep or stationary; 1—active, but with no consistent stereotyped sniffing or rearing; 2—predominantly active but with bursts of stereotyped patterns of sniffing or rearing; 3—stereotyped activity such as sniffing or rearing over a large area; 4—stereotyped activity such as sniffing, rearing, and /or grooming maintained in one location; 5—stereotyped behavior maintained in one location with repetitive gnawing and/or licking of the self or cage; and 6—continual gnawing and licking of the self and/or cage.

Histology

After completion of all experiments, rats were sacrificed with an overdose of sodium pentobarbital. After the NMS experiment, rats were perfused with saline followed by formalin. Brains were removed and placed in a formalin solution saturated with sucrose for 48 h. Brains were frozen to −20°C in a cryostat and sectioned at 40 μm. Sections were stained with cresyl violet and coverslipped.

After the lesion experiments, rats were perfused with phosphate-buffered saline (pH 7.4) and then with 500 ml of 4% paraformaldehyde (BDH). Brains were removed and stored for 24 h in the 4% paraformaldehyde fixative, then transferred to a 0.25% (wt/vol) phosphate-buffered solution for an additional 24 h. Brains were then flash frozen at −70°C and sectioned at 40 μm. Coronal sections were mounted on gelled slides. Nicotinamide adenine dinucleotide phosphate (NADPH) (Sigma) histochemistry was performed using the procedure of Vincent et al. (53). Lesions and injection sites were identified by the presence of reactive gliosis and cholinergic cell loss as revealed by NADPH-diaphorase histochemistry. The lesions were reconstructed by tracing the maximum extent of lesion damage onto atlas sections (45).

Statistics

Locomotor measures were analyzed by two-factor, repeated-measure ANOVAs, with drug treatment and time as the factors. Post hoc analysis of ANOVA was performed with Scheffe's Test and Tukey's Honestly Significant Difference Test. Stereotypy ratings were analyzed with Wilcoxan Signed-Ranks tests for repeated measures.

RESULTS

Histology

Injection sites for the NMS study were all located in and around LDT, but never more than 1 mm from LDT, as shown for the nine animals with complete histological analysis (Fig. 1A). Five of these sites within 0.5 mm of the boundaries of LDT were labeled "excellent" sites; the other four sites were 0.5–1.0 mm dorsal or lateral to the LDT, and were labeled "adequate" sites.

LDT ibotenate lesions (Fig. 1B) were largely confined to the region of the LDT in the tegmentum, but included damage that extended dorsally into the area of the cuneiform nucleus, central gray, and inferior colliculus, where the wider guide cannulae passed through the tissue. All ibotenate lesions were identifiable by heavy concentrations of reactive gliosis, which typically surrounded the perimeter of the lesioned area around the tip and for a short distance dorsally up the cannula track, in some animals. The lesions typically destroyed 35–95% of NADPH-diaphorase–stained Ch6 cells, and the estimated centers of lesion damage ranged from the most caudal to the most rostral extremes of the LDT. Some lesions were largely confined to the medial Ch6 cell regions, while most lesions ablated primarily lateral Ch6 cells and spared a small percentage of the medially located Ch6 cells. In no cases did ibotenate damage extend to the nearby PPT.

The six rats used in the anatomical control lesioned group were found to have cannula placements within the areas of the cuneiform nucleus, inferior colliculus, and central gray, with no damage to the LDT. Damage was predominantly centered in the areas of the cuneiform nucleus, inferior colliculus, and central gray of the midbrain. This damage closely resembled the damage produced dorsally along the cannula track in the LDT-lesioned rats.

Figure 2A shows a representative ibotenate lesion of the LDT region. NADPH-diaphorase–stained cells have been largely ablated and reactive gliosis is present around the lesioned area. Spared Ch6 cells are visible in the medial part of the LDT. Figure 2B shows intact, densely clustered NADPH-diaphorase–stained cells within the rostral LDT from an anatomical control lesion.

Sham control lesions using the citrate/saline vehicle induced mild to moderate damage in the LDT region resulting in 10–35% estimated loss of NADPH-diaphorase–stained cells. Sham lesions using isotonic saline produced negligible to mild damage to the LDT (0–20%). The minor damage was observed only immediately adjacent to cannula tips, and no reactive gliosis was present, suggesting that these were punc-

FIG. 2. Examples of rat brain sections used to make Fig. 1B and C. (A) Section of a lesioned LDT in the region of the cannula tip of an ibotenate-treated rat. A few intact NADPH- diaphorase labeled Ch6 cholinergic cells are visible on the medial edge of the lesion. (B) A section of an intact LDT, in an anatomical control rat. NADPH-diaphorase labeled CH6 cholinergic cells are visible throughout the LDT. Inset diagram shows anatomical areas from which microphotographs were taken for (A), (B), respectively.

ture wounds typically observed after sham lesions. Similar damage along the cannula tracks was observed in all groups.

