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# Electrophysiological actions of nicotine on substantia nigra single units

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**1** Extracellular recordings of single unit activity were made in the substantia nigra (SN) of chloral hydrate-anaesthetized rats.

**2** Dopaminergic neurones of the pars compacta (SNC) were stimulated by (−)-nicotine bitartrate (1.0 mg $kg^{-1}$) given subcutaneously (s.c.). This action was prevented by the secondary amine mecamylamine HCl (2.0 mg $kg^{-1}$ i.v.) but not by a ganglion-blocking dose of the bisquaternary compound chlorisondamine Cl (0.1 mg $kg^{-1}$ i.v.). Mecamylamine reduced the spontaneous activity of dopaminergic neurones.

**3** Nicotine, when administered intravenously (2–128 μg $kg^{-1}$ cumulative dose), also stimulated dopamine cells and this action was dose-related.

**4** Nicotine, administered intravenously, (2–128 μg $kg^{-1}$ cumulative dose) markedly excited non-dopamine cells in the pars reticulata (SNR) in a dose-related manner. In rats pretreated with chlorisondamine (0.1 mg $kg^{-1}$ i.v.), nicotine induced a small excitatory or depressant action, but the marked excitation was not seen. Mecamylamine (2 mg $kg^{-1}$ i.v.) completely prevented the actions of nicotine.

**5** The results are consistent with a direct excitatory action of nicotine on dopaminergic neurones of the substantia nigra pars compacta. The pronounced excitatory action of systemically administered nicotine on non-dopamine cells of the pars reticulata appears to be of peripheral origin.

## Introduction

There is an abundance of neurochemical evidence for cholinergic markers in the substantia nigra (SN); for example, the SN contains a high-affinity choline uptake system (Massey & James, 1978), and both the substantia nigra pars compacta (SNC) and pars reticulata (SNR) contain appreciable amounts of acetylcholine and choline acetyltransferase (ChAT: see Butcher & Talbot, 1978 for review). Biochemical and behavioural studies suggest that cholinomimetic agents can directly affect neuronal activity in the SN. Thus, intranigral infusion of carbachol or of other cholinomimetics have been shown to reduce striatal dopamine turnover (Javoy *et al.*, 1974), and, in other studies, to produce increases in feeding, responding for food, and stereotyped behaviour (Decsi *et al.*, 1978, Winn *et al.*, 1983). At higher doses of cholinomimetics, ipsiversion or catalepsy occurs (DeMontis *et al.*, 1979; Turski *et al.*, 1984). Physostigmine, an acetylcholinesterase (AChE) inhibitor, produces behavioural changes when administered into the SN (Winn & Redgrave, 1981), consistent with a functional cholinergic system within this structure.

Both major subdivisions of the SN appear to possess a cholinergic input. Within the SNC, dopaminergic neurones do not themselves contain ChAT, but do contain AChE, which is localized in the soma and dendrites (Butcher & Talbot, 1978); these cells may therefore be cholinoceptive (Levey *et al.*,1983). Some of the behavioural changes seen after intranigral infusion of carbachol may result from activation of the ascending nigrostriatal dopaminergic pathway (see Winn *et al.*, 1983). In two studies of the SNC, microiontophoretically applied acetylcholine (ACh) was found to excite presumed dopamine cells in the SNC (Dray & Sraughan, 1976; Lichtensteiger *et al.*, 1982), but others have found no such effect (Aghajanian & Bunney, 1974; Collingridge & Davies, 1981; Pinnock & Dray, 1982). In contrast, there is general agreement that micro-iontophoretically applied ACh excites SNR cells (Aghajanian & Bunney, 1974; Dray

& Straughan, 1976; Collingridge & Davies, 1981; Pinnock & Dray, 1982).

