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Different outcomes after acute and chronic treatment with nicotine in pre-pulse inhibition in Lister hooded rats

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Abstract

Excessive tobacco consumption by schizophrenic patients may be a form of self-medication, and nicotine in tobacco may alleviate deficits in information processing. We tested this hypothesis by determining whether nicotine (acute/chronic) would improve information processing in the rat using pre-pulse inhibition as a model. In study 1, rats were injected with nicotine 10 min prior to placement in startle chambers (0.001–0.1 or 0.03–0.3 mg/kg, s.c.). In study 2, rats were injected with either saline or nicotine (0.4 mg/kg, s.c.) for 21 consecutive days and assessed for locomotor activity, pre-pulse inhibition and changes in [^{3}H]nicotine binding in whole brain. Acutely, nicotine had no effect on pre-pulse inhibition. By contrast, after chronic nicotine treatment, rats demonstrated a robust deficit in pre-pulse inhibition and significant increases in locomotor activity and [^{3}H]nicotine binding. The deficit in pre-pulse inhibition after chronic treatment with nicotine may be the result of non-specific behavioural activation due to increased mesolimbic dopamine release or, possibly, nicotine may rapidly desensitize nicotinic receptors important for normal information processing.

Keywords: (Rat); Nicotine; Pre-pulse inhibition; Schizophrenia; Information processing

1. Introduction

Various studies show that schizophrenics abuse tobacco to a greater extent than other psychiatric and non-psychiatric populations (Dalack et al., 1998). A number of hypotheses have been formulated to account for this observation (Forchuk et al., 1997). Schizophrenics may smoke for psychological reasons, and smoking may be a means of coping with stress, improving concentration and facilitating relaxation or may be a behavioural 'filler'. The psychological benefits of smoking may be particularly important in an institution, although no study has compared the level of smoking by schizophrenics in institutional and community settings. Schizophrenics may smoke to reduce the dysphoria and extrapyramidal side effects induced by neuroleptics. However, haloperidol-induced catalepsy in rats is potentiated by concurrent nicotine treatment (Sanberg et al., 1993; Emerich et al., 1991). Alternatively, schizophrenics may be more prone to tobacco addiction. Not only does nicotine increase mesolimbic dopamine function, but it can cross generalize to other psychostimulants under specific conditions in drug discrimination studies, and rats trained to self-administer amphetamine will also self-administer nicotine (Stolerman and Jarvis, 1995). Furthermore, nicotine potentiates the locomotor activating effects of amphetamine and apomorpine (Suemaru et al., 1993).

It has also been proposed that nicotine in tobacco may alleviate positive, negative and cognitive symptoms in schizophrenia by improving information processing. Schizophrenics are impaired in the ability to suppress irrelevant information and this may account, at least in part, for cognitive deficits and possibly also positive and negative symptoms. One manifestation of the deficit in information processing in schizophrenics is the inability to suppress or gate irrelevant thoughts and sensory stimuli from intruding into consciousness. For example, the amplitude of the P50 auditory-evoked potential is normally decreased on the second exposure to an auditory stimulus.

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In schizophrenics the amplitude of the P50 wave after the second exposure is not suppressed to the same extent as in controls (Adler et al., 1992; Freedman et al., 1987). Smoking and nicotine gum transiently alleviate deficits in gating seen in schizophrenics and their relatives, respectively (Adler et al., 1992, 1993). Similar experiments in rodents also point strongly to an involvement of nicotinic receptors, notably in the hippocampus, in the suppression of auditory evoked potentials (Leonard et al., 1996; Stevens and Wear, 1997; Stevens et al., 1998; Luntz-Leybman et al., 1992).

Another model of information filtering is pre-pulse inhibition, which refers to the ability of a weak stimulus to suppress the normal startle response to an intense stimulus (Davis, 1980). Non-medicated schizophrenics have a deficit in pre-pulse inhibition (Braff et al., 1978). Drugs that increase mesolimbic dopamine activity impair pre-pulse inhibition in rats and this is reversed by antipsychotics (Mansbach et al., 1988; Varty and Higgins, 1995; Hoffman and Donovan, 1994). Nicotine improves pre-pulse inhibition in rats although the effect is strain-, age- and sex-dependent (Faraday et al., 1998, 1999; Acri et al., 1994, 1995; Curzon et al., 1994). This enhancement in rats may be akin to the transient improvement of information processing in schizophrenics discussed above. To the best of our knowledge the effect of nicotine on pre-pulse inhibition in schizophrenics has not been examined.

