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Modulation of Dopamine Efflux in the Nucleus Accumbens after Cholinergic Stimulation of the Ventral Tegmental Area in Intact, Pedunculopontine Tegmental Nucleus-Lesioned, and Laterodorsal Tegmental Nucleus-Lesioned Rats

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Microinjections of the cholinergic receptor agonist nicotine and the cholinesterase inhibitor neostigmine were made into the ventral tegmental area (VTA) of urethane-anesthetized rats, and dopamine (DA) efflux in the nucleus accumbens was measured using *in vivo* chronoamperometry. Dose-dependent increases in the chronoamperometric signals corresponding to increased DA efflux were observed in the nucleus accumbens of normal intact rats after cholinergic stimulation of the VTA. The source of the cholinergic input to the VTA was investigated by making excitotoxic lesions in either the laterodorsal tegmental nucleus (LDTg) or the pedunculopontine tegmental nucleus (PPTg). Compared with sham-operated control animals, which showed the same response as intact, nonlesioned rats, ibotenate lesions of the LDTg attenuated the stimulatory effects of intra-VTA neostigmine on DA efflux in the nucleus accumbens. In contrast, rats with ibotenate lesions of the PPTg showed normal nucleus accumbens DA efflux after intra-VTA injections of neostigmine. Such lesions in the PPTg attenuate DA efflux in the caudate–putamen stimulated by injections of neostigmine into the substantia nigra pars compacta (SNc). The present data show that cholinergic neurons in the LDTg, but not the PPTg, regulate the activity of DA-containing neurons in the VTA, which complements previous data showing that cholinergic neurons in the PPTg regulate DA-containing neurons in the SNc.

Key words: ventral tegmental area; nucleus accumbens; laterodorsal tegmental nucleus; pedunculopontine tegmental nucleus; dopamine; acetylcholine; ibotenate; chronoamperometry; rat

Dopamine (DA)-containing neurons in the substantia nigra pars compacta (SNc) receive cholinergic innervation from the pedunculopontine tegmental nucleus (PPTg). This has been shown by tracing studies (Woolf and Butcher, 1986; Beninato and Spencer, 1987, 1988; Clarke et al., 1987; Tokuno et al., 1988; Gould et al., 1989; Lavoie and Parent, 1994) (but see also Lee et al., 1988), electron microscopic studies (Bolam et al., 1991), and functional examinations (Hernandez-Lopez et al., 1992; Blaha and Winn, 1993). This is consistent with the observation that acetylcholine (ACh), together with its synthetic enzyme choline acetyltransferase (CAT), and degradative enzyme acetylcholinesterase (AChE) are present in the substantia nigra (Jacobowitz and Goldberg, 1977; Butcher and Marchand, 1978; Lehmann and Fibiger, 1978; Greenfield et al., 1980), as are both muscarinic and nicotinic receptors, at which ACh has excitatory actions (Clarke et al., 1985; Lacey et al., 1990; Nastuk and Graybiel, 1991). Behavioral studies also support the hypothesis that cholinergic systems interact with DA neurons in the SNc (Parker et al., 1991, 1993; Winn, 1991).

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DA neurons in the ventral tegmental area (VTA) also are cholinoceptive. Nicotinic receptors are present in the VTA (Clarke and Pert, 1985), and the behavioral effects of cholinergic drugs are consistent with stimulation of these receptors (Druhan et al., 1989). Moreover, systemically administered nicotine increases DA efflux in the nucleus accumbens by acting in part in the VTA (Nisell et al., 1994). However, the source of the cholinergic input to VTA DA neurons has not been investigated in detail. It is unclear to what extent the cholinergic neurons of either the PPTg or the laterodorsal tegmental nucleus (LDTg) innervate the VTA. Ascending fibers from the PPTg appear to run through the VTA, possibly making synaptic contact (Hallanger and Wainer, 1988), but a more prominent innervation from the LDTg has been described previously (Satoh and Fibiger, 1986; Cornwall et al., 1990). In a previous study, we assessed the relationships among the PPTg, SNc, and caudate–putamen (Blaha and Winn, 1993). Injections into the SNc of cholinergic receptor agonists or the anticholinesterase neostigmine increased DA efflux in the ipsilateral caudate–putamen, measured by both *in vivo* electrochemistry and microdialysis. In rats with excitotoxic lesions of the PPTg, however, the amount of caudate–putamen DA efflux stimulated by neostigmine was attenuated, whereas the response to nicotine was enhanced. Because the PPTg lesions spared LDTg cholinergic neurons completely, yet postsynaptic receptor supersensitivity in the SNc developed, it was argued that the cholinergic innervation of the SNc must come almost exclusively from the PPTg.

In these experiments, we have adopted the same rationale to investigate the cholinergic innervation of the VTA. In our previous experiments (Blaha and Winn, 1993), we used both *in vivo* microdialysis and electrochemical techniques to cross-validate results. Having done this, we have chosen to use only *in vivo* electrochemistry in the present study, which was designed to determine the following: (1) whether cholinergic receptor agonists and cholinesterase inhibitors increase DA activity in the nucleus accumbens after microinjection into the VTA; and (2) whether PPTg or LDTg lesions remove cholinergic input to the VTA and consequently diminish the response to AChE inhibitors.

MATERIALS AND METHODS

Subjects. Male hooded Long–Evans rats weighing 350–450 gm were used in all experiments. Animals were housed in individual stainless steel cages at constant room temperature (24°C, 60% relative humidity) and maintained on a 12 hr light/12 hr dark cycle (lights on at 7:00 A.M.). Food and water were available *ad libitum*.

