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Tolerance to nicotine's effects in the elevated plus-maze and increased anxiety during withdrawal

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Abstract

In the elevated plus-maze test of anxiety, nicotine (0.1 mg/kg sc; 30 min after injection) had a significant anxiogenic effect, shown by specific decreases in the percentage of time spent on the open arms and in the percentage of open-arm entries. Tolerance developed to this anxiogenic effect after 7 days of nicotine treatment (0.1 mg/kg/day). Five minutes after an acute injection, nicotine (0.1 mg/kg) was ineffective, but after 7 days of treatment a significant anxiolytic effect, shown by specific increases in the percentage of time spent on the open arms and in the percentage of open-arm entries, emerged. After 14 days of nicotine treatment, tolerance developed to this anxiolytic effect. There was a complete dissociation between the effects of nicotine on the measures of anxiety, and on the locomotor activity as measured by closed-arm entries. No changes in closed-arm entries were found after acute administration of nicotine, but rats tested 30 min after their 7th injection made significantly fewer, and those tested 5 min after their 14th injection made significantly more, entries than their respective controls. Rats that were tested after 24 h withdrawal from six daily nicotine injections showed a significant anxiogenic effect. A low dose of nicotine (5 ng) injected into the dorsal hippocampus was without effect in vehicle pretreated rats, but it was able to reverse the anxiogenic effect found after 24 h of withdrawal from 6 days of nicotine treatment.

Keywords: Nicotine; Anxiety; Locomotor activity; Tolerance; Withdrawal; Dorsal hippocampus

1. Introduction

The effects of nicotine on anxiety are unusual in that it can have both anxiolytic and anxiogenic effects in animal tests (Ouagazzal et al., 1999a; File et al., 1998; Brioni et al., 1993; Cao et al., 1993; Costall et al., 1989; Vale and Green, 1986), in non-smoking volunteers (File et al., 2000a; Newhouse et al., 1990) and in smokers (Netter et al., 1998; Ikard et al., 1969). In the social interaction test of anxiety, the direction of nicotine's effects has been shown to depend on dose, with low doses having an anxiolytic, and high doses an anxiogenic, action (File et al., 1998). These two actions in the social interaction test have been shown to be mediated by distinct brain regions, with the dorsal raphé nucleus mediating an anxiolytic effect (Cheeta et al., 2000a; File et al., 1999) and the dorsal hippocampus and lateral septum mediating anxiogenic effects (Cheeta et al., 2000b; Kenny et al., 2000; Ouagazzal et al., 1999b). The direction of nicotine's effects on anxiety, as measured in the social interaction test, also depends on the time since injection. Nicotine (0.1 mg/kg) had an anxiogenic effect 5 min after injection, but an anxiolytic action after 30 min (Irvine et al., 1999).

In the rat elevated plus-maze test of anxiety, both anxiolytic (Brioni et al., 1994) and anxiogenic (Ouagazzal et al., 1999a) effects have been reported, but in this test the direction of nicotine's effects is not dose-related, since the dose (0.3 mg/kg) that was reported to be anxiolytic by Brioni et al. (1994) fell in the dose-range found to be anxiogenic by Ouagazzal et al. (1999a), and Benwell et al. (1994) found 0.4 mg/kg to be ineffective. Lower doses (0.001–0.1 mg/kg) were shown to be ineffective by Ouagazzal et al. (1999a). These differences in response to an acute injection of nicotine could be due to strain differences and/or differences in the baseline scores.

