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Modulation of Dopamine Efflux in the Striatum following Cholinergic Stimulation of the Substantia Nigra in Intact and Pedunculopontine Tegmental Nucleus–Lesioned Rats

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The effects of microinjections of cholinergic agents into the substantia nigra pars compacta on dopamine (DA) efflux in the anterior dorsomedial striatum of urethane-anesthetized rats were investigated using *in vivo* chronoamperometry and intracerebral microdialysis techniques. A dose-dependent augmentation of DA efflux as evidenced by increases in the chronoamperometric signals was observed in the striatum following nigral microinjections of the cholinergic agonists nicotine or carbachol. Enhancing extracellular concentrations of ACh in the substantia nigra by intranigral infusions of the cholinesterase inhibitor neostigmine also resulted in an increase in the chronoamperometric signal corresponding to DA overflow in the striatum. These stimulatory effects of neostigmine on DA efflux in the striatum were confirmed using *in vivo* microdialysis. Compared to sham-operated control animals, quinolinic acid lesions of the pedunculopontine tegmental nucleus (PPTg) resulted in an attenuation of the stimulatory effects of intranigral neostigmine on DA efflux in the striatum. In contrast, these treatments resulted in an enhancement of striatal DA efflux in response to nigral infusions of the direct ACh receptor agonist nicotine. Combined, these data suggest that PPTg cholinergic neurons are indirectly involved in regulating the activity of the striatum by modulating the activity of DA neurons in the substantia nigra of the rat.

[Keywords: substantia nigra, striatum, pedunculopontine tegmental nucleus, dopamine, ACh, quinolinate, chronoamperometry, dialysis, rat]

ACh, its synthetic enzyme CAT, and degradative enzyme AChE are all present in the substantia nigra (Jacobowitz and Goldberg, 1977; Lehmann and Fibiger, 1978; Beninato and Spencer, 1987; Beninato and Spencer, 1988), as are muscarinic (Nastuk and Graybiel, 1991) and nicotinic (Clarke et al., 1985) cholinergic receptors. Dopamine (DA)-containing neurons in the pars compacta appear to be the most likely targets for cholinergic activity within substantia nigra. These neurons contain (Butcher and Marchand, 1978; Lehmann and Fibiger, 1978) and release (Greenfield et al., 1980) AChE, and electrophysiological studies show that cholinergic stimulation predominantly excites them through both muscarinic and nicotinic receptors (Kemp et al., 1977; Lichtensteiger et al., 1982; Clarke and Pert, 1985; Clarke et al., 1985; Lacey et al., 1990). Behavioral studies also support the hypothesis that there is an interaction between cholinergic systems and DA-containing neurons in the substantia nigra. Microinjection of carbachol, physostigmine sulfate, or ACh/physostigmine mixtures into the anterior substantia nigra has been shown to affect motivated behavior in a manner consistent with the stimulation of DA-containing neurons (Winn and Redgrave, 1979, 1981; Winn et al., 1983; Winn, 1991). Feeding in response to cholinergic stimulation has been shown to be blocked by intranigral atropine (Winn et al., 1983) and by low, nonsedative systemic doses of the DA receptor antagonist haloperidol (Taha and Redgrave, 1984), while unilateral 6-hydroxydopamine lesions that deplete the dorsal striatum of ~50% of its DA content, but spare nucleus accumbens DA, abolish completely the response to unilateral microinjections of carbachol into the ipsilateral substantia nigra (Parker et al., 1991).

Interest in the interaction between ACh and DA within the substantia nigra has increased following demonstrations using CAT immunohistochemistry of a cholinergic projection to nigra from the pedunculopontine tegmental nucleus (PPTg) and possibly also the laterodorsal tegmental nucleus (LDTN) (Woolf and Butcher, 1986; Beninato and Spencer, 1987, 1988; Clarke et al., 1987; Lee et al., 1988; Scarnati et al., 1988; Gould et al., 1989). There is uncertainty, however, about the exact nature of this connection. Cholinergic synapses are invariably found in the pars compacta, and some authors suggest that the PPTg provides an important cholinergic innervation of the substantia nigra (Woolf and Butcher, 1986; Gould et al., 1989) whereas others suggest that cholinergic innervation of this structure is nonexistent (Lee et al., 1988). However, given that there is no other known cholinergic projection to substantia nigra, this last suggestion appears incompatible with the high density of muscarinic and nicotinic receptors in the pars compacta. It has also been demonstrated recently in the ferret that cholinergic fibers in the substantia nigra wrap around the dendrites of pars compacta DA-containing neurons and make multiple synaptic contacts (Bolam et al., 1991). Though it is not clear whether these cholinergic fibers derive from PPTg or LDTN neurons, or indeed both, this gives clear evidence of an interaction between cholinergic and dopaminergic systems in the substantia nigra.

Though these data taken all together suggest that there is a cholinergic projection to the substantia nigra from a site caudal

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to it, and that this projection makes synaptic contact with pars compacta DA-containing neurons, no direct evidence shows that increasing cholinergic transmission in the substantia nigra alters DA activity in the ipsilateral striatum. There are now means available to show this. *In vivo* microdialysis (Ungerstedt, 1984) and *in vivo* electrochemical techniques (Adams and Marsden, 1982) can both be used to assess neurochemical activity. There is at present some debate concerning the relative merits of these two techniques, it having been suggested that the extraction properties of microdialysis might adversely affect the baseline extracellular levels of various molecules (Benveniste et al., 1989; Blaha, 1992). *In vivo* electrochemical techniques in contrast have much less effect on CNS tissue and, if appropriately modified electrodes are used, can be made selective for particular molecular species (Blaha and Lane, 1983; Blaha and Jung, 1991). In the present experiments we have used both of these techniques, though the emphasis has been placed on electrochemical techniques, dialysis being used to validate the measurements made.

The present experiments were designed to test two hypotheses: (1) that cholinergic receptor agonists (either nicotinic or muscarinic) and cholinesterase inhibitors (which increase the level of endogenous ACh) will increase striatal DA activity by exciting pars compacta DA-containing neurons, and (2) that lesions in the PPTg will remove the cholinergic input to the substantia nigra and therefore diminish the response to AChE inhibitors (whose action depends upon the inhibition of hydrolysis of ACh released from intact cholinergic terminals) but enhance the response to direct ACh receptor agonists because of the development of postsynaptic receptor supersensitivity. We have tested these hypotheses by measuring the interstitial concentration of DA in the dorsomedial striatum using chronoamperometry before and after microinjection of cholinergic agents into the ipsilateral substantia nigra, in intact and in PPTg-lesioned rats.

