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Different treatment regimens and the development of tolerance to nicotine's anxiogenic effects

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Abstract

The effects of different treatment regimens were investigated on the development of tolerance to the anxiogenic effect of nicotine (0.45 mg/kg) in the social interaction test of anxiety. Rats received nicotine (0.45 mg/kg/day) by intravenous injections (5 days/week), subcutaneous injections (5 or 7 days/week) or continuous infusion by osmotic minipump. In all groups, 4 days of nicotine treatment resulted in significant decreases in social interaction compared with the vehicle control groups, without changes in locomotor activity, indicating a specific anxiogenic effect. These significant anxiogenic effects persisted even after 4 weeks of treatment although they were less marked, indicating development of partial tolerance. No significant changes in the time spent in social interaction were found when rats were tested undrugged 24 and 72 h after the termination of nicotine treatment. There was no evidence that the treatment regimen affected the rate of development of tolerance, despite very different peak plasma nicotine concentrations.

Keywords: Nicotine; Anxiety; Tolerance; Treatment regimen

1. Introduction

The treatment regimen by which a drug is administered can be an important factor that influences behavioural effects, and is one that is often overlooked. It has been shown that chronic treatment with drugs such as the benzodiazepines, amphetamine and cocaine has differing effects on behaviour depending on the route by which it is administered (Ellison and Morrison, 1981; King et al., 1992; Koff et al., 1994; Fernandes et al., 1999). Tolerance to the behavioural effects of nicotine has been seen after daily subcutaneous (sc), intraperitoneal (ip) or intravenous (iv) injections, constant infusion or intake in drinking water (Stolerman et al., 1973, 1974; Clarke and Kumar, 1983a,b; Marks et al., 1983, 1987; Sparks and Pauly, 1999; Irvine et al., 1999), but at present there is little in the literature investigating the differential effects between these treatment regimens. A study by Morgan and Ellison (1987) showed opposing effects on body weight in female rats when the same dose of nicotine (11.2 mg/kg/day) was administered chronically by subcutaneous injection or pellet infusion. Marks et al. (1987) found that mice exposed to chronic nicotine (4 mg/kg/day over a 1 h period) by discrete pulses (1–4 pulses/h) developed tolerance faster to the acute effects of nicotine on Y-maze activity than those receiving the same dose of nicotine by continuous infusion. However, the up-regulation in [^{3}H]-nicotine binding was the same in the two treatment regimens.

Animal studies have shown that nicotine can have both anxiolytic (Vale and Green, 1986; Costall et al., 1989; Brioni et al., 1993, 1994; Cao et al., 1993; File et al., 1998) and anxiogenic (File et al., 1998; Ouagazzal et al., 1999) effects in tests of anxiety. In the social interaction test, the effects of nicotine have been shown to be dose dependent, with low doses (0.01–0.1 mg/kg) having anxiolytic effects and high doses (0.5 and 1 mg/kg) anxiogenic effects, when tested 30 min after an acute ip injection (File et al., 1998). The effects are also dependent on the time between injection and testing and a low dose (0.1 mg/kg; sc) of nicotine has been found to be anxiogenic 5 and 60 min after injection, but anxiolytic after 30 min (Irvine et al., 1999). Following 7 days of treatment with this low (0.1 mg/kg; sc) dose of nicotine, tolerance developed to both the anxiogenic

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and anxiolytic effects, and 72 h after withdrawal from the nicotine treatment an anxiogenic effect was observed in the social interaction test (Irvine et al., 1999). However, in a recent study an anxiogenic effect was observed in the social interaction test in animals that had been self-administering nicotine (0.45 mg/kg/day, 5 days/week) for 4 weeks and were tested 5 min after their daily self-administration session. In these animals, there was no evidence of an anxiogenic withdrawal response either 24 or 72 h after the last self-administration session (Irvine et al., 2001). Since the two studies differed in both the dose and treatment regimen, it is not possible to say whether tolerance did not develop because the animals were self-administering nicotine or because a higher dose was used.

Thus, the purpose of this study was to examine the effects on the development of tolerance to the anxiogenic effect of a high dose of nicotine using different treatment regimens. In all cases, rats received the same daily dose (0.45 mg/kg/day). One group of rats was passively administered iv doses of nicotine in the same pattern as that used for self-administration (15 infusions of 0.03 mg/kg, totalling 0.45 mg/kg/day, 5 days/week). Two groups received sc injections of 0.45 mg/kg/day, but one received five injections per week and the other received daily injections (seven injections per week). The final group received the same daily dose, but infused at a constant rate by an osmotic minipump. The animals were tested in the social interaction test 5 min after their normal daily nicotine injection or straight from the home cage (for the minipump group), and after 24 and 72 h withdrawal.