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The effects of nicotine (0.1 mg/kg) have not yet been examined in the plus-maze 5 min after injection. The purpose of the present experiment was therefore to examine the effects of nicotine (0.1 mg/kg) 5 and 30 min after injection of a single dose and after a period of chronic treatment. In the social interaction test, after a week of pretreatment, tolerance developed to both the anxiogenic effect observed 5 min after injection and to the anxiolytic effect found at 30 min (Irvine et al., 1999), but to date tolerance has not been tested in the plus-maze. In order to determine whether tolerance was due to an oppositional mechanism (Young and Goudie, 1995), animals were also tested undrugged 24 h after the last of the chronic injections. An oppositional mechanism of tolerance involves the recruitment of processes that oppose the acute effect of a drug, resulting in the appearance of behavioral withdrawal signs when the drug is withdrawn. Increased anxiety has been reported on withdrawal from nicotine in animal tests (Cheeta et al., 2000a; Irvine et al., 1999; Costall et al., 1989), in smokers (Parrott and Garnham, 1998; Parrott et al., 1996; West and Russell, 1985; Shiffman and Jarvik, 1976), and in those withdrawing from nicotine gum (Hughes et al., 1990; Keenan et al., 1989). The dorsal hippocampus has been shown to be a brain region crucial to the development of tolerance to the anxiogenic effect of nicotine in the social interaction test (Irvine et al., 2000a). Although this region does not seem to play a role in the acute effects of nicotine in the plus-maze (Ouagazzal et al., 1999b), it does seem to play a very general role in mediating stress-induced changes in a variety of test situations (File et al., 2000b). We therefore examined whether a low dose of nicotine would be effective when administered to the dorsal hippocampus of rats in 24 h withdrawal from nicotine.

2. Method

2.1. Animals

Male hooded Lister rats (Charles River, Margate, Kent, UK) weighing between 220 and 250 g were housed singly. The animals in the withdrawal study that had undergone surgery were allowed to recover for 4 days prior to the start of chronic injections. Food and water were freely available, and the room in which they were housed was lit with dim light and maintained at 22°C. Lights were on from 0700–1900 h. The experimental procedures carried out in this study were in compliance with the UK Animals (Scientific Procedures) Act 1986 (Home Office Project License Number 70/4041).

2.2. Elevated plus-maze test

The elevated plus-maze was made of wood and consisted of two opposite open arms 50×10 cm, and two opposite equal-sized arms enclosed by 40 cm high walls. The arms were connected by a central 10×10 cm square, and thus the maze formed a ''plus'' shape. The maze was elevated 50 cm from the floor and lit by dim light. A closed-circuit TV camera was mounted vertically over the maze, and the behavior was scored from a monitor in an adjacent room by an observer who was blind to the drug treatment. The number of entries onto, and the times spent on, open and closed arms were recorded by an observer blind to the drug treatment. Four paws into, and two paws out of, an arm defining an arm entry and exit, respectively. The percentage number of open-arm entries [open entries/(open + closed entries) $\times$ 100] was calculated, as was the percentage of time spent on the open arms. The percentage of entries onto, and the percentage of time spent on the open arms of the maze, provide the measure of anxiety, and the number of closed-arm entries provides the best measure of locomotor activity in this test (File, 1992; Pellow et al., 1985). At the end of each trial, any fecal boluses were removed from the maze, which was wiped clean with a damp cloth.

2.3. Surgery

Rats were anaesthetized by inhalation of 3% isofluorane (May and Baker, Dagenham, Essex, UK) in oxygen and positioned in the stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The skull was exposed and the incisor bar adjusted such that bregma and lambda were at the same height. Three indentations were made in the skull to accommodate screws, which, together with the application of dental cement, held the cannulae in place. For bilateral cannulation of the dorsal hippocampus, 7 mm long steel guide cannulae (23 gauge, Cooper's Needle Works, Birmingham, UK) were positioned at 3.3 mm posterior to bregma, ± 2.4 mm lateral, and -1.2 mm vertical, thus siting them 2 mm above the target area (according to the atlas of Paxinos and Watson, 1986). Cannulae were kept patent using 7 mm long stainless steel stylets (30 gauge, Cooper's Needle Works). On the test day, the rats were gently wrapped in a cloth and injected using needles constructed from 30-gauge steel tubing that extended 2 mm below the tip of the in-dwelling cannulae, into the dorsal hippocampus. In order to accustom the animals to handling and to keep the stylets patent, each day following surgery the rats were gently wrapped in a cloth and the stylets were replaced.

