ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 1, pp. 67–80, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00050-0

Comparison of the Behavioural Effects of Infusion of Carbachol and Acetylcholinesterase Into the Rat Substantia Nigra

C. A. HAWKINS[1] AND S. A. GREENFIELD

University Department of Pharmacology, Mansfield Rd., Oxford OX1 3QT, UK

Received 13 August 1995; Revised 20 January 1996; Accepted 24 January 1996

HAWKINS, C. A. AND S. A. GREENFIELD. *Comparison of the behavioural effects of infusion of carbachol and acetylcholinesterase into the rat substantia nigra.* PHARMACOL BIOCHEM BEHAV **55**(1) 67–80, 1996.—It has been postulated for many years that acetylcholinesterase (AChE) may play a nonclassical role in the substantia nigra, unrelated to its ability to hydrolyse acetylcholine. In this study the behavioural effects of unilateral infusion of AChE and a cholinergic agonist, carbachol, were compared. Carbachol induced ipsiversive circling over a very short time scale (minutes), whereas AChE induced contraversive circling, but over a longer time course—10 days. Both agents showed selectivity of response within the substantia nigra: acetylcholinesterase was only effective when infused into the most rostral region of the substantia nigra and its effects were limited to the pars compacta. In contrast, carbachol had effects in both the pars compacta and reticulata, with a graded sensitivity to carbachol in the rostral/caudal plane: infusions into rostral regions induced high rates of circling compared to more caudal areas, suggesting that the cholinergic input to the substantia nigra is not homogenous, but greater in rostral regions. This disparity between the effects of carbachol and AChE would, therefore, suggest that AChE is not exerting its long-term behavioural actions via a cholinergic mechanism, both in terms of time course of the response and the areas within the substantia nigra sensitive to these agents.

Acetylcholinesterase Carbachol Substantia nigra Circling behaviour Pars compacta Pars reticulata Rostral Caudal

OVER 20 years ago it was observed that within the substantia nigra there is a disparity between the relatively low density of the enzyme that synthesises acetylcholine (choline-acetyltransferase, ChAT) compared to the comparatively high concentration of acetylcholinesterase (AChE), the enzyme that degrades it (40,75). It was subsequently discovered that AChE is released in a soluble form from the dendrites of dopaminergic nigrostriatal neurones (31) and appears to have a action upon them that is independent of the hydrolysis of AChE (33,34). Taken together, these observations have led to the idea that AChE plays a role within the substantia nigra that is independent of its cholinergic function. AChE infused unilaterally into the substantia nigra induces behavioural effects indicative of increased activity of the nigrostriatal pathway, namely, stereotypy (81) and circling (32,38). Circling behaviour in response to amphetamine challenge reflects an imbalance in the activity of the two nigrostriatal pathways (76) and has been used as a model to assess the behavioural effects of AChE on the dopaminergic nigrostriatal system of the unlesioned rat.

The suggestion that AChE may have a role independent of the cholinergic system has, in part, been prompted by disproportionately high levels of AChE in the substantia nigra: however, this area does receive a modest cholinergic innervation, thought to originate mainly from the pedunculopontine tegmental nucleus (7,15,28), which makes synaptic contact with cell bodies and dendrites of dopaminergic neurones (10). Activation of this pathway by cholinergic agonists appears to increase activity of the nigrostriatal pathway, manifest as increased dopamine release and turnover in the striatum (9,41). In addition, unilateral cholinergic stimulation of the substantia nigra itself induces circling behaviour (19,21,42,44,82).

[1]To whom requests for reprints should be addressed.

This series of experiments was, therefore, undertaken to try and determine whether the behavioural effects of AChE are occurring via a mechanism involving a reduction in cholinergic activity, or are, indeed, cholinergic independent, by directly comparing the ability of AChE and a cholinergic agonist (carbachol) to induce circling behaviour following unilateral infusion into the substantia nigra. Three aspects of the phenomena were studied: the time course of the response, the direction of circling that was elicited, and the areas within the substantia nigra that were sensitive to these agents.

METHOD

Wistar rats, weighing approximately 250 g, were anaesthetised with Equithesin (3 ml/kg) and guide cannulae (Plastics Products Co., USA) implanted unilaterally in the substantia nigra at the following coordinates: AP: −5.0; L: 2.2; DV: −7.1, according to the atlas of Paxinos and Watson (69). Prior to and following recovery from surgery, all rats were tested for inherent bias in circling. Animals with a mean score of two turns/min or more were discarded.

Circling behaviour in rats was assessed by placing them in a familiar circular glass bowl (12 inches dia.) and the net number of 360° turns/min noted over a 20-min period.

In the first experiment, 1 μl of either 0.9% saline, carbachol in saline (2 μg/μl), or purified AChE in saline (450 U/ml) was infused through an internal cannula (placed within the implanted guide cannula, such that it protruded approximately 1 mm), connected to a 10 μl Hamilton syringe by inert capillary tubing (0.010″ i.d.) and driven by an automatic pump at a rate of 0.1 μl/min. After 10 min the pump was switched off but the internal cannula left in place for a subsequent 2 min. During infusion rats were freely moving in a restricted area. In previous experiments assessing the effects of an infusion of AChE (38,39), the best effects were obtained when animals were given an IP injection of amphetamine (1 mg/kg) immediately following infusion and were tested for circling behaviour 15 min after the injection for 20 min. The amphetamine acted to enhance any disparities between activities of the two nigrostriatal pathways. However, given that carbachol appears to have a very short duration of action (42), this protocol was modified such that the IP injection of amphetamine was given just prior to infusion, and circling behaviour was assessed for 20 min immediately following infusion. Data was expressed as mean number of turns per minute. To ascertain the long-term actions of AChE and carbachol, all rats were challenged with amphetamine on 4 subsequent days and tested for rotation 15 min later as described above. The AChE infused was purified from the commercial preparation of electric eel AChE obtained from Sigma, according to the technique of Massoulie and Bon (63). Activity of AChE was measured using the Ellman assay (25).

In the second experiment, cannulae were placed over a range of AP placements: −4.8, −5.3, or −6.3. An experimental crossover design was used in which animals were infused with either 1 μl saline or carbachol (2 μg/μl) and subsequently with the other treatment. Animals were tested for circling on the day prior to infusion, immediately following infusion and the day after. Animals were infused over 5 min, with the internal cannulae being left in place for a further 1 min, and animals being tested for circling behaviour over the following 20 min. Circling behaviour was tested in the absence of amphetamine.

The data from the above experiment was compared with that obtained retrospectively from animals infused with acetylcholinesterase (100–500 U/ml) or saline (1 μl over 10 min). At the end of the infusion, animals were given an IP injection of amphetamine (1 mg/kg) and tested for circling behaviour 15 min later. On 9 subsequent days, animals were given an IP injections of amphetamine and tested for circling behaviour.

At the end of the experiment, the animals were anaesthetised and perfused with formaldehyde (10% v/v in 0.9% NaCl w/v). The brains were removed and placed in formaldehyde and sucrose for at least 24 h prior to sectioning. Cannulae placements within the substantia nigra were verified by examination of 50 μm frozen cut sections, stained with cresyl violet. For examples of cannulae placements, see Figs. 1a and b. The rostral/caudal position of the cannula was taken from the location of the centre of the cannula.

Data from longitudinal studies of the effect of infusion of substances into the substantia nigra was analysed using two-way analysis of variance, followed by ad hoc one-way analysis of variance across factors. In experiments in which the same animal received both drug and control treatments, data was analysed using paired *t*-tests.

RESULTS

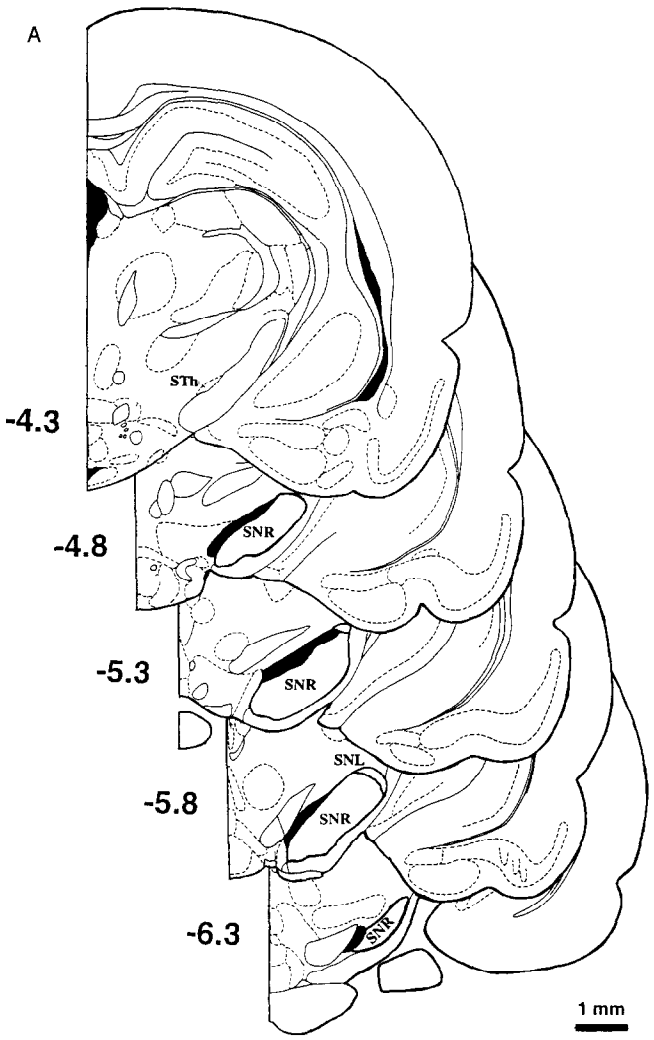
Direct Comparison of Response to Carbachol and AChE

Animals were injected IP with amphetamine, infused with saline ($n = 10$), carbachol ($n = 15$) or acetylcholinesterase ($n = 12$) and tested immediately for circling behaviour (Fig. 2). Ipsiversive circling following carbachol infusion appeared to decrease in a linear manner over 20 min down to control levels, whereas the response to AChE was sustained over this time period. Two-way analysis of variance revealed a significant effect of treatment across the three groups ($F = 6.96$, $p < 0.001$) with significant differences between AChE and saline or carbachol, but no significant differences between carbachol and saline treatment. In no case was there any significant effect of time.