Materials and Methods

Subjects. Male hooded rats (Long–Evans) weighing 250–350 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 12:12 hr light/dark cycle (lights on at 07:00). Food and water were available ad libitum.

Electrochemical studies. Rats were anesthetized with urethane (1 gm/kg, i.p.) and given a single supplemental injection (0.5 gm/kg, i.p.) to maintain a constant level of anesthesia over the course of each experiment. 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 were prepared as previously described (Blaha and Lane, 1983). These electrodes permit *in vivo* measurement of changes in DA efflux without interference from other oxidizable compounds in brain extracellular fluid (Blaha and Jung, 1991). Recording electrodes were implanted stereotaxically into the anterior dorsomedial striatum (coordinates +2.8 mm from bregma, +3.0 mm lateral to midline, and −4.0 mm from dura, with the incisor bar 5 mm above the interaural line; Pellegrino et al., 1979). An Ag/AgCl reference and stainless steel auxiliary electrode combination was placed in contact with contralateral cortical tissue. Repetitive chronoamperometric measurements were conducted with an electrometer (Echempro, Vancouver, Canada). Efflux of DA was monitored by applying a potential pulse for 1 sec from −0.15 V to +0.25 V versus Ag/AgCl to the recording electrode at 30 sec intervals and monitoring the DA oxidation current at the end of each 1 sec pulse. Changes in DA efflux in the dorsomedial striatum observed following drug administration were expressed as a percentage change from baseline and were derived as follows. The baseline current recorded by chronoamperometry includes both Faradaic and residual (background) current. As previously described (Blaha et al., 1990; Blaha and Phillips, 1992), the Faradaic component corresponding to DA oxidation in the dorsomedial striatum was determined by measurement of the maximal decrease in DA oxidation currents from baseline levels following administration of γ-hydroxybutyric acid lactone (GBL) (750 mg/kg, i.p.). GBL administration decreased DA oxidation values in the striatum by −0.83 ± 0.04 nA (100 ± 5%). This mean current value (100% baseline control) thus provided a reference from which to compute percentage increases in DA oxidation current following drug administration. Other techniques used for estimating baseline DA oxidation current values yield similar values [tetrodotoxin, −0.78 ± 0.02 nA (Blaha and Phillips, 1992); side-by-side microdialysis and chronoamperometry, −0.83 ± 0.05 nA (Blaha, 1992)]. 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 expressed as a percentage change with respect to the mean baseline current value given above.

Microdialysis studies. Rats were anesthetized with urethane and mounted in a stereotaxic frame, and body temperature was maintained as described above. A single dialysis probe was implanted the dorsomedial striatum (coordinates +2.8 mm from bregma, +2.8 mm lateral to midline, and −7.0 mm from dura, with the incisor bar 5 mm above the interaural line; Pellegrino et al., 1979). The dialysis probe was of the concentric design consisting of a semipermeable membrane (exposed membrane length, 4 mm; o.d., 340 μm wet; 64,000 MW cutoff; Filtral 12, Hospal-gambro Inc.), PE50 inlet tubing, and fused silica/PE10 outlet tubing (Pfaus et al., 1990). The probe was perfused continuously with Ringer's solution (1.5 mM Na-phosphate buffer containing 147 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, and 1.3 mM $CaCl_2$; pH 7.3) at 5 μl/min using a microinfusion pump (Harvard Pump 22). On-line HPLC analysis of DA in dialysate samples was performed using the method of Damsma et al. (1990). Briefly, dialysate samples from the probe were automatically injected onto an HPLC column (150 × 4.8 mm, Nucleosil 5-C18) at 10 min intervals via an automatic sample valve (Valco Instrument Co. Inc., model EQ36) controlled by an adjustable timer (Valco). Measurement of DA in each 50 μl dialysate sample was quantified by HPLC with electrochemical detection (HPLC-EC). The mobile phase was delivered by a dual-piston pump (Bio-Rad) at 1.5 ml/min and consisted of 0.1 M acetic acid (adjusted to pH 4.1 with Na-acetate), 0.01 mM Na_2EDTA, 0.5 mM sodium octyl sulfate, and 150 ml methanol/liter. Detection of striatal DA was achieved by sequential oxidation and reduction of dialysate samples via a coulometric/amperometric analytical cell (coulometric electrode, +0.35 V; amperometric electrode, −0.25 V; ESA, model 5011). Chromatograms were displayed on a dual pen strip-chart recorder (Kipp). The detection limit of DA on the ESA system was 6 fmol/injection. Basal and stimulated values for DA are presented in terms of percentage change with respect to the average of the last three stable samples (100% baseline, uncorrected for probe recovery) prior to treatment.

Drug microinjections. Drug solutions were backloaded into a 30 gauge stainless steel cannula (90° bevel) connected via PE10 tubing to a 5 μl microsyringe (SGE) mounted in an infusion pump (Sage Instruments). Following stable baseline recordings of at least 60–120 min in both chronoamperometric or dialysis experiments, the infusion cannula was inserted in graduated steps over a 15 min period into the substantia nigra pars compacta (coordinates −2.8 mm from bregma, +2.0 mm lateral to midline, and −8.0 mm from dura, with the incisor bar 5 mm above the interaural line; Pellegrino et al., 1979) ipsilateral to the *in vivo* recording electrode or dialysis probe. Following a further 10 min baseline recording, either neostigmine (0.25 or 0.5 mM), nicotine (0.5 or 5.0 mM), or carbachol (0.5 or 5.0 mM) was injected into the substantia nigra in a volume of 0.5 μl over 2 min. Infusion of 0.5 μl saline (0.9% NaCl) served as the vehicle control. The progress of each microinjection was monitored by noting the movement of a small air bubble placed in the PE10 line. Ten minutes after the infusion, the cannula was slowly retracted from the tissue.