2.4. Drugs and chemicals

For the chronic subcutaneous injections, (−)-nicotine hydrogen tartrate (Sigma, Poole, UK) was dissolved in distilled water, in a volume of 1 ml/kg body weight and a dose of 0.1 mg/kg was used; control animals received equal volume injections of distilled water. For the central injections, (−)-nicotine hydrogen tartrate was dissolved in

artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 126.6 NaCl, 27.4 $NaHCO_3$, 2.4 KCl, 0.5 KH_2PO_4, 0.89 $CaCl_2$, 0.8 $MgCl_2$, 0.48 Na_2HPO_4, and 7.1 glucose, pH 7.4. Injections were 0.5 μl, and were made over a period of 30 s using a CMA/102 microdialysis pump (Biotech Instruments, Stockholm, Sweden) and the needles were left in position a further 30 s to allow drug diffusion; control animals received 0.5 μl infusions of aCSF. All doses are given as free base.

2.5. Behavioral testing

2.5.1. Development of tolerance

Forty-eight animals were randomly allocated to the following drug groups: vehicle, acute nicotine (0.1 mg/kg), and 7 days of nicotine (0.1 mg/kg/day) and in each group half were tested 5 min and half 30 min after injection. Because an anxiolytic effect emerged in the animals that had been treated for 7 days with nicotine and were tested after 5 min, a second group of animals was randomly allocated to the following groups: vehicle ($n=9$) and 14 days of nicotine ($n=8$; 0.1 mg/kg/day) and tested 5 min after the last injection to see if tolerance occurred to the anxiolytic effect after the longer pretreatment period.

2.5.2. Reversal of nicotine withdrawal response in the elevated plus-maze

Animals were randomly allocated to pretreatment with either vehicle or nicotine (0.1 mg/kg/day, sc) for 6 days. On the 7th day, no sc injections were given but rats from both pretreatment groups were randomly assigned to be tested 3 min after a bilateral injection into the hippocampus with aCSF or (−)nicotine (5 ng). The numbers in each group ranged from seven to nine after verification of the cannula placements.

2.6. Histology

At the end of the behavioral testing, the cannulated animals were sacrificed, the brains removed and the injection sites verified histologically (Paxinos and Watson, 1986) by a person blind to drug treatment. Fig. 1, depicting coronal slices through the dorsal hippocampus, shows the site of the injections for the rats whose data were included in the statistical analysis.

2.7. Statistics

The data were analyzed with one-way analyses of variance (ANOVA) and comparisons with individual groups were then made with Fisher's post hoc tests; it is the significance of these that is shown in the figure. Because of the large number of zero scores in the withdrawal group, this group was compared with other groups using Mann–Whitney U tests (although for ease of comparison all the scores in Fig. 3 are presented as means ± S.E.M.).