The time course of the effects of carbachol and AChE were not only different within the minutes immediately after infusion, but also on the days following infusion: on four subsequent days animals were tested for circling behaviour 15 min following an IP injection of amphetamine, but no further infusion of AChE (see Fig. 3). Those animals infused with saline showed very little circling behaviour over this time period. However, carbachol and AChE induced very different responses following their infusion: carbachol-infused animals exhibited ipsiversive circling behaviour on day 1, indicative of decreased activity of the nigrostriatal pathway, but not thereafter; in contrast, acetylcholinesterase stimulated contraversive circling behaviour over all 5 days, reflecting increased activity of the nigrostriatal pathway. Data was analysed using two-way analysis of variance with factors, treatment and day: effect across all three treatments ($F = 2.92$, $p < 0.06$); with a significant difference between AChE and saline ($F = 4.74$, $p < 0.05$) and a trend to a difference between AChE and carbachol ($F = 3.53$, $p < 0.07$). In no case was there a significant effect across day.

Optimal Response to the Effect of Carbachol

In the above experiment, both agents were infused under the same conditions to compare directly the effects of AChE and carbachol, for instance, infusion took place following an IP injection of amphetamine and animals were tested for circling behaviour 15 min after the end of the infusion. However, under these conditions the effects of carbachol were not very



dramatic: circling rates of just 0.2 turns/min were induced over the 20-min test period following infusion. One reason for this could be because the time course of the action of carbachol appears to be extremely short (the effect appears to be over in 10 min, see Fig. 2). It is also possible that the effects of carbachol may have been masked by the amphetamine. Therefore, in subsequent experiments the effects of carbachol and amphetamine were tested separately, with each assessed under optimal conditions for that agent. Carbachol was, therefore, infused over a shorter time period (5 min, with the internal cannula left in place for 1 min) and all testing for circling behaviour took in the absence of amphetamine. This modified protocol precluded a direct comparison of the effects of carbachol with AChE, but did produce very much higher rates of circling behaviour with carbachol. Saline infusion had no effect on circling behaviour, whereas carbachol induced significant circling on the day of infusion (Fig. 4): ($p < 0.001$, paired t-test, relative to preinfusion baseline), but not on the day after infusion.

Regions of the Substantia Nigra Sensitive to Carbachol

Following examination of the cannulae placements, animals were first divided into groups on the basis of the location of their cannulae in the vertical plane within the substantia nigra (see Fig. 5). Infusion of carbachol into the pars compacta, dorsal, or ventral pars reticulata induced significant rates of circling behaviour (paired t-test, relative to saline treatment), whereas infusion of carbachol just above the compacta did not. Analysis of the time course of circling behaviour following infusion into these areas (Fig. 6) showed that circling following infusion above the compacta probably occurred as a result of diffusion of carbachol into the compacta below, given that maximum circling rates occurred 3 min after the end of infusion, whereas infusions directly into the compacta and reticulata maximum elicited circling immediately after infusion and the effects had decayed significantly within 3 min.

Animals were subsequently divided into groups on the basis of the location of their cannulae in the rostral/caudal plane of the substantia nigra (see Fig. 1a). Saline infusion induced no circling behaviour in any location (Fig. 7). In contrast,

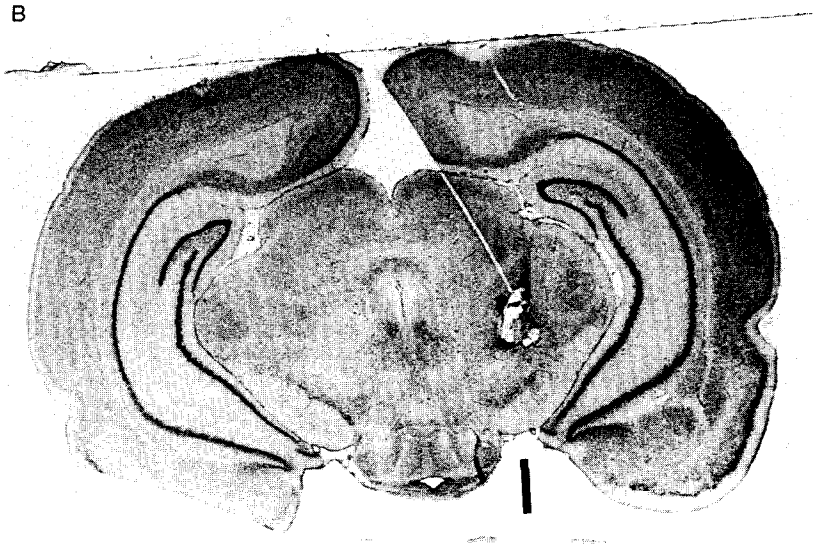


FIG. 1. (A) Drawings based on the atlas of Paxinos and Watson (1982) illustrating the planes of substantia nigra where cannulae tips were placed. The numbers refer to the stereotaxic planes from Bregma. The pars compacta is shaded in black, SNR = pars reticulata, SNL = pars lateralis, STh = subthalamus. (B) A typical cresyl violet-stained section from an animal in which the internal cannula was located within the pars compacta at the AP plane −4.55. Note that the infusion cannula protruded 1 mm beyond the end of the guide cannula, the tip of which is what can be observed in this section. Scale bar = 1 mm.

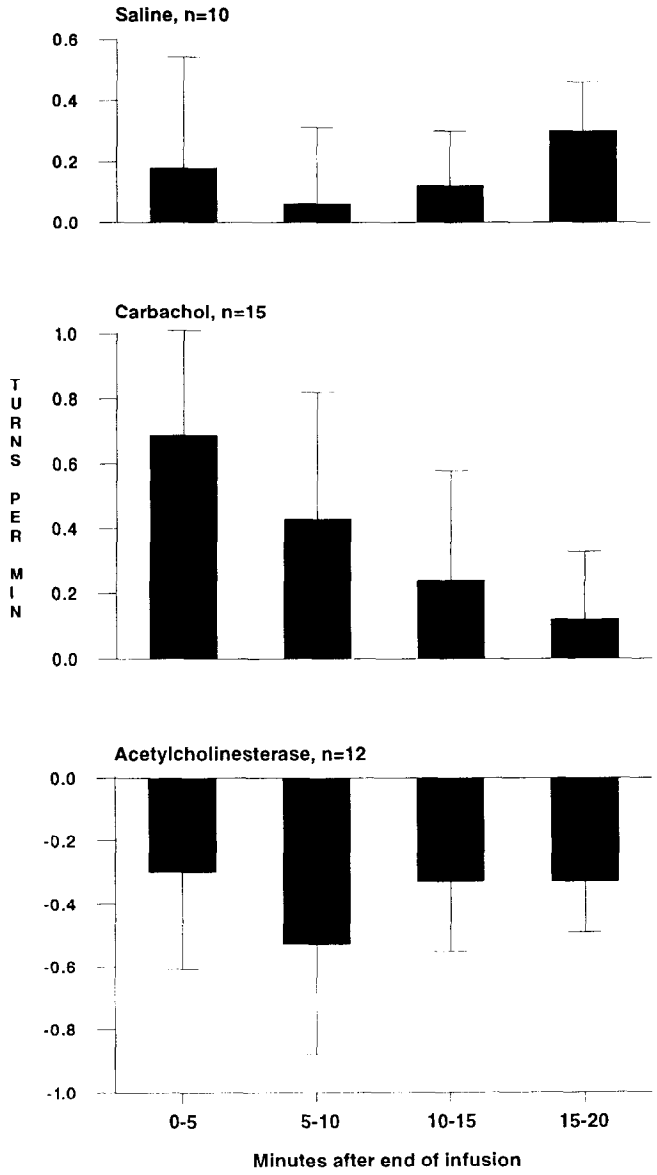


FIG. 2. Effects of saline (n = 10), carbachol (2 mg/ml, n = 15) and AChE (420 U/ml, n = 12) infused unilaterally into the substantia nigra. Animals were given an IP injection of amphetamine immediately prior to infusion and tested for circling behaviour immediately following infusion. Values shown are turns per minute relative to preinfusion baseline circling rates every 5 min following infusion. Negative values indicate contraversive circling, positive values ipsiversive circling.

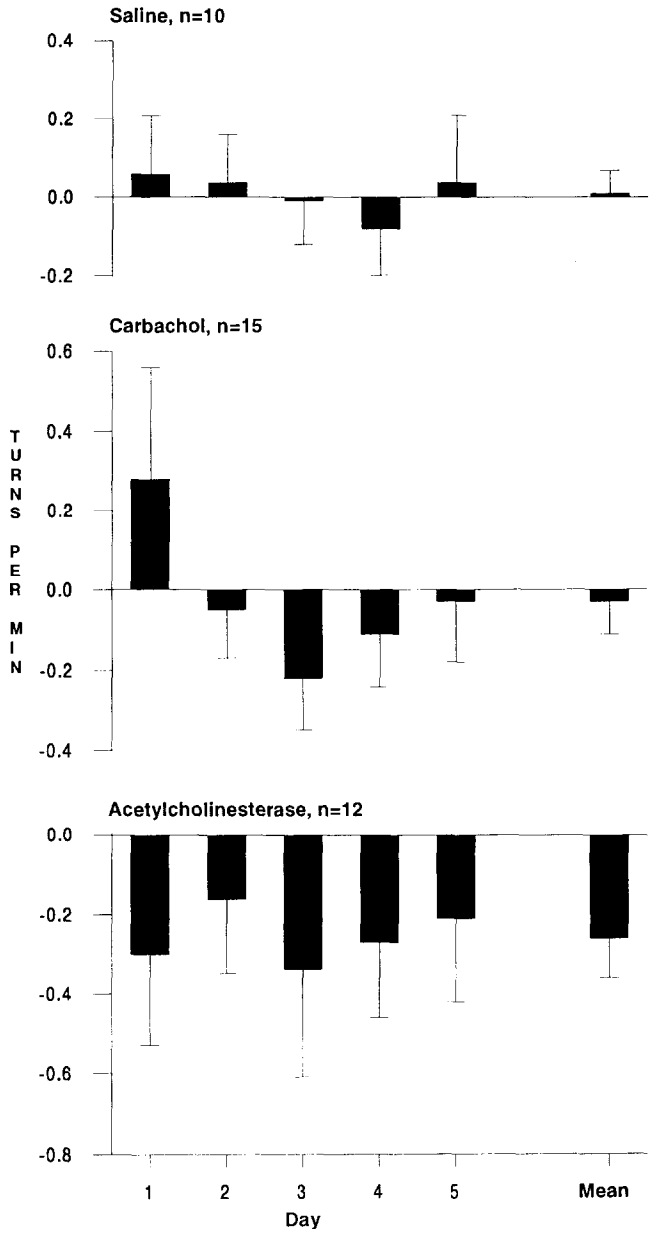


FIG. 3. Effects of saline (n = 10), carbachol (2 mg/ml, n = 15), and AChE (420 U/ml, n = 12) infused unilaterally into the substantia nigra. Animals were given an IP injection of amphetamine immediately prior to infusion on day 1 and tested for circling behaviour over 20 min immediately following infusion. On subsequent days animals were tested for circling behaviour over 20 min, starting 15 min after an IP injection of amphetamine. Values shown are turns per minute relative to preinfusion baseline circling rates ± SEM, plus the mean values from the first 5 days combined. Negative values indicate contraversive circling, positive values ipsiversive circling. There is a significant difference in circling rates between animals treated with AChE and saline, $F = 4.74$, $p < 0.05$, two way analysis of variance).

carbachol infusion induced a clear pattern of circling behaviour dependent on the location of the cannula (anova across AP coordinate: $F = 2.86$, $p < 0.02$): high rates of circling were elicited from rostral regions of the substantia nigra, falling to relatively modest responses in the most caudal areas. A similar pattern was observed when AP placements in the pars compacta or dorsal or ventral reticulata alone were considered (data not shown), with high rates of circling occurring in positions in front of AP −5.55 and relatively low rates of circling behind this location.