The possibility of distinct nicotinic and muscarinic mechanisms in SN has not been investigated extensively. Although the SN has a low density of muscarinic binding sites relative to other brain regions (Nonaka & Moroji, 1984), some of the behavioural effects of intranigral carbachol are reversed by muscarinic antagonists (Decsi *et al.*, 1978; DeMontis *et al.*, 1979; Winn *et al.*, 1983). Within the SN, muscarininc cholinoceptors are found mainly (Cross & Waddington, 1980) or exclusively (Reisine *et al.*, 1979) on non-dopaminergic elements. The excitatory action of microiontophoretically applied ACh on SNR neurones is readily blocked by muscarinic antagonists (Aghajanian & Bunney, 1974; Dray & Straughan, 1976), and one report (Aghajanian & Bunney, 1974) suggests that the nicotinic antagonist dihydro-β-erythroidine may also be effective. In contrast, the SN possesses a dense band of nicotine binding sites located in the pars compacta (Clarke *et al.*, 1984). These nicotinic sites largely disappeared after lesions of the ipsilateral acending dopaminergic pathways were made with the neurotoxin 6-hydroxydopamine (P.B.S. Clarke & A. Pert, unpublished observations). Lichtensteiger *et al.* (1976, 1982) have observed that SNC neurones are greatly excited by nicroiontophoretic application of nicotine and by high doses of the drug given systematically. These findings are significant for at least two reasons: SNC neurones appear to be directly excited by few pharmacological agents (increases in firing rate usually occurring through disinhibition, e.g. Grace & Bunney, 1979); secondly, the magnitude of the nicotinic action suggests a major input to these cells.

The present study comprised an attempt to relate the differential distribution of putative nicotinic cholinoceptors previously observed within the SN (Clarke *et al.*, 1984) with the responses of nigral neurones to systemically administered nicotine. The effects of inravenous nicotine on SNC and SNR single unit activity were compared, and attempts were made to block the actions of nicotine by prior administration of the nicotinic antagonists, chlorisondamine (Plummer *et al.*, 1955) and mecamylamine (Stone *et al*, 1956). Since only the latter readily penetrates centrally (Clarke & Kumar, 1983c), these drugs served to distinguish central and peripheral actions of nicotine.

## Methods

Male Sprague-Dawley rats (150–250g; Taconic Farms, New York) were used. They were housed in a room illuminated from 07 h 00 min to 19 h 00 min, with *ad libitum* access to food and water.

*Recording of single unit activity*

Rats were initially anaesthetized with chloral hydrate 400 mg $kg^{-1}$ i.p. Additional infusions were given as required, but always at least 3 min before the start of recording. The level of anaesthesia was such that a brief, one second footpinch applied by a haemostat produced a small and sluggish limb withdrawal. Rectal temperature was maintained at ± 0.2°C (for a given rat) within the range 36.5 to 37.5°C. Recordings were carried out between 09 h 00 min and 17 h 00 min.

A stereotaxic apparatus was used (Kopf Instruments). Before recording, the scalp and periostium of the rat were reflected and a 3 mm burr hole was drilled over the SN. Single-barrel glass microelectrodes (W-P Instruments, New Haven, CT, type 2B150F: tip diameter approximately 1 μm) were used, containing a 2 M NaCl solution saturated with Pontamine Sky Blue. Electrode impedances were checked directly after filling, and were chosen to be 6–10 MΩ (for SNC recording) or 8–12 MΩ (for SNR), measured at 60 Hz. An electrode was inserted stereotaxically into the region of the substantia nigra using a Kopf Hydraulic microdrive. Extracellular electrode potentials were passed through a high impedance amplifier and monitored on an oscilloscope and audiomonitor. Spike activity was recorded continuously on a pen recorder (Gould Instruments, USA), and aggregated counts were printed every 10 s.

In view of the electrical and physical characteristics of the microelectrodes, it is unlikely that axon spikes were detected; indeed, spike activity was not encountered within known fibre pathways such as the medial lemniscus and corpus callosum. SNC units were initially identified as previously described (Aghajanian & Bunney, 1973). They were selected with a mean spontaneous firing rate of 2–8 spikes $s^{-1}$, and few units were encountered outside this range. SNR units were initially identified by location (within 1000 μm ventral to SNC cells), and by spike duration (less than 1.0 ms; Hommer & Pert, 1983). SNR units with spontaneous firing rates between 5 and 25 spikes $s^{-1}$ were selected for recording. This range is consistent with previously recorded values (e.g., Guyenet & Aghajanian, 1978; Ruffieux & Schultz, 1980). SNR units were inhibited by chloral hydrate anaesthesia, and the mean spontaneous firing rate presented below (approximately 12 spikes $s^{-1}$) is lower than some values found previously, reflecting the level of anaesthesia employed. Units were assessed for responsiveness to footpinch applied three times at one minute intervals for one second with a haemostat. At the end of the experiment, dopamine cells were further identified by intravenous infusion of apomorphine (0.1 mg $kg^{-1}$), which greatly inhibited firing, followed one minute later by haloperidol (0.1 mg $kg^{-1}$ i.v.).

*Histology*

For histological identification of the recording site, dye was ejected iontophoretically at 25 μA for 15 min. The resulting dye spot, approximately 300 μm diameter, served to localize the site of the electrode tip within the nigral subnuclei. Brains were removed and stored in formalin. Coronal sections, 32 μm thick, were taken through the substantia nigra at −16°C, and thaw-mounted on gelatin-coated slides for light microscopic examination.