Locomotion-induced by N-*Methyl-scopolamine in LDT*

Unilateral injections of NMS (2.5–10 μg; $n = 9$ at all doses) increased horizontal locomotion in a dose-dependent manner. Group results are shown in Fig. 3. Locomotor activity was increased by a mean of 4% above saline baseline levels following 2.5 μg of NMS, by 60% following the 6 μg dose, and by 112% following the 10 μg dose. NMS significantly increased locomotion above saline levels at the 6 μg, $F(5, 22) = 5.78$, $p < 0.05$, and 10 μg doses, $F(1, 22) = 5.24$, $p < 0.05$, but not at the 2.5 μg dose. There was no significant interaction between time and treatment.

The cannula sites in LDT were divided into "excellent" sites, within 0.5 mm ($n = 5$) of LDT, and "adequate" sites, within 0.5–1.0 mm of LDT ($n = 4$). Subsequent analysis revealed that for "excellent" LDT sites, locomotor activity was greatly increased in a dose-dependent fashion. At the 6 μg dose, locomotor counts were increased by 85% ($F = 2.38$, NS), and at 10 μg, counts were increased by 223% over saline levels ($F = 6.20$, $p < 0.05$). "Adequate" LDT sites showed no significant increase in locomotor activity. These results suggest that the NMS injections acted locally (within 1 mm of the tip), and that the site of action for inducing locomotion was in the LDT.

Scopolamine-Inducted Locomotion in LDT Lesioned Animals

Rats were generally active for several minutes following saline injections, either before or after LDT lesions (Fig. 4). LDT lesions did not reduce locomotion observed after handling and saline injection.

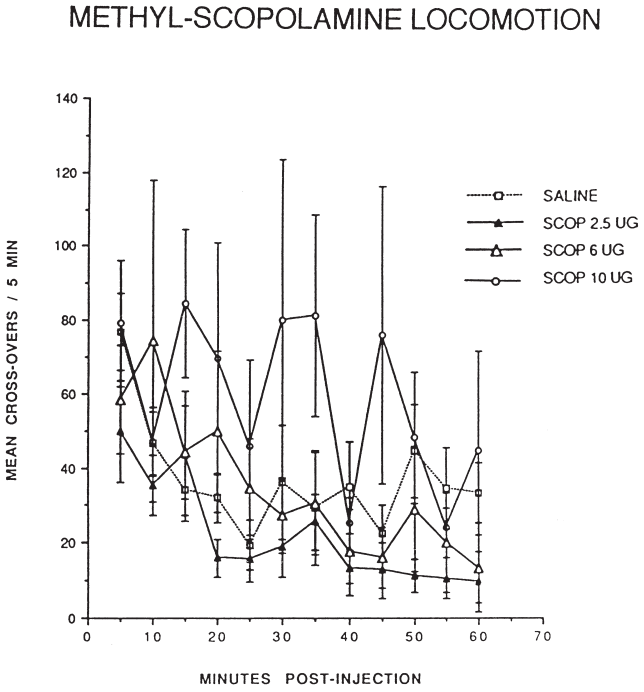

FIG. 3. Locomotion induced by unilateral NMS injections (2.5–10 μg) in LDT. Locomotion is measured for 60 min postinjection by the mean number of beam crosses in each 5- min interval. Error bars are standard errors of the mean in this and all subsequent figures.

Before lesions, scopolamine (1.5 mg/kg, IP) increased locomotion throughout the 60-min session, by a mean of 82% above baseline levels, prelesion. Statistical analysis revealed a significant main effect for scopolamine upon locomotion $F(5, 440) = 107$, $p < 0.0001$, and a significant interaction between time and treatment, $F(35, 440) = 13.2$, $p < 0.0001$. Post hoc comparisons indicated that scopolamine significantly increased locomotion above saline levels ($p < 0.001$) at all test times, prelesion, 7 days, and 28 days postlesion. Also, 7 days postlesion scopolamine levels were significantly lower than prelesion scopolamine levels ($p < 0.0001$), and scopolamine-induced locomotion levels 28 days postlesion were significantly lower than prelesion scopolamine levels ($p < 0.05$). No significant differences were found between saline conditions.

When animals were injected with scopolamine 7 days postlesion, scopolamine-induced locomotion was attenuated by 68% over the 60-min test session compared to saline. Scopolamine-induced locomotion was attenuated by 35% from prelesion levels 28 days postlesion. The attenuation of scopolamine-induced locomotion was calculated by subtracting the total number of crossovers for saline (R-Sal) from the total crossovers in the pre- and postlesion scopolamine conditions (R- SCOPpost and R-SCOPpre) and taking the ratio: (R-SCOPpost − R-Sal) / (R-SCOPpre − R-Sal). The mean of the pre- and postlesion saline conditions was used for R-Sal, because there was no reliable difference between these.