This graded relationship between circling response and AP location does not appear to occur as a result of diffusion of carbachol from caudal areas to a rostral region that is sensitive to the drug: analysis of the kinetics of the response following infusion shows a similar time course in all sensitive areas of

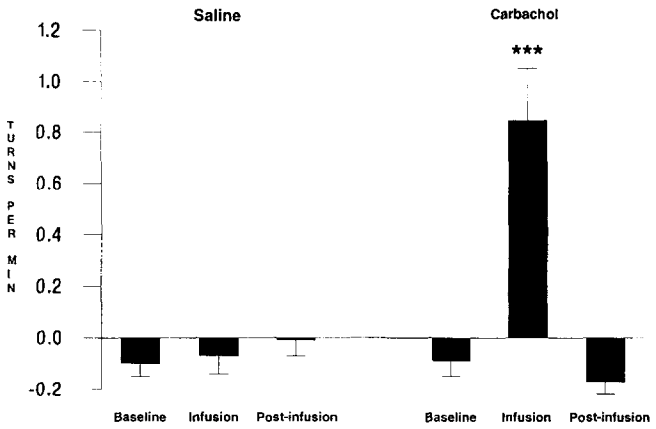


FIG. 4. Effects of saline and carbachol (n = 46, crossover design) infused unilaterally into the substantia nigra. Animals were tested for circling behaviour over 20 min the day before infusion (baseline), immediately after infusion, and the day following infusion. Values shown are turns per minute ± SEM: negative values indicate contraversive circling, positive values ipsiversive circling. ***$p < 0.001$, relative to baseline, paired t-test.

the substantia nigra (Fig. 8). There is no delay in circling response in more caudal regions (which is what would have been expected if the carbachol had to diffuse to a more rostral site to produce its effect) but simply a diminished response over the same time course (AP −5.8) or little response at all (AP −6.05, −6.3).

Regions of the Substantia Nigra Sensitive to Acetylcholinesterase

This pattern of sensitivity to carbachol was then compared to that seen with AChE. Circling data from a total of 234 animals (86 infused with saline and 146 infused with AChE) was collected from previous experiments and are collated in Fig. 9. Animals had been given an IP injection of amphetamine immediately following infusion and were tested for circling behaviour 15 min later. On subsequent days animals were simply tested for circling behaviour 15 min following an IP injection of amphetamine. No significant effect was observed on day 1, immediately following infusion with AChE, compared to saline controls, but significant rates of contraversive circling were observed on subsequent days. To study the areas of the substantia nigra sensitive to AChE, therefore, the mean value for circling behaviour over 10 days was used, rather than circling behaviour on day 1.

Circling behaviour in relation to location of the cannulae in the vertical plane can be seen in Fig. 10. Although there is a trend to an effect of AChE when it is infused above the compacta, only when infused directly into the pars compacta itself does AChE elicit significant rates of circling over the 10 days following infusion, compared to the relevant saline controls. There is no effect, compared to controls, when AChE is infused into either the dorsal or ventral half of the reticulata. However, a contraversive response is sometimes noted following saline infusion into this region (see Fig. 10). This may result from the fact that during infusion the internal cannula has been pushed down through the compacta layer and may itself have caused some damage to these neurones, resulting in a long-term circling response.

Figure 11 shows the same data analysed in terms of rostral/caudal location of the cannulae. Although circling rates in all

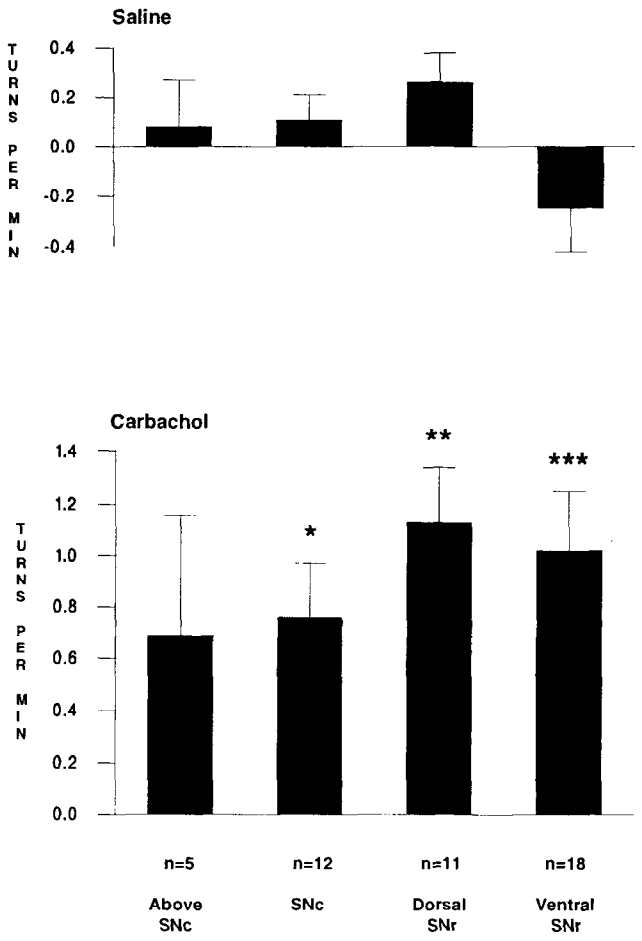


FIG. 5. Circling rates over 20 min following infusion of carbachol or saline into the substantia nigra (n = 46, crossover experimental design) in relation to vertical placement of the cannula within or above the substantia nigra. Values shown are turns/min ± SEM over 20 min, relative to preinfusion baseline rates. Negative values indicate contraversive circling, positive values ipsiversive circling. *, **, ***$p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, relative to corresponding value for saline treatment, paired t-test.

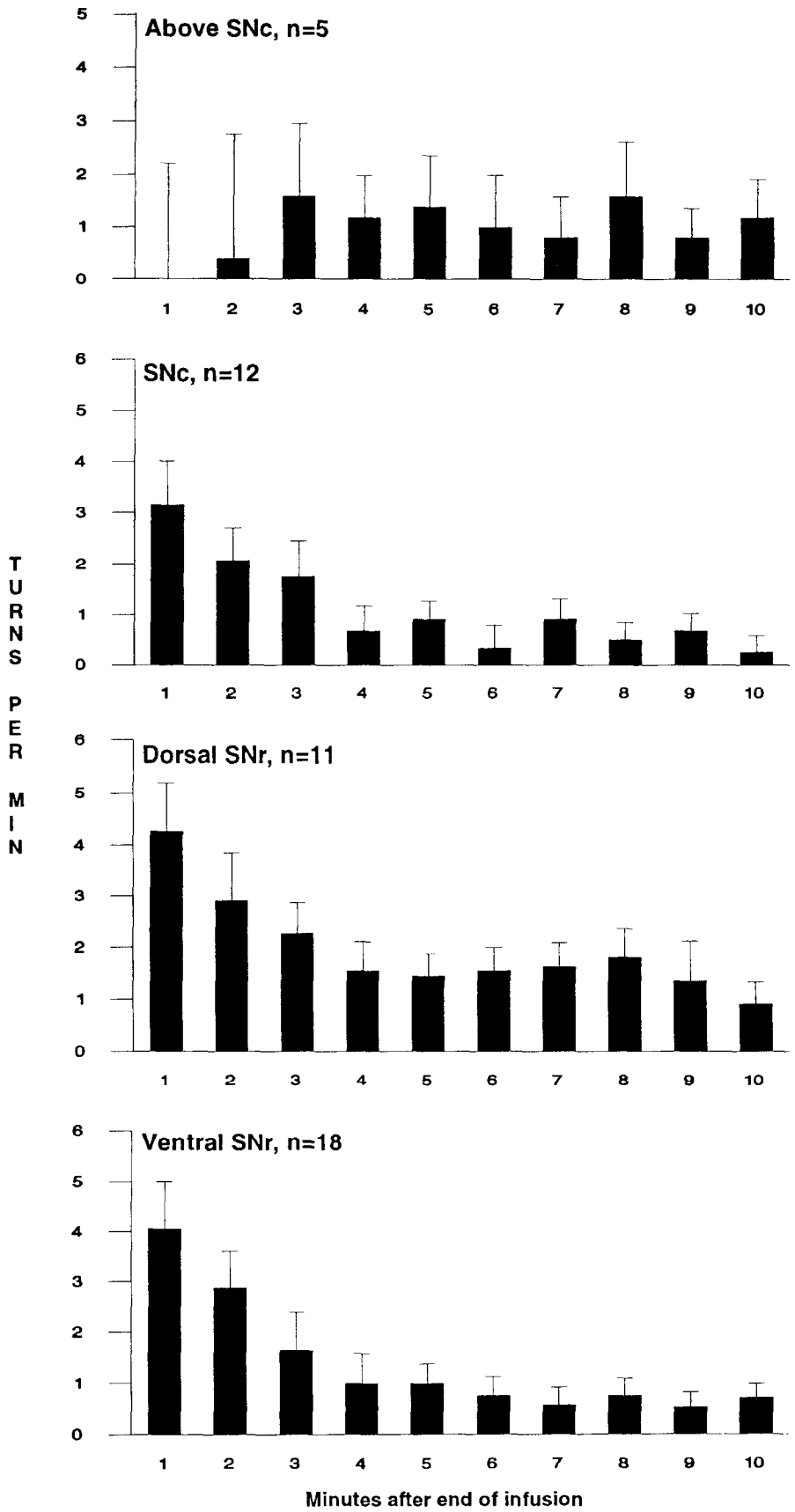
areas were increased following AChE infusion (compared to saline controls) only when the cannula was placed at the very rostral region of the substantia nigra (AP −4.55) was there a significant difference between circling rates induced by saline and AChE ($p < 0.02$, Student's t-test). A similar pattern was observed when data from cannulae placements within the pars compacta alone were analysed (data not shown).