Electrochemical studies. Rats were anesthetized with urethane (2 gm/kg, i.p.) and placed in a stereotaxic frame. Body temperature was maintained at 37°C with a temperature-controlled water-heating pad (American Hospital Supplies, McGraw Park, IL). Stearate-modified graphite paste recording electrodes [prepared as described previously (Blaha and Lane, 1983)], which allow *in vivo* measurement of changes in DA efflux without interference from other oxidizable compounds in brain extracellular fluid (Blaha and Jung, 1991), were implanted stereotaxically into each nucleus accumbens (coordinates: +1.5 mm from the bregma, ±1.3 mm from the midline, −6.5 mm from the dura mater, with skull level) (Paxinos and Watson, 1986). An Ag/AgCl reference and a stainless steel auxiliary electrode combination were placed in contact with cortical tissue 4 mm posterior to the bregma. Repetitive chronoamperometric measurements were made using an electrometer (Echempro, Vancouver, BC, Canada), and DA efflux was monitored by applying a potential pulse from −0.15 to +0.25 V versus Ag/AgCl to the recording electrode for 1 sec at 30 sec intervals and monitoring the DA oxidation current at the end of each 1 sec pulse. Changes in DA efflux in the nucleus accumbens observed after drug administration were expressed as percent change from baseline and were derived as in Blaha and Winn (1993). Drug-induced changes in DA oxidation current were calculated as absolute current values taken from the preinjection baseline of each drug to the observed peak effect and were expressed as percent change with respect to the mean baseline current value given above (Blaha et al., 1990; Blaha and Phillips, 1992).

Drug microinjections. Drug solutions were back-loaded into a 30 gauge stainless steel cannula (90° bevel) connected via PE10 tubing to a 5.0 μl microsyringe (Scientific Glass Engineering, Ringwood, Australia) mounted in an infusion pump (Harvard Pump 22). After stable baseline recordings from both electrodes of 60–120 min, the infusion cannula was inserted in graduated steps over a 15 min period into the VTA (coordinates: +3.4 mm from the interaural line, +1.0 mm lateral to midline, −8.0 mm from the dura mater, with skull surface level) (Paxinos and Watson, 1986). After an additional 10 min baseline recording, neostigmine (0.5 or 1.0 mM; Sigma, St. Louis, MO), nicotine (0.2 or 2.0 mM; Sigma), or saline (0.9% NaCl) was microinjected into the VTA in a volume of 0.25 μl over 2 min. Microinjection progress was monitored by observing the movement of a small air bubble deliberately placed in the PE10 line; 10 min after infusion, the cannula was retracted slowly out of the brain.

Excitotoxic lesions of the LDTg and PPTg. Rats were anesthetized with 30 mg/kg sodium pentobarbitone (Somnotol, 65 mg/ml sodium pentobarbital; MTC Pharmaceuticals, Cambridge, Ontario, Canada) and placed in a stereotaxic frame. Unilateral lesions of the LDTg were made by injection of 20 nmol of ibotenic acid (Cambridge Research Biochemicals, Cheshire, UK; 0.4 μl, 0.05 M) with a 1 μl syringe (Scientific Glass Engineering) mounted on the stereotaxic frame. Ibotenate was dissolved in phosphate buffer, pH 7.4, and the final pH of the solution was adjusted with 2 M NaOH to 7.2. The infusions were made using a step-down procedure of 0.02 μl/10 sec with an additional 300 sec *in situ* to allow for toxin diffusion before retraction of the needle. Care was taken to ensure that the beveled face of the needle always pointed rostrally. The injection needle was angled 20° vertical to ensure that the cannula did not penetrate the cerebral aqueduct or fourth ventricle. The following stereotaxic coordinates were used: anteroposterior, +0.2 mm from interaural line; lateral, +3.3 mm from midline; ventral, −7.2 mm from skull surface; with skull level (Paxinos and Watson, 1986). To make lesions in the PPTg, two injections were made at the following stereotaxic coordinates: (1) anteroposterior, +0.8 mm from interaural line; lateral, +1.6 mm from midline; ventral, −7.0 mm from skull surface; and (2) anteroposterior, +1.5 mm from interaural line and ±1.7 mm from midline; ventral, −7.8 mm from skull surface; with skull level (Paxinos and Watson, 1986). The infusions were made using the step-down procedure described above. At each site in the PPTg, rats received an injection of 24 nmol (0.2 μl × 0.12 M) of ibotenic acid. Control rats were injected with phosphate buffer vehicle only (using either LDTg or PPTg lesion parameters). All rats were observed in the immediate postoperative period; excitotoxic lesions were followed by postural deviation, barrel rolling, forepaw treading, and rotation, which generally lasted <2 hr. When this activity stopped, rats were returned to the home-cage room. Determination of DA efflux in the nucleus accumbens in response to VTA cholinergic stimulation (neostigmine, 0.5 mM) was examined using standard procedures 15–19 days after the lesions had been made.

Histological analysis. For the nonlesioned rats, after completion of each acute electrochemical experiment the brain was removed and placed in 10% buffered formalin. After fixation, 50 μm sections were cut on a cryostat and stained for Nissl substance with cresyl violet. The PPTg-, LDTg-, and sham-lesioned rats were perfused under deep anesthesia with phosphate-buffered saline followed by 4% *p*-formaldehyde in 0.1 M phosphate buffer. Two parallel sets of 50 μm sections were cut 200 μm apart through the PPTg and LDTg (from the posterior substantia nigra to the posterior locus ceruleus). One set of sections was stained using cresyl violet for the determination of lesion volumes, and a second set of sections was stained for nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase using a modification of the procedure of Vincent and Kimura (1992). The locations of cannulae tracks in all rats were determined from Nissl-stained sections. Silhouettes of lesions were drawn onto representative sections of the rat brain (Swanson, 1992), and the number of NADPH diaphorase-positive neurons in the PPTg, LDTg, and subpeduncular tegmental nucleus was counted. All histological assessment was conducted by M.P.L., blind with respect to lesion condition.

Statistical analysis. Statistical analysis of data was undertaken using analysis of variance with Tukey's post hoc tests.

RESULTS

Cannulae and electrode placements

Figure 1 shows representative placements of cannulae in the VTA, where there was close clustering of injection sites, and electrochemical electrodes in the nucleus accumbens.