The extent of Ch6 cell loss as revealed by NADPH-diaphorase histochemistry was not closely related to the attenuation of scopolamine-induced locomotion. For example, one rat displaying the greatest extent of NADPH-diaphorase cell

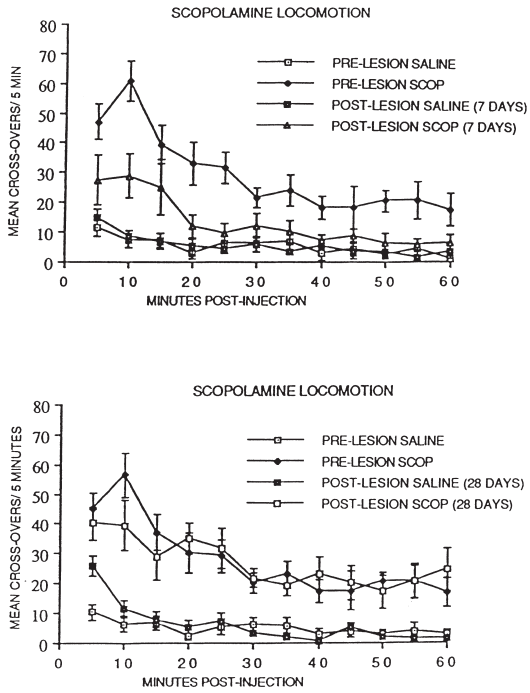

FIG. 4. Locomotion induced by scopolamine (1.5 mg/kg; IP) in eight rats with ibotenate lesions of LDT. Locomotion is measured by the mean number of crossovers in each 5-min interval. (Above) the effects of saline and scopolamine are shown for tests before and 7 days postlesion. (Below) the effects of saline and scopolamine are shown for tests before (again) and 28 days postlesion.

loss (80%) showed a moderate attenuation of scopolamine-induced locomotion at 7 days postlesion (32%). In contrast, five rats displaying lesser degrees of Ch6 NADPH-diaphorase cell loss (10–35%) showed the strongest attenuation of scopolamine-induced locomotion 7 days postlesion (77–98%).

Scopolamine-Induced Stereotypy in LDT Lesioned Rats

Following scopolamine injections, these same rats typically displayed exaggerated exploratory behaviors and stereotyped behavior patterns, such as sniffing and rearing over large areas of the cage, but stereotypy ratings rarely exceeded level 4 (Fig. 5). Statistical analysis revealed that scopolamine significantly increased stereotypy ratings above saline ratings prelesion ($T = 3.05$, $p < 0.002$), but 7 days postlesion, stereotypy ratings were attenuated by 40% from prelesion levels, and significantly lower than prelesion ratings ($T = 2.54$, $p < 0.01$). Twenty-eight days postlesion stereotypy ratings were attenuated by 57%, and were significantly lower than prelesion levels ($T = 2.31$, $p < 0.02$). The effects of scopolamine on stereotypy were variable both pre- and postlesion, possibly due to the low dose used.

Scopolamine-Induced Locomotion and Stereotypy in Sham-Lesioned Rats

In saline-sham lesioned rats ($n = 7$) (Fig. 6), scopolamine increased locomotion as described above, $F(5, 30) = 18.2$, $p < 0.001$). Scopolamine increased locomotor activity by 80% above saline prelesion, by 66% above saline 7 days postlesion, and 84% above saline 28 days postlesion (Fig. 6). Post hoc analysis revealed that scopolamine increased locomotion above saline levels across all test times ($p < 0.01$). No significant differences were found between pre-, 7, and 28 days postlesion levels of scopolamine-induced locomotion. Thus, saline sham lesions failed to significantly alter scopolamine-induced locomotion.

Scopolamine significantly increased stereotypy levels above saline prelesion ($T = 3.05$, $p < 0.002$), 7 days postlesion ($T = 3.05$, $p < 0.002$), and 28 days postlesion ($T = 3.06$, $p < 0.002$). No significant differences were found between pre-, 7-, and 28-day test sessions, however. Thus, saline sham lesions did not significantly alter scopolamine-induced stereotypy levels.

Citrate/saline control lesions ($n = 6$) reduced scopolamine-induced locomotion slightly, that is, by 40% at 7 days postlesion, and by 22% at 28 days postlesion (Fig. 7). This is consistent with modest damage to LDT (10–35% cell loss) observed in these brains postmortem. Statistical analysis revealed a significant main effect of scopolamine on locomotion, $F(5, 25) = 7.9$, $p < 0.05$. Post hoc comparisons revealed no significant differences between prelesion, 7, and 28 days postlesion for scopolamine-induced locomotion. Scopolamine significantly increased stereotypy above saline prelesion ($T = 3.06$, $p < 0.002$), 7 days postlesion ($T = 3.05$, $p < 0.002$), and 28 days postlesion ($T = 2.84$, $p < 0.04$). There were no significant differences between these scopolamine effects prelesion, 7, and 28 days postlesion.

