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The effects of nicotine on locomotor activity in non-tolerant and tolerant rats

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1 Rats were tested for locomotor activity in photocell cages, for 80 min starting immediately after subcutaneous injection of (−)-nicotine bitartrate or 0.9% w/v NaCl solution (saline). In non-tolerant subjects, nicotine (0.1 to 0.4 mg/kg base) depressed activity and induced ataxia in the first 20 min, but increased activity later in the session; these actions were dose-dependent.

2 Tolerance was studied by comparing rats given nicotine (0.4 mg/kg s.c.) every day with control rats given saline instead. Each week, every subject was tested once with nicotine (0.4 mg/kg) and once with saline. With daily or even weekly injections of nicotine, the initial depressant action of the drug was replaced by a dose-dependent stimulant action which occurred throughout the session. In these tolerant animals, little ataxia was seen except when a larger dose of 0.8 mg/kg was given. Tolerance to the depressant action of nicotine persisted for at least 3 weeks.

3 In non-tolerant subjects, mecamylamine (0.5, 1.0 mg/kg s.c.) prevented the initial depressant action of nicotine (0.4 mg/kg). In tolerant rats, the locomotor stimulant action of nicotine (0.4 mg/kg) was prevented by mecamylamine (0.1, 0.32, 1.0 mg/kg s.c.) in a dose-related way; the quaternary ganglion blocker, hexamethonium (0.2, 1.0, 5.0 mg/kg s.c.) had little or no such effect. Neither mecamylamine nor hexamethonium altered activity when given alone.

4 It is suggested that a few treatments with nicotine can unmask a stimulant action of the drug, probably of central origin, which possibly reflects a stimulation of nicotine receptors.

Introduction

Recent evidence suggests that nicotine is self-administered by animals (Goldberg, Spealman & Goldberg, 1981; Nelson & Cox, 1982), as well as by man. The mechanism by which nicotine acts as a reinforcer remains obscure. Whilst the drug produces various signs of central nervous system stimulation or depression (Gilbert, 1979), the predominant action can be hard to predict. Studies of locomotor activity may help to elucidate the central actions of this drug.

Preliminary studies of the time-course of the effects of nicotine (Kuschinsky & Hotovy, 1943; Rosecrans, 1969) suggest that in non-tolerant rats, nicotine may stimulate activity after an initial period of depression. This is consistent with the results of tests of brief duration carried out at different times after injection (e.g. Stolerman, Fink & Jarvik, 1973; Bättig, Driscoll, Schlatter & Uster, 1976). The first experiment reported here was a dose-response study of the actions of nicotine on locomotor activity in non-tolerant rats in which the time course of the depressant and stimulant effects of the drug was also investigated.

Mecamylamine, a ganglion blocking agent, has been found to prevent both stimulant and depressant behavioural actions of nicotine (Morrison, Goodyear & Sellors, 1969; Barthelemy, Tremblay & Jacob, 1970; Newman, 1972). The second experiment tested whether pretreatment with mecamylamine blocked the acute effects of nicotine on locomotor activity.

Tolerance to the depressant effects of nicotine on locomotor activity is acquired rapidly if three injections are given daily (Stolerman *et al.*, 1973), but it may also occur with daily or twice weekly administration (Morrison & Stephenson, 1972; Stolerman, Bunker & Jarvik, 1974). Once tolerance is established, activity may be increased by the drug (Morrison & Stephenson, 1972). Abstinence from tobacco smoking is accompanied by diverse symptoms in man, but the extent to which these are due to the withdrawal of nicotine is not known (Jaffe & Jarvik, 1978). However, in animals, behavioural changes have been reported following the withdrawal of chronically administered nicotine (Morrison, 1974; Hutchinson & Emley, 1973). The third experiment examined how the effects of nicotine on locomotor

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activity changed over successive tests when rats were given daily injections of the drug for four weeks. During this period, the rats became tolerant to the depressant effects of nicotine, and a stimulant action emerged. The rats were next tested with several doses of nicotine, as in the acute dose-response study (experiment 1). Nicotine was then withdrawn and the levels of activity were measured on subsequent days. Finally, the effects of nicotine were reassessed after 23 days of abstinence, in order to see whether tolerance to the drug could persist.