Although nicotine improves pre-pulse inhibition in rats, some studies show that it can also induce deficits (Faraday et al., 1998, 1999). Although various factors may account for this apparent paradox, a major one may be the strain of rat used (Acri 1994; Faraday et al., 1998, 1999; Curzon et al., 1994). However, improvements in pre-pulse inhibition are not necessarily always demonstrable in the same strain (Acri et al., 1995; Acri, 1994). Furthermore, in many of these studies changes in pre-pulse inhibition are concomitant with changes in startle amplitude that may confound interpretation of a drug effect on pre-pulse inhibition.

Therefore, in the present study we examined the effects of acute and chronic administration of nicotine in Lister hooded rats, a strain not previously tested in studies with nicotine in pre-pulse inhibition. We carried out two studies with acute nicotine using two overlapping dose ranges that have been reported to improve pre-pulse inhibition in Sprague Dawley (0.001 and 0.01 mg/kg, Acri et al., 1994) and Long–Evans (0.03–0.3 mg/kg, Curzon et al., 1994) rats. To test the effects of chronic treatment with nicotine on pre-pulse inhibition, rats received the drug as daily subcutaneous injections rather than via osmotic mini-pumps as in previous studies (Acri et al., 1995; Faraday et al., 1998, 1999). Such intermittent daily injections are known to increase locomotor activity and [^{3}H]nicotine binding (Ksir et al., 1985, 1987; Collins et al., 1990). This chronic treatment regimen may better model the clinical situation where schizophrenic's self-administer nicotine and smoking leads to an increase in [^{3}H]nicotine binding sites in the brains of smokers (Benwell et al., 1988; Leonard et al., 1996). Therefore, we have assessed the effect of chronic treatment with nicotine on pre-pulse inhibition, locomotor activity and [^{3}H]nicotine binding in the rat brain.

2. Methods

2.1. Animals

Lister hooded rats (350–400 g) were used in all studies. Rats were obtained from Charles River (UK) and housed four per cage under a 12-h light/dark cycle (lights on 8:00 a.m.), in temperature (20 ± 2°C) and humidity (55 ± 15%) controlled rooms. Animals had free access to food and water. Testing was between 9:00 a.m. and 5:00 p.m. All animal husbandry and experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and British Home Office guidelines concerning the health and welfare of laboratory animals.

2.2. Drugs

[−]Nicotine di(+)tartrate was obtained commercially (Sigma, UK), and in all experiments was injected subcutaneously at a dose volume of 1 ml/kg in saline. L-[^{3}H]nicotine (*N*-methyl-^{3}H, specific activity 60–87 Ci/mmol) was obtained from NEN Life Science Products (Hounslow, UK). All drug doses are expressed as free base.

2.3. Apparatus

Four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) were used to measure startle amplitude. Each chamber consisted of a Plexiglass cylinder (8.8 cm in diameter) mounted on a frame and held in position by four metal pin legs to a base unit. Movement of the rat within the cylinder was detected by a piezoelectric accelerometer attached below the frame. A loudspeaker (Radio Shack Supertweeter) was mounted 24 cm above the cylinder and provided background white noise and acoustic stimuli (pulse and pre-pulses). The whole apparatus was housed in a ventilated and lit chamber ($39 \times 38 \times 58$ cm: $L \times W \times H$). Presentation of the acoustic stimuli was controlled by a SR-LAB software and an interface unit, which also digitized (0–4095), rectified and recorded the responses from the accelerometer. Mean startle amplitude, the dependent measure, was determined by averaging 150, 1 ms readings taken at the beginning of the pulse stimulus onset. All sound levels in each chamber were calibrated using a noisemeter (Dawe Instruments, Model number 1422C).