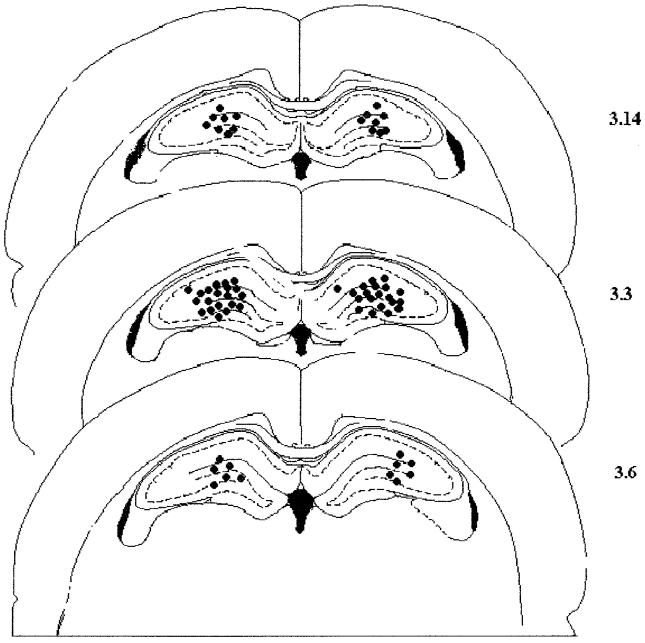

Fig. 1. Diagrammatic representation of coronal sections (3.14 to 3.6 mm posterior to bregma) through the rat brain showing the placements accepted as falling within the dorsal hippocampus (filled circles).

3. Results

3.1. Development of tolerance

In animals that were tested 5 min after subcutaneous injection, there was a significant effect of nicotine (0.1 mg/kg) on the percentage of time spent on the open arm [$F(2,20)=8.9$, $P<.01$] and the percentage of open-arm entries [$F(2,20)=3.5$, $P=.05$]. This arose because, although acute administration was without effect, the rats tested after their seventh injection with nicotine showed a significant increase in both measures ($P<.01$ and $P<.05$, respectively), compared with both the vehicle control group and the acute nicotine group (see Fig. 2). There were no significant effects of nicotine on the number of closed-arm entries [$F(2,20)=1.0$] (see Fig. 2). Thus, a specific anxiolytic effect had emerged after 7 days of chronic treatment when rats were tested 5 min after nicotine (0.1 mg/kg sc) injection. However, after 14 days of pretreatment, tolerance developed to this anxiolytic effect and nicotine was without effect on the percentage of time spent on the open arms [$F(1,15)=0.3$] or the percentage of open-arm entries [$F(1,15)<0.1$] (see Fig. 2). However, it can be seen from Fig. 2 that the animals that had received 14 days of nicotine injections showed a significant increase in the number of closed-arm entries [$F(1,15)=9.9$, $P<.01$] compared with vehicle controls.

In animals that were tested 30 min after injection, there was a significant effect of nicotine on the percentage of time spent on the open arms [$F(2,22)=3.6$, $P<.05$] and the percentage of open-arm entries [$F(2,22)=5.2$, $P<.05$], but in this case the significance arose because of the significant reductions in these measures caused by the acute administration of nicotine ($P<.05$ and $P<.05$, respectively), compared with both the control group of animals and the chronic nicotine group (see Fig. 2). Thus, after seven injections, tolerance had developed to the anxiogenic effect of nicotine The acute administration of nicotine did not change the number of closed-arm entries, but there was a significant effect on the number of closed-arm entries [$F(2,22)=3.7$, $P<.05$], due to the rats tested after their seventh injection having a decrease compared with vehicle controls ($P<.05$, Fig. 2).

3.2. Reversal of nicotine withdrawal response

When rats were withdrawn for 24 h after 6 days of nicotine pretreatment, there was a significant anxiogenic effect, shown by a decrease in the percentage of time spent on the open arms ($U=3$, $P<.01$) and the percentage of open-arm entries ($U=10$, $P<.05$). There was no change in the number of closed-arm entries [$F(1,15)=3.2$]. Bilateral administration of nicotine (5 ng) into the dorsal hippocampus significantly reversed the withdrawal response on both measures ($U=9$, $P<.05$ for percent time and $U=11$, $P<.01$ for percent entries) (see Fig. 3). In the vehicle-pretreated animals, this dose of nicotine administered to the dorsal hippocampus was without effect on the percentage of time spent on the open arms [$F(1,15)=0.3$], the percentage of open-arm entries [$F(1,15)=0.3$], or the number of closed-arm entries [$F(1,15)=0.7$] (see Fig. 3).