The fourth experiment assessed the relative contributions of central and peripheral sites of action to the locomotor stimulation produced by nicotine in tolerant rats. Subjects were pretreated with one of two ganglion blocking drugs, hexamethonium or mecamylamine. Hexamethonium does not readily enter the brain after peripheral administration (Mason, 1980), whereas mecamylamine is believed to act both centrally and peripherally (Bennet, Tyler & Zaimis, 1957).

Methods

Male Lister hooded rats were obtained from OLAC 76 Ltd (Bicester) and were maintained on food and water *ad libitum*. In experiments 1, 3 and 4, the rats were housed in pairs, on a random basis with respect to drug treatment. In experiment 2, rats were housed singly. A normal (08 h 00 min: 20 h 00 min) day-night cycle was imposed by electric lighting.

Apparatus

Test cages (approximate dimensions $30 \times 30 \times 30$ cm) were made of clear perspex with a wire grid floor. Parallel, infra-red photobeams, 23 cm apart, were projected 4 cm away from two opposite walls of the chamber; the beams ran 4.5 cm above the floor. Beam breaks were counted by a solid state programming device, and locomotor activity was measured by the number of times a rat moved from one beam to the other. Data were printed every 10 min by an electromagnetic counter in an adjacent room. Rats were tested for 80 min immediately after injection. Testing was carried out between 10 h 00 min and 15 h 00 min.

Drugs

(−)-Nicotine hydrogen (+)-tartrate (BDH, Poole) was dissolved in 0.9% w/v NaCl solution (saline) and neutralized to pH 7.2 ± 0.2 with NaOH. Mecamylamine HCl (Merck & Co.) and hexamethonium Br (Sigma, Poole) were dissolved in saline. All injections were given subcutaneously into the flank in a volume of 1 ml/kg. Doses are expressed as base. Control injections were of saline.

Analysis of data

Following preliminary analysis, the data were divided into four consecutive periods of 20 min. Multivariate analysis of variance was used, each rat serving as its own control. Analyses of trends were made across dose and time. A dose-dependent effect refers to a significant linear trend across absolute values of dose.

Procedure

(1) *The effects of nicotine on locomotor activity before chronic treatment* Fourteen rats (250–287 g) were used which were naïve to drug and had no previous experience of the apparatus before the start of testing. Each rat received each dose of nicotine (0, 0.1, 0.2, 0.4 mg/kg) once. The order of the drug presentation was based on a Williams square (Cox, 1958), in order to counterbalance carry-over effects, but with two subjects missing from the design. Test days were three or four days apart.

(2) *An attempt to prevent the effects of nicotine on locomotor activity in non-tolerant rats by pretreatment with mecamylamine* Eleven rats (340–585 g) were used which were naïve to drug and had no previous experience of the apparatus. Each rat received each combination of mecamylamine (0, 0.5, 1.0 mg/kg) and nicotine (0 or 0.4 mg/kg) on one occasion only. The six drug combinations were administered according to an incomplete Williams Square design. Test days were spaced three or four days apart. A pretreatment injection preceded the treatment injection by 20 min. Rats were tested immediately after the second injection.

(3) *The effects of nicotine on locomotor activity in rats maintained on a constant daily dose of the drug* Twenty rats were used, weighing between 361 and 464 g at the start of the experiment. Fourteen of these had been used in experiment 1, carried out two months before. The remaining six rats were each tested with saline and nicotine (0.4 mg/kg) once only, at the time of experiment 1. Subjects were randomly allocated to two groups, in which they received a nicotine injection either daily, or once a week at the time of testing.

Phase 1 (Days 1–30): rats were tested at weekly intervals, before and during the period of chronic daily injections. Each rat was tested on Monday and Tuesday, with saline and nicotine (0.4 mg/kg). The order of drug presentation was counterbalanced, so that half the rats in each chronic group received nicotine on Monday, and half received the drug on

Tuesday. On the day after the tests at week 0, daily injections of nicotine, 0.4 mg/kg, were begun; rats in the control group received saline injections instead, and so they were only given nicotine once a week, on the days when they were tested for activity. When the rats in the chronic nicotine group were tested with saline, their dose of nicotine was administered after the session, in order to maintain a constant daily intake of the drug.