In the social interaction test of anxiety, the dependent variable is the time spent in social interaction by pairs of male rats. A decrease in social interaction, without a concomitant decrease in locomotor activity, is interpreted as a specific anxiogenic effect. It is possible to manipulate the anxiety generated in this test by altering light levels and/or the animals' familiarity with the test arena. In this study, the high light, familiar condition of the social interaction test was selected, since it is sensitive to both increases and decreases in anxiety (File, 1980, 1997). Furthermore, this was the test condition in which tolerance was seen to the effects of a low dose of nicotine (0.1 mg/kg) and in which the animals were tested after 4 weeks of self-administration (Irvine et al., 1999, 2001). In order to determine whether there was any evidence for the development of pharmacokinetic tolerance, plasma nicotine concentrations were determined by gas chromatography (Feyerabend and Russell, 1990).

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Olac, Bicester, UK) were individually housed in the same room, maintained at 22°C, with lights (< 50 lx) on from 0700 to 1900 h. Food and water were freely available. At testing, the animals weighed between 300 and 375 g. The experimental procedures carried out in this study were in compliance with the UK Animal Scientific Procedures Act 1986 (Home Office Project License Number 70/4041).

2.2. Nicotine treatment

2.2.1. Intravenous administration

The surgical procedure described by Lane et al. (1992) was used with minor modifications. Rats were anaesthetised by inhalation of 3% isoflurane (May and Baker, Dagenham, Essex, UK) in oxygen and were then implanted with a silastic catheter (inner diameter 0.012 in, outer diameter 0.025 in; Bio Pure Technology, Hampshire, UK) in the right jugular vein. The free end of the catheter was connected to a connector consisting of a modified C313G cannula assembly (Plastic Products, UK) and the resulting unit was mounted to the skull with dental acrylic cement and fixed via three stainless steel screws. Animals were injected iv with 0.1 ml of a solution containing 1 IU/ml heparin (Monoparin, CP Pharmaceuticals, Wrexham, UK). This treatment was repeated every 12 h for 7 days after surgery (period of recovery). After the period of recovery, the animals received infusions of either vehicle or nicotine (0.03 mg/kg/infusion) every min for 15 min, so that the nicotine animals received a total of 0.45 mg/kg/day. The drug solutions were administered at a volume of 0.025 ml during a 3-s period.

2.2.2. Subcutaneous injections

Animals received daily morning injections (5 or 7 days/week) of either nicotine (0.45 mg/kg) or vehicle. All injections were in a volume of 1 ml/kg.

2.2.3. Subcutaneous infusion

Rats were anaesthetised by inhalation of 3% isoflurane (May and Baker) in oxygen and osmotic minipumps (Alzet, USA) delivering 0.45 mg/kg/day nicotine were subcutaneously implanted in the dorsal thoracic area. Animals were monitored daily and the osmotic minipumps manipulated by hand within the subcutaneous pouch to reduce the amount of connective tissue growing around the pump that could impair infusion rate.

2.3. Apparatus

The social interaction test arena was a wooden box 60×60 cm, with 35-cm-high walls and was lit by high light (300 lx). A camera was mounted vertically above the arena and rats were observed on a monitor in an adjacent room by an observer who was blind to the drug treatment. The time spent in social interaction (sniffing, following and grooming the partner, boxing and wrestling) provided the measure of anxiety. The interruption of infrared beams from

photocells mounted in the walls 3.5 cm from the floor, provided an automated measure of locomotor activity (for details see File, 1980).

2.4. Drug

For the iv injections, (−)-nicotine hydrogen tartrate (Sigma, Poole, UK) was dissolved in heparinized saline (0.09% NaCl + 0.5 UI/ml heparin) and for the sc injections and the minipumps it was dissolved in distilled water. Nicotine doses are expressed as milligrams of free base per kilogram of body weight.

2.5. Procedure

In order to familiarise rats with the social interaction test arena, each rat was placed singly in the test arena under high light for a 10 min familiarisation trial on the day prior to testing. For each experiment, animals were allocated to test partners on the basis of weight, such that members of a pair did not differ by more than 10 g. On the test day, each experimental rat was placed together with its unoperated/uninjected partner in the test arena, 5 min after its daily injection or straight from the home cage (for the minipump groups). Social interaction was scored only for interaction that was initiated by the nicotine-treated animal, and was scored for 4.5 min by an observer blind to the drug treatment. All animals were tested in an order randomised for drug treatment, between 0900 and 1230 h. At the end of each trial, any faecal boluses were removed from the test arena, which was cleaned with a damp cloth.