2.4. Experimental procedures

2.4.1. Startle reactivity and pre-pulse inhibition

In study 1, rats were place in the Plexiglass cylinders which were obstructed at either end by two Plexiglass covers, ensuring the rat did not escape. After a habituation period of 5 min during which rats were exposed to background noise (70 dB), the startle session began with the delivery of a single startle stimulus (120 dB, 40 ms). This was then followed by the delivery of 12 times each of the following six trial types in a pseudo-random order: null stimuli (70 dB), startle stimuli (120 dB, 40msec), pre-pulses of 2, 6, 10 or 12 dB above background noise, lasting for 20 ms and occurring 100 ms prior to the startle stimulus. The mean inter-trial-interval was 30 s (range = 20–40 s), and sessions lasted approximately 40 min (Varty and Higgins, 1995).

In study 2, the rats were placed in the startle chambers twice — on days 14 and 21 (see below). Whereas on Day 14 the protocol was as described above, on Day 21 this was modified. There were five blocks of trials with each block containing 2 × null stimuli (70 dB), 2 × startle stimuli (120 dB, 40 ms), and one of each of the following pre-pulses — 2, 4, 8 and 16 dB above background noise — lasting 20 ms and occurring 100 ms prior to the startle stimulus. The mean inter-trial-interval was 10 s (range = 5–15 s), and sessions lasted approximately 12 min (Wilkinson et al., 1994).

2.4.2. Locomotor activity

Rats were placed inside locomotor activity chambers with the following dimensions: 51 (width) × 51 (depth) × 30 (height) cm. The chambers essentially consisted of one set of lateral infrared beams arranged in a 4 × 4 cell array, 10 cm above floor level. Interruption of these infrared beams was taken as a measure of locomotor activity.

2.4.3. [^{3}H]nicotine binding

Binding of [^{3}H]nicotine to whole rat brain was measured by a method adapted from Bhat et al. (1994). Rats were killed by concussion followed by decapitation and removal of the brain. The cerebellum was discarded and the remainder of the brain placed in 10 volumes of ice-cold Kreb–Ringer-HEPES buffer consisting of 50 mM HEPES, 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$ and 1.2 mM $MgSO_4$, adjusted to pH 7.5 with NaOH. The tissues were homogenised using a Ultra-Turrax homogeniser, and the resulting homogenate was centrifuged at 30,000 × g for 30 min. The pellet was re-suspended in 20 volumes of ice-cold Kreb–Ringer-HEPES buffer and re-centrifuged at 30,000 × g for 30 min. The resulting pellet was then re-suspended in 20 volumes of ice-cold distilled water and incubated at 4°C for 60 min. The homogenate was centrifuged at 30,000 × g for 30 min. The pellet was re-suspended in 20 volumes of ice-cold Kreb–Ringer-HEPES buffer and re-centrifuged at 30,000 × g for 30 min. The pellet was re-suspended in assay buffer using an Ultra-Turrax homogeniser for immediate use in the binding. Binding assays were performed in a total volume of 250 μl, containing [^{3}H]nicotine (scatchard analysis ranging from 0.14 to 70 nM), membranes and additional drugs. Non-specific binding was determined using nicotine (100 nM). Following 60 min incubation at 4°C, assays were terminated by rapid filtration using a Brandel Harvester. Radioactivity was assessed using a Beckman LS6500 scintillation counter.

2.5. Experimental protocols

2.5.1. Study 1: effect of acute treatment with nicotine on pre-pulse inhibition

2.5.1.1. Experiment 1. Four groups of rats ($n = 8$) were administered either saline or nicotine (0.001, 0.01 and 0.1 mg/kg, Acri et al., 1994) 10 min prior to being placed in the startle chambers and exposed to auditory stimuli as outlined in Section 2.4.1 (Varty and Higgins, 1995).

2.5.1.2. Experiment 2. Four groups of rats ($n = 10$) were administered either saline or nicotine (0.03, 0.1 and 0.3 mg/kg, Curzon et al., 1994) 10 min prior to being placed in the startle chambers and exposed to auditory stimuli as outlined in Section 2.4.1 (Varty and Higgins, 1995).

2.5.2. Study 2: effect of chronic treatment with nicotine on pre-pulse inhibition

There were two groups of rats ($n = 10$) injected with either saline or nicotine (0.4 mg/kg) once daily (9:00–10:00 a.m.) for 21 consecutive days. During this period, these rats were tested in three procedures, and their weights noted daily.

