

Differential Cholinergic Regulation of Dopamine Release in the Dorsal and Ventral Neostriatum of the Rat: An *in vivo* Microdialysis Study

W. T. O'Connor,¹ K. L. Drew,² and U. Ungerstedt¹

¹Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institute, S171-77 Stockholm, Sweden and ²Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska

We used *in vivo* microdialysis to investigate the effects of local perfusion with the AChE inhibitor neostigmine on the basal and haloperidol evoked increase in dialysate dopamine levels in the dorsolateral and fundus striata of the bilaterally implanted halothane anaesthetized rat. In the absence of neostigmine basal dopamine was consistently higher in the dorsolateral striatum compared with the fundus striati. Local perfusion with neostigmine (10 and 100 μM) increased basal dopamine in the fundus striati compared to the contralateral (control) side but not in the dorsolateral striatum. In the absence of neostigmine haloperidol (0.05–0.5 mg/kg, s.c.) increased dopamine release in both the dorsolateral and fundus striata. However, local perfusion with neostigmine (10 μM) attenuated this increase in the dorsolateral striatum at all doses of haloperidol while only the effect of the highest (0.5 mg/kg) dose of haloperidol was counteracted in the fundus striati. Both the basal and haloperidol (0.25 mg/kg) induced increase in dopamine release in the control (no neostigmine) and neostigmine (+10 μM) treated dorsolateral striata were abolished following local perfusion with tetrodotoxin (1 μM).

The data demonstrate that the introduction of neostigmine into the neostriatum selectively increases basal DA levels in the fundus striati and strongly counteracts the haloperidol evoked DA release in the dorsolateral striatum and thus provide strong evidence for a differential cholinergic regulation of striatal DA release *in vivo*. In addition, we demonstrate that the stimulatory and inhibitory effects of neostigmine operate independently and have a regional specificity within the neostriatum.

[Key words: neostigmine, dopamine, haloperidol, microdialysis, neostriatum, dorsolateral, fundus]

Biochemical and electrophysiological studies on the mechanism of action of neuroleptic drugs provide strong evidence that the antipsychotic effect of these drugs is thought to be mediated via an action on the A₁₀ dopamine (DA) projection to the ventral neostriatum and frontal cortex while the extrapyramidal motor

side effects are thought to be mediated *via* an action on the A₉ DA projection to the dorsal neostriatum (Andén and Stock 1973; Costall and Naylor, 1976; Chiodo and Bunney, 1983; White and Wang 1983). Anticholinergic activity reduces extrapyramidal side effects without decreasing antipsychotic efficacy and may distinguish atypical and typical antipsychotics (Snyder et al., 1974). The purpose of the present study was to investigate the cholinergic regulation of dopamine release within subregions of mammalian striatum.

Until recently the caudate putamen was considered to be a homogenous structure. This region was distinguished from the nucleus accumbens on the basis of their respective A₉ and A₁₀ DA afferents (Dahlstrom and Fuxe, 1964). However it is increasingly evident that the caudate nucleus is not a homogenous structure (Nauta, 1986). Anatomically the ventromedial striatum including the fundus striati (Paxinos and Watson, 1982) and the nucleus accumbens is distinguished from the dorsolateral striatum by afferents originating from limbic structures (e.g., the hippocampus and the amygdala) and from structures implicated in the circuitry of the limbic system (e.g., prefrontal cortex and DA afferents from the ventral tegmental area (A₁₀) (Nauta, 1986). The dorsolateral striatum by contrast, is characterized by glutamatergic afferents originating from the sensorimotor cortex and DA afferents from the substantia nigra (A₉). This striatal subregion receives only sparse inputs of limbic origin (cf. Nauta 1986) and plays a primary role in the “motor circuit” mediating sensorimotor functions particularly the initiation of voluntary motor acts. Regional difference in D₂ receptor density and cholinergic activity has also been reported in the striatum with higher D₂ receptor binding (Joyce and Marshall, 1987), AChE activity (Joyce and Marshall, 1987; Loopuijt 1989) and dialysate ACh levels (O'Connor et al., 1991a; Osborne et al., 1994) in the dorsolateral striatum.

A major function of striatal acetylcholine (ACh) is the regulation of DA release via both nicotinic and muscarinic receptors. Choline acetyltransferase (CAT)-labeled terminals also synapse on tyrosine hydroxylase (TH)-labeled terminals in the dorsal and ventral striatum (Pickle and Chan, 1990). In fact, ACh was the first neurotransmitter shown to affect striatal DA release *in vitro* (Besson et al., 1969) and since then both *in vitro* and *in vivo* studies have provided evidence for a cholinergic modulation of DA release in the neostriatum (Giorguieff et al., 1977; De Bellerroche et al., 1982; Damsma et al., 1988; Xu et al., 1989; Rapier et al., 1990; Farber et al., 1993). Recent binding and autoradiographic studies in mammalian brain demonstrate that the majority (75%) of striatal muscarinic receptors are of the

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Correspondence should be addressed to Dr. W. T. O'Connor at the above address.