2.5.1. Experiment 1: iv administration of nicotine

Rats were randomly allocated to vehicle or nicotine (0.45 mg/kg) groups and within these they were allocated to be tested after an acute injection (vehicle, $n = 5$; nicotine, $n = 11$), after 4 days of injections (vehicle, $n = 7$; nicotine, $n = 8$) or 4 weeks (vehicle, $n = 4$; nicotine, $n = 4$). Animals that had been treated for 4 weeks were then retested undrugged 24 and 72 h later. The small group sizes in the 4-week treatment groups are due to blockade of indwelling catheters over this period.

2.5.2. Experiment 2a: sc injections

Rats were randomly allocated to the following groups: vehicle ($n = 10$) and nicotine (0.45 mg/kg) treatment for 4 days ($n = 10$) or 4 weeks ($n = 20$). Half of each of these groups received either five (Monday–Friday) or seven (every day) injections per week. Immediately after test, four animals from the 4-day nicotine group and four from each of the 4-week nicotine-treated groups (5 and 7 days/week) were taken for determination of plasma nicotine levels. The remaining animals in the chronic nicotine treatment group were retested undrugged 24 and 72 h later with their vehicle controls.

2.5.3. Experiment 2b: sc minipump

Rats were randomly allocated to the following osmotic minipump groups: vehicle ($n = 7$) and 4 days ($n = 10$) or 4 weeks ($n = 10$) of nicotine (0.45 mg/kg/day). Immediately after test, four of the animals from each of the nicotine treated groups were taken for determination of plasma nicotine levels. The remaining animals in the 4-week treatment groups had their osmotic minipumps removed under anaesthesia and were then retested undrugged 24 and 72 h later.

2.5.4. Experiment 2c: determination of plasma nicotine levels

To compare the plasma nicotine levels of the animals that had been treated for 4 days or 4 weeks with nicotine, animals were taken straight from testing, killed by decapitation and trunk blood was taken. The blood was centrifuged for determination of plasma levels of nicotine by gas chromatography, using nitrogen phosphorus detection with detection limit of 100 pg/ml using 100 μl of plasma (Feyerabend and Russell, 1990). Three of the blood samples became contaminated and were therefore excluded from statistical analysis.

2.6. Statistics

For each experiment, the scores were analysed by one-way analyses of variance (ANOVA) and comparisons between individual groups were then made with Fisher's post hoc tests. Because of the low numbers of animals that

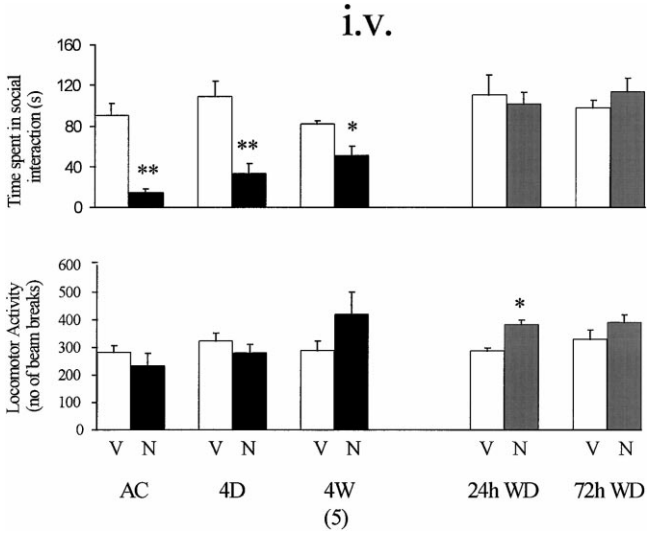

Fig. 1. Mean (±S.E.M.) time (seconds) spent in social interaction (top panel) and locomotor activity (number of beam breaks; bottom panel) made by rats tested 5 min after acute (AC), 4 days (4D)or 4 weeks (4W, five injections per week) of intravenous vehicle or nicotine (0.45 mg/kg/day), and 24 and 72 h after withdrawal (WD) from 4 weeks of nicotine treatment. Rats were tested in the high light familiar (HF) test condition. * $P < .05$ and ** $P < .01$ compared with the vehicle control.

were tested after 4 weeks of iv nicotine, the scores were compared using Mann–Whitney *U* tests (although for ease of comparison all the scores in Fig. 1 are presented as means ± S.E.M.). The plasma concentrations of nicotine were also assessed by Mann–Whitney *U* tests due to the low number of animals used.

3. Results

3.1. Tolerance and withdrawal after 4 weeks of iv nicotine

It can be seen from Fig. 1 that an acute dose of nicotine significantly decreased the time spent in social interaction [$F(1,14) = 56.3$, $P < .00001$], indicating an anxiogenic effect. There were still significant anxiogenic effects after 4 days [$F(1,13) = 19.6$, $P < .001$] and 4 weeks ($U = 0$, $P < .05$) of nicotine treatment. Thus, although some tolerance appeared to have occurred it was not complete. There were no significant changes in locomotor activity [$F(1,14) = 0.5$, $F(1,13) = 0.7$ and $U = 4$, respectively]; see Fig. 1.