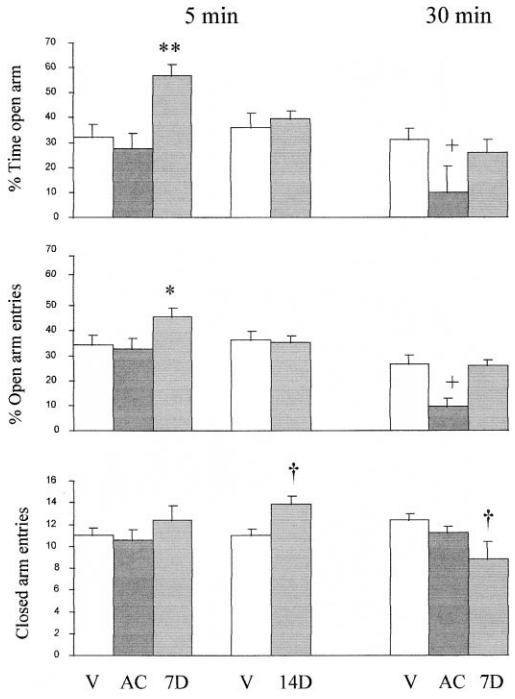

Fig. 2. Mean (±S.E.M.) percentage time spent on open arms, percentage open-arm entries, and number of closed-arm entries in the plus-maze 5 and 30 min after subcutaneous injection of vehicle (V), acute nicotine (AC; 0.1 mg/kg), or 7 days of nicotine (7D, 0.1 mg/kg) and 5 min after subcutaneous injection of vehicle or 14 days of nicotine (14D; 0.1 mg/kg). $*P<.05$ and $**P<.01$ compared with the vehicle control and acute nicotine group, $^{+}P<.05$ compared with the vehicle control and chronic nicotine group, $^{\dagger}P<.05$ compared with the vehicle control.

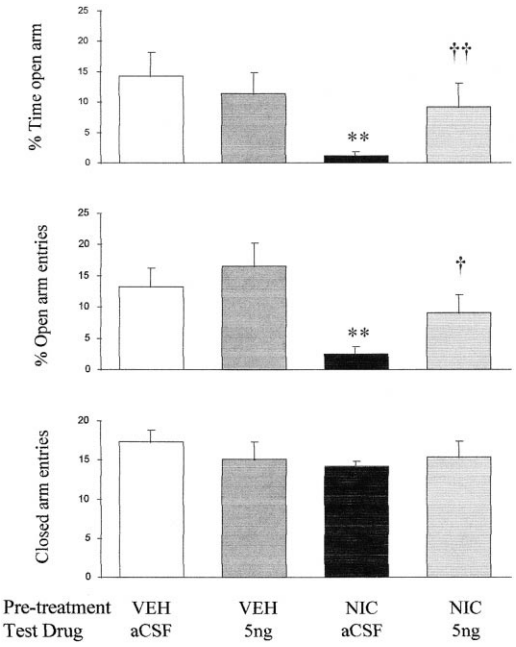

Fig. 3. Mean (±S.E.M.) percentage of time spent on open arms, percentage of open-arm entries, and number of closed-arm entries in the plus-maze in animals pretreated for 6 days with vehicle or nicotine (0.1 mg/kg/day; sc) and tested 24 h later, 3 min after bilateral dorsal hippocampal injections of vehicle (aCSF) or nicotine (5 ng). $*P<.05$ and $**P<.01$ compared with the vehicle control (VEH, aCSF), $^{\dagger}P<.05$ and $^{\dagger\dagger}P<.01$ compared with the withdrawal group (NIC, aCSF).