Effects of intra-VTA nicotine and neostigmine on DA efflux in the nucleus accumbens

As shown in Figure 2, *A* and *B*, microinjection of 0.2 and 2.0 mM nicotine into the VTA increased the chronoamperometric signals corresponding to DA efflux in the nucleus accumbens, in contrast to microinjections of saline, which were without effect. DA efflux in the nucleus accumbens reached maximal increases of 132 ± 4 and 276 ± 14% (Table 1) with respect to baseline levels (0.95 nA, 100%) within 50 and 85 min after injection, respectively. The total duration of action for these effects was 90 and 180 min for 0.2 and 2.0 mM, respectively. Microinjection of the anticholinesterase neostigmine into the VTA resulted in potent enhancement of the chronoamperometric signals recorded in the nucleus accumbens (Fig. 2*C*,*D*; Table 1). Maximal increases in accumbens DA efflux of 580 ± 36 and 1044 ± 49% were observed within 95 and 50 min after microinjection of nicotine into the VTA at doses of 0.5 and 1.0 mM, respectively. The total duration for the stimulatory effects of 0.5 mM neostigmine on nucleus accumbens DA efflux was 210 min. At the higher dose of 1.0 mM neostigmine, the response remained elevated at approximately half-maximal over the course of the experiment.

Effects of mesopontine ibotenate lesions

Figure 3 shows schematic representations of the largest and smallest PPTg and LDTg lesions, and Figure 4 shows representative

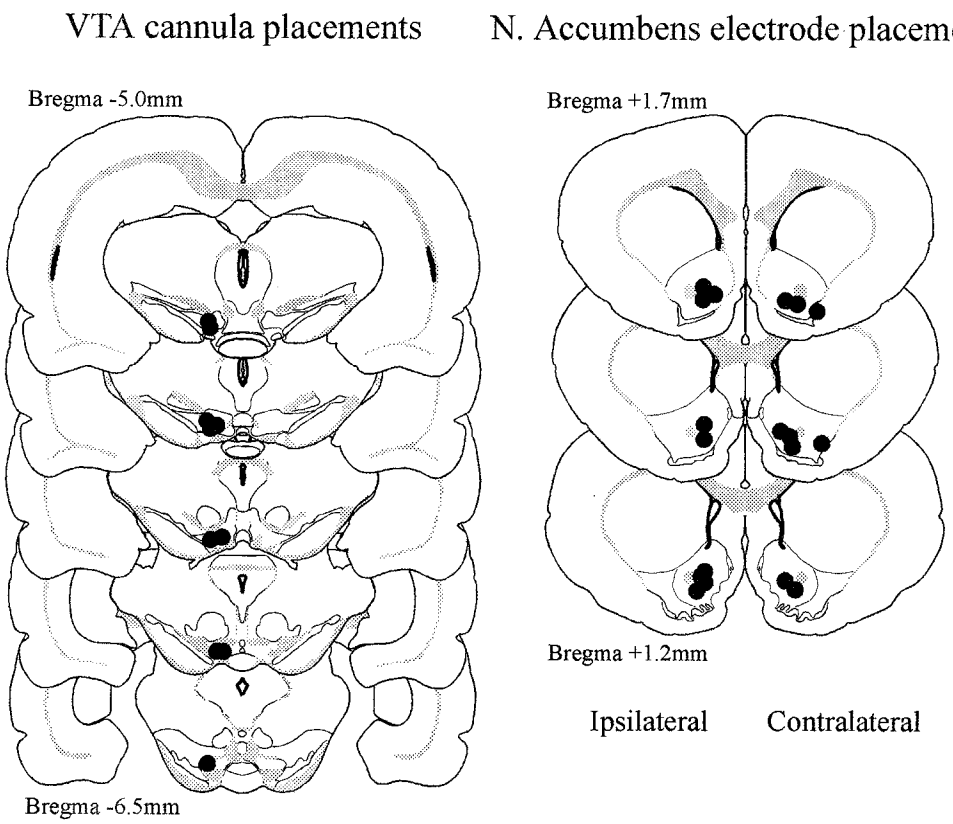

Figure 1. Left, Sections show the placement of microinjection cannulae in the VTA. Placements were tightly clustered; those shown indicate the anteroposterior, mediolateral, and dorsoventral spread of the sites. *Right*, Sections show the placement of electrochemical electrodes in the nucleus accumbens (sections from Swanson, 1992).

photomicrographs of LDTg and PPTg lesions. Together, these figures show that there was no overlap between the two lesions. PPTg lesions extended from a point just caudal to the substantia nigra and extended caudally almost to the parabrachial nuclei. There was no invasion of the central gray and no damage in the substantia nigra, although neurons were lost from other structures adjacent to the PPTg. Damage in these structures (deep mesencephalic nucleus, cuneiform nucleus, and retrorubral nucleus) was never complete and varied among rats, making it unlikely that this partial and inconsistent damage affected the results of these experiments. LDTg lesions, in contrast, tended to be contained within the central gray matter, with leakage into the tissue immediately above and below the superior cerebellar peduncle in the cuneiform and medial parabrachial nuclei in some cases. As with the PPTg, damage to these structures was only partial and was not consistent across all rats. Table 2 presents counts of diaphorase-positive neurons in the PPTg, LDTg, and subpeduncular tegmental nucleus (SPTg) in the three groups of rats, and average lesion volumes were computed from analysis of Nissl-stained sections. The diaphorase counts show clearly that ibotenate directed at the PPTg destroyed neurons there but not in the LDTg, whereas ibotenate injected into the LDTg destroyed neurons there but not in the PPTg. Loss of diaphorase-positive neurons was seen in the SPTg after some LDTg lesions but never after PPTg lesions. The lesions made in the PPTg were similar in size to those reported previously (Dunbar et al., 1992; Rugg et al., 1992; Inglis et al., 1994a,b); to the best of our knowledge, excitotoxic lesions of the LDTg have not been reported previously.