Scopolamine-Induced Locomotion and Stereotypy in Anatomical Controls

In inferior colliculus/cuneiform nucleus lesioned rats ($n = 6$), scopolamine increased locomotion as described for the other scopolamine tests, $F(5, 21) = 20.2$, $p < 0.001$; $p < 0.01$ at all test times. Scopolamine increased locomotion by 86%

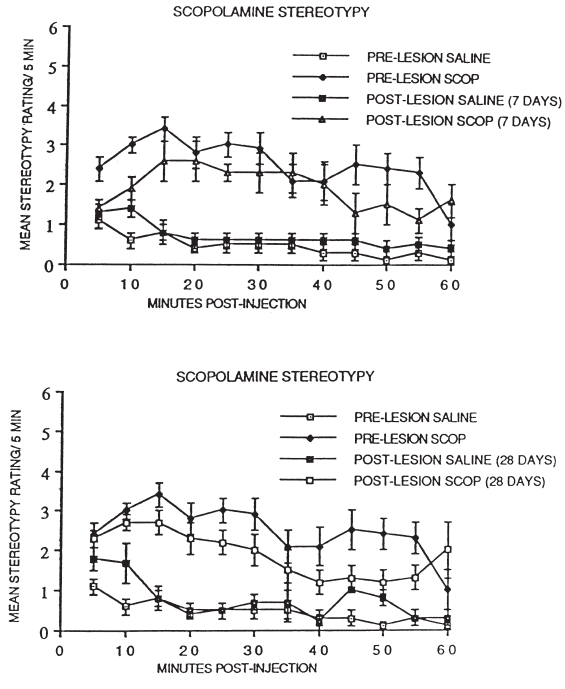

FIG. 5. Stereotypy ratings following scopolamine (1.5 mg/kg; IP) injections in the same eight rats with ibotenate lesions of the LDT as in Fig. 4. (Above) effects of saline and scopolamine are shown before and 7 days postlesion. (Below) effects of saline and scopolamine are shown before and 28 days postlesion.

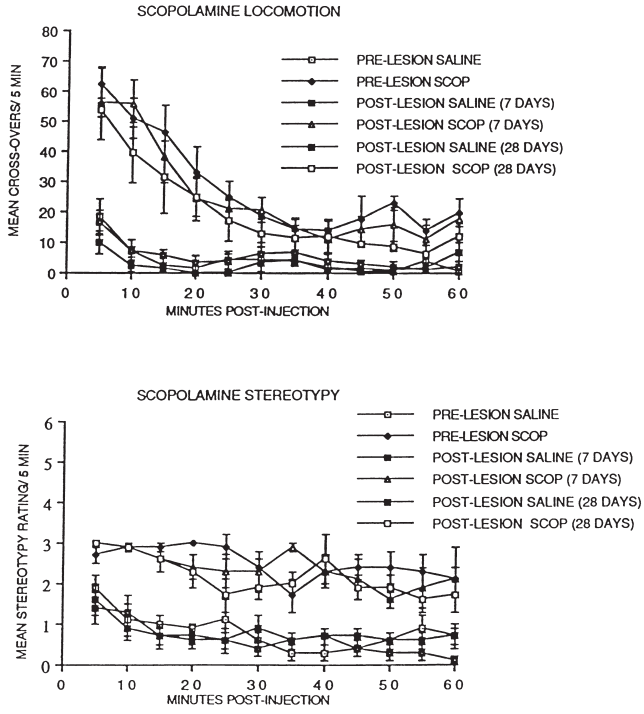

FIG. 6. Effects of saline and scopolamine in saline sham-lesioned rats on scopolamine-induced locomotion (above) and stereotypy (below).

above saline levels prelesion; 80% above saline levels at 7 days postlesion, and 86% above saline levels when tested 28 days postlesion. No significant differences were found between pre-, 7-, and 28-day postlesion levels of scopolamine-induced locomotion. Scopolamine increased stereotypy ratings above saline levels prelesion ($T = 3.06$, $p < 0.002$), at 7 days postlesion ($T = 3.05$, $p < 0.002$) and at 28 days postlesion ($T = 3.06$, $p < 0.002$). No significant differences were found between pre-, 7-, and 28-day post lesion stereotypy ratings. Thus, control lesions of the cuneiform nucleus and inferior colliculus failed to alter scopolamine-induced locomotion or stereotypy.

