Locomotor activity in rats after administration of nicotinic agonists intracerebrally

¹C. Reavill & ²I.P. Stolerman

Department of Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

- 1 Nicotine (0.13 and 0.4 mg kg⁻¹, s.c.) increased the ambulatory component of locomotor activity in rats previously exposed to the drug. Nicotine did not increase repeated movements reliably.
- 2 An infusion of either nicotine (8 µg) or the potent nicotinic agonist cytisine (4 µg) into the ventral tegmental area of the forebrain increased ambulation but not repeated movements.
- 3 An infusion of nicotine or cytisine into the nucleus accumbens, striatum, dorsal hippocampal formation or motor thalamus did not increase ambulatory or repeated movements.
- 4 Mecamylamine (0.1–1.0 mg kg⁻¹, s.c.) blocked increases in locomotor activity produced by an infusion of nicotine or cytisine into the ventral tegmental area.
- 5 The locomotor activity produced by systemically administered nicotine may be mediated, in part, through nicotinic receptors located in the ventral tegmental area of the mesolimbic dopamine system.

Introduction

Nicotine produces a wide range of behavioural changes, many of which are mediated through the central nervous system. Nicotine can enhance locomotor activity in rats, an effect which is more marked in animals that have been previously exposed to the drug (Morrison & Stephenson, 1972; Clarke & Kumar, 1983a, b). The nicotinic antagonist mecamylamine can block this effect of nicotine. Neuroanatomical studies have identified several brain areas which may be implicated in the behavioural effects of nicotine. Binding sites identified with [³H]-(–)-nicotine or [³H]-acetylcholine in the presence of atropine are concentrated, *inter alia*, in the thalamus, ventral tegmental area (VTA), nucleus accumbens, substantia nigra, striatum and molecular layer of the dentate gyrus of the hippocampal formation, (Schwartz *et al.*, 1984; Clarke *et al.*, 1985). As the hyperkinetic effect of dopamine agonists is mediated by the mesolimbic system (Kelly *et al.*, 1975), it is interesting that recent evidence suggests that at least some of the effects of nicotine involve interaction with central dopamine mechanisms of that system (Imperato *et al.*, 1986; Rowell *et al.*, 1987; Clarke *et al.*, 1988; Lapin *et al.*, 1989).

Apart from one short study (Pert & Chieuh, 1986), the effects on locomotor activity of direct infusions of nicotinic agonists into discrete brain areas have not been determined. The present study investigates the effects on locomotor activity of an infusion of nicotine, or the potent but relatively lipophobic nicotinic agonist cytisine, into several brain regions. Rats that were treated chronically with nicotine (s.c.) were used in all studies because the locomotor stimulant effect of nicotine is clearer in such animals (Morrison & Stephenson, 1972; Clarke & Kumar, 1983a, b). The brain areas for investigation were selected because previous evidence showed that they contained large numbers of binding sites for nicotine, and because they have been implicated in locomotor behaviour.

Methods

Animals

Male hooded Lister rats (Olac) were housed individually with free access to food and water. Lights were on from 7 h 30 min to 19 h 30 min.

Apparatus

Photocell activity cages (30 × 30 × 30 cm) were constructed from transparent Perspex sheet with wire mesh floors. Two parallel beams of infra-red light, modulated at a frequency of 7.5 kHz, were located 3 cm from opposite walls, and 4 cm above the floor of each cage. Interruptions of the beams were recorded by a microcomputer in an adjoining room. During each session, two measures of activity were taken: interruptions of either beam that followed interruptions of the other beam were recorded as 'cage crosses' (ambulation), whereas consecutive interruptions of the same beam were recorded as 'repeated moves'. The activity cages used and the measures taken were modified from Clarke & Kumar (1983a, b). The duration of each test session was 1 h, within which data were recorded separately for 6 consecutive periods of 10 min each.

Behavioural procedures

Rats received daily injections of nicotine (0.4 mg kg⁻¹, s.c.) and were placed in the activity cages for 1 h each day. This procedure continued for 5 days a week for at least 2 weeks, to produce tolerance to locomotor depressant effects of nicotine (Morrison & Stephenson, 1972; Clarke & Kumar, 1983a, b) and to familiarise the rats with the cages. In rats which were to receive intracerebral infusions, guide cannulae were then implanted as described below. One week was allowed for recovery from surgery, and the rats then received at least five more daily injections of nicotine (0.4 mg kg⁻¹, s.c.) before tests began.