Phase 2 (Days 33–46): a dose-response study was carried out. Daily injections of nicotine or saline were maintained, and rats were tested on every third day with a dose of nicotine (0, 0.1, 0.2, 0.4, 0.8 mg/kg), in a similar manner to experiment 1. Each rat received each dose of nicotine once. With the exception of the highest dose of nicotine, drugs were given in a counterbalanced order according to a Williams Square design (Cox, 1958). After each test session, rats in the chronic nicotine group were injected with a complementary dose in order to maintain a constant daily intake of the drug amounting to 0.4 mg/kg. The highest dose of nicotine was tested last (Day 46) in order to avoid possible carry-over effects.

Phase 3 (Days 47–71): chronic injections of nicotine or saline were discontinued after day 49. On days 50, 53, 57 and 64 all rats were tested with saline in order to determine activity in withdrawal i.e. when they were 1, 4, 8 and 15 days abstinent from nicotine.

Phase 4 (Days 72, 73): In order to assess any residual tolerance, all subjects were retested after injections of nicotine and saline following 23 days of abstinence.

(4) *Comparison of the effects of pretreatment with hexamethonium or mecamylamine on the locomotor stimulation produced by nicotine* The twenty rats from experiment 3 were randomly allocated to two groups. Twelve rats (438–540 g) were tested with mecamylamine, and eight rats (414–508 g) were tested with hexamethonium. Within each group, one half of the rats had previously received daily injections of nicotine, whilst the remainder had received daily saline. Testing began one week after the last day of experiment 3.

Each rat received each combination of pretreatment (mecamylamine 0, 0.1, 0.32, 1.0 mg/kg or hexamethonium 0, 0.2, 1.0, 5.0 mg/kg) and nicotine (0, 0.4 mg/kg) on one occasion only. The eight drug combinations were administered according to a Williams Square design with incomplete cells. Tests were carried out on Tuesdays and Fridays. The pretreatment injection preceded the nicotine injection by 20 min, as in experiment 2. Rats were tested immediately after treatment injection. On Sundays, an additional injection of nicotine (0.4 mg/kg) in the absence of either antagonist was given to each rat in its home cage in order to maintain tolerance.

Results

(1) *Nicotine and locomotor activity before chronic treatment*

In rats injected with saline, activity declined over successive 20 min periods of the test session ($F = 185.2$, d.f. 1, 13, $P < 0.0001$: see Figure 1). In the first 20 min of the session, activity was diminished in a dose-dependent manner ($F = 15.3$, d.f. 1, 13, $P < 0.005$). Within 2 min of the injection at the two higher doses, the rats became flaccid and tended to lie outstretched on the floor of the cage. Panting was frequently observed. About 5 min after injection, nicotine induced a loss of righting reflex in some animals. During recovery from this phase, there was marked incoordination, and the hind limbs appeared to be most affected.

Between 20 and 40 min after injection, no significant drug effect was observed at any dose. However, in

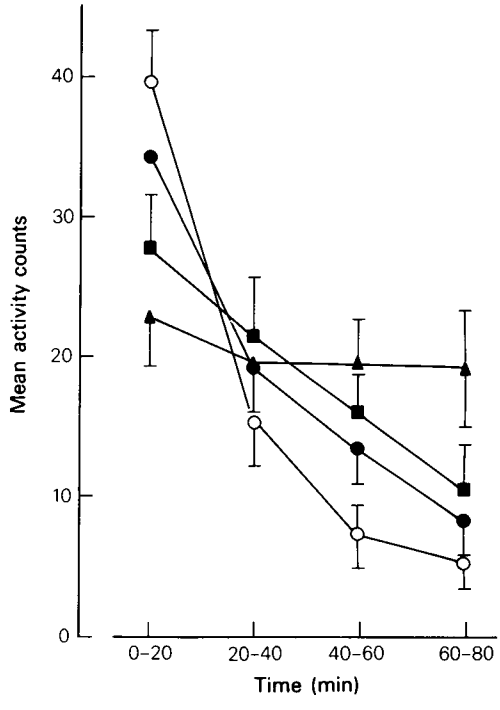

Figure 1 The effects of nicotine on locomotor activity in rats before the start of chronic treatment with the drug. Animals were tested for 80 min, beginning immediately after subcutaneous injection of saline or of nicotine. Nicotine (0.1–0.4 mg/kg s.c. base) reduced activity during the first 20 min and then increased activity relative to the saline baseline. Both effects were significantly dose-related: Saline (○); nicotine 0.1 mg/kg (●), 0.2 mg/kg (■) 0.4 mg/kg, (▲). Each rat received each dose of the drug, and served as its own control. Bars represent one s.e.mean either side of the mean ($n = 14$).

the last two quarters of the session, nicotine increased activity in a dose-dependent way (40–60 min $F = 18.6$, d.f. 1, 13, $P < 0.001$; 60–80 min $F = 17.3$, d.f. 1, 13, $P < 0.005$), above the saline levels of activity which had declined considerably by this time (Figure 1).