If data are considered just from the locus where maximal effects are seen, for instance, −4.55 in the pars compacta, AChE induces net rates of circling of approximately 1.5 turns per minute compared to controls in the same location (AChE −1.23 ± 0.48 turns per minute, n = 8; saline +0.49 ± 0.36 turns per minute, n = 5; $p < 0.05$, Student's t-test). A typical example of such a cannula placement is shown in Fig. 1b.

DISCUSSION

Behavioural Effects of Carbachol

Unilateral cholinergic stimulation of the substantia nigra is usually reported to elicit ipsilateral circling (19,21,44,82).


Above SNc, n=5
SNc, n=12
Dorsal SNr, n=11
Ventral SNr, n=18
TURNS PER MIN
Minutes after end of infusion

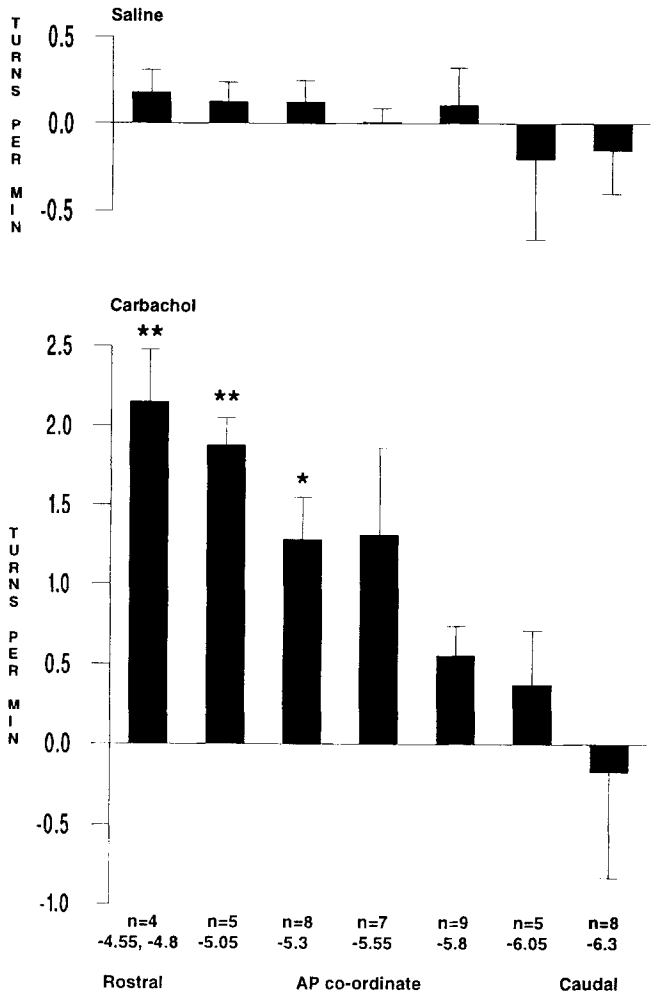


FIG. 7. Circling rates over 20 min following infusion of carbachol or saline into the substantia nigra ($n = 46$, crossover experimental design) in relation to AP coordinate of the cannula. Values shown are turns/min ± SEM over 20 min, relative to preinfusion baseline rates. Negative values indicate contraversive circling, positive values ipsiversive circling. *, **$p < 0.05$, $p < 0.01$ respectively, relative to corresponding value for saline treatment, paired t-test.

However, in a few studies it has been reported that the direction of circling elicited by carbachol infused within the reticulata was dependent on the site where the drug was applied [rostral or caudal in rats (5), medial or lateral in cats (65)]. Consistent with these observations, carbachol infused into the compacta was reported more recently to stimulate ipsiversive circling in rostral regions, but contraversive circling in caudal areas of the compacta (42).

In this study, carbachol elicited ipsiversive circling in both pars compacta and reticulata, and a graded relationship between circling response and AP coordinate was observed, such that high rates of ipsiversive circling were found in rostral areas with a graded decrease to little circling at the caudal end of the substantia nigra. The kinetics of the response indicated that this graded difference was not due to diffusion of the carbachol to a sensitive site in the rostral substantia nigra, because in all areas circling was maximal between 0 and 3 minutes, for instance, there was no delay in response when the carbachol was infused into more caudal regions. This data, therefore, suggests that there is a gradation of cholinoceptivity in the rostral/caudal extent of the substantia nigra.

Cholinergic Receptors Within the Substantia Nigra

Cholinergic input to the substantia nigra occurs predominantly from the pedunculopontine nucleus, which projects to both pars reticulata and compacta (28). While ChAT staining in the substantia nigra is relatively sparse, it is greater in the compacta than reticulata, with ChAT positive synapses abutting onto dopaminergic neurones in the pars compacta and onto dopaminergic dendrites in the pars reticulata (10).

Nicotinic receptors are only found in the pars compacta (17,22) and the presence of nicotinic receptors on dopaminergic neurones in the pars compacta has been clearly demonstrated (16). The situation with muscarinic receptors is less well defined. All three pharmacologically defined subtypes have been identified in the substantia nigra by autoradiography: M_1 and M_2 are located in both pars compacta and reticulata (66) and M_3 receptors primarily in the pars reticulata (26). Molecular biological techniques, however, have identified two further receptor subtypes (m4 and m5), which have a mixed M_1/M_2 profile (11). Only the m5 subtype mRNA has been identified in the cell bodies of neurones in the substantia nigra (79), located within dopaminergic neurones of the pars compacta (77). A significant proportion of muscarinic receptors are thought to occur on nondopaminergic elements, namely afferent terminals, as muscarinic binding sites in rat substantia nigra are only partially depleted when dopamine neurones are lesioned with 6-hydroxydopamine (20,73). This, therefore, suggests that cholinergic receptors occur not only on dopaminergic nigrostriatal neurones, but also presynaptically on neurones projecting to the substantia nigra, for example, on cholinergic afferent terminals from the subthalamus (79) and GABAergic striatonigral terminals (8) regulating release of acetylcholine and GABA, respectively (51,62).

Actions of Carbachol on Dopaminergic Neurones in the Pars Compacta

Acetylcholine applied to presumed dopaminergic neurones in the pars compacta during electrophysiological studies in vivo has been found to be either ineffective (18,70) or excitatory (24,60). Cholinergic stimulation of dopaminergic neurones both in vivo and in vitro increases their firing rate through activation of both nicotinic (15,60) and muscarinic (M_1) receptors (55) and results in increased dopamine release (9) and turnover in the striatum (41).

The evidence would, therefore, suggest that unilateral cholinergic stimulation of nigrostriatal compacta neurones should induce contraversive circling. While there is one report of this predicted direction of circling following infusion of cholinergic agonists (83), the majority of studies (19,21,44,82) confirm the observations reported here, that of ipsiversive circling. This suggests that, in terms of behaviour at least, cholinergic ago-

FIG. 6. Time course of the effects of carbachol infusion in relation to vertical placement of the cannula in or above the substantia nigra. Values shown are turns per minute ± SEM) over 10 min following the end of infusion. Negative values indicate contraversive circling, positive values ipsiversive circling.

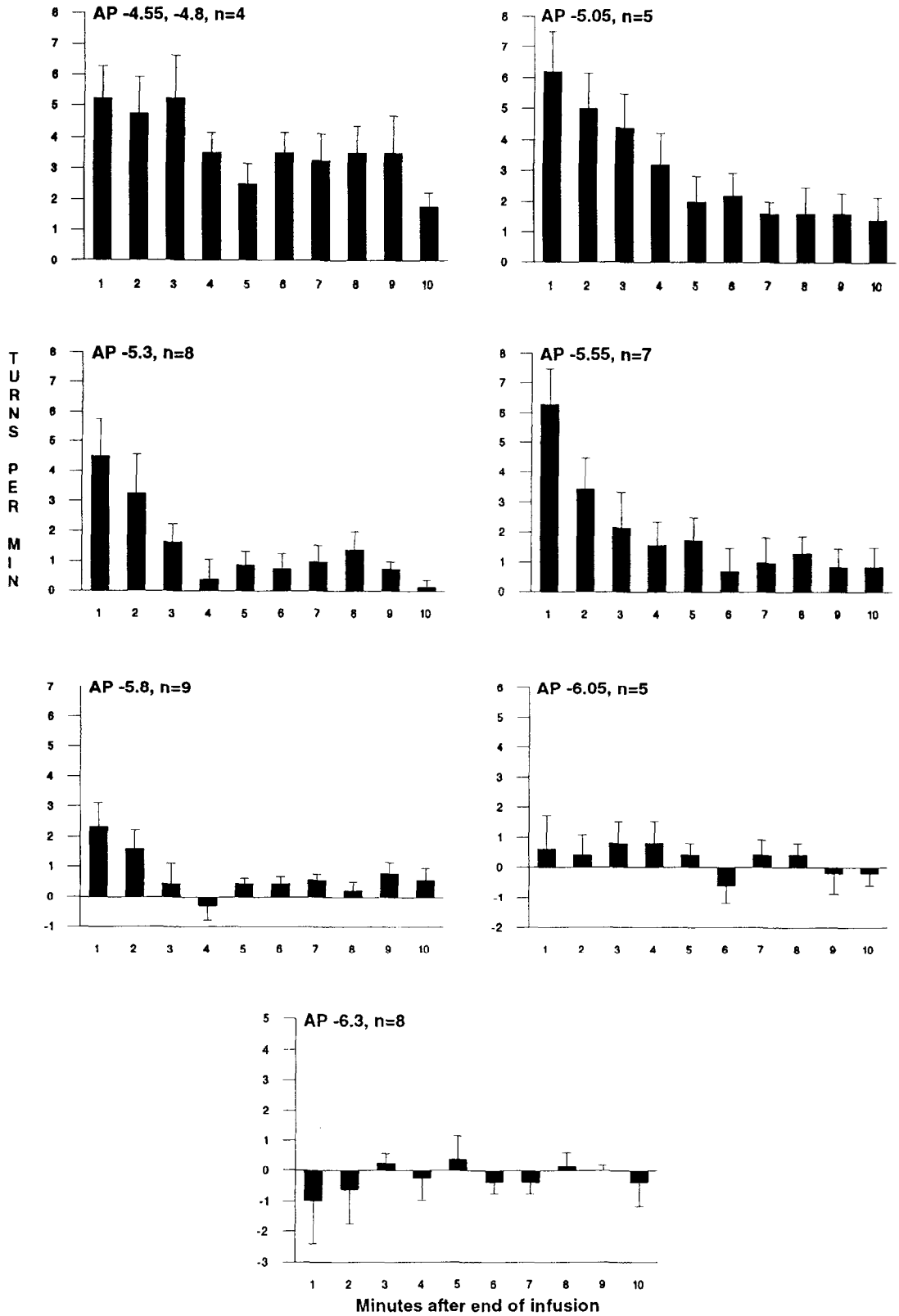


FIG. 8. Time course of the effects of carbachol infusion in relation to AP coordinate of the cannula in the substantia nigra. Values shown are turns per minute ± SEM over 10 min following the end of infusion. Negative values indicate contraversive circling, positive values ipsiversive circling.