Tests of drugs on locomotor activity took place twice weekly (Tuesdays and Fridays), and on the intervening days the rats continued to receive once daily injections of nicotine (0.4 mg kg⁻¹, s.c.). The very few rats in which nicotine increased cage crosses by less than 20% were excluded from these studies. Different doses of test drugs given systemically or intracerebrally were administered in random order and each dose was tested once in each rat. Test sessions began immediately after administrations of drugs and lasted for 1 h. To provide a basis for comparison, each experiment with intracerebral administrations of drugs also included tests with systemic injections of saline (1 ml kg⁻¹, s.c.) or nicotine (0.4 mg kg⁻¹, s.c.).

The effects of nicotine administered systemically were determined first. Saline and three doses of nicotine (0.04 mg kg⁻¹, 0.13 mg kg⁻¹ and 0.40 mg kg⁻¹) were tested in random order, in rats into which intracerebral guide cannulae had not been implanted.

¹ Present address: Smith Kline and French Research Ltd, The Frythe, Welwyn, Herts. AL6 9AR.

² Author for correspondence.

To determine the effects of nicotine or cytosine given intracerebrally, rats were given bilateral infusions of vehicle or a dose of drug into the brain regions detailed below. Normally three doses of each drug were tested. The rats were restrained gently by hand while the drugs were infused through 28 gauge internal cannulae (Plastic Products) connected via polypropylene tubing to 10 μ l Hamilton syringes. The solutions were delivered at a rate of 1 μ l min⁻¹ for 30 s by a Sage Model 341A infusion pump, and the internal cannulae were left *in situ* for a further 1 min before withdrawal. Normally, doses of only one agonist were infused into each brain region and thus, individual rats received no more than 4–5 intracerebral infusions (two per week).

To study the effects of mecamylamine, rats received either saline or mecamylamine systemically (s.c.) 30 min before tests. Mecamylamine was administered in doses of 0.1 mg kg⁻¹, 0.32 mg kg⁻¹ and 1.0 mg kg⁻¹. These doses of mecamylamine were chosen from previous work demonstrating blockade of locomotor activity produced by nicotine (Clarke & Kumar, 1983a, b). Then, either saline, nicotine or cytosine was infused intracerebrally, through guide cannulae aimed at the ventral tegmental area (VTA), immediately before 1 h tests. A single intracerebral dose of nicotine (8 μ g) or cytosine (3.2 μ g) was used in these experiments, selected from the results of the earlier experiments.

At the conclusion of each experiment, all rats with implanted cannulae were killed by a humane method and brains were removed for histological examination.

Surgery

Tolerant rats were anaesthetized with halothane and held on a Stoelting stereotaxic frame. A hole was drilled in the skull over each hemisphere to facilitate placement of 22 gauge guide cannulae (Plastic Products). The cannulae were adjusted to an angle of 10° from vertical and they were lowered to just above the desired site. The stereotaxic coordinates for the injection sites were based on the atlas of Pellegrino *et al.* (1979) and were as follows: dorsal hippocampus, A3.0, L2.5, V3.0; VTA, A3.0, L1.5, V8.8; nucleus accumbens, A9.0, L2.0, V7.0; motor thalamus, A5.2, L2.0, V6.7; striatum, A8.2, L3.0, V4.7. The cannulae were embedded in acrylic dental cement and anchored to the skull with 4 stainless steel screws (10BA, cheesehead).

Histology

Brains were fixed in 40% formaldehyde/glacial acetic acid/methanol (1:1:8), processed, and embedded in paraffin wax. Serial sections (20 μ m) were cut on a sledge microtome and stained with Luxol fast blue and Mallory's phosphotungstic acid Haematoxylin or cresyl violet. The extent of the infusion sites was examined microscopically and recorded on composite diagrams taken from the atlas of König & Klippel (1963).