As shown in Figure 5, microinjection of 0.5 mM neostigmine into the VTA of LDTg-lesioned rats resulted in an attenuated increase in the chronoamperometric signals in the nucleus accumbens compared with the neostigmine-induced increases in accumbens DA efflux observed in sham-lesioned control rats. Peak neostigmine-induced increases in accumbens DA efflux occurred 90 min after infusion; neither sham-lesioned (591 ± 39%) nor PPTg-lesioned (523 ± 22%) rats differed significantly in their response to VTA neostigmine compared with intact controls (580 ± 36%). DA efflux in the accumbens of LDTg-lesioned rats reached maximal increases of 235 ± 11% within 95 min after injection of neostigmine (Fig. 2*C*; Table 1). The total duration of action for these effects was similar to those observed in intact and sham-lesioned animals (210 min; Fig. 5*A*,*B*).

DISCUSSION

ACh in the VTA and nucleus accumbens DA

The data presented in this paper show that cholinergic stimulation of the VTA increased the DA concentration in the extracellular space of the nucleus accumbens. Both nicotinic receptor stimulation and cholinesterase inhibition with neostigmine produced clear dose-dependent effects. These data are consistent with the observation that VTA neurons have cholinergic receptors on their somatodendritic membranes and that activation of these neurons generally is excitatory (Clarke and Pert, 1985; Greenhof et al., 1986; Lacey et al., 1990; Nisell et al., 1994). These data also are consistent with reports of cholinergic innervation of the VTA from the mesopontine tegmentum (Satoh and Fibiger, 1986; Hallanger and Wainer, 1988; Cornwall et al., 1990). A recent report concerning the cholinergic innervation of the VTA and SNc from the mesopontine tegmentum (Oakman et al., 1995) has shown that there is a bilateral innervation of the VTA principally from the LDTg and an ipsilateral innervation of the SNc originating in central and anterior portions of the PPTg. In effect, taking the

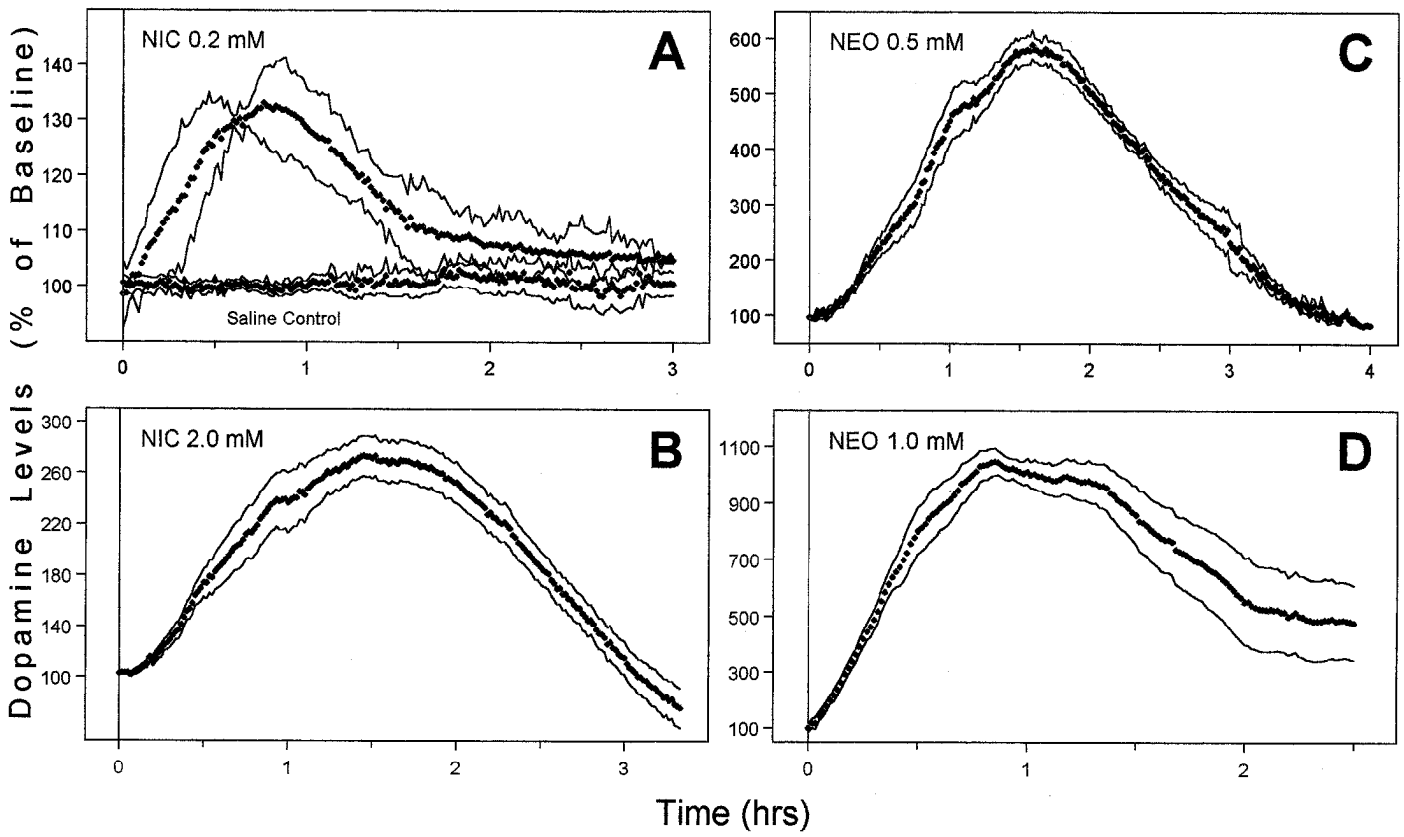

Figure 2. *A*, *B*, Chronoamperometric recordings depict the time courses of the effects of intra-VTA infusions of 0.2 and 2.0 mM nicotine (*NIC*) and saline (0.9% NaCl) on DA efflux in the nucleus accumbens. Effects of nicotine were significant ($p < 0.01$) over 90 min (0.2 mM) and 85 min (2.0 mM). *C*, *D*, Chronoamperometric recordings depict the time courses of the effects of intra-VTA infusions of 0.5 and 1.0 mM neostigmine (*NEO*) on DA efflux in the nucleus accumbens. Effects of neostigmine were significant ($p < 0.01$) over 210 min (0.5 mM) and 240 min (1.0 mM). In all cases, *solid lines* represent the SEM. Chronoamperometric responses at 1 min intervals (rather than the 30 sec intervals at which recordings were made) are presented for clarity.