Excitotoxic lesions of the PPTg. Unilateral lesions of the PPTg were made in six rats. Rats were anesthetized with sodium pentobarbital (Somnotol; 30 mg/kg) and placed in a stereotaxic frame. Injections of quinolinic acid were made with a 1 μl syringe (SGE) mounted on the stereotaxic frame. Care was taken to ensure that the beveled face of the needle always pointed rostrally. Two injections were made into the PPTg at the following stereotaxic coordinates: (1) anterior-posterior +0.8 mm from interaural line, lateral +1.6 mm from midline, and ventral −7.0 mm from skull surface; and (2) anterior-posterior +1.5 mm from interaural line, ±1.7 mm from midline, and ventral −7.5 mm from skull surface, with the skull level (Paxinos and Watson, 1986). The infusions

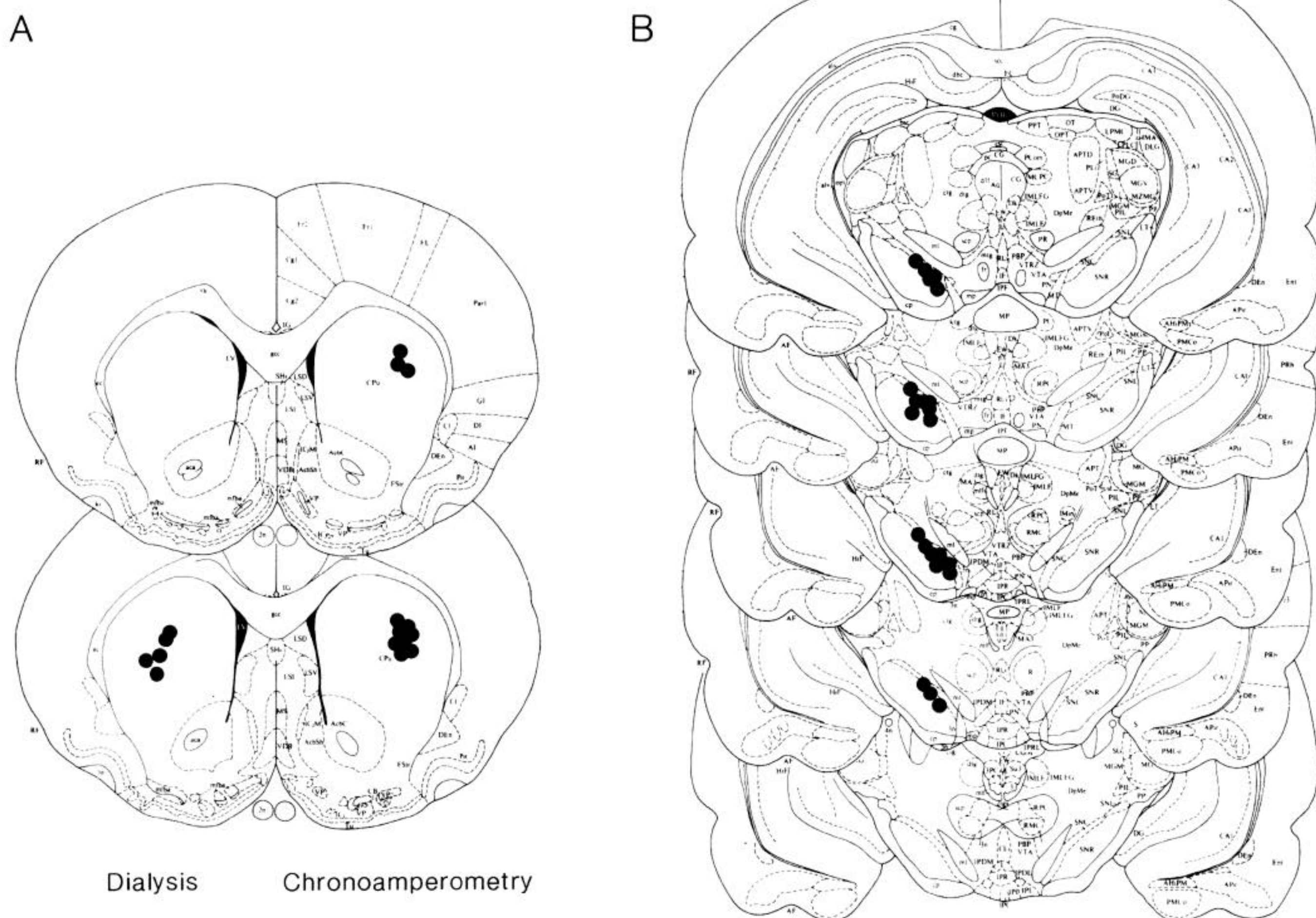


Figure 1. *A*, Representative sections showing the placement of electrochemical electrodes and microdialysis probes in the striatum; *B*, representative sections showing the placement of microinjection cannulas in the substantia nigra. Sections were redrawn from the atlas of Paxinos and Watson (1986).

were made using a step-down procedure of 0.02 μl every 10 sec with an additional 300 sec *in situ* to allow for toxin diffusion prior to retraction of the needle. At each site in the PPTg, rats received an injection of 24 nmol (0.2 μl × 0.12 M) of quinolinic acid; this dose was chosen as a result of previous experiments (Rugg et al., 1992; Dunbar et al., 1992). Quinolinate was dissolved in phosphate buffer (pH 7.4) and the final pH of the solution adjusted with 2 M NaOH to 7.2. Control rats were injected with phosphate buffer vehicle only ($n = 3$). Rats were observed in the immediate postoperative period, and excitotoxic lesions were followed by postural deviation, barrel rolling, forepaw treading, and rotation. These generally lasted for <2 hr, and when convulsive activity had stopped rats were returned to the home cage room. Determination of DA efflux in the dorsomedial striatum in response to nigral cholinergic stimulation was examined in these rats, using the normal procedures, 14–33 d after the lesions had been made. Three PPTg-lesioned rats were tested with intranigral nicotine (0.05 mM) and three with intranigral neostigmine (0.25 mM); three sham-lesioned rats were also tested with intranigral neostigmine (0.25 mM).

Chemicals. Dopamine hydrochloride, carbamylcholine chloride (carbachol), nicotine hydrogen (+)-tartrate, and neostigmine methylsulfate were obtained from the Sigma Chemical Co. Quinolinic acid was obtained from Research Biochemicals, Inc. All other chemicals of reagent or analytical grade quality were purchased from various commercial sources.

Histological analysis. After completion of each acute experiment, the rats brain was removed and placed in 10% buffered formalin. After fixation, 40 μm sections were cut on a bench microtome and stained for Nissl substance with cresyl violet. The placements of electrochemical recording electrodes, microdialysis probes, and nigral cannulas were determined using a Leitz Diaplan microscope. The positions of quinolinate lesions in the PPTg were also determined from 40 μm sections stained with cresyl violet. Silhouettes of lesions were drawn (by a colleague blind with respect to lesion condition) onto representative sections of the rat brain (Paxinos and Watson, 1986) with the aid of a drawing tube fitted to the microscope.

Statistical analysis. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) for repeated measures of time with drug effects. When the ANOVA indicated a significant effect, a Tukey post hoc test was used to compare drug-induced changes in striatal DA efflux with respect to saline-treated controls or quinolinate-lesioned groups at postinfusion time points indicated below. A value of $P < 0.01$ was selected as indicating statistical significance. Variability was expressed as the mean (±SEM).