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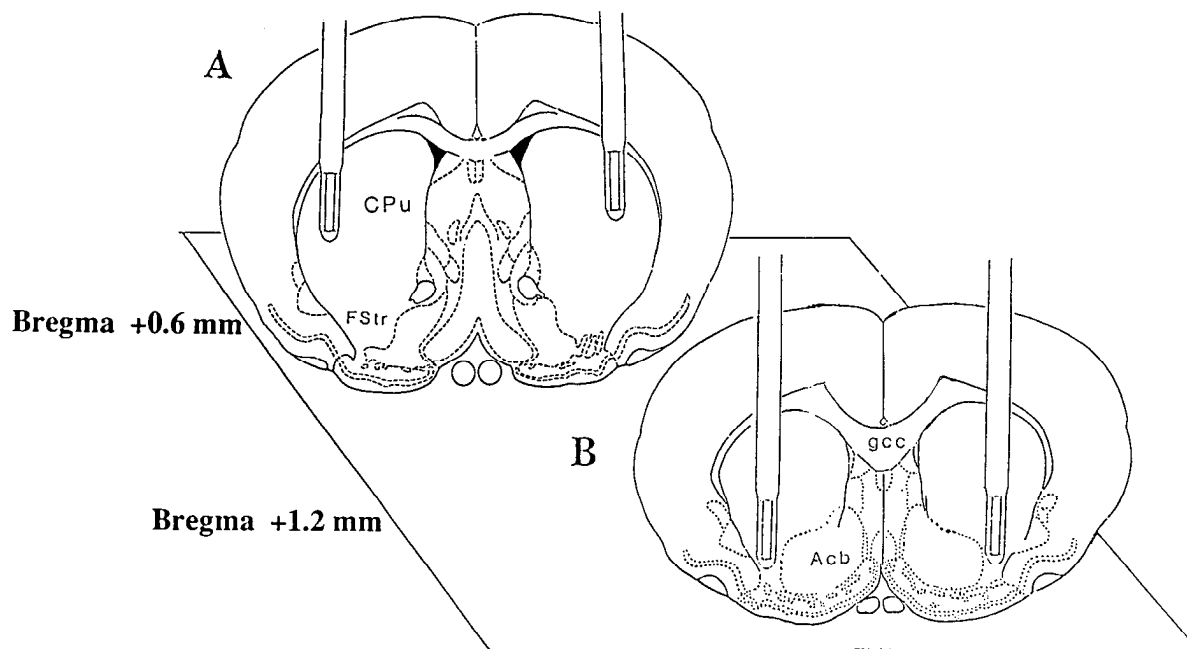


Figure 1. Location of the microdialysis probe in the right (no neostigmine) and left (+ neostigmine) (A) dorsolateral and (B) fundus striata. Abbreviations are as follows: *Acb*, nucleus accumbens; *CPu*, caudate putamen; *FStr*, fundus striati; *gcc*, genu of the corpus callosum. Probes were implanted bilaterally in either the dorsolateral or fundus striata. For full coordinates, see Materials and Methods.

excitatory M_1 , M_3 , and M_5 subtypes and the less numerous and inhibitory M_2 and M_4 subtypes (Hammer and Giachetti, 1982; Olanas et al., 1982; Cortes and Palacios, 1986; Cortes et al., 1986; Mash and Potter, 1986; Spencer et al., 1986). Both the muscarinic and the less common and stimulatory nicotinic (also five subtypes) receptors are localized both pre- and postsynaptically (Cooper, 1994; Jerusalinski et al., 1995) and converging data suggest that both muscarinic and nicotinic receptors are located on the DA terminals in both dorsal and ventral neostriatum (De Belleruche et al., 1979, 1982; Gurwitz et al., 1980; Suga, 1980; Xu et al., 1989). However, pharmacological agents are not highly selective for particular subtypes, making investigations on the functional significance of the subtypes difficult. Striatal ACh has been shown to facilitate evoked striatal DA release via both nicotinic and muscarinic M_1 receptors. ACh can also reduce evoked striatal DA release (Westfal 1974a,b) via M_2 receptor activation giving rise to the concept of a dual cholinergic regulation of striatal DA release resembling the cholinergic modulation of noradrenaline release in peripheral nerves (Muscholl 1979).

Based on the above findings, we tested the hypothesis for a difference in the cholinergic regulation of DA release in the dorsolateral and ventromedial (fundus) neostriatum. Towards this aim, we monitored the effects of local perfusion with the indirect cholinergic agonist neostigmine, on basal dialysate DA levels and following acute, systemic administration of haloperidol (Christensen et al., 1984; Grabiell et al., 1989) in the dorsolateral and fundus neostriata.

Materials and Methods

Brain microdialysis and surgery. Sixty-two male rats weighing 330–390 gm (Sprague–Dawley, Alab, Sollentuna, Sweden) were used in this study. The animals were allowed free access to water and standard laboratory food in a temperature controlled environment and were kept on a 12hr light/dark cycle. The experimental protocols performed in the present study were approved by the Swedish committee for ethical ex-

periments on laboratory animals, license No. N128. Rats were anaesthetized with halothane (1.5% halothane/air mixture delivered at 1.4 liter/min) and placed in a stereotaxic frame (Kopf). Body temperature was continuously maintained at 37°C using a temperature controller (CMA 150, Carnegie Medicin, AB, Stockholm, Sweden). After exposure of the skull and drilling of a burr hole (1 mm diam), concentric microdialysis probes (CMA 12, O.D. 0.5 mm, 2 mm membrane, Carnegie Medicin AB, Stockholm, Sweden) were stereotaxically implanted into the left and right dorsolateral or fundus striata according to the atlas of Paxinos and Watson (1982) (dorsolateral striatum coordinates, A +0.6, L \pm 3.5, V 5.5; and fundus striati, A +1.2, L \pm 2.7, V 7.5 mm relative to bregma; incisor bar –2.5). Thus, there were two groups of rats under investigation, one group received bilateral probe implants in the dorsolateral striatum and a second group received bilateral probe implants in the fundus striati (Fig. 1). The probes were perfused at a rate of 2 μ l/min with commercial Baxter's Ringer solution (147.0 mm Na⁺, 2.4 mm Ca²⁺, 156 mm Cl⁻, 4.0 mm K⁺, pH 6.0; Baxter A/S, Halden, Norway). Neostigmine (1, 10, or 100 μ M) was included in the perfusion medium on the left side. The collection of perfusate samples for assay commenced 120 min after probe implantation. Samples were collected every 30 min and 40 μ l was analyzed for DA. *In vitro* recovery (mean \pm SEM %) across the probe membrane for DA was 11.5 \pm 0.7. The DA levels reported in the present study are not corrected for recovery (Hsiao et al., 1990).

Experimental protocol. This investigation was divided into three experiments. The effects of local perfusion with neostigmine were studied on (1) basal dialysate DA levels and (2) haloperidol stimulated DA levels in the left dorsolateral and fundus striata while the contralateral side acted as the control. In addition, (3) the tetrodotoxin sensitivity of both the basal and haloperidol induced increase in DA release in the control (no neostigmine) and neostigmine (10 μ M) treated dorsolateral striata was also investigated. The microdialysis probes were connected to a microinfusion pump via a syringe selector (CMA 111) which allowed the appropriate perfusion medium to be selected.