Data analysis

Activity scores for the entire hour of each test were examined by one factor or two factor analyses of variance with repeated measures (Winer, 1971). Multiple comparisons with control groups were made with Dunnett's modified *t* test.

Drugs

All doses were calculated as those of the bases. (–)-Nicotine hydrogen-(+)-tartrate (BDH) was dissolved in distilled water and the pH was adjusted to 7.0 with sodium hydroxide. Mecamylamine hydrochloride (Merck, Sharp & Dohme) was dissolved in distilled water. All systemic injections were subcutaneous in volumes of 1.0 ml kg⁻¹ and the control was 0.9% saline. For intracerebral infusions, aqueous solutions of nicotine or cytosine (Koch-Light) were made isotonic with sodium chloride. Control (vehicle) intracerebral infusions were of isotonic sodium chloride.

Results

Systemic administration of nicotine

Nicotine increased locomotor activity as measured by the number of cage crosses ($F(3, 21) = 9.61, P < 0.001$). The effect was dose-related and significant after 0.13 mg kg⁻¹ ($t(21) = 3.38, P < 0.05$) and 0.4 mg kg⁻¹ of nicotine ($t(21) = 4.96, P < 0.01$). The lowest dose of nicotine (0.04 mg kg⁻¹) did not increase the number of cage crosses above baseline (Figure 1a). Figure 2a shows that nicotine (0.4 mg kg⁻¹) increased cage crosses uniformly throughout the 1 h period of measurement. Although there was a significant overall effect of nicotine on the number of repeated moves ($F(3, 21) = 3.99, P < 0.05$), no individual dose of nicotine differed significantly from saline.

Focal infusions of nicotine or cytosine into brain areas

The results with infusions into the VTA are described first. There were clear overall effects of drug treatments (cage crosses, $F(5, 85) = 6.26, P < 0.001$; repeated moves, $F(5, 85) = 10.1, P < 0.001$). Figure 3a shows that after bilateral infusions of nicotine (8 μ g) into the VTA, the number of cage crosses increased to 178 ± 26 as compared with 112 ± 15 after vehicle ($t(85) = 2.87, P < 0.02$). Figure 2b shows that this effect was clearest during the 20–40 min after infusion. In the same

Figure 1 Locomotor activity in rats exposed to nicotine (0.4 mg kg⁻¹, s.c.) daily for two weeks and then tested twice weekly ($n = 8$). Each dose of nicotine shown was tested once in each rat (●). Activity after an injection of saline is also shown (○). Each point in this and subsequent figures represents the mean of data obtained from 1 h sessions in photocell activity cages. Vertical lines show s.e.mean. (a) Shows results for ambulation; (b) results for repeated moves. Significant differences from saline are marked (* $P < 0.05$; ** $P < 0.01$).

Figure 2 Locomotor activity in three groups of rats exposed to nicotine (0.4 mg kg⁻¹, s.c.) daily. Ambulation (cage crosses) is shown for 6 successive periods of 10 min each, beginning immediately after administration of drug or vehicle. Results are shown for nicotine administered systemically (0.4 mg kg⁻¹, s.c., $n = 8$, ●) and intracerebrally (8 μ g infused bilaterally into ventral tegmental area (VTA), $n = 18$, ■), for cytosine administered intracerebrally (3.2 μ g infused bilaterally into the VTA, $n = 13$, ×), and for saline administered systemically (○) or infused into VTA (□).

Figure 3 Locomotor activity in rats exposed to nicotine (0.4 mg kg^{-1} , s.c.) daily and tested with different doses of nicotine ($n = 18$, ■) or cytisine ($n = 9$, ×) infused into the ventral tegmental area (VTA). Activity is also shown after an infusion of saline into VTA (□), and after an injection of saline (○) or 0.4 mg kg^{-1} of nicotine (●) subcutaneously. Asterisks show significant differences from vehicles (* $P < 0.05$; ** $P < 0.01$).

rats, nicotine (0.4 mg kg^{-1} , s.c.) increased cage crosses to 202 ± 21 as compared with 101 ± 9 after saline (Figure 3a). Nicotine (0.4 mg kg^{-1} , s.c.) also increased the number of repeated moves to 601 ± 45 from 421 ± 31 after saline ($t(85) = 4.38$, $P < 0.01$), but this effect was not present after infusions of nicotine into the VTA (Figure 3b). An infusion of the vehicle into the VTA did not significantly increase either cage crosses or repeated moves in comparison with results after systemically administered saline, either in the preceding experiments with nicotine or those with cytisine that are described next.