Results

Cannula and electrode placements

Figure 1 shows the placements of nigral cannulas (Fig. 1*B*) and the location of the tips of the electrochemical electrodes and microdialysis probes (Fig. 1*A*). It is clear that all of these nigral and striatal placements were in similar situations, and that differences between placements cannot account for any differences present in the data presented below.

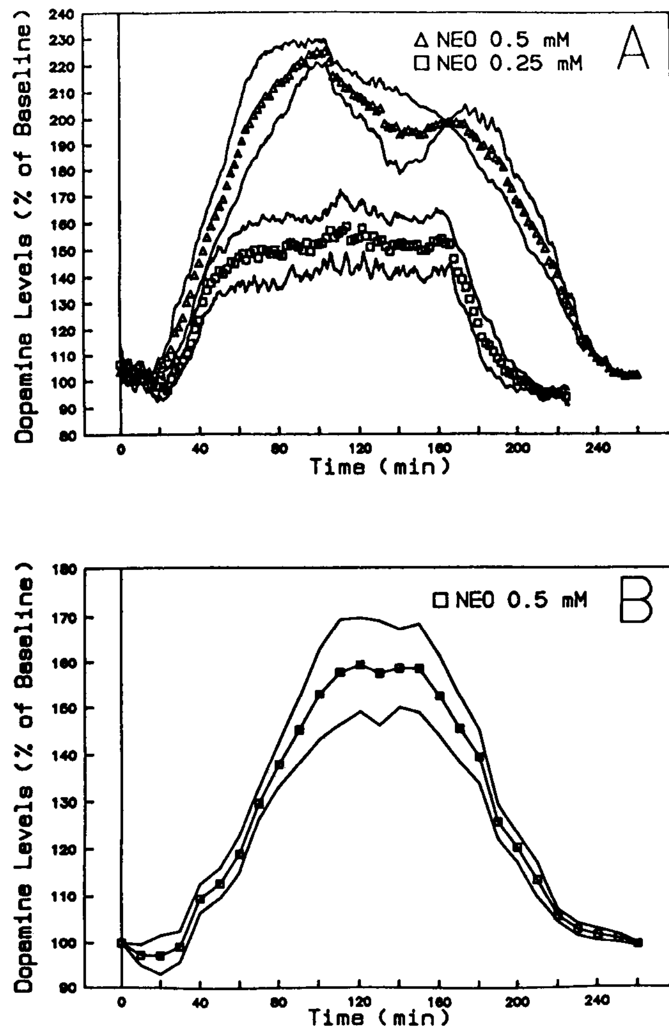


Figure 2. *A*, Chronoamperometric recordings depicting the time courses of the stimulatory effects of intranigral infusions of 0.25 mM and 0.5 mM neostigmine (*NEO*) on DA efflux in the dorsomedial striatum. Effects were significant ($P < 0.01$) between postinfusion intervals of 40–180 min and 40–230 min, respectively. *B*, Effects of intranigral infusion of 0.5 mM neostigmine on the levels of DA in dialysate samples taken every 10 min from the striatum. *Points* represent the mean changes in the chronoamperometric responses ($n = 3$) and dialysate DA concentrations ($n = 5$). *Solid lines* represent the SEM. Changes in DA efflux determined by each technique differed significantly ($P < 0.01$) between postinfusion intervals of 40–230 min. Chronoamperometric responses at 2 min intervals are presented for clarity.

Effects of neostigmine, nicotine, and carbachol on DA efflux in the dorsomedial striatum

As shown in Figure 2*A*, microinjection of the AChE inhibitor neostigmine into the substantia nigra increased the chronoamperometric signals corresponding to DA efflux in the anterior dorsomedial striatum. The onset latencies for these effects were 30 and 25 min for the neostigmine doses of 0.25 and 0.5 mM, respectively. DA efflux in the striatum reached maximal increases of 144 ± 10% and 227 ± 6% (Table 1) with respect to baseline levels (0.83 nA, 100%) within 60 and 100 min, respectively, following injection of neostigmine. The total durations of action for these effects were 210 and 250 min for 0.25 and 0.5 mM, respectively. These temporal profiles are in agreement with the known duration of cholinesterase inhibition by neostigmine (Goodman-Gilman et al., 1980). A similar temporal profile was observed for changes in DA concentrations measured in striatal dialysate samples following substantia nigra microinjection of neostigmine at the dose of 0.5 mM (Fig. 2*B*). Similar to results obtained using chronoamperometry, this dose of neostigmine produced maximal increases in DA efflux within 110 min with a total duration of action of 250 min. However, in comparison with the maximal increase of 227% observed using chronoamperometry, dialysate DA increased only to a level of 159 ± 11% with respect to preinjection baseline control values (Fig. 2*A*,*B*; Table 1).

Microinjection of the cholinergic receptor agonist nicotine into the substantia nigra resulted in pronounced and prolonged increases in chronoamperometric signals recorded in the dorsomedial striatum (Fig. 3*A*,*B*; Table 1). Maximal increases in striatal DA efflux of 155 ± 12 and 512 ± 55% were observed within 90 and 130 min following microinjection of nicotine into the substantia nigra at doses of 0.5 and 5.0 mM, respectively. These facilitatory effects of nicotine on striatal DA efflux were sustained over the course of the chronoamperometric experiment (250–300 min). Microinjection of the muscarinic receptor agonist carbachol also resulted in robust and sustained increases in the chronoamperometric signals recorded in the striatum. Carbachol injections at the doses of 0.5 and 5.0 mM resulted in maximal increases in striatal DA efflux of 132 ± 4% and 331 ± 18% within 60 and 160 min, respectively (Fig. 4*A*,*B*; Table 1). DA efflux remained significantly elevated above baseline levels over the course of these experiments (210–250 min). The facilitatory effects of substantia nigra microinfusions of neostigmine, nicotine, and carbachol on DA efflux in the dorsomedial striatum appear specific, as comparable substantia nigra microinjections of drug vehicle (0.9% saline) failed to alter significantly the chronoamperometric baseline in the dorsomedial striatum recorded over similar time periods (Fig. 4*A*, Table 1).