*Procedure*

(1) *Subcutaneous nicotine and SNC unit activity: dose-response relationship* Baseline firing rates were recorded for at least 5 min before injection of nicotine. Each rat received a single dose of the drug: 0.25 mg kg$^{-1}$ s.c. ($n = 9$), 0.5 mg kg$^{-1}$ s.c. (n = 11), or 1.0 mg kg$^{-1}$ s.c. ($n = 7$). Five minutes after the nicotine injection, apomorphine (0.1 mg kg$^{-1}$ i.v.) was given, followed one minute later by haloperidol (0.1 mg kg$^{-1}$ i.v.).

(2) *Intravenous nicotine and SNC unit activity: dose-response relationship* A cumulative dose-response procedure was employed in each rat ($n = 11$). After an initial baseline period of 3 min or more, rats were injected with saline i.v. (9/11 rats), and all rats then received increasing doses of nicotine (2,2,4,8,16,32,64 μg kg$^{-1}$ i.v.) given at intervals of 3 min. The nicotine solution was diluted so that the injected volume ranged from 0.2 to 1.0 ml kg$^{-1}$. Three minutes after the final infusion of nicotine, apomorphine and haloperidol were administered as described for experiment 1.

(3) *Subcutaneous nicotine and SNC unit activity: effects of pretreatment with nicotine antagonists* After a 5 min period of baseline recording, each rat was given an intravenous pretreatment injection of saline ($n = 14$), chlorisondamine (0.1 mg kg$^{-1}$; $n = 8$), or mecamylamine (2.0 mg kg$^{-1}$; $n = 8$). The infusions were given over a 60 s period, in order to reduce the possibility of central penetration by chlorisondamine (Clarke, 1984). The pretreatment doses were chosen to produce ganglionic blockade (Morrison *et al.*, 1969). Five minutes after the start of pretreatment infusion, rats were injected with nicotine (1.0 mg kg$^{-1}$ s.c.). After a further period of 5 min, apomorphine and haloperidol were administered as described above. In this experiment, the challenge dose of nicotine was chosen on the basis of the results of experiments 1 and 2.

(4) *Intravenous nicotine and SNR unit activity: dose-response relationship* Eleven subjects were used. The same procedure was employed as in experiment 2, except that apomorphine and haloperidol were not given, since these drugs do not serve to identify SNR neurones. In one rat, the highest dose given was 16 μg kg$^{-1}$: in another the highest dose was 32 μg kg$^{-1}$; in all the remaining animals it was 64 μg kg$^{-1}$.

(5) *Intravenous nicotine and SNR unit activity: effects of pretreatment with nicotine antagonists* A procedure resembling that of experiment 3 was used. However, nicotine was administered in a dose of 32 μg kg$^{-1}$ (i.v.), and injections of apomorphine and haloperidol were omitted. In view of the results of experiment 4, only footpinch-sensitive units were tested.

*Data analysis*

Only one cell was recorded from each rat. Mean firing rates were calculated for each rat, beginning one minute after the start of each injection. Drug effects were generally assessed by the mean of individual changes in firing rate, calculated as a percentage of pre-drug baseline, and also in terms of the absolute difference from pre-drug baseline. Both methods gave similar results and all statistical tests described below refer to absolute changes in firing rate. Statistical procedures were carried out using a software package (SPSSX, McGraw-Hill, New York) and included univariate analysis of variance (ANOVA), Pearson produce-moment correlation analysis, and least-squares linear regression. Where appropriate, each rat was used as its own control. All tests of significance are 2-tailed. *A posteriori* tests of significance were carried out using Student's *t* tests; no adjustment was made for the total number of possible comparisons, which was never more than three. Within a given experiment, a random order of drug testing was used.

*Drugs*

(−)-Nicotine hydrogen (+)-tartrate (BDH, Poole, U.K.) was dissolved in 0.9% w/v NaCl solution (saline) and neutralised to pH 7.2 ± 0.2 with 1 M NaOH. Mecamylamine HCl (Merck), chlorisondamine Cl (CIBA-Geigy), apomorphine HCl (Merck) and haloperidol lactate (McNeil Laboratories) were dissolved in saline. All drugs were injected in a volume of 1 ml kg$^{-1}$ unless otherwise stated. Subcutaneous injections were made into the flank. Intravenous administration was made via a 25 gauge hypodermic needle inserted into a lateral tail vein before the start of the experiment. Intravenous infusions of nicotine were of 20–30 s duration. Doses of nicotine, mecamylamine and chlorisondamine refer to the base of the compound; doses of apomorphine and haloperidol are expressed as the salt.