Amphetamine-Induced Locomotion and Stereotypy in LDT-Lesioned Rats

Amphetamine (3 mg/kg, IP) increased locomotion and stereotypy ratings. Generally, locomotion increased within 10 min following injections, and was maintained for the duration of the test session (Fig. 8). Statistical analysis revealed a significant main effect for amphetamine upon locomotion $F(5, 385) = 30.3$, $p < 0.0001$, and a significant interaction between treatment and time, $F(30, 385) = 4.6$, $p < 0.0001$. Amphetamine increased locomotion above saline levels by 72% prelesion, by 55% 7 days postlesion, and by 48% 28 days postlesion. Thus, amphetamine-induced locomotion was slightly attenuated 7 and 28 days following LDT lesions. Post hoc analysis revealed that amphetamine significantly increased locomotion levels above saline prelesion, but was slightly but significantly attenuated 7 and 28 days following LDT lesions ($p < 0.005$). Rats displayed locomotor sensitization to amphetamine at both 7 and 28 days postlesion during the first 10-min postinjection.

Amphetamine increased stereotyped behaviors significantly above saline levels prelesion ($T = 2.98$, $p < 0.003$), at 7 days postlesion ($T = 3.05$, $p < 0.002$), and 28 days postlesion ($T = 3.06$, $p < 0.002$). No significant differences were found between pre-, 7-, and 28-day amphetamine-induced stereotypy ratings. Stereotypy ratings were generally variable and rarely exceeded level 4 stereotypy (Fig. 9). It was observed that rats displayed more exploratory behaviors, such as sniffing and rearing, and more frequent orofacial stereotypies, such as repetitive licking of the self and tongue protrusions, than following scopolamine. Prelesion amphetamine increased stereotypy ratings by 69% above saline levels, by 64% at 7 days postlesion, and by 71% at 28 days postlesion.

Stereotyped behaviors were increased by amphetamine more than scopolamine, reaching level 4 in most cases. Also, stereotypy ratings increased more gradually throughout the first 20 min following amphetamine than for scopolamine, then stayed higher throughout the 60-min session.

Amphetamine-Induced Locomotion and Stereotypy in Saline Sham-Lesioned Rats

Amphetamine (3 mg/kg, IP) increased locomotion and stereotypy in sham-lesioned rats ($n = 7$) above saline by 83% prelesion, by 57% at 7 days postlesion, and by 32% at 28 days postlesion (Fig. 10). Thus, amphetamine-induced locomotion was attenuated at 7 and 28 days postlesion, similar to LDT lesioned animals, but more strongly. Animals displayed locomotor sensitization to amphetamine at 7 and 28 days postlesion that was pronounced during the first 10-min postinjection.

Statistical analysis revealed a main effect of amphetamine on locomotor activity $F(5, 385) = 38.5$, $p < 0.0001$, and a sig-

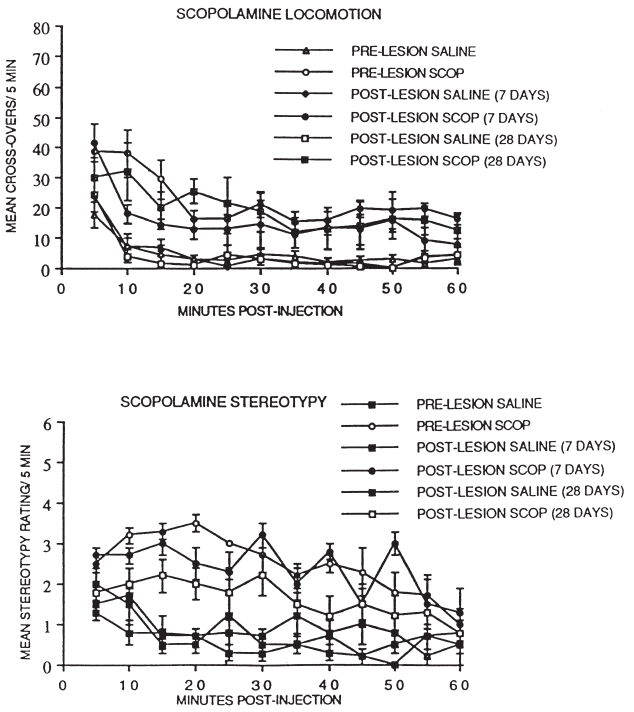

FIG. 7. Effects of citrate/saline lesions of LDT on scopolamine-induced locomotion (above) and stereotypy (below).