The effects of cytisine infused into the VTA were tested in different rats. There was an overall effect of drug treatments ($F(5, 40) = 3.45$, $P < 0.05$). Figure 3c shows that a $4 \mu\text{g}$ dose of cytisine increased the number of cage crosses to 153 ± 21 as compared with 86 ± 15 after vehicle ($t(40) = 2.75$, $P < 0.05$). Figure 2c shows that this effect was clearest during the 20–40 min after infusion. Cytisine in doses of $2 \mu\text{g}$ or $8 \mu\text{g}$ did not increase the number of cage crosses significantly, although inspection of Figure 2 shows that there were no substantial differences between the results after the three doses of cytisine. Systemic administration of nicotine (0.4 mg kg^{-1} , s.c.) increased ambulation to 155 ± 16 as compared with 87 ± 6 crosses after saline (Figure 3c). Neither an infusion of cytisine into the VTA, nor an injection of nicotine (0.4 mg kg^{-1} , s.c.) systemically, increased repeated moves (Figure 3d). In a separate experiment in other rats ($n = 14$), cytisine ($3.2 \mu\text{g}$) infused into the VTA increased cage crosses to 181 ± 18 , as compared with 124 ± 13 after vehicle ($t(13) = 3.04$, $P < 0.01$).

An infusion of nicotine ($2\text{--}8 \mu\text{g}$) into the nucleus accumbens did not increase the number of cage crosses in comparison with infusion of vehicle into the same area. However, analysis of variance showed an overall effect due to drug treatments ($F(5, 55) = 13.2$, $P < 0.001$), and nicotine (0.4 mg kg^{-1} , s.c.) enhanced ambulation (Figure 4a). Similarly, Figure 4b shows that an infusion of cytisine ($0.32\text{--}3.2 \mu\text{g}$) into the nucleus accumbens did not alter locomotor activity in comparison with vehicle, whereas there was an overall effect due to drug treatments ($F(5, 50) = 9.27$, $P < 0.001$), and nicotine (0.4 mg kg^{-1} , s.c.) increased the number of cage crosses. An

Figure 4 Locomotor activity in rats exposed to nicotine (0.4 mg kg^{-1} , s.c.) daily and tested with different doses of nicotine (■) or cytisine (×). (a) and (b) Show effects of an infusion of nicotine and cytisine into nucleus accumbens ($n = 11\text{--}12$). (c) Shows the effect of an infusion of nicotine into striatum ($n = 9$). Activity is also shown after an infusion of saline into striatum or accumbens (□), and after an injection of saline (○) or 0.4 mg kg^{-1} of nicotine (●) subcutaneously. Asterisks show significant differences from saline (** $P < 0.01$).

infusion of the vehicle intracerebrally did not significantly increase either cage crosses or repeated moves in comparison with results after systemically administered saline, either in the preceding experiments with infusions into the nucleus accumbens or with infusions into other regions as described below.

An infusion of nicotine ($2\text{--}8 \mu\text{g}$) into the striatum did not significantly increase the number of cage crosses in comparison with infusions of vehicle ($n = 9$). However, Figure 4c shows that in the same rats, there was an overall effect due to drug treatments ($F(5, 40) = 7.08$, $P < 0.001$), and nicotine (0.4 mg kg^{-1} , s.c.) increased cage crosses significantly.