## Results

### *Histology*

All recording sites were found to lie within the following boundaries according to the atlas of Konig & Klippel (1963): L 1.4 to 2.5, A 1.8 to 2.8, V − 2.9 to − 1.8. Presumed dopamine cells were located within or immediately adjacent to the SNC cell layer; non-dopamine cells were located more ventrally in the SNR.

### (1) *Subcutaneous nicotine and SNC unit activity: dose-response relationship*

The mean rates (± s.e.mean) of spontaneous firing before injection of nicotine were as follows: 4.02 ± 0.33 (0.25 mg kg$^{-1}$ group), 4.57 ± 0.40 (0.5 mg kg$^{-1}$ group) and 4.66 ± 0.47 spikes s$^{-1}$ (1.0 mg kg$^{-1}$ group). Spontaneous firing rates did not differ significantly between groups (ANOVA: $F = 0.71$, d.f. 2,24, $P > 0.1$).

Following injection of nicotine (0.25, 0.5, 1.0 mg kg$^{-1}$), the group mean (± s.e.mean) increases in firing rate were, respectively, 0.22 ± 0.12, 0.26 ± 0.19, and 0.78 ± 0.16 spikes s$^{-1}$. A significant excitatory effect was only seen after the highest dose of nicotine; firing rates increased abruptly between 40 and 60 s after injection, and remained elevated for the period of the recording (5 min). Increases of unit activity were accompanied by a decrease in apparent spike height. Two minutes after injection of nicotine (1.0 mg kg$^{-1}$), respiration became markedly altered in several animals, becoming deep and regular (approx. rate 60 per min).

In order to assess the relationship between response to nicotine (1.0 mg kg$^{-1}$ s.c.) and spontaneous firing rate, the appropriate data from experiments 1 and 3 were pooled. Significant negative correlations were found between baseline rate and drug effect, assessed not only by percentage increase in rate ($r = -0.75$, $n = 21$, $P < 0.0001$), but also by the absolute increase in rate ($r = -0.45$, $n = 21$, $P < 0.025$). Hence, nicotine tended to excite slower-firing SNC cells more than faster-firing cells.

### (2) *Intravenous nicotine and SNC activity*

The initial intravenous injection of saline led to a slight

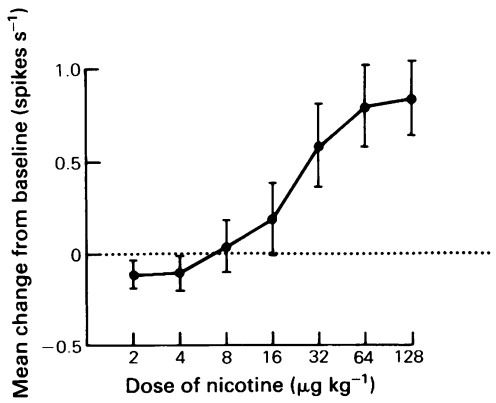


**Figure 1** Change in firing rate of substantia nigra pars compacta (SNC) neurones following intravenous administration of nicotine. Each rat received a series of increasing doses of nicotine. Injections were spaced 3 min apart. The effect of nicotine was assessed by comparing the mean firing rate at a given cumulative dose of nicotine with the mean baseline firing rate (i.e., before any nicotine was given; represented by stippled line). The data are expressed as the group mean (vertical lines show s.e.mean) of the corresponding difference scores ($n = 11$). The mean spontaneous firing rate (baseline) was 4.51 spikes s$^{-1}$.