(1) On days 1 to 5, rats were placed in locomotor activity chambers immediately after injection of either saline or nicotine, without any prior habituation to the chambers. Beam breaks were recorded over a 30-min period. From days 6 to 13, treatment continued with no further testing in locomotor chambers.

(2) On day 14, rats from each group were placed in startle chambers 10 min after treatment with saline or nicotine, and exposed to auditory stimuli as outlined in Section 2.4.1 (Varty and Higgins, 1995). From days 15 to 20, treatment continued with no further testing in any procedure.

(3) On day 21, rats were placed in startle chambers 10 min after treatment with saline or nicotine, and exposed to auditory stimuli as outlined in Section 2.4.1 (Wilkinson et al., 1994).

(4) On day 21, after removal from startle chambers, five rats were randomly chosen from both groups and

[^{3}H]nicotine binding determined in whole brain as outlined in Section 2.4.3 (Bhat et al., 1994).

2.6. Statistics

The effect of nicotine on locomotor activity was analyzed by two-way analysis of variance (ANOVA) (Between groups factor: Treatment; Within group factor: Day). Binding data (B_{max} and K_d) were analyzed by one-way ANOVA with one between group factor: Treatment. For the startle procedure, the two dependent measures were startle response to the 120-dB stimulus and percent pre-pulse inhibition. The latter was calculated according to the following formula: [100 − (startle amplitude on pre-pulse–pulse trials/startle amplitude on pulse alone trials) × 100]. The main effect of Treatment on startle reactivity was analyzed by one-way ANOVA followed by Tukey's HSD. Percent pre-pulse inhibition was analyzed using two-way ANOVA with the factors Treatment and Trial (four levels: 2, 6, 10 and 12 or 2, 4, 8 and 16 dB depending upon the experiment). In the absence of a Treatment × Trial interaction, any main effect of Treatment was followed by Tukey's HSD test. If, however, there was an interaction between Treatment and Trial, then a one-way ANOVA was performed on each pre-pulse–pulse trial separately followed by Tukey's HSD, where appropriate. Locomotor activity, startle reactivity and pre-pulse inhibition were analysed using Statistica software (Tulsa, OK, US). The data from saturation binding experiments was analysed using GraphPad Prism software (San Diego, CA, USA) to determine the binding parameters (dissociation constant, K_d, and the number of binding sites, B_{max}).

Table 1
Effect of acute (s.c.) administration of saline or nicotine on startle response and percent pre-pulse inhibition in Lister hooded rats. Mean ± S.E.M. for startle amplitude to 120 dB auditory stimulus alone and percent PPI collapsed for all pre-pulses. Nicotine was tested twice in separate experiments with overlapping dose ranges ($n = 8$ and 10 per group for experiments 1 and 2 from study 1, respectively). Nicotine had no effect compared to controls in either experiment by one- and two-way ANOVA (see Methods)

Dose of nicotine (mg/kg, s.c.)	Startle response to 120 dB stimulus	Percent pre-pulse inhibition collapsed for pre-pulses 2, 6, 10 and 12 dB above background
Experiment 1		
0.0	319 ± 50	60.0 ± 4.4
0.001	294 ± 86	60.6 ± 4.4
0.01	219 ± 40	50.7 ± 5.3
0.1	182 ± 28	51.9 ± 4.8
Experiment 2		
0.0	170 ± 21	61.6 ± 3.5
0.03	164 ± 17	50.7 ± 4.6
0.1	152 ± 31	52.3 ± 3.8
0.3	215 ± 20	52.7 ± 4.0

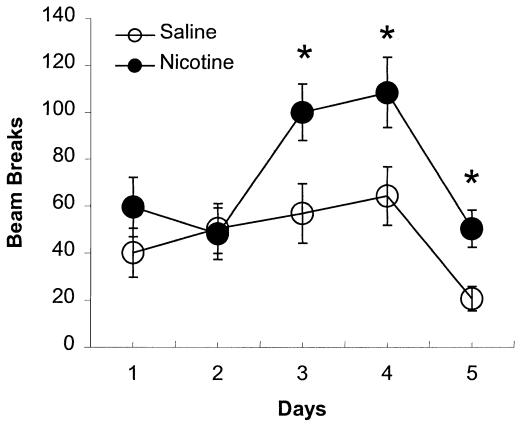

Fig. 1. Effect of daily injections of saline or nicotine (0.4 mg/kg, s.c.) on mean ± S.E.M. beam breaks over 5 consecutive days in rats ($n = 10$). Rats were placed in locomotor activity cages immediately after injections. * $P < 0.05$ compared to saline control, two-way ANOVA and Tukey's HSD test.