Neostigmine. Neostigmine was added to the perfusion medium to inhibit AChE and to elevate synaptic ACh levels. The selected concentration of neostigmine (1, 10, or 100 μ M) was included in the perfusion medium on the left side prior to probe implantation and was infused throughout the experiment. Neostigmine was chosen over physostigmine because of its more local effect in the interstitial compartment by virtue of its poor lipophilicity and its lack of sensitivity to pH and light (Koelle, 1975).

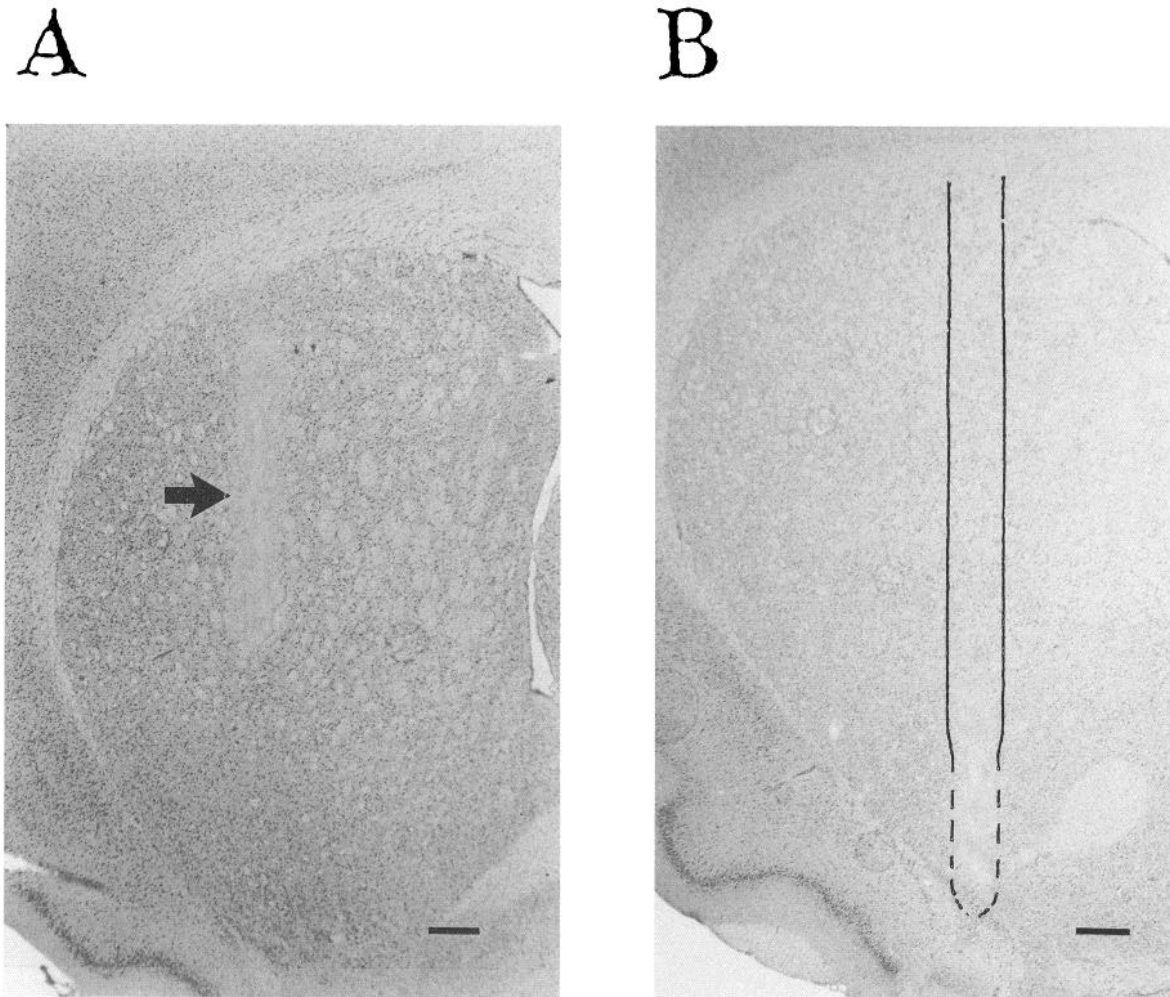


Figure 2. *A*, Photomicrograph from a cresyl violet-stained section through the dorsolateral striatum showing the track left by the microdialysis membrane (arrow). *B*, Section through the fundus striati. The schematic drawing of the probe represents its actual size and position. The dialysis membrane corresponds to the part delimited by the dashed lines. Scale bar: 500 μm .

Haloperidol. At least three stable DA samples were obtained (90 min) after which the selected dose of haloperidol (0.05, 0.25, or 0.5 mg/kg) or haloperidol vehicle was administered as a single subcutaneous injection in the hind limb area.

Tetrodotoxin. The reversible antagonist of voltage dependent Na^+ channels, tetrodotoxin, (TTX) was perfused locally into both the control and neostigmine treated dorsolateral striata in order to study the firing dependence of the basal and haloperidol induced increases in dialysate DA levels. TTX (1 μM) was included in the perfusion medium on both the control and neostigmine (10 μM) treated sides after three stable basal DA values (90 min) were recorded and continued to be perfused until the end of the experiment (270 min). 90 mins following the start of TTX perfusion, either haloperidol (0.25 mg/kg, s.c.) or vehicle was administered.

Dopamine analysis. The perfusate samples (40 μl) were placed into a refrigerated micro-sampler/autoinjector (CMA200/240) and were automatically injected. Reverse phase HPLC with electrochemical detection was used to assay DA. The method has been described previously (Drew et al., 1989). The composition of the mobile phase was 0.132 M NaH_2PO_4 , 9 mM EDTA, 1.2 mg sodium octane sulfonate, and 12% methanol, pH = 3.8. The flow rate of the mobile phase was 0.5 ml/min. The limit of detection was 0.02 pmol DA.

Verification of cannula placement. At the end of each experiment rats were killed with an overdose of halothane. The brain was removed and cut on a Leitz freeze microtome (Germany) and the placement of the probe verified by microscopic examination (Fig. 2). Those animals displaying either improper probe implantation or excessive damage were omitted from the study.