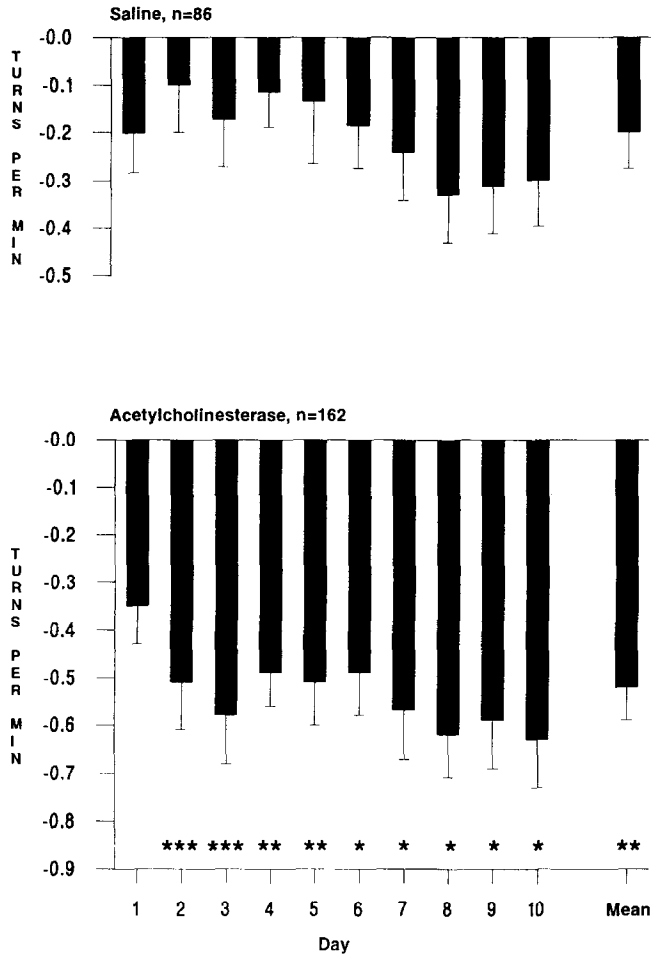


FIG. 9. Effects of saline (n = 86) and AChE (100–500 U/ml, n = 146) infused unilaterally into the substantia nigra. Immediately following infusion on day 1 animals were given an IP injection of amphetamine and tested for circling behaviour 15 min later. On 9 subsequent days animals were given an IP injection of amphetamine and tested for circling behaviour 15 min later. Values shown are turns per minute relative to preinfusion baseline circling rates ± SEM, plus the mean values from the first 10 days combined. Negative values indicate contraversive circling, positive values ipsiversive circling. *, **, ***$p <$ 0.05, $p < 0.01$, $p < 0.001$, respectively, relative to corresponding value for saline treatment, Student's t-test.

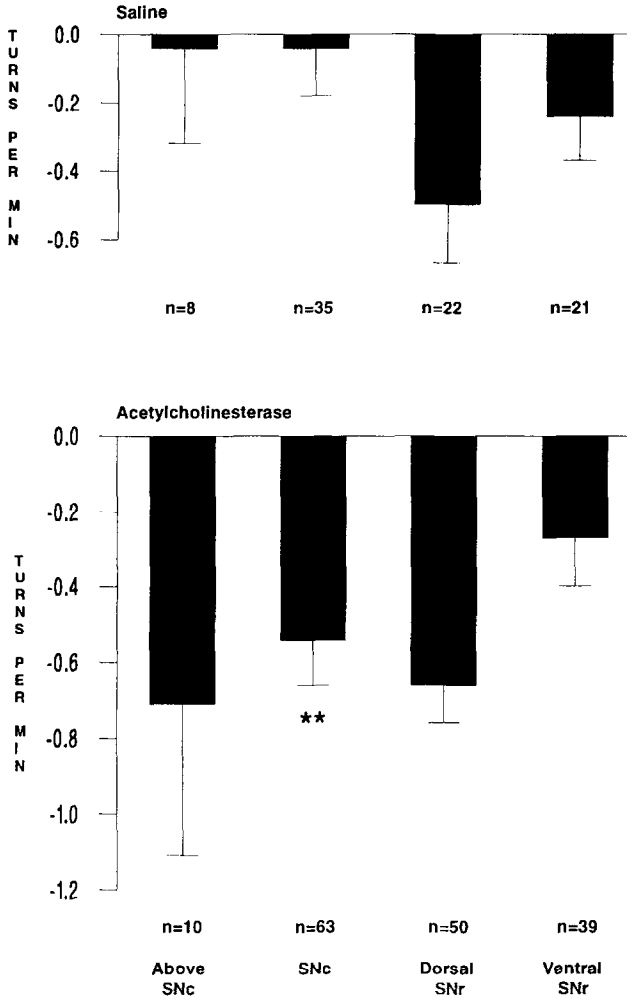


FIG. 10. Mean circling rates over 10 days following infusion of acetylcholinesterase or saline into the substantia nigra, in relation to vertical placement of the cannula (see legend to Fig. 9 for protocol). Values shown are turns/min ± SEM relative to preinfusion baseline rates. Negative values indicate contraversive circling, positive values ipsiversive circling. **$p < 0.01$, relative to saline treatment, Student's t-test.

nists are not acting directly to excite dopaminergic nigrostriatal neurones, but may have some indirect mechanism of action.

Nigrostriatal dopaminergic neurones release dopamine from their dendrites (14) and acetylcholine enhances such release from dendrosomes of the rat substantia nigra (80) via a receptor of the M_1 type (62). It is possible, therefore, that the effect of carbachol in the rostral compacta is indirect, stimulating the release of dendritic dopamine, which, by hyperpolarising dopaminergic neurones (55,67), would decrease their firing and induce ipsiversive circling. Irregularly firing dopaminergic neurones within the rostral segment of the rat pars compacta are more sensitive to the inhibitory actions of iontophoretically applied dopamine than burst-firing and regular-firing cells in the most caudal region of the compacta (74), which could be one explanation for the rostral/caudal difference in sensitivity to carbachol observed in the present study.

Alternatively, carbachol may not be acting directly on nigrostriatal neurones via M_1 receptors but presynaptically on afferents to the pars compacta to regulate release of transmitter via M_2 receptors. Stimulation of these two receptor types produces opposing behavioural effects on the activity of nigrostriatal dopaminergic neurones (27): activation of M_1 receptors increases DA metabolism in the neostriatum, whereas activation of M_2 appears to decrease it. Carbachol is a mixed M_1/M_2 agonist, but has greater affinity for the M_2 receptor subtype (71). Some non-M_1 type muscarinic autoreceptors are located on nigral cholinergic afferent terminals and mediate inhibition of acetylcholine release (62). A reduction in cholinergic activity as a result of administration of cholinergic agonists could also, therefore, explain the decrease in activity of the nigrostriatal pathway and consequent ipsiversive circling.

Within the substantia nigra it has been demonstrated that

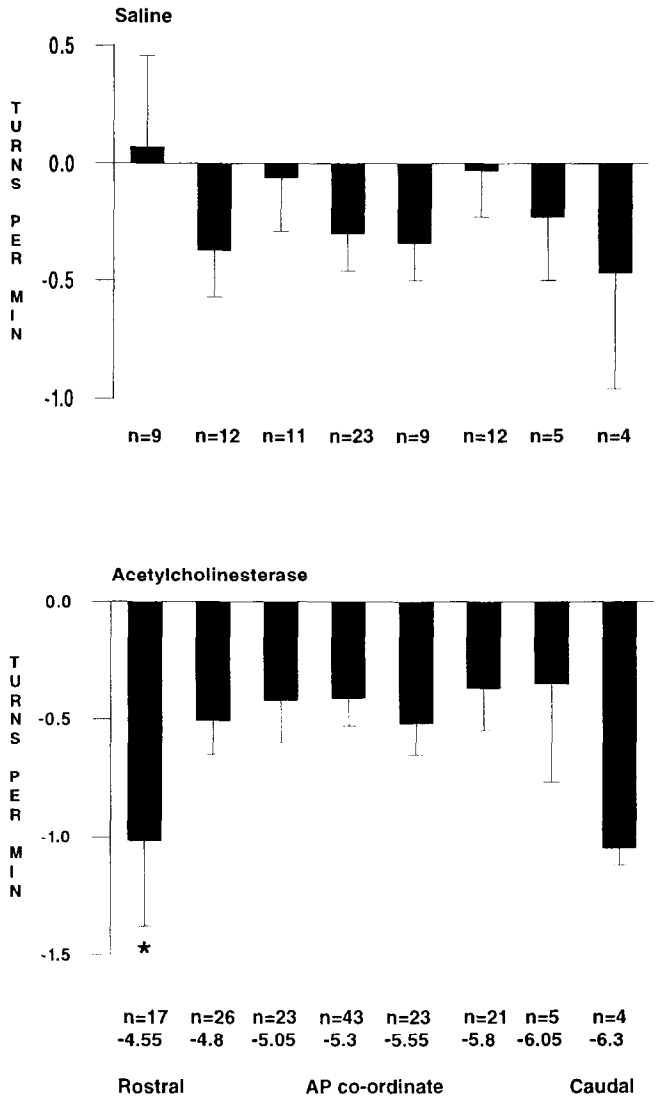


FIG. 11. Mean circling rates over 10 days following infusion of acetylcholinesterase or saline into the substantia nigra, in relation to AP placement of the cannula (see legend to Fig. 9 for protocol). Values shown are turns/min ± SEM relative to preinfusion baseline rates. Negative values indicate contraversive circling, positive values ipsiversive circling. *$p < 0.05$, relative to saline treatment, Student's t-test.

there are muscarinic receptors located presynaptically on striatonigral terminals, regulating the release of the transmitter GABA (50): application of nicotine to nigral slices increases spontaneous release of GABA (51). Striatonigral neurones project back onto nigrostriatal neurones as part of a negative feedback loop, so increased GABA release would lead to a decrease in activity of dopaminergic neurones projecting to the striatum (and, hence, ipsiversive circling). Striatonigral neurones also project onto the GABAergic output neurones from the nigra, located in the pars reticulata, which themselves project to the thalamus: greater inhibition of these output neurones would result in contraversive circling. The net effect of cholinergic stimulation of GABA release, therefore, depends on whether it is infused into the compacta or reticulata: only those effects following infusion into the compacta are consistent with those observed behaviourally following infusion of carbachol.