Effects of PPTg lesions

Figure 5 shows silhouettes of the largest and smallest lesions made by quinolinate in the PPTg. In every case a significant portion of the PPTg was destroyed, and the lesions were similar to those reported previously in which significant numbers of cholinergic neurons were lost from the PPTg but not the adjacent LDTN (Rugg et al., 1992; Dunbar et al., 1992). Damage was present from the caudal PPTg, on the lateral tip of the superior cerebellar peduncle, down through its length to the caudal edge of the substantia nigra. No significant damage was present in the substantia nigra, though neurons were lost from other structures adjacent to the PPTg, including the deep mesencephalic nucleus, cuneiform nucleus, and retrorubral nucleus. The damage to these structures varied between rats, and none of the structures was ever completely lesioned. It is unlikely, therefore, that this partial and inconsistent damage will have significantly affected the results of these experiments. Reactive gliosis was present in all lesioned tissue, as noted previously (Rugg et al., 1992; Dunbar et al., 1992).

As shown in Figure 6*A*, microinjection of 0.25 mM neostigmine into the substantia nigra of PPTg-lesioned rats resulted in an attenuated increase in the chronoamperometric signals in the dorsomedial striatum as compared to the neostigmine-induced increases in striatal DA efflux observed in intact control rats. Sham-operated control rats did not differ significantly in their response to a substantia nigra microinjection of neostigmine

Table 1. Effects of intranigral microinjections of cholinergic agents on dopamine efflux in the striatum

Drug treatment	Dose (mM)	Maximal change in current (nA)	Change from baseline (100%)	Time (min)	N
SAL	150	−0.01 ± 0.04	99 ± 5	60	4
		+0.01 ± 0.04	101 ± 5	90	
		−0.01 ± 0.02	99 ± 2	100	
		−0.02 ± 0.03	98 ± 3	120	
		+0.03 ± 0.03	103 ± 3	130	
		−0.02 ± 0.04	98 ± 5	150	
		+0.02 ± 0.05	102 ± 6	160	
NEO	0.25	+0.37 ± 0.08	144 ± 10*	60	3
NEO	0.5	+1.05 ± 0.05	227 ± 6*	100	3
NEO-DIA	0.5		159 ± 11***	110	5
NEO-LES	0.25	+0.17 ± 0.03	120 ± 4**	150	3
NIC	0.5	+0.46 ± 0.10	155 ± 12*	90	3
NIC	5.0	+3.42 ± 0.46	512 ± 55*	130	3
NIC-LES	0.5	+0.73 ± 0.06	188 ± 7**	120	3
CAR	0.5	+0.27 ± 0.03	132 ± 4*	60	3
CAR	5.0	+1.92 ± 0.15	331 ± 18*	160	3

Data are the mean (±SEM) maximal changes in DA oxidation current measured from pre-drug injection baseline values normalized to zero current, which were used to compute mean (±SEM) percentage changes in DA efflux with respect to 100% baseline DA oxidation current values (0.8 nA) (see text for details) at postinfusion time intervals corresponding to maximal effects of each drug (neostigmine, NEO; nicotine, NIC; or carbachol, CAR). SAL, saline. DIA, microdialysis; LES, lesioned.

* Significant ($P < 0.01$) drug-induced percentage changes in baseline versus saline (SAL) values at corresponding post infusion time intervals.

** Significant ($P < 0.01$) differences between drug-induced percentage changes in baseline in PPTg quinolinate-lesioned (NEO-LES and NIC-LES) animals versus nonlesioned animals (NEO and NIC), respectively.

*** Significant ($P < 0.01$) differences between the maximum effects of 0.5 mM neostigmine on baseline striatal levels of DA as determined by microdialysis (NEO-DIA) versus chronoamperometry (NEO).

compared to intact controls (peak neostigmine-induced increases in striatal DA efflux 60 min postinfusion corresponded to 148 ± 15%; +0.40 ± 0.12 nA, $n = 3$; data not shown). The onset latencies for neostigmine-induced effects were also markedly extended from 30 min for controls to 60 min for PPTg-lesioned animals. DA efflux in the striatum of PPTg-lesioned rats reached maximal increases of 120 ± 4% within 150 min following injection of neostigmine (Table 1). The total duration of action for these effects was similar to those observed in intact animals (220 min; Fig. 6*A*). In contrast to the attenuating effects of PPTg lesions on neostigmine-induced efflux of striatal DA, substantia nigra microinfusions of 0.5 mM nicotine resulted in an augmentation of the chronoamperometric response in lesioned animals. Maximal increases in striatal DA efflux of 188 ± 7% were observed within 120 min following microinjection of nicotine into the substantia nigra of PPTg-lesioned rats (Fig. 6*B*, Table 1). As in the case of control animals, DA efflux in the dorsomedial striatum remained elevated over the course of the chronoamperometric experiment (240 min).

Discussion

Nigral ACh and striatal DA

The data presented here show that cholinergic stimulation of the anterior substantia nigra increased the concentration of DA in the extracellular space of the dorsomedial striatum. Both nicotinic and muscarinic receptor agonists did this, and although full dose–effect curves were not established, nicotine appeared to be the more potent of the two. Neostigmine also produced a clear effect that was presumably dependent on the inhibition of AChE activity and consequent potentiation of the effects of endogenous ACh released from terminals in the substantia nigra. These data are consistent with the observation that pars compacta neurons contain AChE (Butcher and Marchand, 1978; Lehmann and Fibiger, 1978), which presumably functions normally to inactivate a cholinergic input, and they are consistent also with electrophysiological data showing that both nicotinic and muscarinic agonists excite pars compacta neurons (Clarke et al., 1987; Lacey et al., 1990). The present data also are in agreement with psychopharmacological data suggesting that cholinergic drugs excite pars compacta DA-containing neurons (Winn and Redgrave, 1979, 1981; Taha and Redgrave, 1980; Winn et al., 1983; Parker et al., 1991; Winn, 1991).

In vivo *dialysis and electrochemical measurement of changes in striatal DA concentration*

Although evidence for the facilitatory action of neostigmine on nigrostriatal DA neuronal transmission was provided by both electrochemical and microdialysis techniques, the results differed somewhat in terms of the maximum percentage increase in DA efflux. For example, following intranigral infusion of neostigmine (0.5 mM), a maximum increase in striatal DA efflux reached 159% with microdialysis as compared to 227% with chronoamperometry (see Fig. 2*A*,*B*; Table 1).