Nicotine ($2\text{--}8 \mu\text{g}$) infused into the dorsal hippocampal formation did not increase ambulation ($n = 8$). For example, after nicotine ($8 \mu\text{g}$ bilaterally), the number of cage crosses was 103 ± 14 , as compared with 121 ± 23 after vehicle. However, there was an overall effect of drug treatments ($F(5, 35) = 7.64$, $P < 0.001$) and nicotine (0.4 mg kg^{-1} , s.c.) increased the number of cage crosses to 192 ± 33 , as compared with 96 ± 18 after saline (s.c.). Similarly, cytisine ($0.32\text{--}3.2 \mu\text{g}$) did not increase locomotor activity when infused into the dorsal hippocampal formation ($n = 7$). After cytisine ($3.2 \mu\text{g}$ bilaterally), the number of cage crosses was 112 ± 19 , as compared with 103 ± 17 after vehicle. However, there was an overall effect of drug treatments ($F(5, 30) = 16.7$, $P < 0.001$) and nicotine (0.4 mg kg^{-1} , s.c.) increased the number of cage crosses to 231 ± 30 , as compared with 109 ± 16 after saline (s.c.).

Nicotine ($2\text{--}8 \mu\text{g}$) infused into the motor thalamus did not increase ambulation ($n = 7$). Nicotine ($8 \mu\text{g}$ bilaterally) produced the largest activity scores (116 ± 31 , as compared with 81 ± 23 after vehicle), but the increase was not significant. However, there was an overall effect of drug treatments ($F(5, 30) = 6.87$, $P < 0.001$) and nicotine (0.4 mg kg^{-1} , s.c.) increased the number of cage crosses to 143 ± 24 , as compared with 57 ± 16 after saline (s.c.). Similarly, cytisine ($0.32\text{--}3.2 \mu\text{g}$) did not increase locomotor activity when infused into the motor thalamus ($n = 5$). After cytisine ($3.2 \mu\text{g}$ bilaterally), the number of cage crosses was 76 ± 24 , as compared with 58 ± 13 after vehicle. However, there was an overall effect of drug treatments ($F(5, 20) = 7.03$, $P < 0.01$) and nicotine (0.4 mg kg^{-1} , s.c.) increased the number of cage crosses to 189 ± 40 , as compared with 67 ± 11 after saline (s.c.).

Neither nicotine nor cytisine significantly increased the number of repeated moves when infused into any of the areas under investigation. For this reason, data for repeated moves have been presented only for nicotine administered systemically (Figures 1 and 3), and for infusions of nicotine or cytisine into the VTA (Figure 3).

Effects of mecamylamine

An injection of nicotine (0.4 mg kg^{-1}) systemically markedly increased cage crosses as shown by a two-factor analysis of

Figure 5 Locomotor activity in rats exposed to nicotine (0.4 mg kg^{-1} , s.c.) daily and tested after intracerebral infusions of nicotinic agonists ($n = 6-7$). (a) Effects of three doses of mecamylamine (s.c.) 30 min before an infusion of $8 \mu\text{g}$ nicotine bilaterally into the ventral tegmental area (VTA, ■). (b) Effects of three doses of mecamylamine (s.c.) 30 min before an infusion of $3.2 \mu\text{g}$ of cytosine bilaterally into VTA (×). In both (a) and (b), points above 0 show effects of subcutaneous saline (○), 0.4 mg kg^{-1} (s.c.) nicotine (●), and $8 \mu\text{g}$ nicotine (■) or $3.2 \mu\text{g}$ cytosine (×) infused into the VTA.

variance ($F(1, 77) = 54.3$, $P < 0.01$). There was no overall difference between the activity scores for two subgroups of the rats ($F(1, 11) = 1.19$). Nicotine ($8 \mu\text{g}$, Figure 5a) or cytosine ($3.2 \mu\text{g}$, Figure 5b) infused into the VTA of the subgroups slightly increased cage crosses and the overall effect was significant when compared with results after infusion of vehicle ($F(1, 77) = 6.13$, $P < 0.05$). Nicotine and cytosine did not increase locomotor activity when infused into the VTA after administration of any dose of mecamylamine (largest $F(1, 77) = 1.69$). There was no significant difference between the

effects of mecamylamine in the two groups of rat (interaction $F(7, 77) = 0.91$).