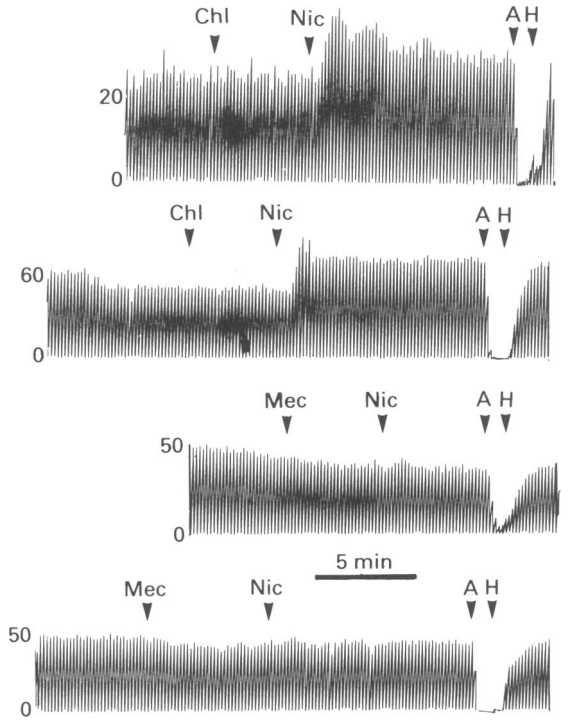


**Figure 2** Ratemeter records showing the responses of single SNC neurones to nicotine, in rats pretreated with mecamylamine (Mec) or chlorisondamine (Chl). Extracellular spikes were aggregated over periods of 10 s (vertical axes). Following a baseline period of 5 min, the ganglion blockers chlorisondamine (0.1 mg kg$^{-1}$) or mecamylamine (2 mg kg$^{-1}$) were given intravenously. Only mecamylamine prevented the excitatory effect of nicotine (Nic; 1.0 mg kg$^{-1}$ s.c.). Each experiment was concluded with i.v. injections of apomorphine (A; 0.1 mg kg$^{-1}$) and haloperidol (H; 0.1 mg kg$^{-1}$) to show that the cells investigated were dopamine cells.

fall in firing rate of $0.29 \pm 0.06$ spikes $s^{-1}$ ($n = 9$). Baseline firing rate was calculated from the 2 min period immediately preceding the first nicotine injection (mean ± s.e.mean rate = $4.52 \pm 0.49$ spikes $s^{-1}$, $n = 11$). For the group as a whole, nicotine stimulated unit activity in a dose-related manner (Figure 1). The data from individual rats were analysed by linear regression of mean firing rate against log cumulative dose (2 to 128 $\mu g\,kg^{-1}$). This analysis indicated a stimulant action that was significant ($P < 0.05$) in 10 out of the 11 cells.

(3) *Subcutaneous nicotine and SNC unit activity: effects of pretreatment*

Baseline rates did not differ significantly among the three groups of rats (ANOVA: group main effect: $F = 1.05$, d.f. 2,27, $P > 0.1$); means ± s.e.mean were as follows: $4.60 \pm 0.34$ spikes $s^{-1}$ (saline group), $4.02 \pm 0.36$ spikes $s^{-1}$ (chlorisondamine group), and $4.00 \pm 0.29$ spikes $s^{-1}$ (mecamylamine group). In the period before nicotine was given, the three pretreatments differed in their effects on spontaneous firing rate (ANOVA: group main effect: $F = 5.04$, d.f. 2,27, $P < 0.02$). Compared to the control (i.e. saline) pretreatment, mecamylamine produced a small (0.31 spikes $s^{-1}$) drop in firing rate (between-group comparison: $t = 3.48$, d.f. 7, $P < 0.01$); chlorisondamine pretreatment alone was without effect (Figure 2). The effect of nicotine on firing rate depended on pretreatment (ANOVA: group main effect: $F = 22.55$, d.f. 2,27, $P < 0.0001$). As shown in Figures 2 and 3, mecamylamine prevented the excitatory effect of nicotine. In contrast, chlorisondamine enhanced this action of nicotine (between-group comparison: $t = 3.17$, d.f. 20, $P < 0.005$).

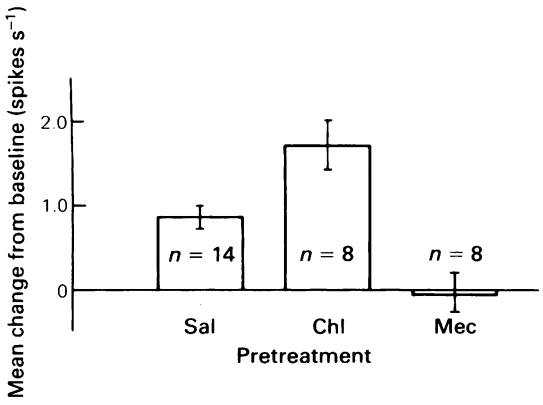


**Figure 3** The effects of pretreatment on nicotine-induced excitation of SNC neurones. After a 5 min baseline period, each rat received an intravenous pretreatment with saline (Sal), chlorisondamine (Chl; 0.1 mg $kg^{-1}$), or mecamylamine (Mec; 2 mg $kg^{-1}$). Five minutes later, nicotine (1.0 mg $kg^{-1}$ s.c.) was given, and recording continued for 5 min. Baseline firing rate did not differ significantly between groups. The effect of nicotine is shown as the mean (with vertical lines representing s.e.mean) difference score (i.e., rate after pretreatment subtracted form post-nicotine rate).