mesopontine cholinergic neurons all together, there is an anterolateral–posteromedial gradient in the innervation of midbrain DA neurons; PPTg neurons tend to innervate the SNc, LDTg, and VTA. The present data add to this finding significantly by indicating functionally that lesions in the LDTg affect cholinergic activity in the VTA. These findings are in contrast to the effects of lesions in the PPTg, which affect cholinergic activity in the SNc to the extent that receptor supersensitivity develops in the SNc after

Table 1. Effects of intra-VTA microinjections of nicotine and neostigmine on DA efflux in the nucleus accumbens

	Ipsilateral nucleus accumbens						Contralateral nucleus accumbens				
Drug treatment	Dose (mM)	Maximal change in current (nA)[a]	Change from baseline (100%)[b]	Time (min)[c]	*n*	Drug treatment	Dose (mM)	Maximal change in current (nA)[a]	Change from baseline (100%)[b]	Time (min)[c]	*n*
SAL	150	−0.02 ± 0.03	98 ± 5	50	4	SAL	150	+0.05 ± 0.07	105 ± 7	50	4
SAL	150	+0.01 ± 0.06	101 ± 6	95	4	SAL	150	+0.11 ± 0.04	112 ± 4	95	4
NIC	0.2	+0.31 ± 0.07	132 ± 4*	50	5	NIC	0.2	+0.09 ± 0.05	109 ± 5	50	5
NIC	2.0	+1.67 ± 0.13	276 ± 14*	85	6	NIC	2.0	+0.48 ± 0.16	151 ± 17*	85	6
NEO	0.5	+4.56 ± 0.34	580 ± 36*	95	5	NEO	0.5	+0.78 ± 0.52	181 ± 27*	95	5
NEO	1.0	+8.97 ± 0.47	1044 ± 49*	50	5	NEO	1.0	+2.20 ± 0.76	332 ± 80*	50	5
NEO-LDTg-X	0.5	+1.28 ± 0.10	235 ± 11*,**	95	6	NEO-LDTg-X	0.5	+0.71 ± 0.12	174 ± 13*	95	6
NEO-PPTg-X	0.5	+4.01 ± 0.21	523 ± 22*	95	4	NEO-PPTg-X	0.5	+1.19 ± 0.47	225 ± 49*	95	4
NEO-Sham-X	0.5	+4.66 ± 0.37	591 ± 39*	95	3	NEO-Sham-X	0.5	+0.91 ± 0.65	196 ± 68*	95	3

[a]Data represent the mean (±SEM) maximal changes in DA oxidation current measured from predrug injection baseline values normalized to zero current. These current values were used to compute [b]mean (±SEM) percent changes in DA efflux with respect to 100% baseline DA oxidation current values (0.95 nA) (for details, see Blaha et al., 1990) at [c]postinfusion time intervals corresponding to maximal effects of each drug. NEO, neostigmine; NIC, nicotine; SAL, saline.

*Significant ($p < 0.01$) drug-induced percent changes in baseline versus SAL values at corresponding postinfusion time intervals.

**Significant ($p < 0.01$) differences between drug-induced percent changes in baseline in LDTg ibotenate-lesioned rats versus sham- and PPTg-lesioned rats.