Histology

Histological examination revealed that the sites into which nicotine was infused in the VTA were concentrated in an area ventral to the red nucleus and intermediate between substantia nigra and interpeduncular nucleus, with occasional encroachment on these areas. Figure 6 illustrates the location of these infusion sites. However, in the study with mecamylamine, the infusion sites for the agonists were located towards the rostral pole of the VTA. Within the nucleus accumbens, infusion sites were predominantly in the ventral and medial sector with some overlap into the ventral striatum (Figure 6). Striatal nicotine injections were confined to the medial striatum lying dorsal to the nucleus accumbens. Infusions into the hippocampus were confined exclusively to the dorsal hippocampus. Thalamic nicotine injection sites were located primarily in the ventral sector of the motor thalamus comprising the ventrolateral, ventral anterior and ventromedial thalamic nuclei (Figure 6). The sites into which cytosine was infused were similar to those for nicotine and histological details are not presented. Data for the small number of rats in which the infusion sites were not located in the targeted areas have been excluded throughout.

Discussion

Previous studies have established that nicotine can increase locomotor activity in rats and the present experiments investigate brain regions that may be involved. Locomotor activity was separated into two components, cage crosses that reflected ambulatory activity, and repeated moves that probably encompassed a wider range of behavioural changes (such as grooming and rearing onto the hind legs). The results with the two measures were different. Nicotine consistently increased cage crosses both when administered systemically and by intracerebral infusion into the VTA. Nicotine did not increase repeated moves reliably when administered systemically, and it never did so when given intracerebrally. Direct observation also suggested that nicotine (s.c.) did not produce stereotypy of the type seen with dopamine agonists. The findings support previous observations that nicotine can increase ambulatory activity but produces only low levels of stereotyped responses (Clarke & Kumar, 1983a, b; Lapin *et al.*, 1987), and emphasize again the importance of using recording methods that distinguish adequately between different components of locomotor activity (Krsiak *et al.*, 1970; Sahgal *et al.*, 1989). Crude measures of activity that confound ambulation with other behaviours may fail to detect effects of nicotine, especially after intracerebral injections.

The findings support the view that nicotine may increase locomotor activity by acting in the VTA. Alone among the brain areas examined, the VTA was sensitive to nicotine and cytosine, as shown by increases in ambulation after infusions of the drugs. The finding that the nicotinic antagonist mecamylamine blocked the effects of the agonists suggests the involvement of nicotinic receptors. A previous study also suggests that infusion of cytosine into the VTA may increase locomotor activity (Pert & Chiueh, 1986). The effects of nicotine and cytosine were generally similar, although cytosine acted at smaller doses which accords with its greater potency as an inhibitor of the binding of [^3H]-nicotine (Clarke *et al.*, 1985; Reavill *et al.*, 1988). The $8 \mu\text{g}$ dose of nicotine needed to enhance locomotor activity would have amounted to about 0.05 mg kg^{-1} if given systemically, which is close to the threshold for stimulation of locomotor activity (Figure 1). With both nicotine and cytosine, there was a delay between infusion and effect, the basis for which is not known. For these and other reasons, there is a need for more precise mapping of the neuroanatomical site through which nicotine acts; the inter-

Figure 6 Composite diagrams showing the sites where nicotine was infused intracerebrally into the ventral tegmental area (VTA) (a), dorsal hippocampal formation (b), motor thalamus (c), striatum (d) and nucleus accumbens (e). Anterior coordinates taken from the atlas of König & Klippel (1963) are shown. In the case of VTA, only a representative selection of injection sites is shown for clarity. All infusions were bilateral.

peduncular nucleus and substantia nigra are near to the VTA, and both of these regions contain many binding sites for [^3H]-nicotine (Clarke *et al.*, 1985). In the experiments with mecamylamine, infusions of the agonists into the rostral pole of the VTA produced less marked increases in ambulation than when the drugs were infused into the central region of the VTA in the preceding experiments.