Data presentation and statistical analysis. Data are reported as percent of basal values (calculated as mean of the two samples before haloperidol). Data are calculated as means \pm SEM ($N = 3-7$). Data were analysed with a two way analysis of variance with region (dorsolateral and fundus) and concentration (neostigmine) and dose (haloperidol) treated as between subject variables and time treated as a within subjects variable. Significant ($P < 0.05$) dose time interactions (DT) were followed by a one way analysis of variance for each dose and subsequent Newman-Keuls post-hoc comparison (Crisp Crunch) or a paired t test where indicated.

Chemicals. Neostigmine methyl sulfate [(3-dimeethylcarbamoylphenyl) trimethyl-ammonium methyl sulfate], (N-2126, Sigma Chemical Company, St. Louis, MO) and tetrodotoxin (TTX) (Janssen Pharmaceutica, Belgium) were dissolved in Ringer's and water, respectively, and stored at -80°C as 1 mM stock and on the day of the experiment was added to the perfusion medium to give the desired concentration. Haloperidol (Haldol, Janssen Pharmaceutica, Beerse, Belgium) was diluted to the required concentrations in water and administered as a single subcutaneous injection in a volume of 1 mg/kg. Haloperidol placebo (Janssen Pharma AB, V. Frölunda) was employed as the vehicle.

Results

There was no difference in basal dialysate DA levels in the dorsolateral or fundus striata between the left and right sides in the bilateral control group (i.e., in the absence of neostigmine) (Table 1). In addition, there was no difference in the maximal

Table 1. The effect of neostigmine on basal dialysate dopamine levels in the dorsolateral and fundus striata

Control	Neostigmine	Dose (μM)	(N)
Dorsolateral striatum			
6.75 \pm 0.99	6.54 \pm 1.41	0	8
7.05 \pm 1.35	8.45 \pm 1.79	1	5
4.52 \pm 1.10	4.12 \pm 0.95	10	7
11.92 \pm 2.72	10.30 \pm 1.72	100	4
Fundus striati			
1.47 \pm 0.43	1.62 \pm 0.45	0	7
4.82 \pm 2.15	3.62 \pm 1.15	1	4
1.20 \pm 0.40	2.72 \pm 0.45*	10	4
6.15 \pm 1.20	9.80 \pm 0.97*	100	3

Values (means \pm SEM) are expressed in nanomolar concentrations and represent the mean of two stable basal levels (60 min). Data are presented as mean \pm SEM. Neostigmine (1, 10, or 100 μM) was included in the perfusion medium on left side. A group of rats without neostigmine on either side acted as the bilateral control in the dorsolateral or fundus striata. Statistical analysis was performed by a two-way ANOVA with side (neostigmine) treated as a within-subjects variable and followed by a paired *t* test. *N* represents the number of animals in each group.

* *P* < 0.05 versus control side (no neostigmine) fundus striati.

haloperidol (0.25 mg/kg) induced increase in dialysate DA levels in the right and left dorsolateral striata (155 \pm 31% and 152 \pm 23%, respectively) and in the right and left fundus striata (174 \pm 30% and 224 \pm 30%, respectively) in this control group. However, basal DA levels were consistently higher in dorsolateral striatum (4.85 \pm 0.07 nM) compared with the fundus striati (1.75 \pm 0.25 nM) (*p* = 0.024) (Table 1). A large variability in dialysate DA levels was observed during the first 120 min of perfusion (not shown), probably reflecting tissue disruption resulting from probe implantation and equilibration between the dialysate and the extracellular fluid compartments. DA release stabilized 120 min after probe implantation and remained constant over the course of the experiment (300 min).

Effect of neostigmine on dialysate dopamine levels

Local perfusion with neostigmine (10 and 100 μM) increased basal DA levels in the fundus striati (*p* = 0.0155) but not the dorsolateral striatum compared to the control (no neostigmine) side (*p* < 0.05) (Fig. 3A,B). Thus, when compared to the control side, only the fundus striati showed a significant neostigmine induced increase in basal DA levels. There was an increase in basal DA levels on both sides in fundus (*p* < 0.001) and dorsolateral striata (*p* < 0.05) at the highest 100 μM concentration of neostigmine compared with the two lower concentrations.

Effect of haloperidol on dialysate dopamine levels

Acute haloperidol administration (0.05, 0.25, and 0.5 mg/kg, s.c.) was associated with an increase in DA release in both the control (no neostigmine) dorsolateral (DT = 0.012) (Fig. 4A) and fundus striata (DT = 0.0102) (Fig. 5A). Dialysate DA levels were elevated in the first fraction (30 min) following haloperidol and continued to rise until the end of the experiment (150 min).

Effect of neostigmine on the haloperidol response

Local perfusion with neostigmine (10 μM) was associated with a profound counteraction of the haloperidol induced increase in DA release in the dorsolateral striatum at all the doses of haloperidol tested (Fig. 4B). In the fundus striati the effects of the highest (0.5 mg/kg, s.c.) dose of haloperidol was significantly