The Actions of Carbachol Within the Pars Reticulata

There is general agreement that microiontophoretically applied acetylcholine excites cells in the pars reticulata (1,18,24,70). This is thought to occur primarily via activation of muscarinic receptors, as nicotinic receptors are only found within the pars compacta (17,22).

Cholinergic terminals within the reticulata synapse onto the apical dendrites of dopaminergic neurones (10). The actions of carbachol infused into the pars reticulata could, therefore, be occurring via cholinergic stimulation of receptors on these dendrites. A direct effect of carbachol in this manner, however, would, as indicated above, result in excitation of nigrostriatal neurones and, hence, contraversive circling. An effect of carbachol on apical dendrites of nigrostriatal neurones must, therefore, be postulated as occurring indirectly via dendritic release of dopamine. Indeed, ipsiversive circling produced by carbachol injected into the pars reticulata is abolished when carbachol is injected simultaneously with the dopamine antagonist, α-flupenthixol (44).

Muscarinic receptors, however, occur predominantly on nondopaminergic elements in the substantia nigra (20,73). In vivo studies have shown that GABAergic projection neurones of the reticulata increase their firing rate when acetylcholine is applied by iontophoresis (18). Given that this output from the pars reticulata to the thalamus is inhibitory in nature, thereby reducing thalamic activation of cortical areas, activation of this pathway by carbachol could, therefore, result in the induction of ipsiversive circling. In addition, there is evidence that reticulata neurones send axon collaterals that synapse with the dopaminergic neurones of the compacta (29,36). Thus, when GABAergic neurones of the reticulata are stimulated, an inhibitory IPSP is elicited in many dopaminergic neurones in the compacta (36), a mechanism that could also contribute to the induction of ipsiversive circling.

While there are, as yet, few studies regarding differential sensitivity to cholinergic agonists in the rostral/caudal extent of the substantia nigra, there is considerable evidence for functional heterogeneity within this domain of the pars reticulata. At the behavioural level, substance P induces contralateral turning when injected into the caudal pars reticulata (45) but ipsilateral turning and asymmetry when injected into the rostral region (5). Similarly, the direction of circling elicited by GABA antagonists is dependent on the site within the reticulata (5) (contralateral in rostral areas and ipsilateral in caudal regions). Finally, opiates stimulate contralateral turning following infusion into rostral regions of the reticulata (43) but ipsiversive circling in caudal regions (6).

In the pars compacta, therefore, carbachol would appear to be acting not directly to excite dopaminergic neurones, but via a number of possible indirect mechanisms. Direct stimulation of GABAergic output neurones in the pars reticulata could explain the ipsiversive circling induced by infusion in this area, but an indirect action on nigrostriatal dendrites is also possible.

Behavioural Effects of Acetylcholinesterase

Acetylcholinesterase also induces circling behaviour when infused into the substantia nigra. However, its actions appear to be very site-selective: AChE only induced significant rates of circling when infused into the pars compacta and not into

the reticulata. In addition, a response was only seen in the most rostral part of the substantia nigra. The pattern of selectivity observed in this study differs somewhat from that observed in a previous preliminary report (38) where there appeared to be a site in the rostral half of the substantia nigra where ipsiversive circling was induced by AChE, in contrast to contraversive circling exhibited in other areas of the substantia nigra. The difference between the two studies may be attributed to the fact that the AChE originally used was not affinity purified, and may, therefore, have contained contaminants responsible for these ipsiversive effects. Indeed, a commercially obtained form of BuChE induced an ipsiversive circling response (unpublished observations), whereas the laboratory-purified preparation had no effect at all (50). An additional consideration may be that the numbers in the original study were relatively small [46] compared to the 234 animals in this current study.

Given the fact that saline itself, or rather needle-induced necrosis, also induces contralateral turning, it might be argued that AChE is simply exacerbating an nonspecific lesion effect. However, these nonspecific lesion effects are greatest in the dorsal part of the substantia nigra pars reticulata, whereas AChE exerts its greatest effects in rostral areas of the pars compacta, suggesting that AChE has a more specific mechanism of action. As acetylcholinesterase only induces significant rates of circling behaviour when infused into the very anterior part of the substantia nigra, it could be argued that AChE is diffusing from here and exerting its effects in the subthalamus, which lies rostral to the substantia nigra. The subthalamus has a projection to the substantia nigra (13,37,52) and cholinergic stimulation of this pathway reduces activity of the nigrostriatal pathway (64). AChE could, therefore, be acting in the subthalamus to reduce cholinergic activation of this pathway, resulting in increased activity of the nigrostriatal pathway and, hence, contraversive circling. However, analysis of cannulae placements outside the substantia nigra at an AP coordinate of −4.3 shows that AChE infused into this area has no effect (mean rates of circling over 10 days following infusion: AChE -0.18 ± 0.3, $n = 24$; saline -0.27 ± 0.26, $n = 10$). Thus, the behavioural effects of AChE do appear to be limited to the substantia nigra, or more specifically the most rostral part of the substantia nigra in the region of the pars compacta. The effects of AChE also appear to be specific to the pars compacta and not either the dorsal or ventral reticulata.

Biochemical (59) and light microscopy studies (12) have suggested the AChE is located primarily in dopaminergic nigrostriatal neurones within the compacta and the dendrites of these neurones that project down into the pars reticulata (12). Within the pars compacta of the squirrel monkey, distribution of AChE is not homogenous, but discrete patches of AChE-rich and AChE-poor compartments have been identified (46). The rostral/caudal distribution of AChE staining has not been studied to any extent, but Jimenez-Castellanos and Graybiel (47) have reported a caudal 'densocellular' subdivision of the substantia nigra pars compacta, corresponding to a uniquely AChE-poor zone, with densely packed cell bodies staining heavily for tyrosine hydroxylase.

AChE is released from dendrites of nigrostriatal neurones (32): such release from brain slices is thought to occur from the more dorsal regions of the substantia nigra, close to the cell bodies of pars compacta neurones (61). Jones (48), however, found no difference in spontaneous release of AChE in vivo between pars compacta and pars reticulata, although there was a significant difference between rostral and caudal regions of the substantia nigra, with almost twice as much AChE being released from rostral regions compared to caudal. This, therefore, suggests that AChE is stored in and released from neurones within the compacta, particularly rostral regions, and presumably has an action locally within this particular area of the substantia nigra.

In addition to the histochemical and cytoarchitectural specialisations of the pars compacta (46,47), there is now also mounting evidence for functional heterogeneity. Electrophysiological studies conducted in vivo and in vitro have shown that substantia nigra dopaminergic neurones constitute a heterogeneous population. Extracellular recordings performed in anaesthetised rats have demonstrated that dopaminergic neurones exhibit at least three firing patterns: burst, regular, and irregular (74). Moreover, these cell types are not uniformly distributed along the rostral–caudal extent of the pars compacta. Burst-firing neurones are most frequently observed in the caudal half of the compacta, whereas irregular-firing cells are more abundant in the rostral half. Similarly, intracellular recordings performed in midbrain slices of guinea pig have revealed the existence of two subsets of purported dopaminergic neurones that differ both in firing pattern and also in their anatomical localisation within the rostral–caudal extent of the compacta (68). Dopaminergic neurones that show a rhythmic discharge pattern are more abundant in the caudal portions of the compacta, whereas those exhibiting phasic discharge predominate in rostral regions.

Electrophysiological studies in the guinea pig have identified that the phasic cells are particularly sensitive to AChE (78): these neurones are concentrated within the rostral part of the nucleus at the level of the mamillary body (which equates with the AP level of −4.8 in the rat). Morphologically, these neurones possess long apical dendrites running into the pars reticulata. However, sectioning the reticulata from the compacta, and, hence, removing the apical dendrites from these neurones did not abolish their sensitivity to AChE (35), suggesting an action on the more proximal part of the dendritic tree or the soma itself. However, caution must be exercised in extrapolating from these short-term electrophysiological actions of AChE to the long-term behavioural effects observed in the study reported here, where effects can take longer than an hour to be manifest completely.

Evidence from a wide range of completely different types of study, therefore, support the hypothesis that the rostral part of the compacta is more sensitive to the actions of AChE than caudal regions. However, the reason why its action should apparently be so limited to the very rostral edge of the substantia nigra is not so evident, but suggests that in this location there is a small subpopulation of pars compacta neurones peculiarly sensitive to the actions of AChE. The fact that the areas of the substantia nigra sensitive to AChE are so proscribed could explain why the response to AChE infusion has often been variable: the proportion of cannulae in the required location may often be small and no overall effect of AChE is registered.

Comparison of the Behavioural Effects of Carbachol and Acetylcholinesterase

The circling responses elicited by carbachol and AChE are in opposite directions, so the actions of AChE could, therefore, most readily be explained by a reduction of cholinergic input to the substantia nigra. However, the responses differ in two important respects; namely, time course and potency within the substantia nigra.

When the two agents were compared directly under identi-

cal experimental conditions, the duration of the effects induced by these two agents was very different; first, carbachol induces circling behaviour very rapidly and its effect is virtually finished approximately 10 min after the end of infusion, with a linear decrease in response over this time frame. In contrast, the response to AChE is maintained at a steady rate over the 20 min following infusion. Similarly, the effects with carbachol are only observed on the day of infusion and not on subsequent days, i.e., the effects are acute, whereas circling behaviour following infusion of AChE was maintained over the 5 days in which the animals were tested, and, indeed, has previously been observed to last for at least 40 days (38). This profile suggests that carbachol is acting via activation of acetylcholine receptors, whereas the duration of action of AChE is commensurate with a more modulatory mode of action. If AChE were acting via hydrolysis of acetylcholine, this extended time course is hard to explain.

These two agents also exhibited a very different time course when they were each assessed independently under their own optimal conditions (immediately following infusion for carbachol, but 15 min after an IP injection given immediately after infusion for AChE): carbachol had a maximum effect in the minute following the end of infusion, and the response was virtually over within 3 min. There was no circling on the following day. AChE, in contrast, did not induce a significant change in circling behaviour until day 2, and this response was maintained over all subsequent days tested.

These two agents also differed in their site sensitivity within the substantia nigra. The substantia nigra exhibited a graded response to carbachol, in terms of rostral/caudal extent, with high rates of circling being elicited in rostral regions of the substantia nigra and diminishing low levels of circling at the caudal end of the substantia nigra. A different pattern of response was observed when AChE was infused. In this case, a contraversive response was only seen in the most rostral part of the substantia nigra. In addition, AChE only induced significant rates of circling when infused into the pars compacta, and not into the reticulata, whereas carbachol induced circling when infused into either the pars compacta, or dorsal or ventral halves of the pars reticulata. The greater range of sensitivity to carbachol cannot be attributed to its diffusion because kinetic analysis of all responses indicated that maximum rates of circling were observed within 1 min.