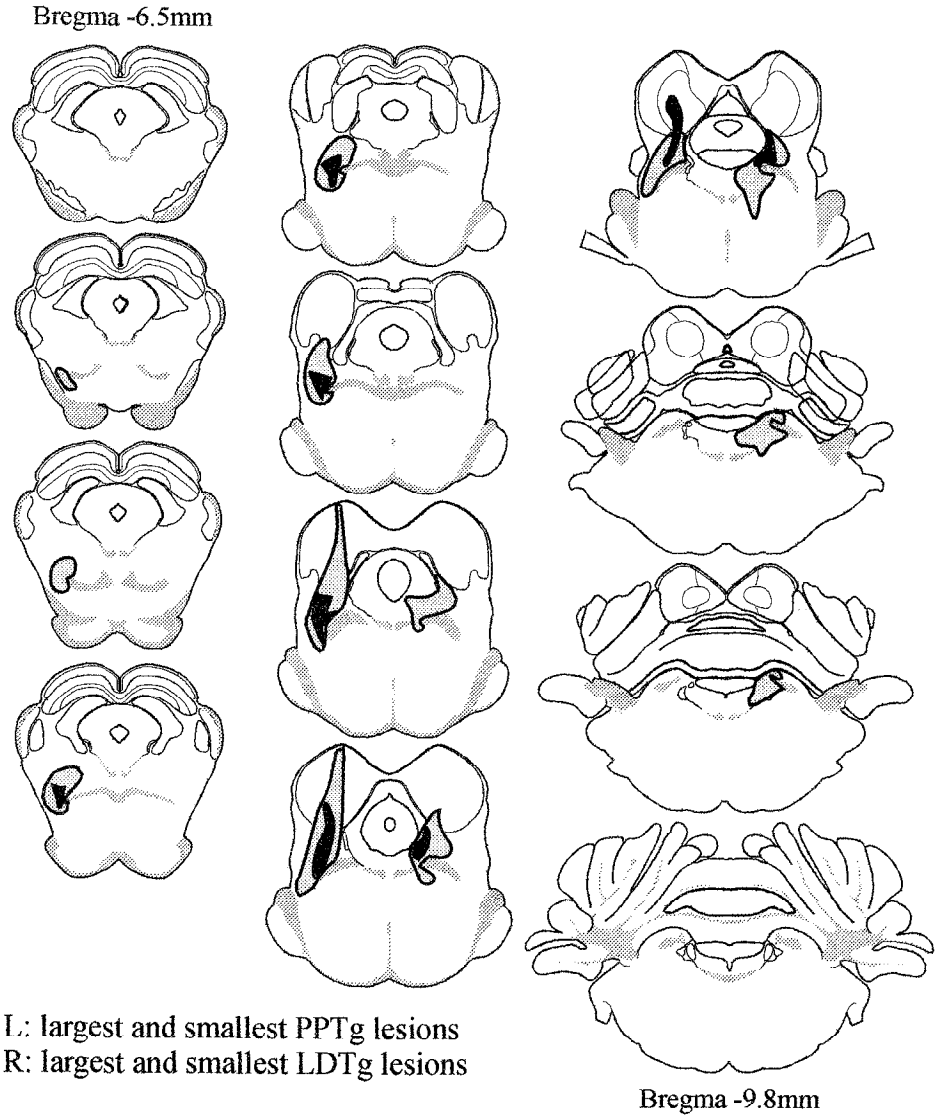

Figure 3. Silhouettes of the largest and smallest PPTg (*left*) and LTDg (*right*) lesions shown on serial sections reproduced from the atlas of Swanson (1992). For convenience, all PPTg lesions are shown in the left hemisphere. Note that the lesions of PPTg and LDTg are entirely separated, the former lying lateral and anterior to the latter.

lesions selective for the PPTg, but not LDTg (Blaha and Winn, 1993). In the present study, PPTg lesions had no effect on cholinergic activity in the VTA.

Neuroanatomical and methodological considerations

Excitotoxins have been used in several previous studies to make lesions in the PPTg (Jones and Webster, 1988; Webster and Jones, 1988; Rugg et al., 1992; Inglis et al., 1994a,b; Olmstead and Franklin, 1994) but, to the best of our knowledge, an excitotoxic lesion aimed exclusively at the LDTg has not been reported previously. The anatomical data presented here show clearly that entirely separate ibotenate lesions can be made in the LDTg and PPTg. In no case did we observe damage in one site after lesion of the other. The SPTg, a structure incorporated into the PPTg by some authors but classified as separate by others, was not affected by PPTg lesions, but spillage of toxin from the periaqueductal gray after LDTg lesions produced some loss of SPTg neurons. Further refinement of the LDTg lesion parameters should reduce this effect. Neither PPTg nor LDTg lesions were complete. In the case of the PPTg, loss of neurons was maximal in the compact portion of the nucleus, around the superior cerebellar peduncle, with the damage becoming less marked as the nucleus descends toward the substantia nigra. In the LDTg, there generally was complete damage in the central portion of the nucleus and less complete damage at the anterior and posterior poles. It is clear, however, that the lesions were entirely separate, and it is the separation of the two lesions that is the most important consideration. It is this differentiation that allows clear conclusions to be drawn concerning the separate innervations made by these two structures.

NADPH diaphorase is a nitric oxide synthase (Hope et al., 1991) found in many central nervous system neurons. In the mesopontine tegmentum, it is localized almost exclusively in cholinergic neurons in the PPTg, SPTg, and LDTg; in several studies, therefore, it has been used to label cholinergic neurons. We have shown a strong correlation between counts of neurons stained for NADPH diaphorase and those processed immunohistochemically to show CAT-positive neurons (Inglis et al., 1994a). The neuronal counts shown in Table 2 are raw, taken from 50 μm sections cut every 200 μm through the mesopontine tegmentum, but estimates of the total number of cholinergic neurons present made from the control data yield figures very close to those reported previously—~1600 neurons in both the PPTg and the LDTg (Rye et al., 1987; Rugg et al., 1992).

Unilateral cholinergic stimulation of the VTA produced clear and significant effects on DA efflux not only in the ipsilateral