3. Results

3.1. Study 1: effect of acute treatment with nicotine on pre-pulse inhibition

In experiment 1, nicotine (0.001–0.1 mg/kg) had no effect on either startle amplitude or percent pre-pulse inhibition (Table 1). There was no effect of Treatment ($F[3,28] < 1$), a significant effect of Trial ($F[3,84] = 165$, $P < 0.01$), but no Treatment × Trial interaction ($F[9,84] < 1$) on pre-pulse inhibition. Similarly, in experiment 2 using a different nicotine dose range (0.03–0.3 mg/kg), there

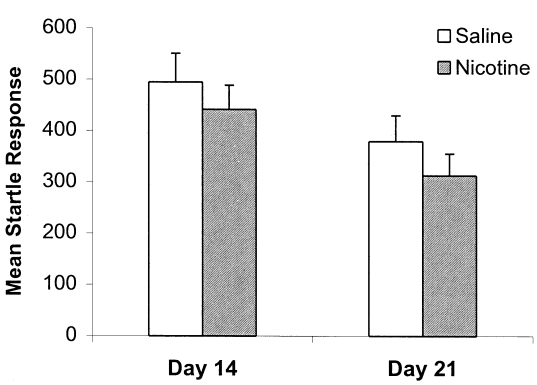

Fig. 2. Effect of chronic treatment with saline or nicotine (0.4 mg/kg, s.c.) on mean ± S.E.M. startle amplitude in rats ($n = 10$). The figure shows the effect of saline and nicotine on startle response in rats after 14 and 21 consecutive daily injections. The same rats that were tested on day 14 continued to receive nicotine for another 7 days and were tested again on day 21. On both days 14 and 21, rats were placed in startle chambers 10 min after injection. No significant effects of nicotine were detected compared to control (one-way ANOVA).

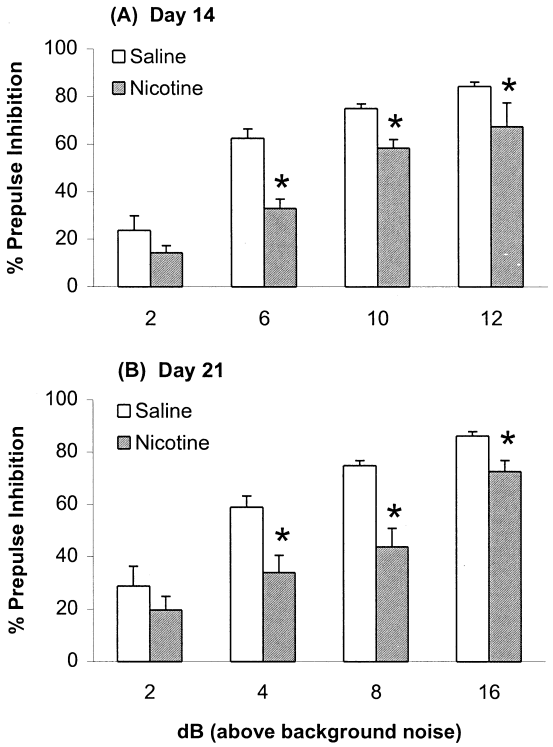

Fig. 3. Effect of chronic treatment with saline or nicotine (0.4 mg/kg, s.c.) on mean ± S.E.M. percent pre-pulse inhibition in rats ($n = 10$). The figure shows the effect of nicotine after 14 (A) and 21 (B) consecutive once daily injections. The same rats that were tested on day 14 continued to receive nicotine for another 7 days and were tested again on day 21. On both days 14 and 21, rats were placed in startle chambers 10 min after injection. $^{*}P < 0.05$ compared to saline control, by two-way ANOVA and Tukey's HSD test.

was no effect of Treatment ($F[3,36] = 2.1$), a significant effect of Trial ($F[3,108] = 176$, $P < 0.01$) and no Treatment × Trial interaction ($F[9,108] < 1$) on pre-pulse inhibition, nor an effect of nicotine on startle amplitude (Table 1).