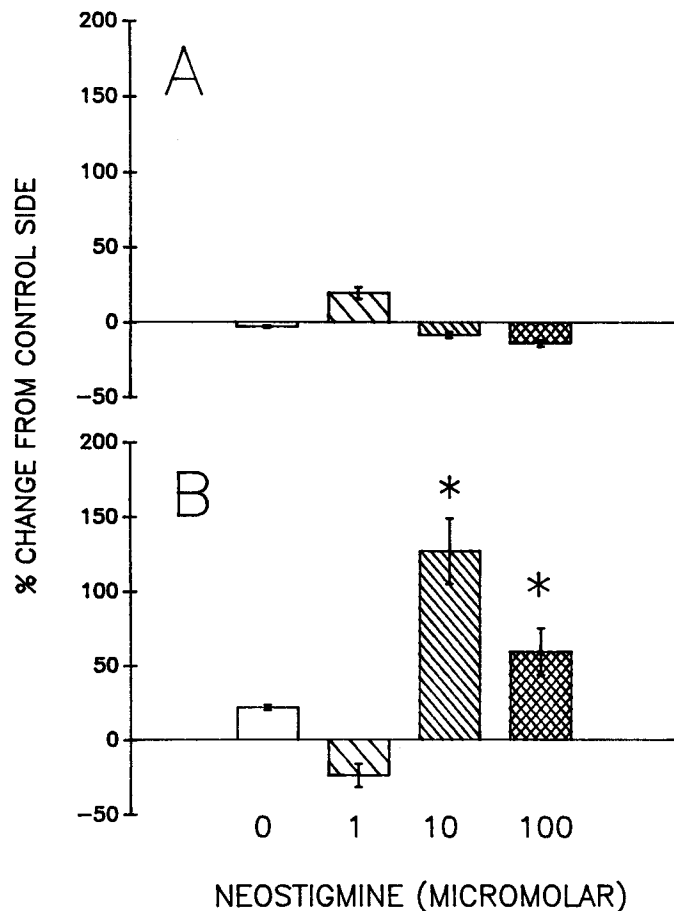


Figure 3. Effect of local perfusion with neostigmine on basal dialysate dopamine (DA) levels in (A) the dorsolateral striatum and (B) the fundus striati of halothane anesthetized rats in 30 min Ringer's perfusate fractions. The perfusate contained either 1 μM (wide hatch), 10 μM (narrow hatch) or 100 μM (cross hatch) neostigmine which was included in the perfusion medium on the left side prior to probe implantation and continued to be perfused until the end of the experiment. A group of rats without neostigmine on either side acted as the bilateral control (open bar) in the dorsolateral or fundus striata. Data are expressed as percent change from the basal levels on the contralateral control side. The change in dialysate DA levels are expressed as percent change from the levels on the control (no neostigmine) side. See Table 1 for absolute DA levels. Neostigmine significantly increased basal DA levels in the fundus striati compared to the control (no neostigmine) side. Each data point (*N* = 3–7) per group represents the mean \pm SEM. Statistical analysis was performed by a paired Student's *t* test. *, *P* < 0.05 versus the control (no neostigmine) side.

counteracted by neostigmine but this inhibition was not observed at the lower the 0.05 and 0.25 mg/kg doses of haloperidol (Fig. 5B). When present, the counteraction of the haloperidol induced increase in DA release by neostigmine was already evident in the first fraction (30 min) following haloperidol and was still present at the end of the experiment (250 min).

Intrastriatal perfusion with TTX (1 μM) on both the control (no neostigmine) and left (10 μM neostigmine) sides decreased basal dialysate DA levels by 70% within two fractions (60 min) (*P* < 0.001) and blocked the effect of haloperidol (0.25 mg/kg s.c.) on dialysate DA levels both in the absence and presence of neostigmine in the perfusion medium (Fig. 6A,B).

Discussion

This study was undertaken to test the hypothesis for a difference in the cholinergic regulation of DA release in the dorsolateral

and fundus neostriata which may be relevant to the antipsychotic efficacy and neuroleptic properties of antipsychotic drugs. Toward this aim we used *in vivo* microdialysis (Ungerstedt, 1991) to monitor the effects of local perfusion with neostigmine on both basal and haloperidol evoked DA release in the acutely implanted halothane anaesthetized rat. The results suggest a dorsolateral/ventromedial heterogeneity in the neostriatum which markedly influences how basal and haloperidol-induced DA release respond in the presence of increased cholinergic tone.

Basal dialysate dopamine levels

We report a regional difference in basal dialysate DA levels within the neostriatum with consistently higher levels in the dorsolateral striatum compared to the fundus striati. This difference is consistent with previous findings (O'Connor et al., 1989; Drew et al., 1990) and strengthens the evidence for a dorsolateral-ventromedial heterogeneity in DA tone within the neostriatum (Joyce and Marshall 1985; Nauta, 1986; Grenhoff et al., 1988).

Effect of neostigmine on dialysate dopamine levels

Local perfusion with the 10 μ M and 100 μ M concentrations of neostigmine increased basal DA levels in the fundus striati but not in the dorsolateral striatum suggesting that in contrast to the well documented DA regulation of ACh release in the dorsal and ventral striatum (Bertorelli and Consolo, 1990; Damsma et al., 1990; Stoof et al., 1992; Di Chiara et al., 1994) activation of local cholinergic receptors in these striatal subregions differentially regulates basal DA release. The highest (100 μ M) concentration of neostigmine increased basal DA in both striatal subregions compared to the two lower concentrations (Table 1). However, due to the large inter-group difference in basal DA levels it is not possible to determine if this was an effect of neostigmine per se.

Previous microdialysis studies report a diminished ability of concentric (and U-shaped) cannulae to detect extracellular ACh from the dorsal striatum compared with the transversal design (Damsma, 1987a, 1988). Thus, higher (e.g., 10 mM) concentrations of the inhibitor are generally required in studies utilizing the concentric probe (Damsma et al., 1987b; Marien and Richards, 1990; Herrera-Marschitz 1992). A previous set of characterization studies using a similar microdialysis preparation demonstrated that the 10 mM concentration of the inhibitor was the minimum concentration required for the detection of a haloperidol induced increase in ACh release in the fundus striati (O'Connor et al., 1991a).

Large inter-group differences in basal DA levels from the dorsolateral striatum have been reported previously (see Osborne et al., 1991 for discussion) and probably account for the large number of reports that present data as percent of control. The presentation of the raw data graphically highlights the difficulties in attempting quantitative comparison between experimental groups that present different basal transmitter levels and emphasize the importance of choosing the appropriate control. In contrast to the large inter-group variability in basal DA levels, the within group variability (i.e., control side vs neostigmine side) was low demonstrating that basal DA levels do not differ between the left and right sides.