A Nonclassical Role for Acetylcholinesterase in the Substantia Nigra

These temporal and spatial differences in response to carbachol and AChE can be taken as evidence that AChE is not exerting its long-term effects via the cholinergic system. Further evidence to support this view is the observation that butyrylcholinesterase (which also hydrolyses acetylcholine) does not induce any long-term behavioural changes (39). The way in which AChE does exert its sustained action is not entirely clear, but recent evidence suggests that it is taken up into cells (23), where it may exert an intracellular role that would be consistent with effects of long duration and the fact that the maximum response to AChE is not observed until the day following infusion. Recently, it has been suggested that AChE exerts long-term nonclassical actions in the hippocampus via an induction of long-term potentiation of synaptic transmission in CA1 pyramidal cells (4). A similar potentiation of the nigrostriatal pathway could underlie the effects observed here. It is possible that the small nonsignificant effect induced immediately after infusion is attributable to a classical cholinergic action (reducing activity of the cholinergic system) with long-term nonclassical effects only occurring over a longer time course.

There is now considerable evidence that AChE exerts nonclassical roles both in the substantia nigra [see (30)], other brain areas [both adult (3) and developing brain (49,57)], nonmammals (72), and even in nonneuronal tissue (53,54). There is also increasing support for the notion that AChE is exerting at least some of these effects by a mechanism that is independent of its catalytic site, inasmuch as similar effects are not seen with BuChE (32,39,56), and persist in the presence of inhibitors of the catalytic site (34,58). This nonclassical action has been shown most clearly in studies of neurite outgrowth in chick nerve cells (58) , where stimulation of neurite outgrowth by AChE is blocked only by inhibitors of the enzyme acting at a peripheral site, and not those acting directly at the catalytic site, thereby implicating the peripheral site in these nonclassical actions of AChE. A similar conclusion was reached by Jones et al. (49), studying the stimulation of neurite growth by AChE in organotypic cell cultures of substantia nigral neurones. Given this putative role of AChE as some form of trophic factor, it is interesting to note that brain-derived neurotrophic factor (BDNF), when infused unilaterally into the substantia nigra, also induces contraversive circling in the presence of amphetamine (2).

In summary, these results suggest that acetylcholinesterase is able to induce long-term behavioural effects in the pars compacta, indicative of increased nigrostriatal activity, and that this action is not occurring via a cholinergic mechanism. Neurones sensitive to this nonclassical action of AChE are located at the very rostral edge of the substantia nigra. Carbachol, in contrast, appears to cause circling in the opposite direction to AChE, but via a mechanism that does not seem to involve direct excitation of nigrostriatal dopaminergic neurones. In both pars reticulata and pars compacta, there is a graded sensitivity to carbachol, with maximum effects exhibited rostrally. The site selectivity within the substantia nigra for both these agents is further evidence for the heterogeneous nature of this key brain region.

ACKNOWLEDGEMENTS

C.A.H. is funded by the McDonnell-Pew Centre for Cognitive Neuroscience, Oxford. AChE was kindly purified by Dr. T. Budd. We would like to thank Ms. L. Guiver for processing the histology and Mrs. L. Annetts for photography.

REFERENCES

1. Aghajanian, G. K.; Bunney, B. S. Central dopaminergic neurons: Neurophysiological identification and responses to drugs. In: Usdin, E.; Snyder, S. H., eds. Frontiers in catecholamine research. Oxford: Pergamon Press; 1973:643–648.
2. Altar, C. A.; Boylan, C. B.; Jackson, C.; Hershenson, S.; Miller, J.; Wiegand, S. J.; Lindsay, R. M.; Hyman, C. Brain-derived neurotrophic factor augments rotational behaviour and nigrostriatal dopamine turnover in vivo. Proc. Natl. Acad. Sci. USA 89:11347–11351; 1992.
3. Appleyard, M. E. Noncholinergic functions of acetylcholinesterase. Trends Neurosci. 15:485–490; 1993.
4. Appleyard, M. E. Acetylcholinesterase induces long-term potenti-

ation in CA1 pyramidal cells by a mechanism dependent on metabotropic glutamate receptors. Neurosci. Lett. 190:25–28; 1995.
5. Arnt, J.; Scheel-Kruger, J. GABAergic and glycinergic mechanisms within the substantia nigra: Pharmacological specificity of dopamine-independent contralateral turning behaviour and interactions with other neurotransmitters. Psychopharmacology (Berlin) 62:267–277; 1979.
6. Bache, S.; Moller-Nielsen, I. Turning behaviour in rats after intranigral injection of GABA agonists and antagonists and opiate agonists and antagonists. Abstr 1543, Seventh Int. Congress of Pharmacology, Paris; 1978.
7. Beninato, M.; Spencer, R. F. A cholinergic projection to the rat substantia nigra from the pedunculopontine tegmental nucleus. Brain Res. 412:169–174; 1987.
8. Bernard, V.; Normand, E.; Bloch, B. Phenotypical characterisation of the rat striatal neuron expression of muscarinic receptor genes. J. Neurosci. 12:3591–3600; 1992.
9. Blaha, C. D.; Winn, P. Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and pedunculopontine tegmental nucleus-lesioned rats. J. Neurosci. 13:1035–1044; 1993.
10. Bolam, J. P.; Francis, C. M.; Henderson, Z. Cholinergic input to dopaminergic neurons in the substantia nigra: A double immunocytochemical study. Neuroscience 41:483–494; 1991.
11. Buckley, N. J.; Bonner, T. I.; Buckley, C. M.; Brann, M. R. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. Mol. Pharmacol. 35:469–476; 1989.
12. Butcher, L. L.; Woolf, N. J. Monoaminergic-cholinergic relationships and the chemical communication matrix of the substantia nigra and neostriatum. Brain Res. Bull. 9:475–492; 1982.
13. Chang, H. T.; Kita, H.; Kitai, S. T. The ultrastructural morphology of the subthalamo-substantia nigral axon terminals intracellularly labelled with horseradish peroxidase. Brain Res. 299:182–185; 1984.
14. Cheramy, A.; Leviel, V.; Glowinski, J. Dendritic release of dopamine in the substantia nigra. Nature 289:537–542; 1981.
15. Clarke, P. B. S.; Hommer, D. W.; Pert, A.; Skirboll, L. R. Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: Neuroanatomical and electrophysiological evidence. Neuroscience 23:1011–1019; 1987.
16. Clarke, P. B. S.; Pert, A. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res. 348:355–358; 1985.
17. Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A. Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H] acetylcholine, [^{3}H] nicotine, and [^{125}I]-α-bungarotoxin. J. Neurosci. 5:1307–1315; 1985.
18. Collingridge, G. L.; Davies, J. The influence of striatal stimulation and putative neurotransmitters on identified neurons in the rat substantia nigra. Brain Res. 212:345–359; 1981.
19. Costall, B.; Naylor, R. J.; Olley, J. E. Catalepsy and circling behaviour after intracerebral injections of neuroleptic cholinergic and anticholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain. Neuropharmacology 11:645–663; 1972.
20. Cross, A. J.; Waddington, J. L. [^{3}H] quinuclidinyl benzylate and [^{3}H] GABA receptor binding in rat substantia nigra after 6-hydroxydopamine lesions. Neurosci. Lett. 17:271–275; 1980.
21. De Montis, G. M.; Olianas, M. C.; Serra, G.; Tagliamonte, A.; Scheel-Kruger, J. Evidence that substantia nigral GABAergic-cholinergic balance controls posture. Eur. J. Pharmacol. 53:181–190; 1979.
22. Deutch, A. Y.; Holliday, J.; Roth, R. H.; Chun, L. L. Y.; Hawrot, E. Immunohistochemical localisation of a neuronal nicotinic acetylcholine receptor in mammalian brain. Proc. Natl. Acad. Sci. USA 84:8697–8701; 1987.
23. Dickie, B. G. M.; Budd, T. C.; Vaux, D.; Greenfield, S. A. Uptake of acetylcholinesterase by neurons in the substantia nigra. Eur. J. Neurosci. 7:351–357; 1995.
24. Dray, A.; Straughan, D. W. Synaptic mechanisms in the substantia nigra. J. Pharmacol. Pharmacol. 28:400–405; 1976.
25. Ellman, G. L.; Courtney, D.; Andres, V.; Fetherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88–95; 1961.
26. Frey, K. A.; Howland, M. M. Quantitative autoradiography of muscarinic cholinergic receptor binding in the rat brain: Distinction of receptor subtypes in antagonist competition assays. J. Pharmacol. Exp. Ther. 263:1391–1400; 1992.
27. Gongora-Alfaro, J. L.; Hernandez-Lopez, S.; Martinez-Fong, D.; Brassart, J. L.; Aceves, J. Activation of substantia nigral M_1 and M_2 muscarinic receptors produced opposing effects on striatal 3,4-dihydroxyphenylacetic acid measured by in vivo voltammetry. Brain Res. 54:329–332; 1991.
28. Gould, E.; Woolf, N. J.; Butcher, L. L. Cholinergic projection to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. Neuroscience 28:611–623; 1989.
29. Grace, A. A.; Bunney, B. S. Paradoxical GABA excitation of substantia nigral dopaminergic cells: Indirect mediation through reticulata inhibitory neurons. Eur. J. Pharmacol. 59:211–218; 1979.
30. Greenfield, S. A. A noncholinergic action of acetylcholinesterase (AChE) in the brain: From neuronal secretion to the generation of movement. Cell. Mol. Neurobiol. 11:55–77; 1991.
31. Greenfield, S. A.; Grunewald, R. A.; Foley, P.; Shaw, S. G. Origin of various enzymes released from the substantia nigra and caudate nucleus: Effects of 6-hydroxydopamine lesions of the nigrostriatal pathway. J. Comp. Neurol. 214:87–92; 1983.
32. Greenfield, S. A.; Chubb, I. W.; Grunewald, R. A.; Henderson, Z.; May, J.; Portnoy, S.; Weston, J.; Wright, M. C. A noncholinergic function for acetylcholinesterase in the substantia nigra: Behavioural evidence. Exp. Brain Res. 54:513–520; 1984.
33. Greenfield, S. A.; Jack, J. J. B.; Last, A. T. J.; French, M. An electrophysiological action of acetylcholinesterase independent of its catalytic site. Exp. Brain Res. 70:441–444; 1988.
34. Greenfield, S. A.; Nedergaard, S.; Webb, C.; French, M. Pressure ejection of acetylcholinesterase within the guinea pig substantia nigra has nonclassical actions on the pars compacta cells independent of selective receptor and ion channel blockade. Neuroscience 29:21–25; 1989.
35. Hajos, M.; Greenfield, S. A. Differential actions of acetylcholinesterase on the soma and dendrites of dopaminergic substantia nigra neurons in vitro. Brain Res. 585:416–420; 1992.
36. Hajos, M.; Greenfield, S. A. Topographic heterogeneity of substantia nigra neurons: Diversity in intrinsic membrane properties and synaptic inputs. Neuroscience 55:919–934; 1993.
37. Hammond, C.; Deniau, J. M.; Rizk, A.; Feger, J. Electrophysiological demonstration of an excitatory subthalamosubstantia nigral pathway in the rat. Brain Res. 151:235–244; 1978.
38. Hawkins, C. A.; Greenfield, S. A. Noncholinergic action of exogenous acetylcholinesterase in the rat substantia nigra. I: Possible dose-related effects on motor behaviour. Behav. Brain Res. 48:153–157; 1992.
39. Hawkins, C. A.; Greenfield, S. A. Recombinant acetylcholinesterase has behavioural effects in the rat substantia nigra not attributable to its enzymatic activity. Neurosci. Lett. 197:203–207; 1995.
40. Henderson, Z.; Greenfield, S. A. Does the substantia nigra have a cholinergic innervation? Neurosci. Lett. 73:109–113; 1987.
41. Hernandez-Lopez, S.; Gongora-Alfaro, J. L.; Martinez-Fong, D.; Aceves, J. A cholinergic input to the substantia nigra pars compacta increases striatal dopamine metabolism measured by in vivo voltammetry. Brain Res. 598:114–120; 1992.
42. Hernandez-Lopez, S.; Gongora-Alfaro, J. L.; Martinez-Fong, D.; Rosalles, M. G.; Aceves, J. Cholinergic stimulation of rostral and caudal substantia nigra pars compacta produced opposite effects on circling behaviour and striatal dopamine release measured by brain microdialysis. Neuroscience 62:441–447; 1994.
43. Iwamoto, E. T.; Way, E. L. Circling behaviour and stereotypy induced by intranigral opiate microinjections. J. Pharmacol. Exp. Ther. 203:347–359; 1977.
44. James, T. A.; Massey, S. Evidence for a possible dopaminergic link in the action of acetylcholine in the rat substantia nigra. Neuropharmacology 17:687–690; 1978.
45. James, T. A.; Starr, M. S. Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat. J. Pharm. Pharmacol. 29:182–182; 1977.
46. Jimenez-Castellanos, J. J.; Graybiel, A. M. Subdivisions of the