3.2. Study 2: effect of chronic treatment with nicotine on pre-pulse inhibition

Nicotine had no effect on body weight gain over time compared to controls. Mean body weights on Days 1 and 21, respectively, were: saline, 449 ± 8.3 and 466 ± 11; nicotine, 450 ± 9.9 and 458 ± 8.1.

3.2.1. Locomotor activity (days 1–5)

The effect of nicotine on locomotor activity was highly significant, but varied across the 5 days (Fig. 1). There was a significant Treatment × Day interaction ($F[4,72] = 4.3$, $P < 0.01$). One-way ANOVA's at each day showed that nicotine significantly increased the number of beam breaks on days 3–5 ($F[1,18]$ range: 14–46, $P < 0.001$ in each case).

3.2.2. Pre-pulse inhibition (day 14)

Eight days after the last locomotor activity session, rats were placed into startle chambers for the first time. Fig. 2 shows that nicotine treatment had no effect on startle amplitude (Treatment: $F[1,18] < 1$). However, there was a main effect of Treatment ($F[1,18] = 23.2$, $P < 0.01$) and a significant Treatment × Trial interaction on the percent pre-pulse inhibition measure ($F[3,54] = 4.1$, $P < 0.01$, Fig. 3A). One-way ANOVA's for each trial type showed a significant deficit in percent pre-pulse inhibition after nicotine when pre-pulses 6, 10 and 12 dB above background noise preceded the startle stimulus ($F[1,18]$ range: 16.7–27.2, $P < 0.01$ in each case).

3.2.3. Pre-pulse inhibition (day 21)

Seven days after the first pre-pulse inhibition study, rats were again placed into startle chambers. Again pre-pulse inhibition was assessed, but using a modified protocol (see Methods). Nevertheless, the profile of nicotine's effects remained the same. Nicotine had no effect on startle

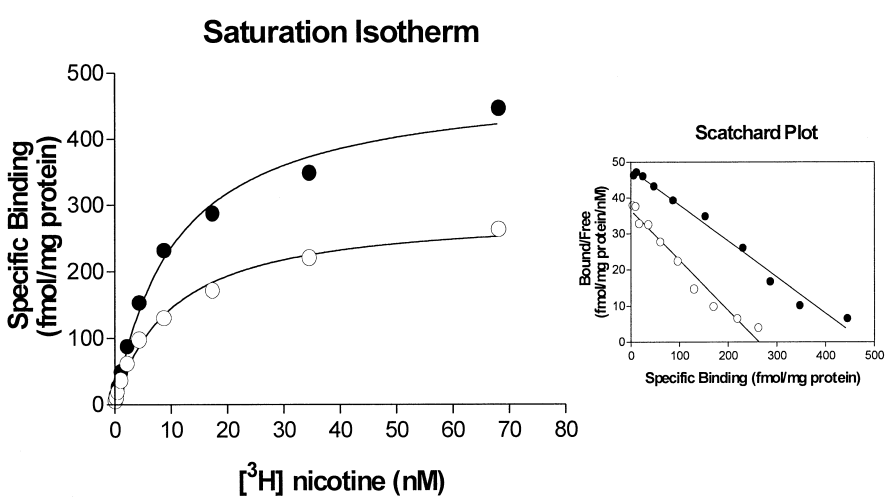

Fig. 4. Saturation analysis and derived scatchard plots for [^{3}H]nicotine binding to whole brain homogenates, from rats treated (s.c.) with saline (○) or nicotine (●) for 21 consecutive days ($n = 5$).