4. Discussion

Tolerance developed rapidly to the anxiogenic effect of nicotine (0.1 mg/kg) in the elevated plus-maze and, thus, after 1 week this dose no longer had an anxiogenic effect 30 min after injection. This is similar to the rapid development of tolerance to the anxiogenic effect of this dose observed in the social interaction test 5 min after injection (Irvine et al., 1999). However, the effects in the plus-maze differed from those in the social interaction test in that, acutely, this dose was ineffective 5 min after injection and an anxiolytic effect emerged when the rats were tested 5 min after their seventh injection. This is in concordance with previous reports showing nicotine to have anxiolytic effects in the plus-maze after 14–15 days of nicotine treatment (Ericson et al., 2000; Bhattacharya et al., 1995). Although the dorsal raphé

nucleus has been shown to be a site mediating the anxiolytic effect of an acute dose of nicotine in the social interaction test, it as yet unknown what brain structure(s) mediates the anxiolytic effect in the plus-maze. Tolerance also developed rapidly to this anxiolytic effect, with a further seven daily injections. Tolerance to the anxiolytic effects in the social interaction test, observed 30 min after an acute dose of 0.1 mg/kg, also developed after seven injections (Irvine et al., 1999). Thus, tolerance develops much more rapidly to the anxiolytic effects of a low dose of nicotine than it does to the anxiolytic effect of benzodiazepines, which normally takes 21 days to develop (Fernandes and File, 1999; Chopin et al., 1993; File et al., 1987; Treit, 1985; Vellucci and File, 1979).

There was a dissociation in the time-course of changes in locomotor activity, as measured by closed-arm entries, and the measures of anxiety. Thus, in the rats tested 5 min after injection, there were no changes in locomotor activity when the anxiolytic effect emerged at 7 days, but after 14 days when there were no changes in the measures of anxiety there was evidence of locomotor stimulation. This increase in locomotor activity after chronic nicotine treatment is in accordance with other studies (Ericson et al., 2000; Clarke and Kumar, 1983a,b). In the rats tested 30 min after injection, there were no changes in locomotion after acute treatment, when nicotine had an anxiogenic effect, but a reduction in locomotor activity occurred after 7 days, when there was no change in the measures of anxiety.

When rats were tested 24 h after the last of six daily injections, they showed a significant anxiogenic effect, as has been previously reported (Bhattacharya et al., 1995). This anxiogenic effect was not accompanied by any change in locomotor activity which is consistent with other studies that have measured locomotor activity 24 h after withdrawal of nicotine (Robinson et al., 1994; Helton et al., 1993). The studies in which a decrease in locomotor activity was found at 24 h after withdrawal of nicotine used higher doses and longer periods of treatment (Hildebrand et al., 1999; Fung et al., 1996). As was reported by Bhattacharya et al. (1995), we observed no somatic signs of withdrawal in our animals, but the fact that the anxiogenic effect could be reversed by an injection of nicotine strengthens the interpretation that it is a withdrawal response. An anxiogenic effect was not found at this time-point in the social interaction test (Irvine et al., 2000a), but does occur at 72 h (Irvine et al., 1999) and can be reversed by a subcutaneous injection of nicotine (Cheeta et al., 2000a), again suggesting it is a withdrawal response. In the mouse black–white crossing test, an anxiogenic response was observed 8–96 h after withdrawal from 14 days of twice daily nicotine (0.1 mg/kg/day; Costall et al., 1989). Thus, the duration and timing of these withdrawal responses may depend both on the particular test and on the duration of treatment. However, it is clear that they can be observed following a relatively short period of treatment with a low dose of nicotine. Again, this contrasts with the effects of the benzodiazepines, where increased anxiety is usually only observed after withdrawal from 3 weeks of treatment (Ward and Stephens, 1998; Andrews et al., 1997; Chopin et al., 1993; File and Andrews, 1991; File et al., 1987, 1991).