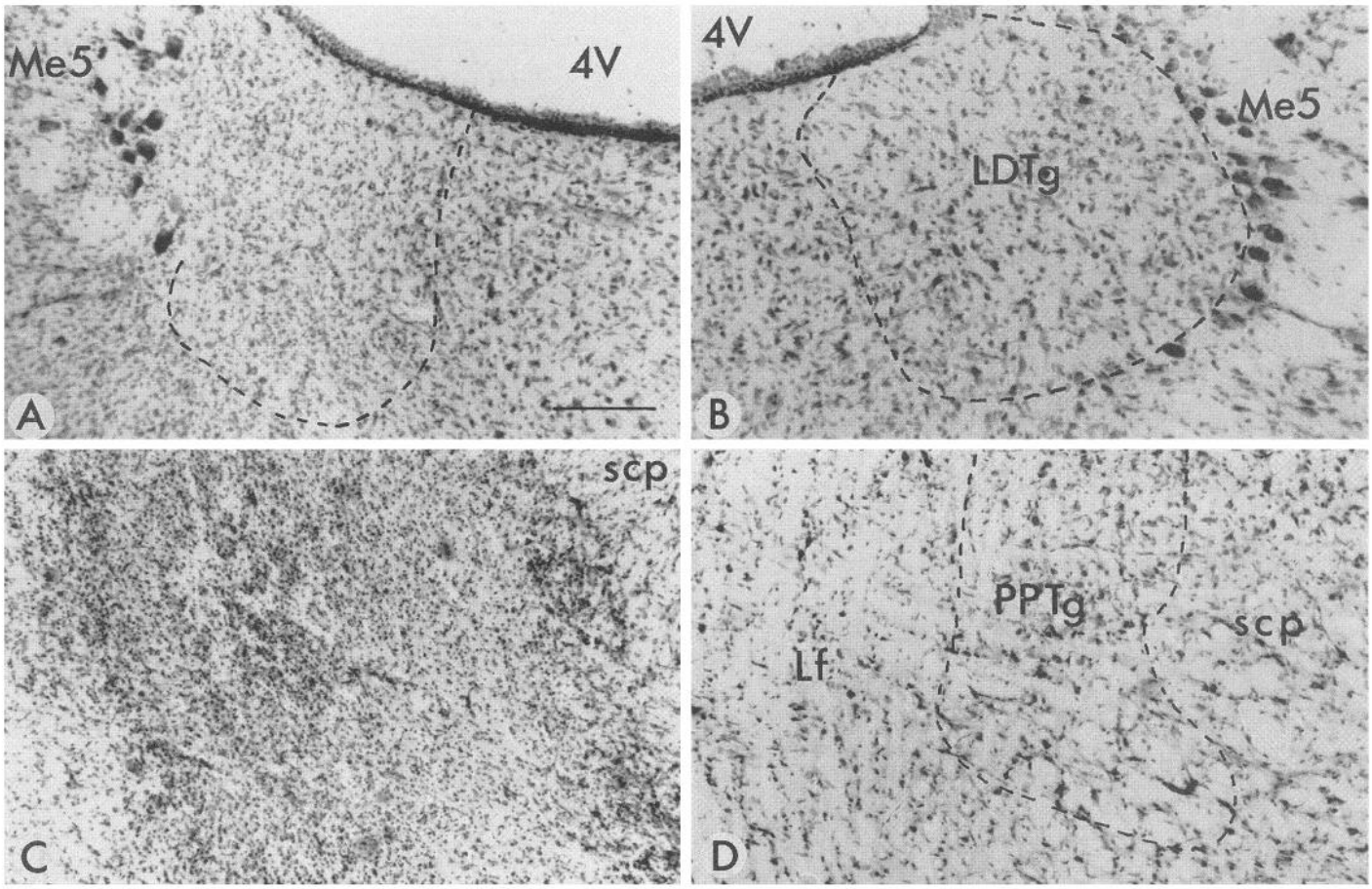

Figure 4. Representative photomicrographs showing Nissl-stained sections of LDTg- and PPTg-lesioned tissue and contralateral nonlesioned tissue. *A*, LDTg-lesioned tissue (area enclosed by the *line*). Note the survival of Me5 neurons around the edge of the central gray, the presence of reactive gliosis in the LDTg, and the absence of large cholinergic neurons (compare with *B*). Scale bar, 15 μm (standard for all photomicrographs). *B*, LDTg control tissue. The area of the LDTg is approximated by the *line*. Note the presence of many large neurons in this area. NADPH diaphorase staining shows these to be nitric oxide synthase-positive and, therefore, presumably cholinergic. *C*, PPTg-lesioned tissue dominated by dense, reactive gliosis throughout the area. *D*, non-PPTg-lesioned tissue shows the position of the PPTg in relation to the superior cerebellar peduncle and lemniscal fibers. *4V*, fourth ventricle; *LDTg*, laterodorsal tegmental nucleus; *Lf*, lemniscal fibers; *Me5*, mesencephalic trigeminal nucleus; *PPTg*, pedunculopontine tegmental nucleus; *scp*, superior cerebellar peduncle.

nucleus accumbens, but also in the contralateral nucleus accumbens. Several studies have suggested the existence of a crossed VTA–accumbens pathway (Swanson, 1982; Björklund and Lindvall, 1986). The stimulation seen on the contralateral side in our experiments could represent activation of a crossed connection or could be accounted for by spread of drug to the contralateral VTA. It is worth considering that crossed VTA-to-nucleus accumbens fibers would have been deafferented by lesions and, consequently, PPTg and LDTg lesions should have had the same effects on the ipsilateral and contralateral accumbens. The failure to find any effects of PPTg or LDTg lesions on the contralateral response indicates that this finding most likely was attributable to diffusion of drug from the injected VTA to the contralateral side. The doses used appear to be quite large, but several points need to be

Table 2. Mean (±SEM) NADPH diaphorase-positive cell counts and lesion volumes for PPTg-, LDTg-, and sham-lesioned rats

	Lesion site			
Brain site	Control	LDTg	PPTg	Lesion volume (mm^3)
LDTg	413.1 ± 19.0	196.4 ± 47.0	440.5 ± 53.9	1.55 ± 0.43
	(100 ± 5%)	(48 ± 11%)	(107 ± 13%)	
PPTg	383.0 ± 26.1	383.8 ± 40.8	211.3 ± 42.3	4.81 ± 1.01
	(100 ± 7%)	(100 ± 11%)	(55 ± 11%)	
SPTg	153.3 ± 10.9	107.0 ± 12.8	179.0 ± 36.4	ND
	(100 ± 7%)	(70 ± 8%)	(117 ± 24%)	

These are the mean numbers of neurons counted on 50 μm sections taken through the mesopontine tegmentum at 200 μm intervals. Estimates of the total populations of cholinergic neurons derived from these counts (~1600 in PPTg and LDTg) are similar to those reported previously (Rye et al., 1987; Rugg et al., 1992). ND, not determined. Figures in parentheses are percentages of appropriate control values (100%). *Significant ($p < 0.01$) differences compared with appropriate control values.