Table 2
Effect of chronic treatment with saline or nicotine (s.c.) for 21 consecutive days on [^{3}H]nicotine binding to whole rat brain ($n = 5$). Mean ± S.E.M for the number of binding sites (B_{max}) and dissociation rate constant K_d of [^{3}H]nicotine binding sites

Treatment	Number of [^{3}H]nicotine binding sites (B_{max}) in fmol/mg protein	Dissociation rate constant for [^{3}H]nicotine binding sites (K_d) in nM
(1) Chronic saline (s.c.)	270 ± 13.9	10.1 ± 0.7
(2) Chronic nicotine (0.4 mg/kg, s.c.)	462 ± 74.7[a]	10.3 ± 2.3

[a] $P < 0.05$ compared to the saline control, by one-way ANOVA and Tukey's HSD test.

amplitude (Treatment: $F[1,18] = 1$; Fig. 2). For the pre-pulse inhibition parameter there was again a main effect of Treatment ($F[1,18] = 11.3$, $P < 0.01$) and a significant Treatment × Trial interaction ($F[3,54] = 3.6$, $P < 0.01$, Fig. 3B). One-way ANOVA's for each trial type showed a significant deficit in percent pre-pulse inhibition after nicotine when pre-pulses 4, 8 and 16 dB above background noise preceded the startle stimulus ($F[1,18]$ range: 9–17.7, $P < 0.01$ in each case).

3.2.4. [^{3}H]nicotine binding

Fig. 4 shows the saturation curves, and derived scatchard plots, for [^{3}H]nicotine binding to whole rat brain homogenates from rats treated with saline or nicotine for 21 consecutive days. One-way ANOVA demonstrated a significant increase in the number of [^{3}H]nicotine binding sites (B_{max}) in the brains of rats treated with 0.4 mg/kg nicotine compared to saline treated rats ($F[1,8] = 6.4$, $P < 0.03$). By contrast, there was no significant difference ($F[1,8] < 1$) between the two groups in terms of dissociation rate constants (K_d). Means ± S.E.M. for both B_{max} and K_d are summarized in Table 2.

3.2.5. Correlations

There were significant negative (Pearson's) correlation coefficients between mean locomotor activity for days 3–5 and mean pre-pulse inhibition, collapsed for all pre-pulse trials, on Days 14 ($r = -0.54$, $P < 0.01$, Fig. 5A) and 21 ($r = -0.46$, $P < 0.04$, Fig. 5B). Further, preliminary correlations based only on those rats for which binding data was generated, showed (i) significant positive correlations between B_{max} and mean locomotor activity for days 3–5 ($r = 0.7$, $P < 0.02$), and (ii) significant negative correlations between B_{max} and mean pre-pulse inhibition, collapsed for all pre-pulse trials, on Days 14 ($r = -0.68$, $P < 0.03$) and Day 21 ($r = -0.66$, $P < 0.04$).

4. Discussion

Nicotine had different effects on pre-pulse inhibition in Lister hooded rats depending upon whether it was administered acutely or chronically. Acute administration had no effect on pre-pulse inhibition or baseline startle (0.001–0.3 mg/kg). By contrast, in rats that had been administered nicotine (0.4 mg/kg) chronically for 14 days there was a deficit in pre-pulse inhibition without a change in baseline startle. This deficit persisted after 21 days of nicotine administration, again with no change in baseline startle. This chronic treatment regimen also resulted in an increase in locomotor activity and [^{3}H]nicotine binding sites in whole rat brain.

No study has previously assessed the effects of nicotine on pre-pulse inhibition in Lister hooded rats. Acutely, nicotine improves pre-pulse inhibition in both Sprague–Dawley and Long–Evans rats (Acri et al., 1994; Curzon et al., 1994). In Long–Evans rats, nicotine improved pre-pulse inhibition with no effect on baseline startle amplitude, whereas in Sprague–Dawley rats, at some doses nicotine improved pre-pulse inhibition but also increased baseline startle (0.01 mg/kg). In the current study, no significant