(2) *Mecamylamine, nicotine, and locomotor activity in non-tolerant rats*

In the absence of mecamylamine, nicotine reduced locomotor activity in the first 20 min ($F = 24.3$, d.f. 1, 10, $P < 0.001$), but in contrast to Experiment 1, it did not significantly increase activity later in the session. Mecamylamine, when given alone, exerted no significant effect, either in individual quarters or over the session as a whole. However, both doses of mecamylamine completely prevented the depressant action of nicotine.

Thus in the first 20 min, only in rats tested with nicotine alone was activity significantly different from that of undrugged rats; the group mean scores (±s.e.mean) were respectively 13.9 ± 1.7 and 30.7 ± 3.7. This 'behavioural antagonism' is illustrated by an interaction between the effects of nicotine and mecamylamine ($F = 15.4$, d.f. 1, 10, $P < 0.005$). Mecamylamine also blocked the ataxia and prostration induced by nicotine.

(3) *Chronic administration of nicotine and locomotor activity*

Phase 1: Activity in saline test sessions across four weeks of daily injections During this phase, all rats were tested at weekly intervals with saline as well as with nicotine. In rats which were not maintained on daily nicotine injections, activity scores were stable across successive weekly saline tests. In contrast, there was a slight but consistent fall in the saline activity scores of the rats receiving nicotine every day. This decline was significant both in absolute terms, and in comparison with the control rats (linear trend over weeks $F = 6.10$, d.f. 1, 18, $P < 0.05$).

Phase 1: Changes in the effects of nicotine on activity across four weeks of daily injections Before chronic injections were started (week 0), nicotine exerted similar effects on activity to those found in Experiment 1. Activity was reduced in the first 20 min ($F = 54.2$, d.f. 1, 18, $P < 0.0001$), and was later enhanced (40–60 min $F = 7.78$, d.f. 1, 18, $P < 0.05$; 60–80 min $F = 16.0$, d.f. 1, 18, $P < 0.001$, see Figure 2).

Tolerance developed rapidly over successive weeks to the depressant actions of nicotine, and at the same time the drug produced a less pronounced and briefer ataxia. Within a week of daily injections of nicotine, the initial depressant action (0–20 min) of this drug had been replaced by locomotor stimulation