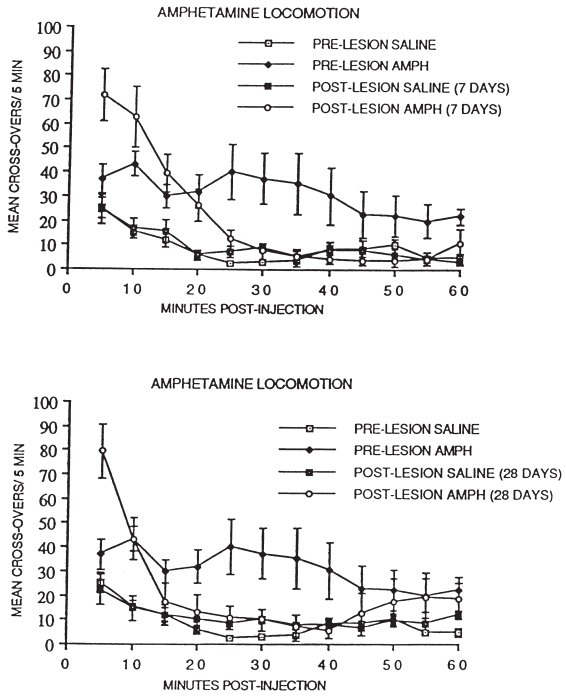

FIG. 8. Locomotion induced by amphetamine (3 mg/kg; IP) in rats with ibotenate lesions of LDT. (Above) effects of saline and amphetamine before and 7 days postlesion. (Below) effects of saline and amphetamine before and 28 days postlesion.

nificant interaction between treatment and time, $F(30, 385) = 8.3$, $p < 0.0001$. Amphetamine significantly increased locomotor activity above saline levels 7 days postlesion ($p < 0.01$), but at 28 days postlesion, amphetamine did not significantly increase locomotion above saline levels. Amphetamine-induced locomotion at 7 and 28 days postlesion was significantly lower than prelesion amphetamine levels ($p < 0.0001$).

Amphetamine increased stereotypy in sham-lesioned rats ($n = 7$) (Fig. 11) similar to that described above. Amphetamine increased stereotypy ratings above saline significantly, prelesion ($T = 2.93$, $p < 0.003$), 7 days postlesion ($T = 2.98$, $p < 0.002$), and 28 days postlesion ($T = 2.93$, $p < 0.003$). Amphetamine-induced stereotypy ratings at 7 and 28 days postlesion were significantly higher than prelesion levels ($p < 0.003$, at both times). Amphetamine increased stereotypy ratings 73% above saline levels prelesion; 79% at 7 days postlesion and 85% at 28 days postlesion. Thus, amphetamine-induced stereotypy increased steadily over repeated injections in the sham-lesioned animals.

DISCUSSION

Consistent with previous reports (25,36,37,46,50), scopolamine (1.5 mg/kg, IP) and amphetamine (3 mg/kg, IP) increased locomotion and stereotypy. A similar increase in locomotion resulted from unilateral injections of NMS (6 μg) into the LDT, suggesting that blockade of muscarinic receptors near LDT is sufficient to induce this effect. NMS did not increase locomotion if the cannula was beyond 0.5–1 mm dorsal or lateral to the LDT, however. Because NMS does not diffuse readily in brain tissue (15,54), the locomotion likely results from an action of NMS in LDT. Furthermore, destruction of Ch6 cells with the neurotoxin ibotenate, attenuated scopolamine-induced locomotion by 68% 7 days postlesion, and by 35% 28 days postlesion. Attenuation of scopolamine-induced locomotion was not observed in sham-lesioned or inferior colliculus/cuneiform nucleus lesioned rats. These results suggest an important, but not exclusive role for the LDT in the mediation of locomotion induced by systemic antimuscarinics.

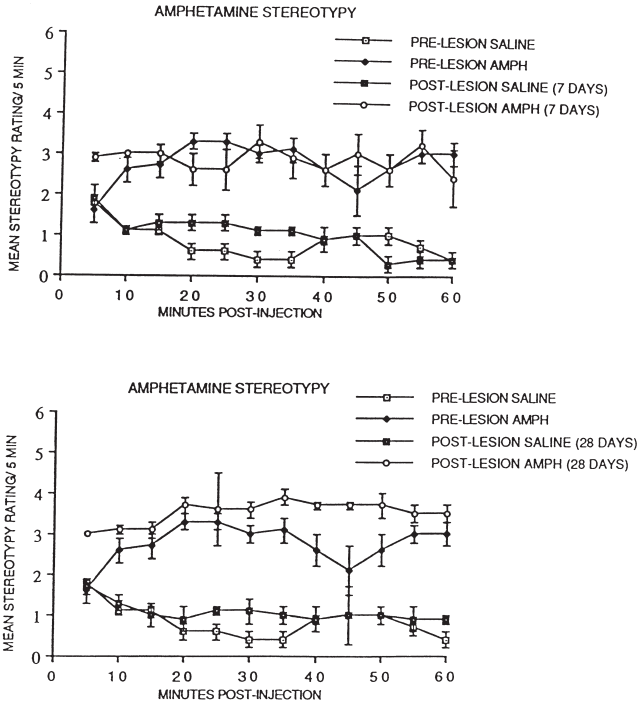

FIG. 9. Stereotypy induced by amphetamine (3 mg/kg; IP) in seven rats with ibotenate lesions of the LDT. (Above) effects of saline and amphetamine before and 7 days postlesion. (Below) effects of saline and amphetamine before and 28 days postlesion.