primate substantia nigra pars compacta detected by acetylcholinesterase histochemistry. Brain Res. 437:349–354; 1987.
47. Jimenez-Castellanos, J. J.; Graybiel, A. M. Subdivisions of the dopamine-containing A8–A9–A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. Neuroscience 23:223–242: 1987.
48. Jones, S. A. 'On-line' monitoring of acetylcholinesterase secretion in the basal ganglia in relation to the generation of movement. D. Phil. Thesis, Oxford University; 1992.
49. Jones, S. A.; Holmes, C.; Budd, T. C.; Greenfield, S. A. The effect of acetylcholinesterase and its inhibitors on the outgrowth and survival of rat midbrain dopaminergic neurons in organotypic slice culture. Cell Tissue Res. 279:323–330; 1995.
50. Kayadjanian, K.; Gioanni, H.; Menetrey, A.; Besson, M. J. Muscarinic receptor stimulation increases the spontaneous [^{3}H]GABA release in the rat substantia nigra through muscarinic receptors localised on striatonigral terminals. Neuroscience 63:989–1002; 1994.
51. Kayadjanian, N.; Retaux, S.; Menetrey, A.; Besson, M. J. Stimulation by nicotine of the spontaneous release of ^{3}H-gamma aminobutyric acid in the substantia nigra and in the globus pallidus of the rat. Brain Res. 49:129–135; 1994.
52. Kanazawa, I.; Marshall, G. R.; Kelly, J. S. Afferents to the rat substantia nigra studied with horseradish peroxidase with special reference to fibres from the subthalamic nucleus. Brain Res. 115: 485–491; 1976.
53. Klegeris, A.; Budd, T. C.; Greenfield, S. A. Acetylcholinesterase activation of peritoneal macrophages is independent of catalytic activity. Cell. Mol. Neurobiol. 14:87–96; 1994.
54. Klegeris, A.; Korkina, L.; Greenfield, S. A. A possible interaction between acetylcholinesterase and dopamine molecules during autoxidation of the amine. Free Radic. Biol. Radiat. Med. 18:223–230; 1995.
55. Lacey, M. G.; Mercuri, N. B.; North, R. A. On the potassium conductance increase by $GABA_B$ and dopamine D_2 receptors in rat substantial nigra neurones. J. Physiol. 401:4370–453; 1989.
56. Last, A. T. J.; Greenfield, S. A. Acetylcholinesterase has noncholinergic neuromodulatory actions in the guinea-pig substantia nigra. Exp. Brain Res. 67:445–448; 1987.
57. Layer, P. G.; Alber, R.; Rathjen, F. G. Sequential activation of butyrylcholinesterase in rostral half somites and acetylcholinesterase in motoneurones and myotomes preceding growth of motor axons. Development 102:387–396; 1988.
58. Layer, P. G.; Weikert, T.; Alber, R. Cholinesterases regulate neurite growth of chick nerve cells in vitro by means of a noncnzymatic mechanism. Cell Tissue Res: 273:219–226; 1993.
59. Lehmann, J.; Fibiger, H. C. The localisation of acetylcholinesterase in the corpus striatum and substantia nigra of the rat following kainic acid lesion of the corpus striatum: A biochemical and histochemical study. Neuroscience 4:217–225; 1979.
60. Lichtensteiger, W.; Hefti, F.; Felix, D.; Huwyler, T.; Melamed, E.; Schlumpf, M. Stimulation of nigrostriatal dopamine neurons by nicotine. Neuropharmacology 21:963–968; 1982.
61. Llinas, R. R.; Greenfield, S. A. On-line visualisation of dendritic release of acetylcholinesterase from mammalian substantia nigra neurons. Proc. Natl. Acad. Sci. USA 84:3047–3050; 1987.
62. Marchi, M.; Augliera, A.; Codignola, A.; Lunardi, G.; Fedele, E.; Fontana, G.; Raiteri, M. Cholinergic modulation of [^{3}H] release from dendrosomes of rat substantia nigra. Naunyn Schmiedebergs Arch. Pharmacol. 344:275–280; 1991.
63. Massoulie, J.; Bon, S. Affinity chromatography of Acetylcholinesterase: The importance of hydrophobic interactions. Eur. J. Biochem. 68:531–539; 1976.
64. Mintz, I.; Hammond, C.; Guibert, B.; Leviel, V. Stimulation of the subthalamic nucleus enhances the release of dopamine in the rat substantia nigra. Brain Res. 376:406–408; 1986.
65. Nashold, B. S.; Urbaniak, J. R.; Hatcher, M. A. Chemical stimulation of red nucleus, substantia nigra and basis pedunculi in alert cats. Neurology 15:604–612; 1965.
66. Nastuk, M. A.; Graybiel, A. M. Pharmacologically defined M_1 and M_2 muscarinic cholinergic binding sites in the cat's substantia nigra: Development and maturity. Dev. Brain Res. 61:1–10; 1991.
67. Nedergaard, S.; Hopkins, C.; Greenfield, S. A. Do nigrostriatal neurones possess a discrete dendritic modulatory mechanism? Exp. Brain Res. 69:444–448; 1988.
68. Nedergaard, S.; Greenfield, S. A. Subpopulations of pars compacta neurons in the substantia nigra: The significance of qualitatively and quantitatively distinct conductances. Neuroscience 48: 423–437; 1992.
69. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
70. Pinncock, R. D.; Dray, A. Differential sensitivity of presumed dopaminergic and nondopaminergic neurons in rat substantia nigra to electrophoretically applied substance P. Neurosci. Lett. 29: 153–158; 1982.
71. Potter, L. T.; Flynn, D. D.; Hanchett, H. E.; Kalinoski, D. L.; Luber-Narod, J.; Mash, D. C. Independent M_1 and M_2 receptors: Ligands, autoradiography and functions. Trends Pharmacol. Sci. Suppl. 4:22–31; 1983.
72. Peretz, B. The modulatory effect of acetylcholinesterase on neuronal activity is dependent on age in *Aplysia*. Netherlands J. Zool. 44:395–404; 1994.
73. Reisine, T. D.; Nagy, J. I.; Beaumont, K.; Fibiger, H. C.; Yamamura, H. I. The localisation of receptor binding sites in the substantia nigra and striatum of the rat. Brain Res. 177:241–252; 1979.
74. Shepard, P. D.; German, D. C. Electrophysiological and pharmacological evidence for the existence of distinct subpopulations of nigrostriatal dopaminergic neuron in the rat. Neuroscience 27: 537–546; 1988.
75. Silver, A. The biology of the cholinesterases. Amsterdam: Elsevier; 1974:117–303, 428–431.
76. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367:1–48; 1971.
77. Vilaro, M. T.; Palacios, J. M.; Mengod, G. Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci. Lett. 114:154–159; 1990.
78. Webb, C.; Greenfield, S. A. Noncholinergic effects of acetylcholinesterase in the substantia nigra: A possible role for an ATP-sensitive potassium channel. Exp. Brain Res. 89:49–58; 1992.
79. Weiner, D. M.; Levey, A. I.; Brann, M. R. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc. Natl. Acad. Sci. USA 87:7050–7054; 1990.
80. Westerink, B. H. C.; de Boer, P.; Santiago, M.; De Vries, J. B. Do nerve terminals and cell bodies of nigrostriatal dopaminergic neurons of the rat contain similar receptors? Neurosci. Lett. 167:109–112; 1994.
81. Weston, J.; Greenfield, S. A. Application of acetylcholinesterase to the substantia nigra induces stereotypy in rats. Behav. Brain Res. 18:71–74; 1985.
82. Wolfarth, S.; Duiska, E.; Golembiowska-Kikitin, K.; Vetulani, J. A role of the polysynaptic system of substantia nigra in the cholinergic-dopaminergic equilibrium in the central nervous system. Naunyn Schmiedebergs Arch. Pharmacol. 302:123–131; 1978.
83. Wolfarth, S.; Wand, P.; Sontag, K. L. The effects of intrasubstantia nigral injections of picrotoxin and carbachol in cats with a lesioned nigrostriatal pathway. Neurosci. Lett. 11:197–200; 1979.