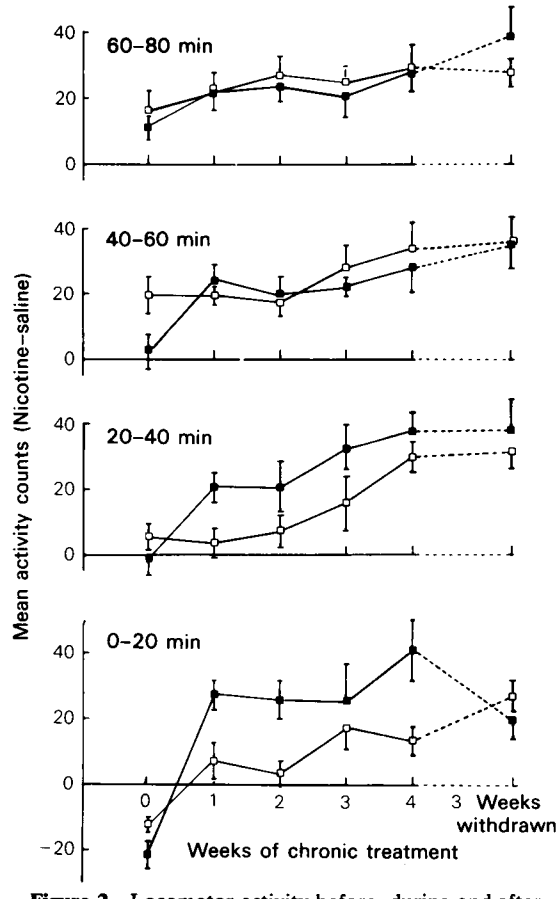

Figure 2 Locomotor activity before, during and after chronic treatment with nicotine. Every week, each rat was tested with nicotine 0.4 mg/kg and with saline (■). In addition, animals in the chronic nicotine group received the same dose of nicotine after the saline test and on days between tests. The rats in the other group (□) received only nicotine (0.4 mg/kg) once weekly, on one of their test days. On all other days they were injected with saline. Represented on the vertical axis is the group mean (± s.e.mean) difference of scores between nicotine and saline tests. With repeated testing, the depressant action of nicotine waned (0–20 min), and concurrently, a stimulant action appeared and became more pronounced, especially in rats given the drug daily. Tolerance to the depressant action of nicotine persisted three weeks after withdrawal of the drug. Bars represent one s.e.mean either side of the mean ($n = 10$).

(Figure 2); this change was highly significant ($F = 93.0$, d.f. 1, 18, $P < 0.0001$). The rats which were given nicotine once weekly (i.e. only on the days when they were tested) showed a similar but smaller change; tolerance was found after a single dose of nicotine in these animals, even though a week of saline injections was interposed between tests ($F = 15.1$, d.f. 1, 18, $P < 0.005$). After the first week, nicotine stimulated activity in the first 20 min

(Figure 2). This action was more marked in the group receiving daily nicotine than in the controls given nicotine only weekly, even after 4 weeks.

The interval from 20 to 40 min after injection corresponded, in non-tolerant rats, to a period of transition between the depressant and stimulant phases of the drug action. However, over successive weeks, a pronounced stimulant action emerged. After the first week, this stimulant action was more marked in the rats receiving daily nicotine than in the control group receiving saline injections instead ($F = 7.83$, d.f. 1, 18, $P < 0.05$, Figure 2). The stimulant action of nicotine (40–80 min), which was already apparent before the start of daily injections, increased gradually across subsequent weekly tests (linear trend over weeks: $F > 4.59$, d.f. 1, 18, $P < 0.05$ for either quarter of the session). This occurred at the same rate in the two groups of subjects (Figure 2).

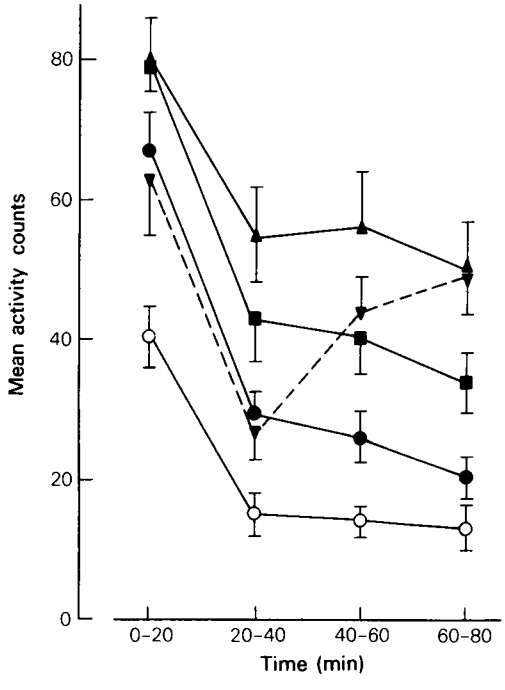

Figure 3 The effects of nicotine on locomotor activity in tolerant rats which had been repeatedly tested with the drug. Except at the highest dose of 0.8 mg/kg, nicotine stimulated activity in a significant dose-related way throughout the session. Saline (○); nicotine 0.1 mg/kg (●), 0.2 mg/kg (■), 0.4 mg/kg (▲), 0.8 mg/kg (▼). This figure may be compared to Figure 1, showing the results of similar tests before chronic treatment. The highest dose did not reduce activity, even in the first 20 min where it produced ataxia. Each rat received each dose of the drug. Bars represent one s.e.mean either side of the mean ($n = 20$).

Phase 2: Dose-response study in tolerant rats At this stage of the experiment, the effect of nicotine on locomotor activity did not differ significantly between those rats which had received daily drug injections and those which had received nicotine only weekly. This was the case even in the first 20 min ($F = 1.22$, d.f. 3, 16). The data were therefore pooled.

In the dose range 0–0.4 mg/kg, nicotine increased activity in a dose-dependent way ($F = 51.6$, d.f. 1, 18, $P < 0.001$), which did not differ between the quarters of the session (Figures 3 and 4).