The TTX sensitivity of basal dialysate DA levels in both the control (no neostigmine) and neostigmine treated dorsolateral striata confirms the findings of previous similar studies (Drew et al., 1989; Santiago and Westerink, 1990) and suggests that a

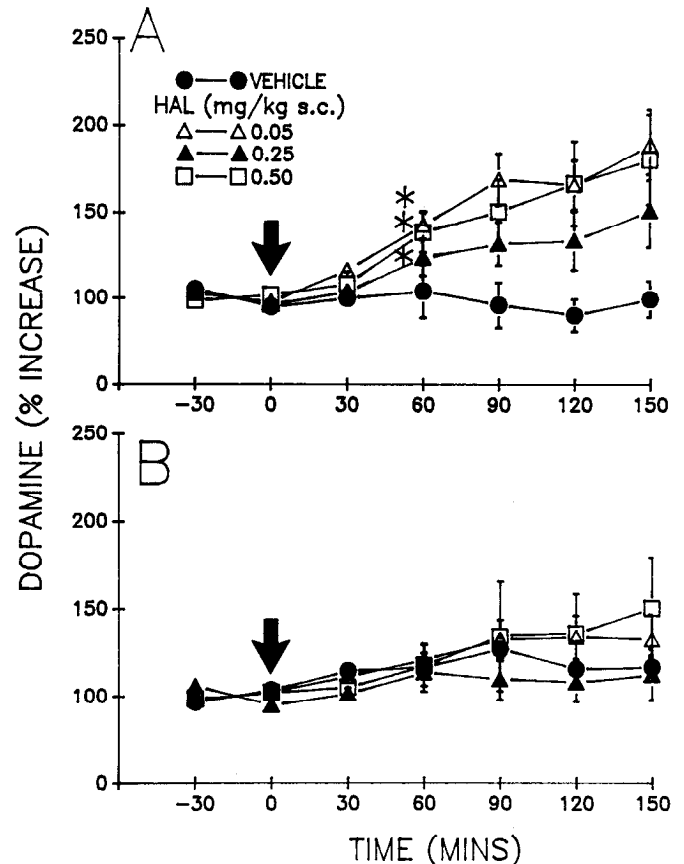


Figure 4. The effect of local perfusion with neostigmine (10 μ M) on the haloperidol (0.05, 0.25, and 0.5 mg/kg s.c.) induced increase dialysate dopamine (DA) levels in the dorsolateral striatum of halothane anaesthetized rats in 30 min Ringer's perfusate fractions. The perfusion medium on the left side (B) contained neostigmine which was included prior to probe implantation and continued to be perfused until the end of the experiment. The contralateral side (A) acted as the control. Haloperidol was administered following two stable baseline levels (commencing 120 min after probe implantation). The vertical arrow indicates the time of haloperidol or vehicle injection. The increase in dialysate DA levels are expressed as a percent of the mean of two basal levels prior to haloperidol. Each data point ($N = 4-7$ per group) represents the mean \pm SEM. Basal dialysate DA on the control (no neostigmine) side were (in nanomolar); 2.10 ± 0.37 , 4.55 ± 1.10 , and 3.37 ± 0.65 for the 0.05, 0.25, and 0.5 mg/kg s.c. treated groups and were maximally increased to 4.07 ± 0.87 , 7.75 ± 2.27 , and 6.40 ± 1.62 , respectively, after haloperidol. Basal dialysate DA levels in the presence of 10 μ M neostigmine were; 3.10 ± 0.17 , 4.12 ± 0.95 , and 4.45 ± 0.45 for the 0.05, 0.25, and 0.5 mg/kg s.c. treated groups and were maximally increased to 4.12 ± 0.37 , 4.42 ± 0.80 , and 6.82 ± 1.42 , respectively, 180 min after haloperidol. Statistical analysis was performed by a two-way ANOVA followed by a Newman-Keuls post-hoc comparison (Crisp Crunch) or a *t* test where indicated. *, $P < 0.05$ versus prehaloperidol levels.

large fraction of basal dialysate DA levels observed in the present study is derived from impulse dependent neuronal release.

The neostigmine induced increase in basal DA levels in the fundus striati is in general agreement with *in vitro* and *in vivo* (push-pull) studies demonstrating that locally applied ACh increases spontaneous DA release in rat and cat striatum (Bartholini et al., 1975; Giorguieff et al., 1977; De Bellerocche et al., 1982; Lehman and Langer, 1982; Rapier et al., 1990) and with *in vivo* microdialysis studies which report an increase in basal DA levels following local perfusion with neostigmine (10 μ M) (Farber et al., 1993), the M_1 receptor agonist AF102B (Xu et al., 1989)