The animals that were withdrawn for 24 and 72 h from 4 weeks of nicotine administration did not differ from their saline control group in the time they spent in social interaction ($U = 8$ and $U = 5$, respectively), see Fig. 1. However, the animals tested 24 h after withdrawal from nicotine showed a significant increase in locomotor activity compared with saline controls ($U = 0$, $P < .05$), but this had disappeared by 72 h ($U = 4$), see Fig. 1.

Table 1
Mean (± S.E.M.) locomotor activity (number of beam breaks) made by rats after vehicle (V), 4 days (4D) or 4 weeks (4W, five or seven injections per week) of nicotine (0.45 mg/kg/day) administration, either by sc injection or minipump infusion, and 24 and 72 h after withdrawal from 4 weeks of nicotine

	Locomotor Activity	
	Subcutaneous	Minipump
Tolerance		
V	491.4 ± 19.1	455.1 ± 19.0
4D	412.3 ± 30.7	455.7 ± 31.0
4W (5)	468.1 ± 24.1	–
4W (7)	490.6 ± 25.5	476.7 ± 23.8
24-h Withdrawal		
V	454.0 ± 30.2	411.1 ± 36.0
4W (5)	425.8 ± 27.0	–
4W (7)	490.2 ± 28.0	516.5 ± 26.4*
72-h Withdrawal		
V	423.6 ± 24.4	395.9 ± 33.7
4W (5)	509.7 ± 33.6	–
4W (7)	444.5 ± 60.3	455.7 ± 23.2

* $P < .05$ compared with the vehicle control.

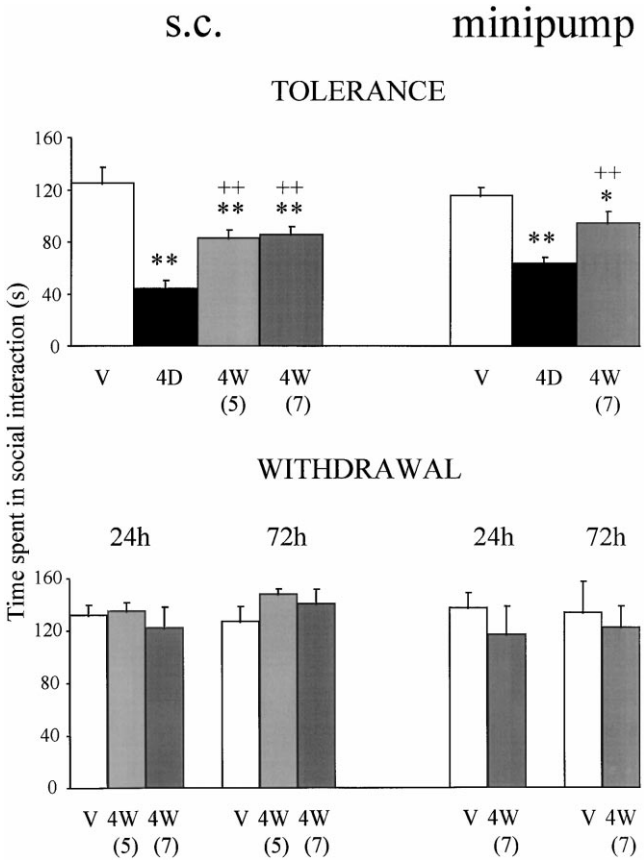

Fig. 2. Mean (± S.E.M.) time (seconds) spent in social interaction made by rats after vehicle (V) or 4 days (4D) and 4 weeks (4W, five or seven injections per week) of nicotine (0.45 mg/kg/day) administration, either by sc injection or minipump infusion (top panel), and 24 and 72 h after withdrawal from 4 weeks of nicotine treatment (bottom panel). * $P < .05$ and ** $P < .01$, compared with the vehicle control, and $^{++}P < .01$, compared with the 4-day group.

3.2. Tolerance and withdrawal after 4 weeks of sc nicotine injections

In animals tested 5 min after a sc injection of nicotine (0.45 mg/kg) there was a significant effect of nicotine on the time spent in social interaction [$F(3,36) = 15.3$, $P < .00001$], and post hoc analysis showed that this was due to the scores from both the 4-day and 4-week (5 and 7 days/week) nicotine groups being significantly lower than those of vehicle controls ($P < .01$ for both groups; see Fig. 2). However, the animals that had been chronically treated with nicotine for 5 or 7 days/week had significantly higher scores than those that had received nicotine for 4 days ($P < .01$ for both groups), suggesting that partial tolerance had developed. There were no significant effects of nicotine on locomotor activity [$F(3,36) = 2.2$]; see Table 1.