The higher dose of 0.8 mg/kg produced marked ataxia in all rats and the effect resembled that seen after the administration of 0.4 mg/kg in non-tolerant animals. As the session progressed, nicotine 0.8 mg/kg began to stimulate locomotor activity and this action did not decline within the 80 min session (Figure 3).

Phase 3: Abstinence Activity increased over successive tests with saline when daily injections were stopped (linear trend over days $F = 5.97$, d.f. 1, 18, $P < 0.05$), and there was no difference between the two groups of rats, either in this respect or in their general appearance.

Phase 4: Residual tolerance When retested 23 days after daily maintenance injections were stopped, both groups were stimulated by nicotine in the first 20 min after injection (Figure 2). This drug effect was clearly different from the effect of nicotine before daily injections were started, i.e. on days 1 and 2 ($F = 48.8$, d.f. 1, 18, $P < 0.0001$). The levels of activity obtained after three weeks of abstinence resembled those found in tolerant rats after four weeks of daily injections.

(4) *Nicotine and locomotor stimulation in tolerant rats: pretreatment with hexamethonium or mecamylamine*

In the absence of either pretreatment drug, nicotine increased activity (0–80 min) in both groups of tolerant rats, (those subjects assigned to testing with mecamylamine, $t = 4.43$, d.f. 11, $P < 0.001$; with hexamethonium, $t = 9.72$, d.f. 7, $P < 0.0001$). Activity was stimulated throughout the session, including the first 20 min.

When given alone, neither mecamylamine nor hexamethonium significantly altered activity ($F = 0.25$, d.f. 3, 9; $F = 0.83$, d.f. 3, 5, respectively; see Figure 5), and this did not change over successive quarters of the session.

The locomotor stimulant action of nicotine was reduced in a dose-dependent way by mecamylamine ($F = 27.6$, d.f. 1, 11, $P < 0.0005$); the highest dose

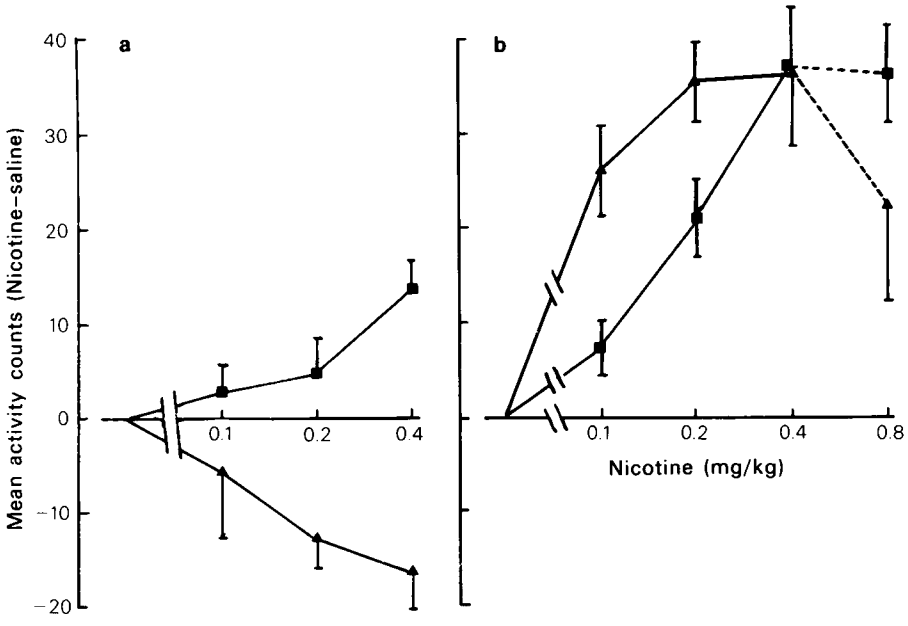

Figure 4 Changes in the effects of nicotine on locomotor activity in rats following repeated testing with the drug. This figure shows the mean difference (± s.e.mean) of activity scores between nicotine tests and the saline test (▲) 0–20 min; (■) 60–80 min. (a) Shows data from Experiment 1, before chronic treatment $n = 14$; absolute scores are given in Figure 1; (b) refers to Experiment 3, in tolerant rats ($n = 20$) (cf. Figure 3). The dose-related depressant action (0–20 min) was replaced, after repeated testing with nicotine, by a dose-related stimulant action.

completely prevented the stimulant action. In contrast, hexamethonium reduced the stimulant action of nicotine only slightly (Figure 5), and this was significant only at the intermediate dose of 1.0 mg/kg ($P < 0.05$). The analysis of variance confirmed the lack of interaction between the effects of hexamethonium and nicotine, either in the session as a whole, or in the first 20 min.