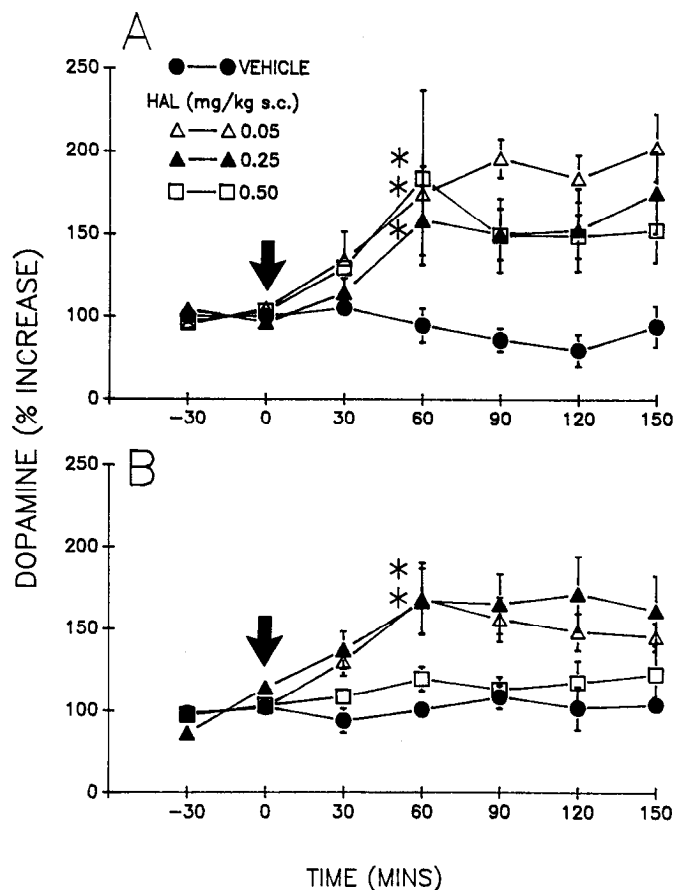


Figure 5. The effect of local perfusion with neostigmine ($10 \mu\text{M}$) on the haloperidol (0.05, 0.25, and 0.5 mg/kg s.c.) induced increase dialysate dopamine (DA) levels in the fundus striati of halothane anaesthetized rats in 30 min Ringer's perfusate fractions. The perfusion medium on the left side (B) contained neostigmine which was included prior to probe implantation and continued to be perfused until the end of the experiment. The contralateral side (A) acted as the control. Haloperidol was administered following two stable baseline levels (commencing 120 min after probe implantation). The vertical arrow indicates the time of haloperidol or vehicle injection. The increase in dialysate DA levels are expressed as a percent of the mean of two basal levels prior to haloperidol. Each data point ($N = 3-4$ per group) represents the mean \pm SEM. Basal dialysate DA levels on the control (no neostigmine) side were (in nanomolar); 2.02 ± 0.35 , 1.20 ± 0.40 , and 1.75 ± 0.4 for the 0.05, 0.25, and 0.5 mg/kg s.c. treated groups and were maximally increased to 3.90 ± 0.52 , 2.40 ± 1.1 , and 3.20 ± 1.07 , respectively, after haloperidol. Basal dialysate DA levels in the presence of $10 \mu\text{M}$ neostigmine were: 2.02 ± 0.15 , 2.72 ± 0.45 , and 4.62 ± 1.50 for the 0.05, 0.25, and 0.5 mg/kg s.c. treated groups and were maximally increased to 3.42 ± 0.52 , 6.37 ± 1.87 , and 5.27 ± 1.95 , respectively, after haloperidol. Statistical analysis was performed by a two-way ANOVA followed by a Newman-Keuls post-hoc comparison (Crisp Crunch) or a t test where indicated. *, $P < 0.05$ versus pre-haloperidol levels.

and systemic nicotine (Damsma et al., 1988) from an area of the striatum incorporating both the dorsal and ventral subregions and implies that this release is occurring primarily in the ventral subregion of the neostriatum. The results of the present study are also consistent with reports that both systemic and local perfusion with nicotine increases DA release in the nucleus accumbens (Imperato et al., 1986).

The selective stimulatory effects of neostigmine on basal DA release in the fundus striati may reflect the regional variability in AChE activity reported within the neostriatum (Joyce and Marshall, 1987; Loopuijt 1989) and/or a direct action of ACh

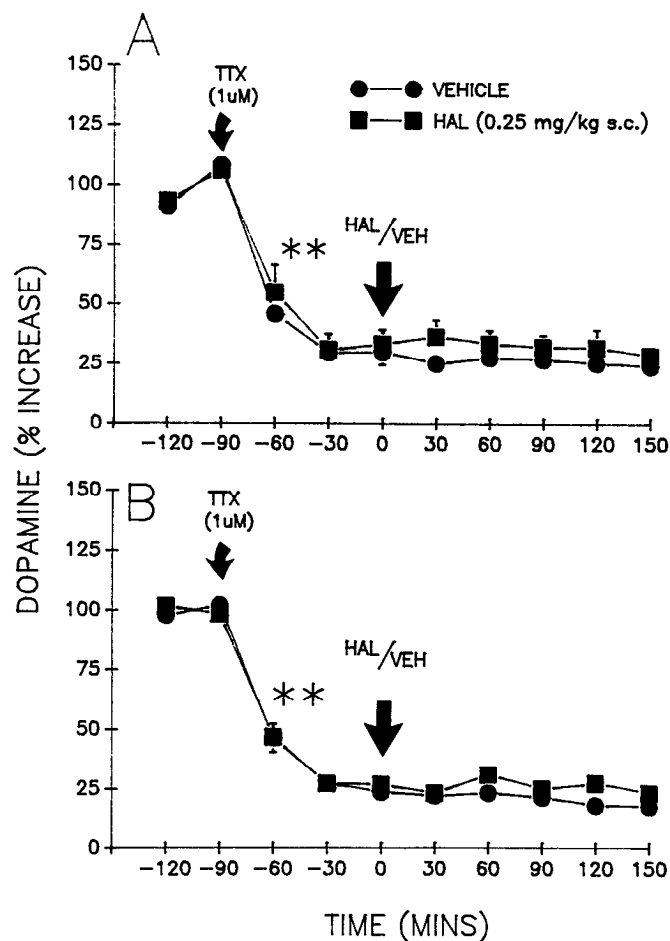


Figure 6. The effect of local perfusion with $1 \mu\text{M}$ tetrodotoxin (TTX) on basal and haloperidol (0.25 mg/kg s.c.) induced increase dialysate dopamine (DA) levels in the control (no neostigmine) and contralateral neostigmine ($10 \mu\text{M}$) treated dorsolateral striata of halothane anaesthetized rats in 30 min Ringer's perfusate fractions. The perfusion medium on the left side (B) contained neostigmine which was included prior to probe implantation and continued to be perfused until the end of the experiment. The contralateral side (A) acted as the control. TTX was locally perfused following two stable DA levels (commencing 120 min after probe implantation) and continued to be perfused until the end of the experiment. Haloperidol was administered 90 min after the onset of perfusion with TTX. The vertical arrow indicates the time of haloperidol or vehicle injection. Basal dialysate DA levels on the control (no neostigmine) side were (in nanomolar); 6.17 ± 0.95 and 4.80 ± 0.97 for the vehicle and 0.25 mg/kg s.c. treated groups and were reduced to 1.77 ± 0.30 and 2.17 ± 0.82 , respectively, 90 min after onset of perfusion with TTX. Basal dialysate DA levels in the presence of $10 \mu\text{M}$ neostigmine were: 7.10 ± 1.32 and 4.05 ± 0.65 for the vehicle and 0.25 mg/kg s.c. treated groups and were reduced to 1.30 ± 0.15 and 1.12 ± 0.30 , respectively, 90 min after onset of perfusion with TTX. The decrease in dialysate DA levels are expressed as a percent of the mean of two basal levels prior to TTX. Each data point ($N = 4-8$ per group) represents the mean \pm SEM. Statistical analysis was performed by a two way analysis of variance ANOVA followed by a Newman-Keuls post-hoc comparison (Crisp Crunch) or a t test where indicated. **, $P < 0.001$ versus pre-TTX levels.