Differences between these two techniques in terms of the area of tissue sampled, sampling intervals, and proportion of basal extracellular DA sensitive to TTX following probe implantation into tissue may account for some of the observed discrepancies. For example, the surface area of the cylindrical 4 mm × 0.34 mm dialysis probe (427×10^{-4} cm^2) exposed to striatal tissue is approximately 240 times greater than that of the area of the ~0.15 mm (o.d.) planar surface of the electrochemical electrode (1.8×10^{-4} cm^2). Thus, in comparison to the electrochemical

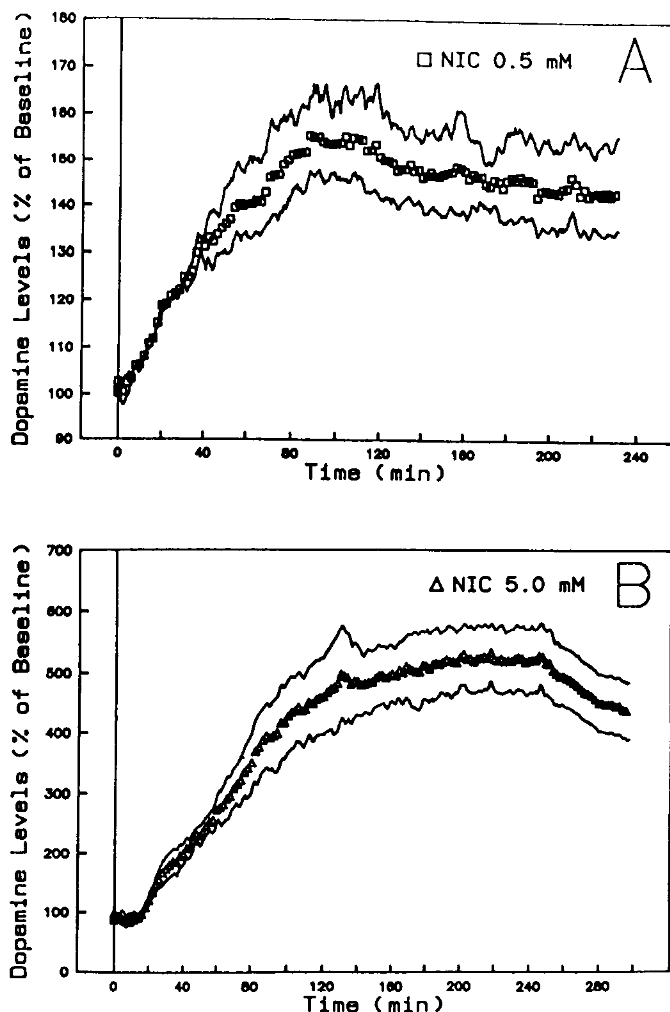


Figure 3. Chronoamperometric recordings depicting the time courses of the effects of intranigral injections of 0.5 mM (*A*) and 5.0 mM (*B*) nicotine (*NIC*) on DA efflux in the dorsomedial striatum. *Points* represent the mean changes in the chronoamperometric responses, and *solid lines*, the SEM. Effects achieved significance ($P < 0.01$) 20 and 30 min following drug infusion, respectively. For clarity, only chronoamperometric measurements taken at 2 min intervals are shown.

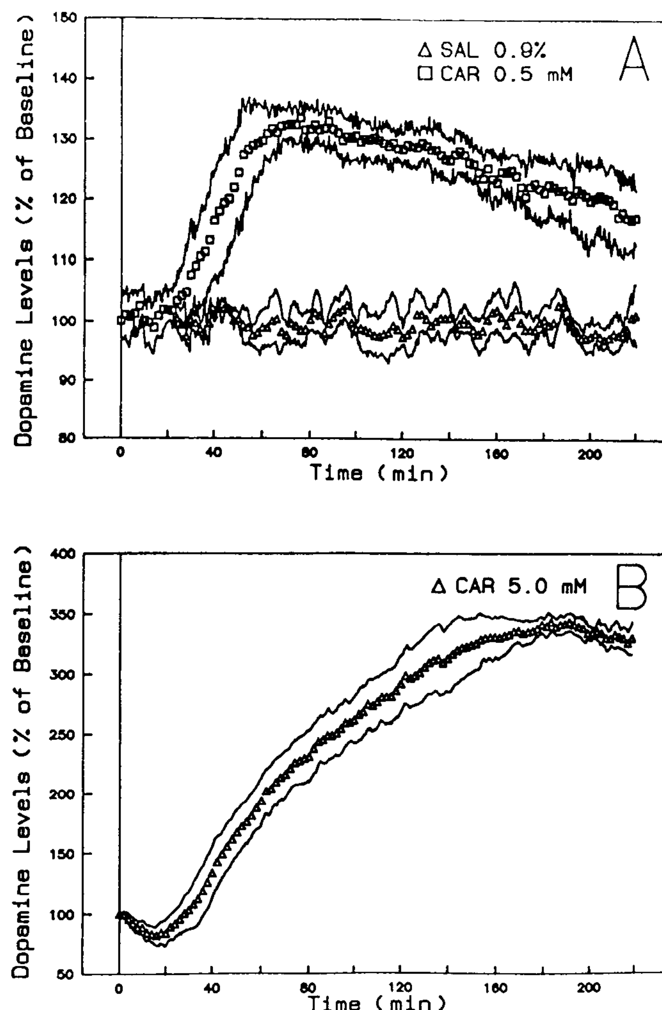
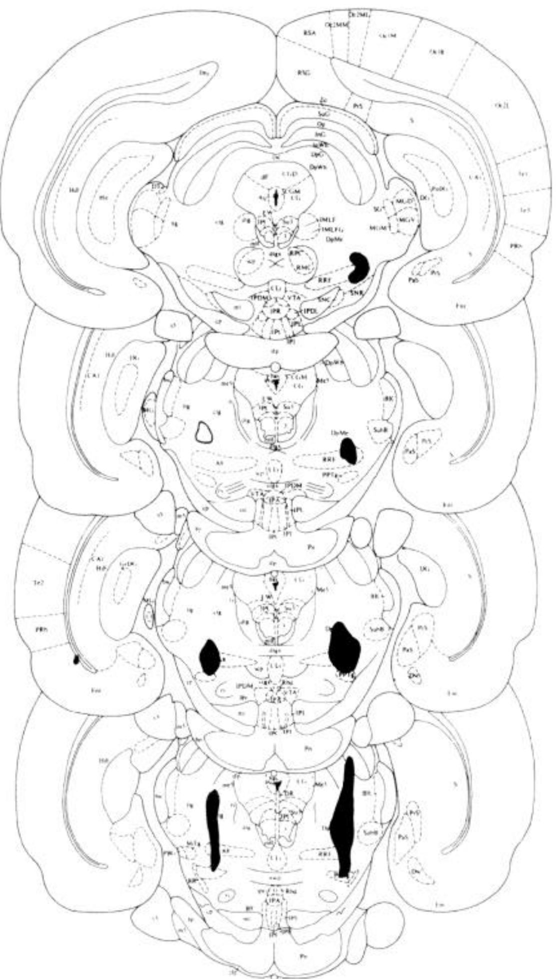
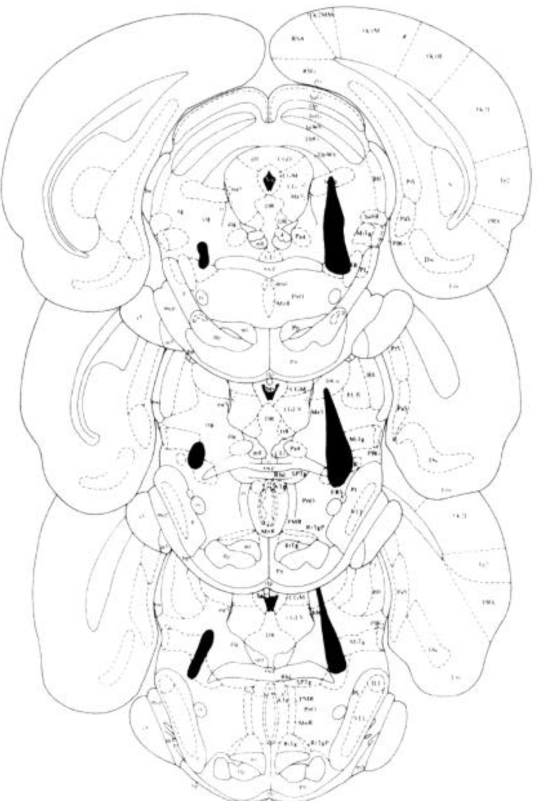