Discussion

Before daily injections were started, nicotine initially depressed, and then, generally, enhanced locomotor activity. Both effects were dose-related. These findings are consistent with the majority of previous studies. Reduced activity has been typically found when large doses of the drug are given shortly before a brief test session (e.g. Stolerman *et al.*, 1973); increased activity may occur during prolonged test sessions (Bovet, Bovet-Nitti & Oliverio, 1967; Pradhan, 1970) or in tests of a few minutes duration given at least 30 min after injection (e.g. Bättig *et al.*, 1976).

The rapid onset of ataxia and prostration has been described previously (Stolerman *et al.*, 1973; Clarke & Kumar, 1981) and coincides with high concentrations of nicotine in the brain and blood (Rosecrans & Schechter, 1972). Such motor disturbances may account for the initial depressant actions of nicotine that have been observed in a variety of testing procedures (e.g. Morrison *et al.*, 1969; Pradhan & Bowling, 1971). Small intravenous doses of nicotine markedly suppress spinal reflexes (Schweitzer & Wright, 1938). The same action lasting several minutes was also observed following subcutaneous injection of 0.4 mg/kg nicotine in rats anaesthetized with chloralose (J.D. Stephenson and P.B.S. Clarke, unpublished observations).

Activity levels in rats tested with saline declined as the session progressed, raising the possibility that the biphasic effect of nicotine resulted from a changing behavioural baseline, rather than from a change in drug action as such. However, rats responding for intracranial stimulation or for water may show little or no decline in responding within a session, and in such cases nicotine has nevertheless been found to exert a biphasic action (Morrison, 1967; Clarke & Kumar, 1981).

Mecamylamine, which was without effect when given alone, completely prevented the ataxia and locomotor depressant effects of nicotine. Mecamylamine has also been found to block the inhibition of reflexes by nicotine in spinal preparations (Tang & Yim, 1965) and to prevent nicotine-induced locomotor depression in mice (Barthelemy

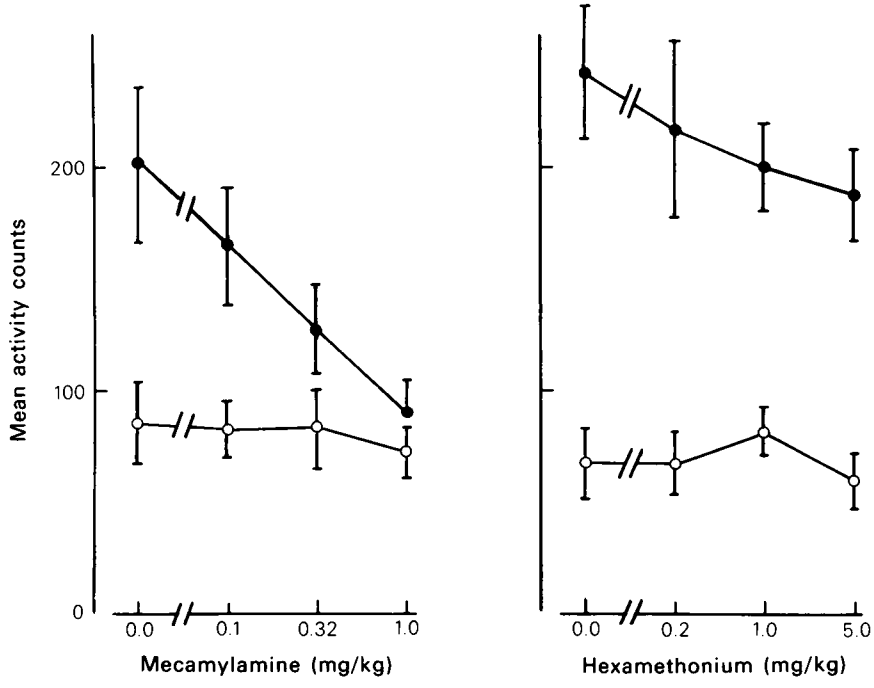

Figure 5 The effects of pretreatment on nicotine-induced locomotor activity in tolerant rats. Each rat was tested with each dose combination of pretreatment (mecamylamine or hexamethonium depending on group) and saline (○) or nicotine (●, 0.4 mg/kg). Whereas mecamylamine reduced the locomotor stimulant action of nicotine in a dose-related way, hexamethonium had little or no effect. For mecamylamine $n = 12$; for hexamethonium $n = 8$. Bars represent one s.e.mean either side of the mean.

et al., 1970). In the second experiment, nicotine did not significantly increase activity later in the session; possibly the presence of mecamylamine in preceding sessions within the balanced design had protected the rats from becoming tolerant to the depressant effects of nicotine.