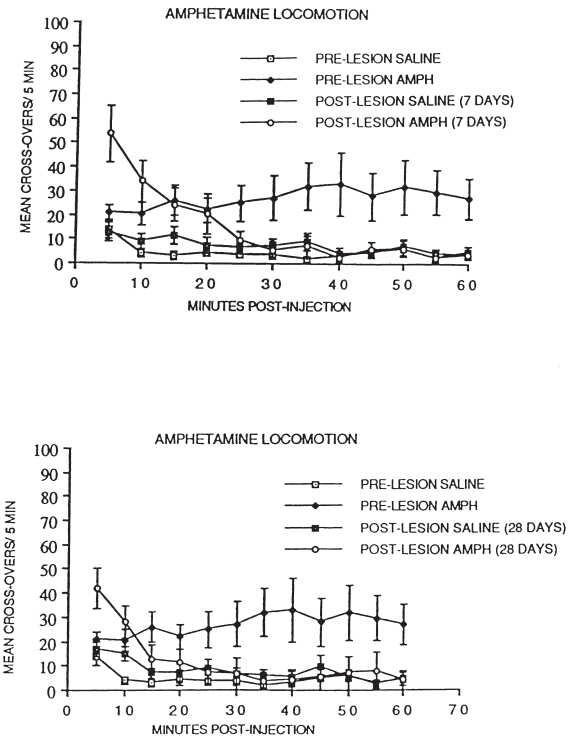

FIG. 10. Locomotion induced by amphetamine (3 mg/kg; IP) in seven rats with saline sham lesions of the LDT. (Above) effects of saline and amphetamine are shown before and 7 days postlesion. (Below) effects of saline and amphetamine are shown before and 28 days postlesion.

Muscarinic receptors of the m2 and m3 subtype are found in high concentrations in LDT and PPT (32,35,52). Muscarinic agonists, including carbachol, hyperpolarize cholinergic and noncholinergic cells of the PPT and LDT (30,33,47), and the muscarinic antagonist, atropine, depolarizes Ch6 cholinergic cells in slice (31). By blocking the inhibitory actions of these muscarinic receptors, scopolamine increases the excitability of LDT cholinergic neurons.

Evidence suggests that LDT neurons activate VTA dopamine neurons via cholinergic terminals (4). Anatomically, these cholinergic terminals make monosynaptic contacts with dendrites and soma of dopaminergic cells (7), and are the only known cholinergic inputs (58). Both LDT and PPT project to the ventral tegmental area (VTA) and substantia nigra (SN) (3,13,14,41), but the LDT projects more heavily to the VTA region and less heavily to SN (41). Injections of cholinergic agonists, such as the anticholinesterase neostigmine, into the VTA increase dopamine efflux in the nucleus accumbens (5). Ibotenate lesions of the LDT, but not PPT, blocked nucleus accumbens dopamine efflux in response to neostigmine in VTA (5).

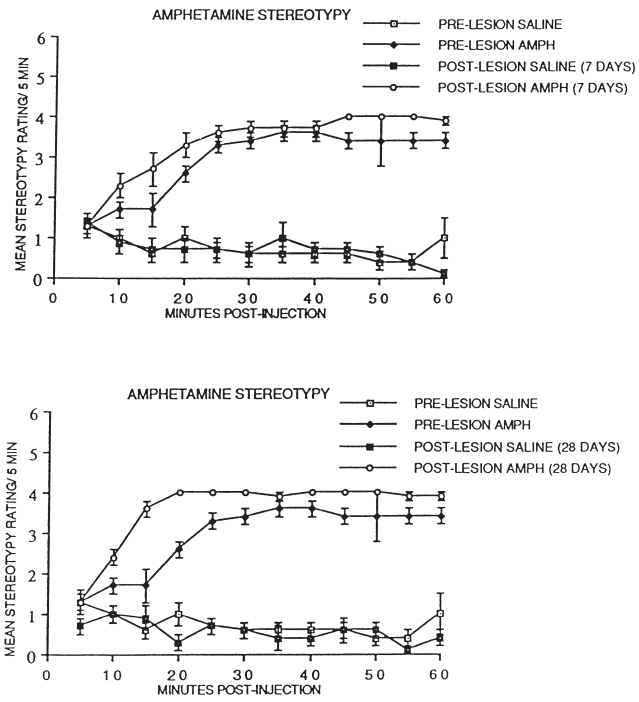

FIG. 11. Stereotypy induced by amphetamine (3 mg/kg; IP) in seven rats with saline sham lesions of the LDT. (Above) effects of saline and amphetamine are shown before and 7 days postlesion. (Below) effects of saline and amphetamine are shown before and 28 days postlesion.