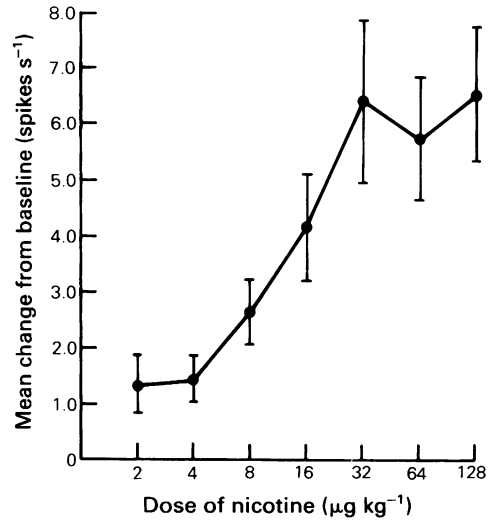


**Figure 4** Change in firing rate of substantia nigra pars reticulata (SNR) neurones following intravenous administration of nicotine. Each rat received a series of increasing doses of nicotine. Injections were spaced 3 min apart. The effect of nicotine was assessed by comparing the mean firing rate at a given cumulative dose of nicotine with the mean baseline firing rate (i.e., before any nicotine was given). The data are expressed as a the group mean (vertical lines show s.e.mean) of the corresponding difference scores ($n = 11$). The mean spontaneous firing rate was 11.8 spikes $s^{-1}$.

(4) *Intravenous nicotine and SNR unit activity*

The initial injection of saline failed to affect firing rate. Baseline firing rate was defined as in experiment 2: mean ± s.e.mean = $11.80 \pm 1.37$ spikes $s^{-1}$ ($n = 11$). Nicotine increased unit activity in a dose-related manner (see Figure 4), and linear regression (performed as in experiment 2) indicated that all 11 units were significantly sitmulated by nicotine ($P < 0.02$ for each cell). In the group as a whole, even the lowest dose of 2 $\mu g\,kg^{-1}$ was effective (paired $t$ test: $t = 3.90$, d.f. 10, $P < 0.005$, Figure 4). Of the 11 units tested, 8 were excited by the footpinch. Footpinch-sensitive cells appeared to be more sensitive to low doses of nicotine (Figure 5), and in these cells, the excitatory effect appeared rapidly, about 30 s after the start of the nicotine infusion. Cells that were insensitive to footpinch showed a gradual rise in firing rate with successive doses.

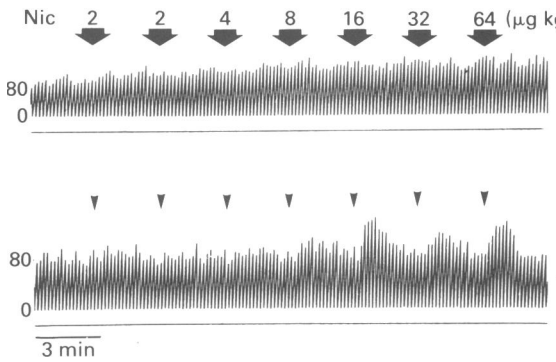


**Figure 5** Ratemeter recordings of SNR single unit activity. Traces from two rats are shown. Each rat received a series of increasing doses of nicotine (Nic; i.v.) given at 3 min intervals. The top trace refers to a neurone which did not respond to noxious footpinch; firing rate increased gradually during the period of nicotine administration. The lower trace shows an injection-linked excitatory action of nicotine in a neurone which was footpinch-sensitive.

(5) *Intravenous nicotine and SNR unit activity: effects of pretreatment*

Baseline rates did not differ significantly among the three groups of rats (ANOVA: $F = 0.54$, d.f. 2,21, $P > 0.1$); means ± s.e.mean were as follows: 11.62 ± 1.30 (saline group), 12.96 ± 1.94 (chlorisondamine group) and 10.71 ± 1.28 spikes $s^{-1}$ (mecamylamine group). Before nicotine administration, the pretreatment injections did not differentially affect spontaneous firing rate (ANOVA: group main effect: $F = 1.97$, d.f. 2,21, $P > 0.1$). Mecamylamine clearly blocked the excitatory effect of intravenous nicotine (Figure 6). Among rats pretreated with chlorisondamine, there were great individual differences in the response to nicotine; in some rats, nicotine produced a small but clear excitation, but in others, a marked depressant action occurred (Figure 6).