on stimulatory nicotinic and muscarinic M_1 receptors located on DA terminals in this striatal subregion (Xu et al., 1989; 1990). In addition, the fact that extracellular ACh levels are consistently higher in the dorsal compared to the ventral striatum (O'Connor et al., 1991a; Osborne et al., 1994) indicates that stimulatory muscarinic and nicotinic receptors may be already maximally activated in this striatal subregion. However, the fact that anti-

cholinergic drugs have little effect on basal DA levels in the neostriatum either *in vitro* (Memo et al., 1988; Meltzer et al., 1994) or *in vivo* (Damsma et al., 1988) suggest the cholinergic regulation of basal DA release in this striatal subregion may be of a phasic rather than a tonic nature. Differences in the affinity and/or density of stimulatory cholinergic receptor subtypes in the neostriatum may also play a role. The selective facilitation of basal DA release in the fundus striati implies a cholinergic amplification of DA transmission which may be relevant in motivated and goal directed behaviors as well as providing *in vivo* evidence for a role of limbic DA in the central action of ACh.

Effect of haloperidol on dialysate dopamine levels

DA release in both the control (no neostigmine) dorsolateral and fundus striata were increased following all the doses of haloperidol tested. In addition, there was a similarity in the dose response profile in both striatal subregions. In the first fraction (30 min) following haloperidol, dialysate DA levels were elevated and continued to increase until the end of the experiment (150 min). Taken together, these observations suggest that the presynaptic D₂ autoreceptor appears to be primarily involved in the regulation of striatal DA release (Westerink and deVries, 1989) and despite the reported difference in D₂ receptor density (Joyce and Marshall, 1987), has a similar activity in both the dorsal and ventral neostriatum. The magnitude of the haloperidol induced increase in DA release in the dorsolateral striatum was less than that observed in chronically implanted awake rats (Imperato and Di Chiara 1985; Osborne et al., 1994) and probably reflects the different methodological approaches employed, for example, probe design, anesthesia, perfusion medium etc. The TTX sensitive increase in DA release following haloperidol confirms the findings of a similar study by Drew et al. (1990) and suggests that the impulse activity of the nigrostriatal dopamine neurons is a prerequisite for the effect of haloperidol on striatal dopamine release (Bunney et al., 1973; Westerink and deVries, 1989).

The effect of neostigmine on the haloperidol response

Local perfusion with neostigmine counteracted the effects of all doses of haloperidol on DA release in the dorsolateral striatum but this was only observed at the highest dose of haloperidol in the fundus. This is consistent with *in vitro* studies demonstrating that locally applied ACh counteracts ³H-amine release evoked by potassium, electric field and nicotine stimulation in rat neostriatal slices (Westfall et al., 1974a,b) and with recent microdialysis studies indicating that activation of striatal muscarinic M₂ receptors located at least in part on DA terminals, inhibits DA release evoked by nerve stimulation (see Cheselett, 1984; Xu et al., 1989) and implies that this inhibition occurs dorsally (Javoy et al., 1974; Westfall et al., 1974a,b). Striatal ACh and DA also interact indirectly by converging on the medium spiny GABA neuron (Pickel and Chang, 1980) and a differential distribution of both muscarinic receptor subtypes has been recently described in the neostriatum, with increased expression of the stimulatory M₁ subtype on enkephalin containing neurons and inhibitory M₂/M₄ subtypes on substance P containing neurons (Gerfen et al., 1990; Le Moine et al., 1991; Bernard et al., 1992). In addition, local perfusion with neostigmine (10 μM) and systemic haloperidol increases GABA release in the dorsolateral striatum (Drew et al., 1990; O'Connor et al., 1991b, 1992; Osborne et al., 1994). Taken together, these findings suggest that the differential inhibitory action of ACh in the neostriatum seen

in our experiments may also reflect the existence of either different subpopulations of GABA neurons exhibiting a different sensitivity to ACh or different neuronal loops (Xu and Kato, 1988).

Differential cholinergic regulation of dopamine release in the dorsal and ventral neostriatum. Possible mechanisms and implications

In addition to blocking the D₂ receptor and thus diminishing D₂ mediated transmission, haloperidol also blocks the D₂ autoreceptor resulting in an elevation of synaptic DA levels which may exert a partial overcoming effect on DA transmission by competing with haloperidol for at least a portion of postsynaptic D₂ receptors. In the present study we demonstrate that this haloperidol induced increase in synaptic DA levels is decreased following local perfusion with neostigmine in the dorsolateral striatum which leads to a further impairment in DA transmission in this striatal subregion.

Considered in the light of evidence implicating a decrease in DA transmission in the dorsolateral striatum with the mediation of extrapyramidal side effects (Costall and Naylor, 1976), the present findings may help to relate the haloperidol induced increase in striatal ACh release (Bertorelli and Consolo 1990; Damsma et al., 1990; O'Connor et al., 1991a) with the extrapyramidal motor side effects due to the ability of striatal ACh to further impair DA transmission in this striatal subregion and explains why neostigmine may worsen haloperidol induced extrapyramidal motor side effects. Furthermore, the amelioration of haloperidol induced extrapyramidal motor side effects and other "striatal dopamine deficiency syndromes" (Hornykeiwicz, 1973) such as Parkinson's disease, and tardive dystonias by anticholinergics may be due in part, to a restoration of DA transmission in this striatal subregion by antagonizing the inhibitory effect of ACh and may explain why the newer atypical neuroleptics such as clozapine and thioridazine which have potent anticholinergic activity possess few extrapyramidal motor side effects. Furthermore, the relative lack of effect of neostigmine on the haloperidol induced increase in DA release in the fundus striati is consistent with the fact that anticholinergics do not impair antipsychotic efficacy. Taken together, these observations strengthen the evidence that the ventral striatum is the site of antipsychotic action of neuroleptics whereas the extrapyramidal motor side effects are produced by actions on the more dorsal regions of the striatum.

In conclusion, we provide *in vivo* evidence for a differential cholinergic regulation of DA release in the neostriatum and demonstrate that the facilitatory and inhibitory actions of neostigmine operate independently and have a regional specificity within the neostriatum.

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