The animals that were withdrawn for 24 and 72 h from 4 weeks of nicotine administration did not differ from their saline control group in the time they spent in social interaction [$F(2,19) = 0.4$ and $F(2,19) = 0.9$, respectively; see

Table 2
Median plasma nicotine levels following 4 days (4D) or 4 weeks (4W, five or seven injections per week) of nicotine administered by either sc injections or constant infusion

Treatment group	Plasma nicotine levels (ng/ml)	*n*
Subcutaneous injections		
4D	123.1**	4
4W (5)	105.9	3
4W (7)	134.3**	4
Subcutaneous infusions		
4D	5.7	3
4W (7)	9.6	3

** $P<.01$ compared to the same treatment period in the animals that received constant infusion of nicotine.

Fig. 2] or in their locomotor activity [$F(2,19)=1.1$ and $F(2,19)=1.3$, respectively]; see Table 1.

3.3. Tolerance and withdrawal after 4 weeks of sc minipump nicotine infusion

Animals receiving treatment via osmotic minipumps showed a significant effect of nicotine on the time spent in social interaction [$F(2,24)=13.76$, $P<.0001$], and post hoc analysis showed that the scores from the animals treated for 4 days ($P<.01$) and 4 weeks ($P<.05$) with nicotine were significantly decreased compared with the vehicle controls, see Fig. 2. However, the animals that had been treated for 4 weeks had significantly higher scores than those that had received nicotine for 4 days ($P<.01$), suggesting that again partial tolerance had developed. There were no significant effects of nicotine on locomotor activity [$F(2,24)=0.21$], see Table 1.

The animals that were withdrawn for 24 and 72 h from 4 weeks of nicotine administration did not differ from their saline control group in the time they spent in social interaction [$F(1,11)=0.7$ and $F(1,11)=0.1$, respectively; see Fig. 2]. However, the animals tested 24 h after withdrawal from nicotine showed a significant increase in locomotor activity compared with saline controls [$F(1,11)=5.2$, $P<.05$), but this had disappeared by 72 h [$F(1,11)=2.0$]; see Table 1.

3.4. Plasma nicotine levels

There was no significant difference in the plasma concentration of nicotine between rats treated for 4 days or 4 weeks with nicotine in either of the sc injection groups (five and seven times per week) or in the minipump infusion group, see Table 2. Thus, there was no evidence for any development of pharmacokinetic tolerance. Table 2 also shows that the plasma nicotine concentration at testing was significantly higher in both the sc injection groups than in the minipump group ($U=0$, $P<.05$ in both cases). This confirms the higher peak concentrations produced by intermittent injections.

4. Discussion

Our results have clearly shown that after 4 days of nicotine treatment (0.45 mg/kg/day) there were decreases in social interaction, without changes in locomotor activity, suggesting specific anxiogenic effects. These effects were very similar, regardless of whether nicotine was given by intravenous injection, subcutaneous injection or infused by minipump. Our plasma nicotine concentrations after subcutaneous injections are similar to those reported by Shoaib and Stolerman (1999) after intravenous self-administration of nicotine, but these levels were very different from the plasma concentrations in the minipump group. The plasma concentrations in our minipump group are similar to those found by Rowell and Li (1997) following minipump infusions of 0.6 mg/kg/day. Our results therefore suggest that, at least after 4 days of treatment, the anxiogenic effects were not related to the plasma nicotine concentration and perhaps it is simply necessary to reach a certain threshold concentration to see an anxiogenic effect. After an acute dose of nicotine and after 10 days of treatment, brain concentrations of nicotine are threefold higher than in plasma (Manser and Mattila, 1975; Rowell and Li, 1997). It is possible that this difference is further enhanced after 4 weeks of treatment and perhaps to a greater extent in the continuous infusion group, although this was not the case after 10 days of treatment (Rowell and Li, 1997).