The incidence of a withdrawal response in the opposite direction to the acute effects of a drug is an indication of an oppositional mechanism of tolerance. Thus, benzodiazepines initially have an anxiolytic effect, tolerance develops to this and an anxiogenic response is seen on drug withdrawal. A similar pattern can be seen following repeated injections with the anxiogenic drug pentylenetetrazole, where an anxiolytic effect is seen on withdrawal (File et al., 1996a). This is not the pattern seen in the elevated plus-maze or the black–white crossing test (Costall et al., 1989) where both the acute response to nicotine and that seen during withdrawal are in the same direction, i.e., increased anxiety. Furthermore, a withdrawal response was observed after 6 days of treatment, at the same time that an anxiolytic effect could be seen in response to a nicotine injection. In the social interaction test, tolerance was observed after 6 days to the anxiogenic effect, but in this test no withdrawal response was found at the 24-h time-point. It therefore seems unlikely that an oppositional mechanism underlies the development of tolerance to the anxiogenic effects of nicotine in any of the animal tests thus far investigated.

Whilst a decremental mechanism of tolerance (i.e., a change that results in a drug having less effect, but which is not manifest in the absence of the drug) can account for the development of tolerance, without the occurrence of a withdrawal response, it alone cannot explain the incidence of a withdrawal response in the same direction as the acute effect. One possibility is that the withdrawal response is mediated by changes in a neural system different from that which is manifesting the changes of tolerance. This possibility is strengthened by our finding that although dorsal hippocampal administration of nicotine was without effect in the control animals, it was able to reverse the effects of nicotine withdrawal. In the present experiment, we investigated only a single dose of nicotine, but other studies have shown a wide range of doses to be ineffective in the plus-maze (Cheeta et al., 2000b; Ouagazzal et al., 1999a). One area that mediates the anxiogenic effects of acute nicotine is the lateral septum (Cheeta et al., 2000b) and there seems to be a reciprocal inhibition between the dorsal hippocampus and the lateral septum in mediating behavior in the plus-maze. The 5-HT_{1A} receptors have been implicated in this anxiogenic effect, because the coadministration into the lateral septum with the 5-HT_{1A} antagonist WAY 100,635 reversed the anxiogenic effect of nicotine (Cheeta et al., 2000c). Thus, when the baseline scores are low (e.g., 10% open-arm entries), a 5-HT_{1A} receptor agonist administered to the dorsal hippocampus has an anxiolytic effect (Menard and Treit, 1998), whereas with higher baseline scores (30%) neither benzodiazepines nor a 5-HT_{1A} receptor agonist has any action (Gonzalez et al., 1998; File et al., 1996b). In contrast, when baseline scores are high an anxiolytic effect

can be seen after administration of a 5-HT_{1A} receptor agonist to the lateral septum (Cheeta et al., 2000b), whereas it is without effect if baseline scores are low (Menard and Treit, 1998). The dorsal hippocampus has been implicated in the anxiogenic effect that can be detected in the plus-maze after restraint stress (Netto and Guimaraes, 1996; McBlane and Handley, 1994; Titze-de-Almeida et al., 1994) and in the stress-induced decrease in locomotor activity (Carli et al., 1993). It is therefore possible that the anxiogenic response observed during nicotine withdrawal is an example of a wide range of stress-induced responses in which the dorsal hippocampus plays a role. Thus, the nicotine–cholinergic system in this brain region may be implicated in several stressful situations and withdrawal from nicotine may have been stressful because it constituted a major change in state. It would be interesting to see whether dorsal hippocampal administration of nicotine could reverse the anxiogenic effect seen after restraint stress in the elevated plus-maze.

The results of this and previous studies show that chronic administration of a low dose of nicotine results in the rapid development of tolerance to its acute anxiogenic effects and an anxiogenic response on drug withdrawal. However, when rats self-administer a high dose of nicotine (0.45 mg/kg/day) an anxiogenic effect is still seen even after 4 weeks of treatment and no withdrawal responses are seen 24 and 72 h after the last dose (Irvine et al., 2000b). Future studies are necessary to detect whether tolerance does develop to the effects of this high dose and whether passive vs. self-administration is a crucial factor.

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