The chronic experiment shows that tolerance develops rapidly to the behavioural depressant actions of nicotine, and this is consistent with previous reports (Stitzer, Morrison & Domino, 1970; Domino & Lutz, 1973). Tolerance may appear within minutes or hours of a single injection (Domino, 1965; Stolerman *et al.*, 1974). However, tolerance involving short intervals between injections, termed tachyphylaxis (Domino, 1965) or acute tolerance (Stolerman *et al.*, 1973) appears to subside after a few hours, and thus may be different from tolerance which is seen at test intervals of a day or more. In the present study, little or no tolerance appeared to have been lost after three weeks of abstinence from nicotine. Stolerman *et al.* (1973) found diminished, but detectable, residual tolerance to nicotine after 80 days of withdrawal.

The prolonged tolerance observed here was unlikely to have been due to an alteration in nicotine concentrations in body tissues. A similar maintenance regime produced little or no increase in the metabolism of nicotine for up to 10 days (Turner, 1977). Several studies have shown little or no change in brain uptake of nicotine following multiple pretreatment injections of the drug (e.g. Rosecrans, 1972), even when residual tolerance to the locomotor depressant action was found (Corfield-Summer & Stolerman, 1978). Equally, tolerance to the locomotor stimulant action of nicotine has not been found (Kuschinsky & Hotovy, 1943; Morrison & Stephenson, 1972; Bättig *et al.*, 1976), although this action should also be attenuated by any metabolic tolerance. Some authors have speculated that tolerance to the behavioural depression may be learnt (Stitzer *et al.*, 1970; Domino & Lutz, 1973; Corfield-Sumner & Stolerman, 1978). This suggestion is consistent with the development of persistent tolerance with spaced injections of the drug.

In Experiment 3, the locomotor stimulant action seemed to emerge in parallel with the development of tolerance to the depressant action. This process was hastened by additional injections of the drug in the home cage. Previous studies have indicated that the locomotor depressant action of nicotine may be prevented by prior injections of the drug in the home cage (Morrison & Stephenson, 1972; Stolerman *et al.*, 1973). However, the findings of Schlatter &

Bättig, (1978) suggest that a locomotor stimulant action emerges with increasing familiarity with the testing apparatus, and may not depend on previous exposure to the drug in the home cage.

There was only slight evidence for an abstinence syndrome during or after the period of daily nicotine injections. Rats receiving nicotine daily became less active over successive weeks when tested with saline 24 h after their previous injection of drug, and their baseline levels of activity tended to recover after nicotine injections were discontinued. As reported before (Stolerman *et al.*, 1973; Morrison, 1974), signs of withdrawal were not readily seen.

In tolerant rats, mecamylamine blocked the stimulant action of nicotine, whilst hexamethonium had a negligible effect. These ganglion blocking drugs differ in their relative penetration of the CNS, and although comparative studies in the rat do not appear to have been carried out, they act with similar potencies in the periphery in the cat (Stone, Torchiana, Navarro & Beyer, 1956). Mecamylamine has the longer duration of action at least in the cat (Stone *et al.*, 1956), but kinetic studies in the rat (Levine, 1960) suggest that the failure of hexamethonium to block the stimulant action of nicotine was not due to its rapid breakdown. Hence, nicotine appears to act at a central site to produce hyperactivity, in common with certain other behavioural actions (e.g. Morrison *et al.*, 1969; Spealman, Goldberg & Gardner, 1981). *In vitro* studies suggest that mecamylamine directly blocks nicotinic cholinoceptors, at least at autonomic ganglia (Ascher, Large & Rang, 1979). Neither mecamylamine nor hexamethonium affected locomotor activity when given alone, suggesting tentatively that nicotine increases locomotor activity through a stimulation rather than a blockade of central receptors.

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