The minipump group had plasma concentrations that would be associated with a single injection of a low dose of nicotine, which would have an anxiolytic, rather than an anxiogenic effect (File et al., 1998; Irvine et al., 1999). This suggests that following 4 days of continuous infusion tolerance had developed to the anxiolytic action and that there was sensitisation to the anxiogenic effects. Certainly, rapid tolerance does develop to the anxiolytic effects (Irvine et al., 1999; Cheeta et al., 2001), but sensitisation to the anxiogenic effects has not previously been seen. The results of the present study strongly suggest that it takes more than 4 weeks for complete tolerance to develop to the anxiogenic effect of this relatively high dose of nicotine, but that the route and manner of nicotine administration is relatively unimportant to the rate of tolerance development. This is in contrast to the rapid rate of development of tolerance to the low dose of nicotine (0.1 mg/kg). The anxiolytic effect of this dose of nicotine is mediated by stimulating the 5-HT_{1A} autoreceptors in the dorsal raphé nucleus (Cheeta et al., 2001; File et al., 1999), whereas the anxiogenic effects of high doses are mediated by stimulation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus (Kenny et al., 2000) and the lateral septal nucleus (Cheeta et al., 2000). It is therefore possible that different mechanisms and/or rates of tolerance operate in the different brain regions. The brain region mediating the anxiogenic effect of 0.1 mg/kg, that is observed 5 min after injection, is unknown at present.

Our results showing persistent anxiogenic effects following 4 weeks of nicotine treatment (0.45 mg/kg/day) support

our previous findings that animals that had been self-administering nicotine (0.45 mg/kg/day) for 4 weeks had an anxiogenic response in the social interaction test when they were tested 5 min after their usual self-administration session. This is important because it shows that a dose of nicotine can cause anxiety and be rewarding. Corrigall and Coen (1989) had suggested that higher doses of nicotine are not self-administered because of their aversive properties. Whilst this is likely in the proconvulsant range and possibly with an extreme level of anxiety, our results raise the possibility that a milder anxiogenic effect is actually rewarding. A very similar argument for the rewarding effects of cocaine has been proposed by Gedders (2001), who has shown that the release of the stress hormones CRH and corticosterone are necessary for cocaine self-administration. The dose of amphetamine that is self-administered is also one that has anxiogenic effects (Lin et al., 1999; Carroll and Lac, 1997; File and Hyde, 1979).

After 4 weeks of treatment, the anxiogenic effects persisted, but there was development of partial tolerance. This is unlikely to be due to the development of pharmacokinetic tolerance, since there was no significant reduction in nicotine concentrations after 4 weeks of treatment. Several pharmacodynamic mechanisms are possible. An oppositional mechanism of tolerance is one that involves the progressive recruitment of processes that oppose the acute effect of the drug. Thus, following withdrawal of the drug, these processes work unopposed and a behavioural response is seen in the opposite direction of the acute drug effect (Young and Goudie, 1995). We found no evidence for an oppositional mechanism, since no anxiolytic withdrawal responses were observed at either 24 or 72 h after the end of nicotine treatment. However, it is possible that the absence of a withdrawal response was because tolerance had not fully developed. An alternative mechanism of tolerance is a decremental one, in which the behavioural impact of a drug is reduced, but which is without behavioural consequence in the absence of the drug (Young and Goudie, 1995). Our pattern of results would fit with a decremental process, such as receptor desensitisation, which has been observed in vitro with very low nicotine concentrations (Benecherif et al., 1995; Grady et al., 1994; Rowell and Hillebrand, 1994; Marks et al., 1993). However, in an in vivo study receptor desensitisation was only found with plasma nicotine concentrations of 24–87 ng/ml, and not with a concentration of 9 ng/ml (Benwell et al., 1995). Since our minipump group had a concentration of 9.6 ng/ml our results suggest that receptor desensitisation might occur at this concentration in at least some brain regions.

There are also clear differences in the responses that can be seen on withdrawal from chronic treatment with the low and high doses of nicotine. Following 7 days of treatment with the low dose, an anxiogenic effect was seen 72 h after the last dose (Irvine et al., 1999). Following the 4 weeks of treatment with the high dose, there were no changes in social interaction at either 24 or 72 h in the present study or when rats were self-administering nicotine (Irvine et al., 2001). It had been speculated that the lack of a withdrawal response in the rats that had been self-administering nicotine was due to their having had previous experience of 72-h withdrawal periods each weekend. However, this does not seem to be the crucial factor since there was no change in social interaction following withdrawal in the rats with daily injections or with constant infusions of nicotine. Our results suggest that kindling of an anxiogenic response does not occur after repeated nicotine withdrawals. This has also been found following repeated benzodiazepine withdrawals, although kindling of convulsions does occur (Ward and Stephens, 1998). Furthermore, whilst repeated administration of the benzodiazepine partial inverse agonist FG 7142 kindles seizures, it does not kindle anxiety (Taylor et al., 1988). The separation of anxiety and seizures was discussed by Pellow (1985). The only changes that we found when testing during withdrawal from the 4 weeks of nicotine treatment was that in two of the groups (iv injections five times per week and continuous minipump infusion) there was a significant increase in locomotor activity. However, it would not seem correct to interpret the increased locomotor activity as a true withdrawal response, since locomotor activity was not decreased by either acute or 4 days of nicotine treatment. Furthermore, sensitisation to a locomotor stimulant effect, as measured in photocell activity cages, has been found (Zubaran et al., 2000) after 3 weeks of nicotine treatment (0.4 mg/kg/day). Whilst this was not detected in the conditions of the social interaction test, there was a trend towards increased locomotor activity in the 4-week intravenous nicotine group. The lack of locomotor sensitisation could be because two animals are present in the social interaction test and this could modify nicotine's effects on locomotor activity, as has been found for chlorpromazine (File and Pope, 1974). Locomotor depressant effects have been found in the social interaction test following an injection of 0.5 mg/kg nicotine, but this was in the hooded Lister strain of rat. It is possible that there is a strain difference in sensitivity to these effects.