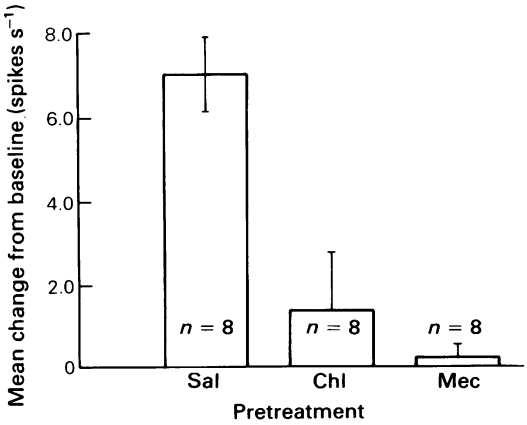


**Figure 6** The effects of pretreatment on nicotine-induced excitation of SNR neurones. After a 5 min baseline period, each rat received an intravenous pretreatment with saline (Sal), chlorisondamine (Chl; 0.1 mg $kg^{-1}$), or mecamylamine (Mec; 2 mg $kg^{-1}$). Five minutes later, nicotine (32 μg $kg^{-1}$ i.v.) was given, and recording continued for 5 min. Baseline firing rate did not differ significantly between groups. The effect of nicotine is shown as the mean (with vertical lines representing s.e.mean) difference score (i.e., rate after pretreatment subtracted from post-nicotine rate).

## Discussion

*Nicotine and SNC unit activity*

This study was initiated by the observation of putative nicotinic cholinoceptors in substantia nigra pars compacta (Clarke *et al.*, 1984), and one of its aims was to investigate possible functional correlates. Dopamine cells in the SNC were found to be excited by nicotine, given either subcutaneously or intravenously. When the drug was given by the subcutaneous route, a high dose (1.0 mg $kg^{-1}$) was required in order to obtain a convincing excitatory action. By way of comparison, in unanaesthetized rats, dose-related behavioural effects are encountered in a dose range of 0.05 to 0.8 mg $kg^{-1}$ s.c. base (Clarke & Kumar, 1983a,b). In the present study, nicotine (1.0 mg $kg^{-1}$ s.c.) typically increased firing rates by 10–40%. Lichtensteiger *et al.* (1976) observed a twofold increase in rate at this dose, so that presumed dopamine cells were firing well in excess of 10 spikes $s^{-1}$. These investigators (Lichtensteiger *et al.*, 1976; 1982) employed urethane rather than chloral hydrate to anaesthetize their animals, and possibly the excitant action of nicotine is affected by the choice of anaesthetic. Their identification of dopamine cells was less rigorous than in the present study, and it seems possible that a significant proportion of their presumed dopaminergic neurones were non-dopaminergic nigral cells.

When nicotine was administered intravenously, the magnitude of the peak drug effect was similar to that seen after subcutaneous injection. However, intravenous nicotine produced a more variable response, with some units showing little excitation. Administered by either route, nicotine is rapidly taken up into the brain, and the occurrence of central effects within one or two minutes of subcutaneous injection has been noted previously (Clarke & Kumar, 1983a).

Are SNC neurones excited by a central action of nicotine? In this regard, we found that the bisquaternary ganglion blocker chlorisondamine, in a dose sufficient to protect peripheral nicotinic receptors of

the ganglionic type, failed to reduce the stimulant effect of nicotine, but rather enhanced it. At this dose, chlorisondamine is unlikely to exert significant central actions (Clarke, 1984). In contrast, the centrally-active ganglion blocker mecamylamine prevented the nicotine-induced excitation. The central nervous system contains nicotinic cholinoceptors of the ganglionic type, and it has been argued that these receptors correspond to the high-affinity nicotine binding sites that are found in brain (Clarke *et al.*, 1985). Since dopamine cells of the SNC possess a high density of these binding sites (see Introduction), it seems likely that systemically administered nicotine acts directly on dopamine-containing cells in the SNC, as suggested by microiontophoretic data (Lichtensteiger *et al.*, 1982).

In the third experiment, mecamylamine reduced the spontaneous firing of SNC cells within the five minute period before nicotine was administered; chlorisondamine had no effect. Other investigators have shown that direct application of AChE depresses SNC unit activity (Greenfield *et al.*, 1981), whereas the AChE inhibitor physostigmine has the opposite effect (Lichtensteiger *et al.*, 1976). These findings suggest that endogenous acetylcholine may tonically excite SNC neurones via nicotinic cholinoceptors. A similar conclusion has been drawn from biochemical studies of striatal dopamine metabolism following the administration of nicotinic antagonists (Ahtee & Kaakkola, 1978).