Figure 4. Chronoamperometric recordings showing the time courses of the stimulatory effects of intranigral injections of 0.5 mM (*A*) and 5.0 mM (*B*) carbachol (*CAR*) on DA efflux in the dorsomedial striatum. Effects were significant ($P < 0.01$) 40 min following CAR infusion at each dose tested. Note the lack of effect of intranigral injections of the drug vehicle (*SAL*; 0.9% saline) on the baseline chronoamperometric response recorded in the striatum (*A*). *Points* represent the mean changes in the chronoamperometric responses, and *solid lines*, the SEM. For clarity, only chronoamperometric measurements taken at 2 min intervals are presented.

electrode, the larger surface area of the dialysis probe results in a relatively greater volume of the striatum sampled by the dialysis method. In this regard, it is worth noting that DA innervation of striatum from the substantia nigra runs a dorsoventral–rostrocaudal gradient that is reflected by a similar gradient in striatal tissue content of DA (Glowinski et al., 1966). Given the neurochemical heterogeneity of the striatum, the response of the nigrostriatal DA system to nigral cholinergic stimulation may also show a degree of pharmacological heterogeneity. Additionally, HPLC-EC measurements of individual dialysate samples represent a change in extracellular DA concentrations integrated over the entire intersampling period of 10 min, whereas individual chronoamperometric measurements represent changes integrated over a much smaller sampling period (30 sec).

Recent findings indicate that the baseline DA signals detected by each method differ in TTX sensitivity. Under similar experimental conditions, dialysate DA levels have been shown to exhibit a reduction of 75% following local TTX administration, indicating that at least 25% of the dialysate DA baseline signal is non-neuronally mediated (Blaha, 1992). In contrast, striatal DA baseline levels determined by *in vivo* chronoamperometry exhibited complete TTX sensitivity. The precise basal level of extracellular DA present in the striatum is a matter of considerable controversy. A number of microdialysis studies have estimated basal DA concentration here at between 0.01 and 0.05 μM, while electrochemical experiments have yielded values between 0.025 and 1.0 μM. Recent *in vitro* examinations of the properties of dialysis probes have, however, suggested that these probes significantly reduce the concentrations of dialyzable substances in the surrounding extracellular fluid (Benveniste et al., 1989). The extraction properties of microdialysis may therefore contribute to a significant reduction in the apparent basal concentrations of dialyzed substances, which may explain why microdialysis estimates of basal interstitial concentrations are much lower than those measured by *in vivo* electrochemical tech-





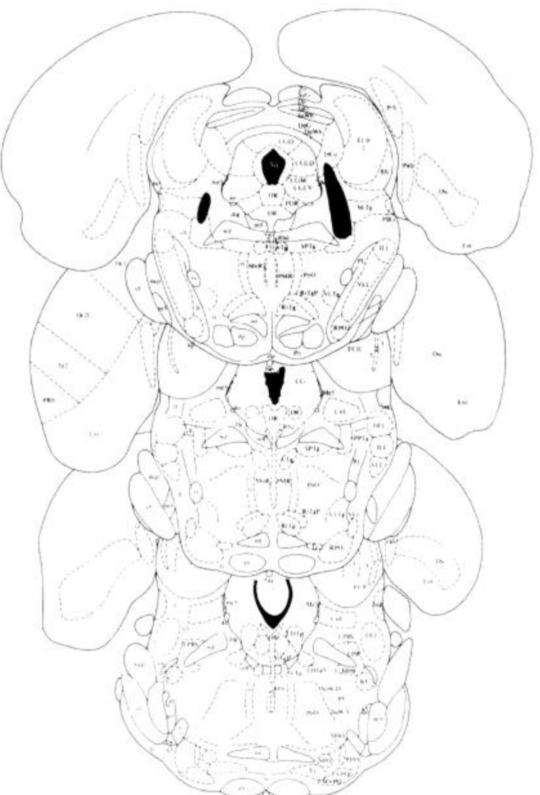


Figure 5. Silhouettes of the largest and smallest quinolinate lesions in the PPTg. The *shaded area* represents the lesioned tissue; within this area reactive gliosis was present and neurons lost (sections redrawn from the atlas of Paxinos and Watson, 1986).