In conclusion, the results of the present study show, somewhat surprisingly, that the treatment regimen did not affect the rate of development of tolerance to the anxiogenic effects of a high dose of nicotine, despite strikingly different levels of peak plasma concentration, and despite differences in the patterning of nicotine treatment.

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References

Benecherif M, Fowler K, Lukas RJ, Lippiello PM. Mechanism of up-regulation of neuronal nicotinic acetylcholine receptors in clonal cell lines and primary cultures of fetal rat brain. J Pharmacol Exp Ther 1995; 275:987–94.

Benwell MEM, Balfour DJK, Birrell CE. Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine. Br J Pharmacol 1995;114:454–60.

Brioni JD, O'Neill AB, Kim DJB, Decker MW. Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus-maze test. Eur J Pharmacol 1993;238:1–8.

Brioni JD, O'Neill AB, Kim DJB, Buckley MJ, Decker MW, Arneric SB. Anxiolytic-like effects of the novel cholinergic channel activator ABT-418. J Pharmacol Exp Ther 1994;271:353–61.

Cao W, Burkholder T, Wilkins L, Collins AC. A genetic comparison of the behavioral actions of ethanol and nicotine in the mirrored chamber. Pharmacol, Biochem Behav 1993;45:803–9.

Carroll ME, Lac ST. Acquisition of iv amphetamine and cocaine self-administration in rats as a function of dose. Psychopharmacology 1997; 129:206–14.

Cheeta S, Kenny PJ, File SE. The role of 5-HT_{1A} receptors in mediating the anxiogenic effects of nicotine following lateral septum administration. Eur J Neurosci 2000;12:3797–802.

Cheeta S, Irvine EE, Kenny PJ, File SE. The dorsal raphé nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. Psychopharmacology. (in press).

Clarke PBS, Kumar R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharmacol 1983a;78:329–37.

Clarke PBS, Kumar R. Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br J Pharmacol 1983b;80:587–94.

Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 1989;99:473–8.

Costall B, Kelly ME, Naylor RJ, Onaivi ES, Wong EH, Smith WL, Whiting RM, Eglin RM. The actions of nicotine and cocaine in the mouse model of anxiety. Pharmacol, Biochem Behav 1989;33:197–203.

Ellison G, Morrison W. Opposed stages of continuous amphetamine administration: parallel alterations in motor stereotypies and in vivo spiroperidol accumulation. Eur J Pharmacol 1981;74:207–14.

Fernandes C, Arnot MI, Irvine EE, Bateson AN, Martin IL, File SE. The effect of treatment regimen on the development of tolerance to the sedative and anxiolytic effects of diazepam. Psychopharmacology 1999;145:251–9.

Feyerabend C, Russell MAH. A rapid gas–liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. J Pharm Pharmacol 1990;42:450–2.

File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 1980;2: 219–38.

File SE. Animals measures of anxiety. In: Crawley J, Gerfen C, McKay R, Rogawski MA, Sibley D, Sklonick P, editors. Current protocols in neuroscience. New York: Wiley, 1997. pp. 8.3.1–8.3.15.

File SE, Hyde JRG. A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilizers and of stimulants. Pharmacol, Biochem Behav 1979;11:65–9.

File SE, Pope JH. The action of chlorpromazine on exploration in pairs of rats. Psychopharmacologia 1974;37:249–54.

File SE, Kenny PJ, Ouagazzal A-M. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav Neurosci 1998;112:1423–9.

File SE, Cheeta S, Kenny PJ, Ouagazzal AM, Gonzalez LE. Roles of the dorsal raphé nucleus, lateral septum and dorsal hippocampus in nicotine's effects on anxiety. Soc Neurosci Abstr. 1999;25:1981.

Gedders NE. The HPA axis and cocaine reinforcement. Psychoneuroendocrinology. (in press).

Grady SR, Marks MJ, Collins AC. Desensitization of nicotine stimulated [^{3}H] dopamine release from mouse striatal synaptasomes. J Neurochem 1994;62:1390–8.