An excitatory nicotinic action of SNC neurones may be clinically relevant, since nicotine has been found to alleviate the immobility associated with Parkinson's disease (Moll, 1926), a condition that is associated with degeneration of the ascending dopamine pathways. This stimulant action of nicotine may bear directly on the epidemiological observation that Parkinsonism is less prevalent among tobacco smokers than among non-smokers (Kessler, 1973).

*Nicotine and SNR unit activity*

To our knowledge, the effects of nicotine on SNR unit activity have not been examined previously. SNR cells were found to be much more responsive than SNC cells to intravenous nicotine. Even the lowest dose of nicotine ($2\,\mu g\,kg^{-1}$) was effective in exciting SNR cells. Effects of similar potency have been found previously; intravenous doses of 5 to $20\,\mu g\,kg^{-1}$ may produce marginal hypertension (Ikushima *et al.*, 1982), as well as a number of central actions, such as excitation of Renshaw cells, depression of spinal reflexes, and electroencephalographic activation (Domino, 1973). Unit doses of around $10\,\mu g\,kg^{-1}$ (base i.v.) are self-administered by monkeys and humans (Henningfield & Goldberg, 1983) as well as by rats (Cox *et al.*, 1984).

Both mecamylamine and chlorisondamine prevented the pronounced excitatory action of nicotine on SNR cells, suggesting that nicotine stimulates these neurones mainly or exclusively via a direct action on peripheral cholinoceptors of the ganglionic type. Given in the absence of an antagonist, nicotine had a particularly marked effect on those SNR cells that were excited by noxious footpinch. However, there are several reasons to suggest that these phenomena are independent; for example, SNC cells that decreased their firing rate in response to footpinch did not do so in response to nicotine infusion. In addition, SNR units responded promptly to footpinch, but responded to nicotine with a delay of 30 s from the start of the infusion. In unanaesthetized rats, the activity of SNR neurones is related to the state of behavioural arousal (Miller *et al.*, 1983), and low doses of nicotine commonly increase behavioural and electrocortical indices of arousal (Domino, 1973, Clarke & Kumar, 1983a). However, these effects of nicotine occur through a direct central action (Domino, 1973, Clarke & Kumar, 1983c) and are thus distinguishable from the excitatory effects of the drug on SNR neurones.

Among animals pretreated with chlorisondamine, the effects of nicotine on SNR unit activity were highly variable, and could be either inhibitory or mildly excitatory. These mixed effects were presumably of central origin since they occurred in the presence of peripheral blockade and failed to occur in rats pretreated with mecamylamine. Nicotine is usually excitatory when applied iontophoretically to single units (Krnjević, 1975), and so the central actions of nicotine on SNR cell firing during peripheral blockade may well have been indirect. This conclusion accords with the low density of putative nicotinic receptors in SNR shown by receptor autoradiography (Clarke *et al.*, 1984).

Recently, a cholinergic input to substantia nigra has been proposed, arising from the rostral pontine tegmentum (McGeer & McGeer, 1984). This region includes the pedunculopontine and parabrachial nuclei, which contain many cholinergic neurones (Mesulam *et al.*, 1983). Local injection of the excitotoxins kainic acid and folic acid produced remote pathological changes in the SN, reflected by decreases in tyrosine hydroxylase (TH) and glutamic acid decarboxylase (GAD). Pretreatment with the muscarinic antagonist scopolamine prevented the reduction of GAD but not of TH. Considerable amounts of GAD are contained within the SNR (Ribak *et al.*, 1976), and evidence from several sources points to a muscarinic cholinergic excitatory input into SNR (see Introduction). The pathology occurring in SNC could not be attributed to a muscarinic mechanism; the possibility of a nicotinic mechanism was not investigated. Recently, Scarnati *et al.* (1984) found that electrical stimulation of the pedunculopontine nucleus leads to a short latency, orthodromic activation of cells in both SNC

and SNR, which is probably monosynaptic. The response of SNC units was unaffected by the administration of atropine, but again, the possible actions of nicotinic antagonists were not examined (Scarnati, 1984).

In conclusion, the autoradiographic distribution of putative nicotinic cholinoceptors in the substantia nigra (Clarke *et al.*, 1984) suggests that dopaminergic neurones of the pars compacta should be directly excited by nicotine, whereas the drug should have little or no direct effect on SNR cells. The results of our electrophysiological experiments, presented here, are consistent with this prediction.

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