The neurochemical mechanisms by which nicotine produces locomotor stimulation are still unclear, but evidence has accumulated that some of its effects are mediated preferentially through the dopaminergic mesolimbic system. The importance of the dopaminergic projection from the VTA to the nucleus accumbens in locomotor activity is well established (Pijnenburg & Van Rossum, 1973; Kelly *et al.*, 1975). Degeneration of forebrain dopamine pathways produced by 6-hydroxydopamine reduces the numbers of binding sites for [^3H]-(-)-nicotine on dopamine cell bodies and terminals (Clarke & Pert, 1985). Release of dopamine *in vitro* and *in vivo* occurs with lower concentrations of nicotine in the nucleus accumbens than in the striatum (Imperato *et al.*, 1986; Rowell *et al.*, 1987; Rapier *et al.*, 1988). Evidence from studies of the nicotine discriminative stimulus and of rotational behaviour also support the view that nicotine interacts with dopamine mechanisms (Reavill & Stolerman, 1987). An important recent study shows that the locomotor activation produced when nicotine is administered systemically is accompanied by increased dopamine utilisation in some limbic areas, and that it is attenuated by 6-hydroxydopamine lesions of the nucleus accumbens (Clarke *et al.*, 1988). These findings suggest that the locomotor activity produced by nicotinic agonists infused into the VTA may also involve the mesolimbic dopamine system. It was surprising that infusions of the agonists into the nucleus accumbens did not stimulate ambulation. However, although acute systemic injection of nicotine can increase release and metabolism of dopamine in the nucleus accumbens, chronic exposure to nicotine (as in the present study) can attenuate the effect (Lapin *et al.*, 1989).

There appears to be a close relationship between binding sites for [^3H]-(-)-nicotine and dopamine nerve terminals in the striatum, but the experiments showed that focal infusion

of nicotine into the striatum did not enhance locomotor activity. Striatal dopamine mechanisms are associated more with stereotyped behaviours than with locomotor activity which is mediated mainly through the mesolimbic system (Fog *et al.*, 1967; Kelly *et al.*, 1975).

The autoradiographic study of Clarke *et al.* (1985) showed that large areas of the thalamus, including the motor thalamus, exhibited some of the greatest numbers of binding sites for [^3H]-nicotine in rat brain. The motor thalamus, comprising the ventrolateral and ventral anterior nuclei, is the main thalamic relay centre between two major motor areas, the basal ganglia and cerebellum, which project to the motor cortex (Massion, 1976/77). However, the present results do not provide any evidence that nicotine produces locomotor stimulation by an action in the motor thalamus.

Recent evidence has shown that hippocampal projections to the nucleus accumbens play a role in motor stimulation in the rat, as blockade of glutamate mechanisms in the nucleus accumbens attenuates the locomotor activity produced by carbachol injection into the dentate gyrus (Mogenson & Nielsen, 1984). The hippocampus may also contribute to some behavioural effects of nicotine in the rat; an infusion of nicotine into the dorsal hippocampus of rats trained to discriminate nicotine from saline in two-bar operant conditioning procedures can produce effects like those of nicotine injected systemically (Meltzer & Rosecrans, 1981; Reavill & Stolerman, unpublished data). However, the present results indicate that an infusion of nicotine or cytosine into the dorsal hippocampus does not increase locomotor activity and thus, they suggest that different brain areas may mediate different behavioural effects of nicotine. However, for the present, only the VTA and the mesolimbic dopamine system have been linked to the activating effect of nicotine on ambulation.