Irvine EE, Cheeta S, File SE. Time-course of changes in the social interaction test of anxiety following acute and chronic administration of nicotine. Behav Pharmacol 1999;10:691–7.

Irvine EE, Bagnalasta M, Marcon C, Motta C, Tessari M, File SE, Chiamulera C. Nicotine self-administration and withdrawal: modulation of anxiety in the social interaction test in rats. Psychopharmacology. 2001;153:315–320.

Kenny PJ, Cheeta S, File SE. Anxiogenic effects of nicotine in the dorsal hippocampus are mediated by 5-HT_{1A} and not by muscarinic receptors. Neuropharmacology 2000;39:300–30.

King GR, Jyner C, Lee T, Kuhn C, Ellingwood ER. Intermittent and continuous cocaine administration: residual behavioral states during withdrawal. Pharmacol, Biochem Behav 1992;43:243–8.

Koff JM, Shuster L, Miller LG. Chronic cocaine administration is associated with behavioral sensitization and time-dependent changes in striatal dopamine transporter binding. J Pharmacol Exp Ther 1994; 268:277–82.

Lane JD, Pickering CL, Hooper ML, Fagan K, Tyers MB, Emmett-Oglesby MW. Failure of ondansetron to block the discriminative or reinforcing stimulus effects of cocaine in the rat. Drug Alcohol Depend 1992;30: 151–62.

Lin HQ, Burden PM, Christie MJ, Johnston GAR. The anxiogenic-like and anxiolytic-like effect of MDMA on mice in the elevated plus-maze: a comparison with amphetamine. Pharmacol, Biochem Behav 1999;62: 403–8.

Manser R, Mattila MJ. Nicotine induced tremor and antidiuresis and brain nicotine levels in the rat. Med Biol 1975;53:169–76.

Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and cholinergic receptors. J Pharmacol Exp Ther 1983;226:806–16.

Marks MJ, Stitzel JA, Collins AC. Influence of kinetics of nicotine administration on tolerance development and receptor levels. Pharmacol, Biochem Behav 1987;27:505–12.

Marks MJ, Farnham DA, Grady SR, Collins AC. Nicotine receptor function determined by stimulation of rubidium efflux from rat brain synaptasomes. J Pharmacol Exp Ther 1993;264:542–52.

Morgan MM, Ellison G. Different effects of chronic nicotine treatment regimens on body weight and tolerance in the rat. Psychopharmacology 1987;91:236–8.

Ouagazzal A-M, Kenny PJ, File SE. Modulation of anxiety on trials 1 and 2 in the elevated plus-maze after systemic and hippocampal administration of nicotine. Psychopharmacology 1999;144:54–60.

Pellow S. Can drug effects on anxiety and convulsions be separated? Neurosci Biobehav Rev 1985;9:55–74.

Rowell PP, Hillebrand JA. Characterization of nicotine-induced desensitization of evoked dopamine release from rat striatal synaptasomes. J Neurochem 1994;63:561–9.

Rowell PP, Li M. Dose–response relationship for nicotine-induced up-regulation of rat brain nicotinic receptors. J Neurochem 1997;68:1982–9.

Shoaib M, Stolerman IP. Plasma nicotine and cotinine levels following intravenous nicotine self-administration in rats. Psychopharmacology 1999;143:318–21.

Sparks JA, Pauly JR. Effects of continuous oral administration on brain nicotinic receptors and responsiveness to nicotine in C57BI/6 mice. Psychopharmacology. 1999;145–53.

Stolerman IP, Fink R, Jarvik ME. Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacologia 1973;30:329–42.

Stolerman IP, Bunker P, Jarvik ME. Nicotine tolerance in rats: role of dose and dose interval. Psychopharmacologia 1974;34:317–24.

Taylor SC, Johnston AL, Wilks LJ, Nicholass JM, File SE, Little HJ.

Kindling with the betacarboline FG7142 suggests separation between changes in seizure threshold and anxiety-related behaviour. Neuropsychobiology 1988;19:195–201.

Vale AL, Green S. Effects of chlordiazepoxide, nicotine and D-amphetamine in the rat potentiated startle model of anxiety. Behav Pharmacol 1986;7:138–43.

Ward BO, Stephens DN. Sensitisation of withdrawal signs following repeated withdrawal from a benzodiazepine: differences between measures of anxiety and seizure sensitivity. Psychopharmacology 1998; 135:342–52.

Young AM, Goudie AJ. Adaptive processes regulating tolerance to behavioural effects of drugs. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press, 1995. pp. 733–42.

Zubaran C, Shoaib M, Stolerman IP. The development and expression of locomotor sensitization to nicotine in the presence of ibogaine. Behav Pharmacol 2000;11:431–6.