It is possible that scopolamine-induced locomotion is mediated in part through mesolimbic dopaminergic pathways, as reviewed in the introduction. Injections of 6-hydroxydopamine into the nucleus accumbens, however, failed to block scopolamine-induced locomotion (25). The LDT and neighboring locomotor regions send projections directly to brain stem structures involved in the generation of rhythmic limb movements (7,16,20,22). It is, thus, possible that anticholinergic blockade of LDT muscarinic receptors excites descending locomotor pathways from the LDT in addition to ascending projections to limbic midbrain structures in the tegmentum.

Ibotenate Lesions

It has recently been reported that ibotenate lesions to the LDT are highly effective in inducing cholinergic cell loss and produce compact lesions that may destroy 80–90% of cholinergic cells in this nucleus (24). Ibotenate lesions in the present study were highly effective in ablating Ch6 cells. Furthermore, ibotenate has been shown to spare fibres of passage in lesioned areas (56), suggesting that the lesion effects in this study are not likely to result from damage to fibers of passage in the LDT region.

Anatomical control lesions of the cuneiform nucleus, inferior colliculus, or central gray did not significantly reduce scopolamine-induced locomotion or stereotypy. Therefore, the effects of LDT lesions are not due to damage from the cannula track dorsal and lateral to LDT. Furthermore, LDT lesions did not attenuate saline-induced locomotor activity. Thus, the attenuation of scopolamine-induced locomotor activity was not due to any motoric disabilities from the lesions.

When animals were tested 28 days postlesion, scopolamine-induced locomotion had recovered to 65% of prelesion levels. This suggests that, while LDT is important for mediation of scopolamine-induced locomotion, other systems can compensate for ibotenate lesions of LDT in the mediation of this effect. One such recovery mechanism may involve the recovery of choline acetyltransferase (ChAT) activity in VTA and SN postlesion. Fujimoto et al. (17) reported an 85% reduction in ChAT activity in the VTA following ibotenate lesions of the PPT, followed by a maximal recovery of ChAT activity at 28 days postlesion. This recovery time coincides with the 28-day recovery of scopolamine-induced locomotor activity following LDT lesions in the present study.

Scopolamine-induced stereotypy was significantly attenuated at both 7 and 28 days postlesion, relative to prelesion levels. This may suggest a role for Ch6 cells in the mediation of scopolamine-induced stereotypy, but this effect was not as strong as the effect on scopolamine-induced locomotion. It is possible that stereotyped behaviors were less apparent in the present study due to the relatively low dose of scopolamine used (1.5 mg/kg, IP).

Amphetamine-Induced Locomotion and Stereotypy

Amphetamine (3 mg/kg, IP) increased locomotion and stereotyped behaviors in rats similar to that seen with scopolamine. After the second and third injections of amphetamine, LDT and saline-sham lesioned rats displayed behavioral sensitization to amphetamine as manifest in more intense stereotypy (levels 4 and 5) and increased locomotion peaking in the first 10 min following the second and third injections By contrast, while sham-lesioned rats displayed significant stereotypy sensitization at both 7 and 28 days postlesion, LDT lesioned rats failed to show stereotypy sensitization 7 days postlesion and at 28 days postlesion, LDT lesioned rats did not show stereotypy sensitization as strongly as did sham-lesioned rats.

Recent reports suggest that cholinergic systems may interact with dopaminergic substrates in the mediation of behavioral sensitization to amphetamine. Ohmori et al. (42) reported that behavioral sensitization to methamphetamine was blocked by pretreatment with scopolamine. Yui et al. (60) reported that repeated administrations of methamphetamine paired with scopolamine potentiated behavioral sensitization to methamphetamine when compared to methamphetamine injections alone. Scopolamine also potentiated apomorphine-induced stereotypy in rats (9).

Several investigators have proposed that behavioral sensitization to amphetamine is initiated by receptor alterations in the VTA (10,27,51,57), and that the expression of behavioral sensitization to amphetamine is mediated by the nucleus accumbens (10,26). Thus, destruction of the LDT cholinergic input to VTA dopamine cells may, in turn, alter the development of amphetamine sensitization. Therefore, the interaction between scopolamine and dopamine agonists in sensitization may result from the LDT activation of VTA dopamine neurons.

LDT lesions altered amphetamine-induced locomotor activity only slightly in the present study. Previous, PPT lesions did not inhibit locomotion or behavioral sensitization induced by amphetamine (23,43,49).

CONCLUSIONS

The present study suggests that blockade of muscarinic receptors in the LDT is sufficient to induce locomotion. Also,

the integrity of LDT neurons is important for the expression of scopolamine-induced locomotion and stereotypy. Amphetamine-induced locomotion depends less on LDT neurons, but LDT inputs to VTA may be involved in the expression of stereotypy sensitization to amphetamine.

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