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References

- CLARKE, P.B.S., FU, D.S., JAKUBOVIC, A. & FIBIGER, H.C. (1988). Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. *J. Pharmacol. Exp. Ther.*, **246**, 701–708.
- CLARKE, P.B.S. & KUMAR, R. (1983a). The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br. J. Pharmacol.*, **78**, 329–337.
- CLARKE, P.B.S. & KUMAR, R. (1983b). Characterization of the locomotor stimulant action of nicotine in tolerant rats. *Br. J. Pharmacol.*, **80**, 587–594.
- CLARKE, P.B.S. & PERT, A. (1985). Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. *Brain Res.*, **348**, 355–358.
- CLARKE, P.B.S., SCHWARTZ, R.D., PAUL, S.M., PERT, C.B. & PERT, A. (1985). Nicotinic binding in rat brain: autoradiographic comparison of [^3H]acetylcholine, [^3H]nicotine, and [^{125}I]- α -bungarotoxin. *J. Neurosci.*, **5**, 1307–1315.
- FOG, R.L., RANDRUP, A. & PAKKENBERG, H. (1967). Aminergic mechanisms in corpus striatum and amphetamine-induced stereotyped behaviour. *Psychopharmacologia*, **11**, 179–183.
- IMPERATO, I., MULAS, A. & DI CHIARA, G. (1986). Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur. J. Pharmacol.*, **132**, 337–338.
- KELLY, P.H., SEVIOUR, P.W. & IVERSEN, S.D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.*, **94**, 507–522.
- KONIG, J.F.R. & KLIPPEL, R.A. (1963). *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: Williams & Wilkins Co.
- KRSIAK, M., STEINBERG, H. & STOLERMAN, I.P. (1970). Uses and limitations of photocell activity cages for assessing effects of drugs. *Psychopharmacologia*, **17**, 258–274.
- LAPIN, E.P., MAKER, H.S., SERSHEN, H., HURD, Y. & LAJTHA, A. (1987). Dopamine-like action of nicotine: lack of tolerance and reverse tolerance. *Brain Res.*, **407**, 351–363.
- LAPIN, E.P., MAKER, H.S., SERSHEN, H. & LAJTHA, A. (1989). Action of nicotine on accumbens dopamine and attenuation with repeated administration. *Eur. J. Pharmacol.*, **160**, 53–59.
- MASSION, J. (1976/77). The thalamus in the motor system. *Appl. Neurophysiol.*, **39**, 222–238.
- MELTZER, L.T. & ROSECRANS, J.A. (1981). Investigations on the CNS sites of action of the discriminative stimulus effects of arecoline and nicotine. *Pharmacol. Biochem. Behav.*, **15**, 21–26.
- MOGENSEN, G.J. & NIELSEN, M.A. (1984). A study of the contribution of hippocampal-accumbens-subpallidal projections to locomotor activity. *Behav. Neural Biol.*, **42**, 38–51.
- MORRISON, C.F. & STEPHENSON, J.A. (1972). The occurrence of tolerance to a central depressant action of nicotine. *Br. J. Pharmacol.*, **46**, 151–156.
- PELLEGRINO, L.J., PELLEGRINO, A.S. & CUSHMAN, A.J. (1979). *A Stereotaxic Atlas of the Rat Brain*. New York, London: Plenum Press.
- PERT, A. & CHIUH, C.C. (1986). Effects of intracerebral nicotinic agonists on locomotor activity: involvement of mesolimbic dopamine. *Soc. Neurosci. Abstr.*, **12**, 250.4. P.917.
- PIJNENBURG, A.J.J. & VAN ROSSUM, J.M. (1973). Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. *J. Pharm. Pharmacol.*, **25**, 1003–1005.
- RAPIER, C., LUNT, G.G. & WONNACOTT, S. (1988). Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. *J. Neurochem.*, **50**, 1123–1130.
- REAVILL, C. & STOLERMAN, I.P. (1987). Interaction of nicotine with dopaminergic mechanisms assessed through drug discrimination and rotational behaviour in rats. *J. Psychopharmacol.*, **1**, 264–273.

- REAVILL, C., JENNER, P., KUMAR, R. & STOLERMAN, I.P. (1988). High-affinity binding of [³H](–)-nicotine to rat brain membranes and its inhibition by analogues of nicotine. *Neuropharmacology*, **27**, 235–241.
- ROWELL, P.P., CARR, L.A. & GARNER, A.C. (1987). Stimulation of [³H]dopamine release by nicotine in rat nucleus accumbens. *J. Neurochem.*, **49**, 1449–1454.
- SAHGAL, A., KEITH, A.B., LLOYD, S. & EDWARDSON, J.A. (1989). [D-TRP¹¹]-Neurotensin, unlike α-flupenthixol, may not block amphetamine-induced hyperactivity in rats. *Neuropharmacology*, **28**, 283–289.
- SCHWARTZ, R.D., LEHMANN, J. & KELLAR, K.J. (1984). Presynaptic nicotinic cholinergic receptors labelled by [³H]acetylcholine on catecholamine and serotonin axons in brain. *J. Neurochem.*, **42**, 1495–1498.
- WINER, B.J. (1971). *Statistical Principles in Experimental Design*. 2nd edn. New York: McGraw-Hill.

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