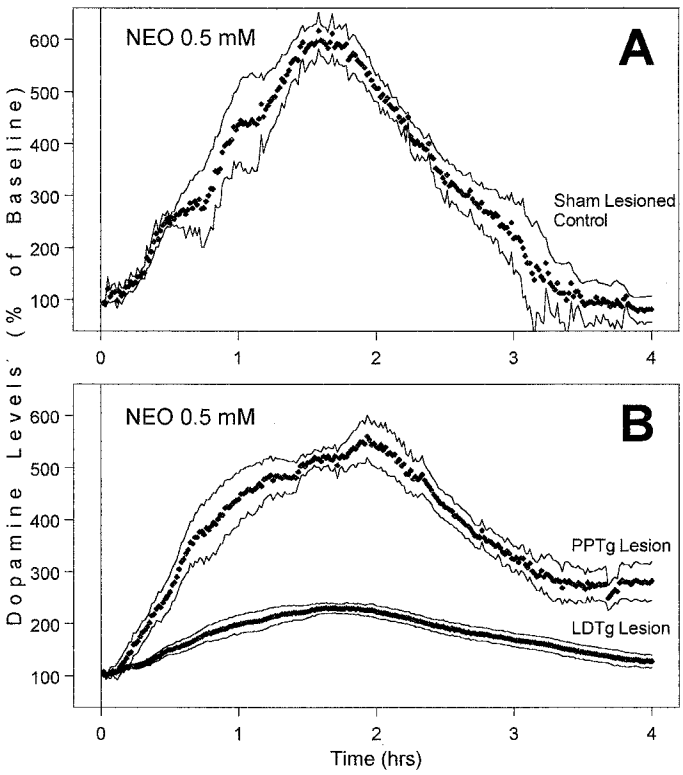

Figure 5. *A*, Chronoamperometric recordings show the time courses of the stimulatory effects of intra-VTA injections of 0.5 mM neostigmine (*NEO*) in sham-lesioned rats. *B*, Chronoamperometric recordings show the time courses of the stimulatory effects of intra-VTA injections of 0.5 mM neostigmine (*NEO*) in PPTg- and LDTg-lesioned rats. Effects of neostigmine differed significantly ($p < 0.01$); sham-lesioned and PPTg-lesioned rats did not differ from each other (or from intact rats treated with 0.5 mM neostigmine), but LDTg-lesioned rats differed from all others. *Points* represent the mean changes in the chronoamperometric responses, and *solid lines* represent the SEM. Chronoamperometric responses at 1 min intervals (rather than the 30 sec intervals at which recordings were made) are presented for clarity.

considered. First, the doses are compatible with those used in the SNc (Blaha and Winn, 1993). Second, a large portion of microinjected fluid is lost rapidly from the brain (Myers and Hoch, 1978). Third, behavioral studies using these doses have produced predictable increases in normal behavior (Winn, 1991; Parker et al., 1993), consistent with the DA efflux observed in anesthetized rats. This suggests that similar electrochemical results could be obtained in both conditions.

One point of contrast between the previous study, in which DA efflux in the caudate–putamen was measured after cholinergic stimulation of the SNc, and the present study concerns the difference in response to nicotine and neostigmine. Slightly different doses of nicotine were used in the two studies, but the effects on striatal DA efflux essentially were similar. In contrast, the effects of neostigmine in the VTA were much greater than those in the SNc. The maximal percentage effect of the lowest dose of neostigmine (125 pmol) injected into the SNc was 144%; for the highest dose (250 pmol), this effect was 227%. In contrast, the VTA maximal percentage effects were 580 and 1044% (125 and 250 pmol, respectively). It is not clear why neostigmine had a fourfold greater effect on accumbens DA efflux compared with the caudate–putamen. The density of the cholinergic innervation of the VTA compared with the SNc, the volume or concentration of ACh present, and the efficiency of AChE in the VTA all are possible causes for these differences. An explanation in terms of receptor differences is less likely given the similar effects of nicotine after microinjection into either the VTA or the SNc.

Mesopontine cholinergic innervation of midbrain DA neurons

The importance of this work has been emphasized by recent suggestions that, in some cases at least, there are increases in the numbers of mesopontine cholinergic neurons in the brains of schizophrenics (Garcia-Rill et al., 1995) (but see also Zweig et al., 1994) and, as has been indicated by others (Yeomans, 1995), such changes have implications for the regulation of midbrain DA neurons. Mesopontine cholinergic neurons generally are considered to be part of the ascending reticular activating system (Mesulam et al., 1989; Harrison et al., 1990), and many studies show that they have a role in the maintenance of sleep and arousal (Semba et al., 1990; Steriade et al., 1990; Harrison et al., 1990). In particular, they regulate the state of the thalamus, aiding the transition from burst-firing to single-spiking modes of operation (Steriade and Llinas, 1988; Steriade et al., 1990; Kamondi et al., 1992; Williams et al., 1994), and are thought to be crucial in the control of behavioral state. In addition to controlling thalamic operations, the previous and present data suggest that mesopontine cholinergic neurons also regulate midbrain DA neurons, an operation that is consistent with behavioral state control, given that midbrain DA neuronal activity has been associated with behavioral activation and response to novel stimuli (Romo and Schultz, 1990; Schultz and Romo, 1990; Ljungberg et al., 1992). Although a cholinergic presence in both the VTA and the SNc has been recognized for many years, the functional data we have presented argue strongly that it is the LDTg cholinergic neurons that impact on the VTA, whereas PPTg cholinergic neurons affect the SNc, and that this separation is almost complete. This is paralleled by the cholinergic innervation of the thalamus. Although not as sharply defined, it is clear that PPTg cholinergic neurons innervate sensory and motor nuclei of the thalamus (such as the geniculate nuclei, several of the lateral nuclei, and the ventrobasal complex) strongly, whereas LDTg neurons provide the bulk of the innervation of limbic nuclei such as the mediodorsal nucleus (Hallanger et al., 1987). We speculate, therefore, that although the ascending cholinergic neurons of the PPTg and LDTg innervate similar sites (thalamic nuclei and midbrain DA neuron), there is an anatomical separation such that PPTg neurons are associated with "sensorimotor" systems, whereas the LDTg neurons are associated with "limbic" systems. In further experiments, we will determine what functional differentiation exists between these cholinergic systems and the extent to which the innervations of thalamus and midbrain are collateralized.

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