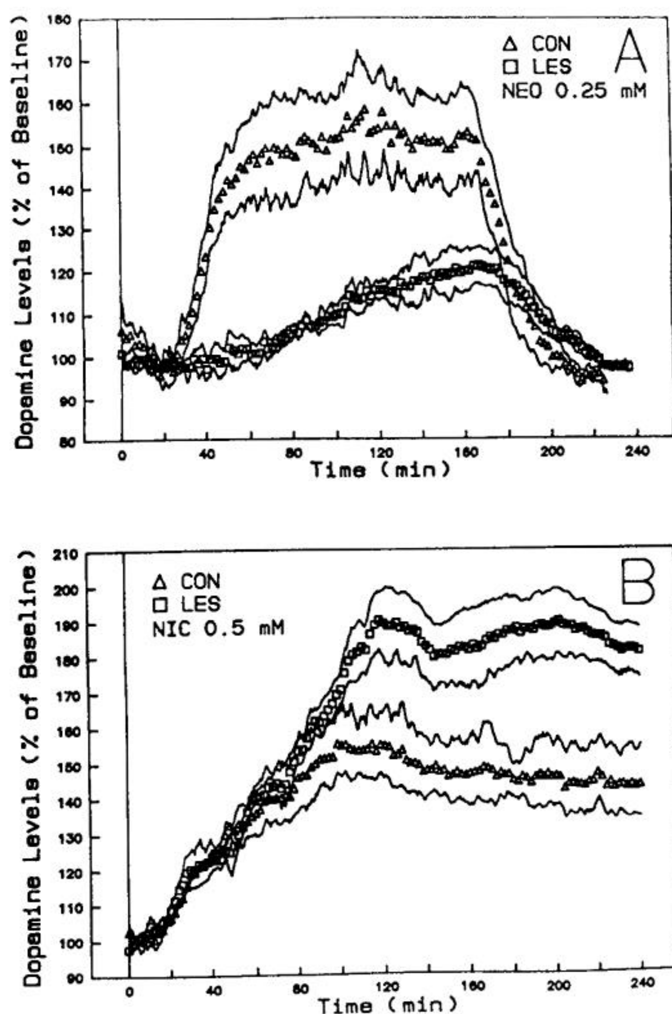


Figure 6. Chronoamperometric recordings showing the time courses of the stimulatory effects of intranigral injections of 0.25 mM neostigmine (*A*) and 0.5 mM nicotine (*B*) on DA efflux in the dorsomedial striatum before (*CON*) and after (*LES*) quinolinic acid lesions of the PPTg. *Points* represent the mean changes in the chronoamperometric responses, and *solid lines*, the SEM. Effects of neostigmine (*LES*) and nicotine (*LES*) differed significantly ($P < 0.01$) from drug effects in nonlesioned animals between postinfusion intervals of 40–160 and 120–240 min, respectively. Chronoamperometric responses at 2 min intervals are presented for clarity.

niques. Given the technical and methodological differences between dialysis and *in vivo* electrochemical techniques, and the fact that the striatum is heterogeneously populated with DA terminals, it is perhaps not too surprising that some differences in the profiles of the magnitude of the effects of neostigmine were observed. Apart from the above differences, the results obtained with the two techniques are complementary and indicate that endogenous cholinergic stimulation of the substantia nigra leads to an augmentation of DA neurotransmission in the striatum.

PPTg and substantia nigra

Considerable controversy has surrounded the identification of a cholinergic input to the substantia nigra, principally because of the mismatch in the volumes of ACh, CAT, and AChE present. This mismatch is probably accounted for by AChE having two separate functions: membrane-bound AChE hydrolyzes ACh whereas released AChE may have a noncholinergic function (Greenfield, 1980). Studies using CAT immunohistochemistry have demonstrated that a cholinergic projection to substantia nigra originates in the PPTg, and possibly the LDTN, although the nature of this projection has been disputed (Woolf and Butcher, 1986; Lee et al., 1988; Gould et al., 1989). The present data are consistent with the hypothesis that the PPTg makes a significant cholinergic innervation of the pars compacta. Previous work established that quinolinate lesions of the PPTg made in the same way as here reduce the number of CAT-positive neurons in the PPTg by ~75% (Dunbar et al., 1992). Other excitotoxins also reduced the number of CAT-positive neurons, but quinolinate differed from them in having less impact on noncholinergic neurons in this area (Rugg et al., 1992; Dunbar et al., 1992). That these lesions had an effect on a cholinergic projection to the substantia nigra is confirmed by the significant reduction in the striatal DA response to intranigral neostigmine, an agent that acts presynaptically. Had there been no cholinergic projection to the substantia nigra from the PPTg, or had the projection been noncholinergic, the response to neostigmine would not have been affected. Moreover, the fact that responses to nicotine were enhanced suggests that postsynaptic receptor supersensitivity had developed, strengthening the argument in favor of a cholinergic input to nigra from the PPTg having been destroyed. It is also of interest to note that the fact that responses to neostigmine were so affected, and the fact that supersensitivity developed, suggest that the bulk of the cholinergic projection to nigra comes from the PPTg. The LDTN was not affected by lesions placed in the PPTg: had there been a significant innervation from this site, it is unlikely that the lesion-induced effects would have been so great. Chronoamperometric studies examining the effects of specific LDTN lesions in the present paradigm would further clarify this issue.

The PPTg is one of very few extrastriatal sources of nigral innervation able to excite pars compacta DA-containing neurons. While the functions of the PPTg have not been systematically investigated in any detail, most authors agree that these cholinergic neurons are part of the ascending reticular activating system (Mesulam et al., 1989; Harrison et al., 1990). A number of studies suggest that they may have a role in the maintenance of sleep and arousal (Harrison et al., 1990; Semba et al., 1990), and that in particular they may help regulate the state of the thalamus, shifting it from burst-firing to single-spiking modes of operation (Steriade and Llinas, 1988; Steriade et al., 1990). The present data are not inconsistent with the hypothesis that the cholinergic neurons of the PPTg are involved in the maintenance of sleep and arousal, but they further suggest that, as well as regulating the operational state of the thalamus, they may control the basal firing of nigrostriatal DA neurons in order to regulate activity in the striatum. These processes are likely to be functionally related: the active waking state requires not only appropriate thalamocortical activity, but also an operational basal ganglia. Further studies will concentrate on elucidating the neurochemical consequences of the action of ACh in the substantia nigra, and on defining the behavioral processes that these cholinergic neurons influence.

It has been known for some time that cholinergic neurons are lost from the PPTg, and possibly the LDTN, in Parkinson's disease and supranuclear palsy (Lloyd et al., 1975; Hirsch et al., 1987; Zweig et al., 1987, 1989; Jellinger, 1988; Halliday et al., 1990). Additional interest in the PPTg has been provided by the extraordinary finding of increased numbers of cholinergic

neurons in the PPTg of schizophrenic patients (Karson et al., 1991). The data presented here indicate clearly that cholinergic neurons in the PPTg influence the activity of nigrostriatal DA-containing neurons. It may therefore be possible to suggest that PPTg cholinergic neurons could be contributing to at least some of the symptoms of schizophrenia by driving DA neurons at source in the substantia nigra pars compacta. It will be of interest to determine in future studies exactly how mesopontine cholinergic systems might contribute to these various psychopathological disorders.

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