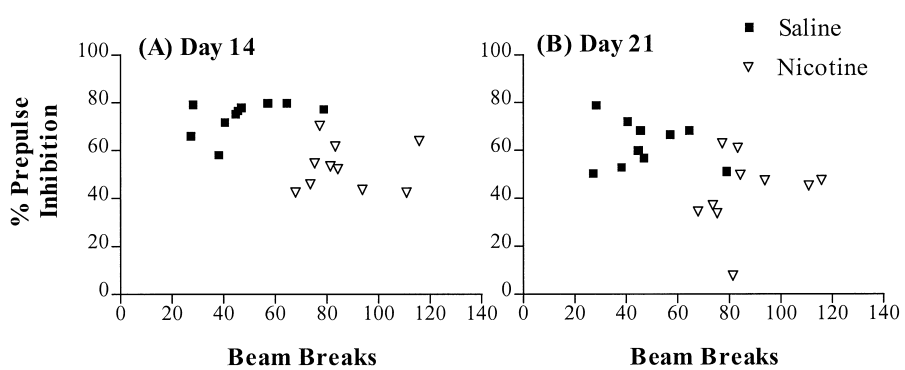

Fig. 5. Correlation of percent pre-pulse inhibition with locomotor activity in rats treated with saline or nicotine. The correlation's are between mean locomotor activity during days 3–5 of treatment and mean pre-pulse-inhibition, collapse for all pre-pulse trials, measured on days 14 (A) and 21 (B). Separate groups of rats ($n = 10$) were administered (s.c.) saline or nicotine for 21 consecutive days. Locomotor activity was measured for the first 5 days immediately after daily treatment and the same rats were tested for pre-pulse inhibition on days 14 (A) and 21 (B) 10 min after treatment (see Methods).

effect of acute nicotine administration on pre-pulse inhibition or baseline startle was observed up to a dose of 0.3 mg/kg. The combined findings of the experiments in study 1 suggest that acutely nicotine tended to decrease startle at low doses (0.001–0.1 mg/kg), and increase it at higher doses (0.3 mg/kg, Table 1). This is inconsistent with the change in startle amplitude reported by Acri et al. (1994), with increases at low doses (0.01 mg/kg) and decreases at very high doses ($\geq$ 0.5 mg/kg).

Chronic treatment with nicotine induced clear deficits in pre-pulse inhibition, on days 14 and 21 of treatment, with no effect on baseline startle. The experimental protocols differed slightly on days 14 and 21, suggesting the deficits were not dependent upon the method used and are a robust finding. Our results differ from previous studies showing that chronic treatment with nicotine improves pre-pulse inhibition in Sprague–Dawley rats (Acri et al., 1995; Faraday et al., 1999). However, the findings concur with those of Faraday et al. (1998, 1999), who reported that nicotine impaired pre-pulse inhibition in Long–Evans rats. An important distinction between our data and previous results is that the nicotine-induced deficits in pre-pulse inhibition we observed were entirely independent of effects on baseline startle. The pre-pulse inhibition deficit following chronic treatment with nicotine in the Long–Evans strain and improvement in Sprague–Dawley rats occurred concurrently with a decrease and increase in startle, respectively (Acri et al., 1995; Faraday et al., 1998, 1999). Therefore, the effects on pre-pulse inhibition in the studies of Acri et al. (1994) and Faraday et al. (1998, 1999) may be secondary to nicotine-induced changes in baseline startle. However, it is possible that by day 14 tolerance developed to any effect of nicotine on startle in the current study. While possible, it is clear that there was no effect of acute treatment with nicotine on the startle response in study 1.

Chronic treatment with nicotine also increased locomotor activity by day 3 of administration. This effect persisted until day 5, and, while we did not determine whether the effect was maintained until day 21, previous studies have demonstrated that this is a robust and long lasting effect (Stolerman et al., 1973, 1974). Furthermore, after the second pre-pulse inhibition experiment, nicotine-treated rats that were sacrificed had an increased number of [^{3}H]nicotine binding sites in whole brain. This is also a robust effect of chronic treatment with nicotine and is consistent with previous reports (Ksir et al., 1985; Schwartz and Kellar 1983; Collins et al., 1990). Like previous authors, we have demonstrated a significant correlation between increased locomotor activity and [^{3}H]nicotine binding (Marks et al., 1983; Ksir et al., 1985). However, since we only measured locomotor activity for the first 5 days and assessed binding on Day 21, we cannot infer a causal relationship between the two measures, although previous literature would appear to suggest that such a relationship exists.

There was a significant correlation between locomotor activity and pre-pulse inhibition deficits on days 14 and 21. Since locomotor activity and [^{3}H]nicotine binding are correlated, it is possible that the increased binding and pre-pulse inhibition deficits are similarly correlated. The increase in locomotor activity after acute and chronic treatment with nicotine is thought to be dependent upon increased dopamine release and activation of dopamine receptors in the nucleus accumbens (Clarke et al., 1988; Benwell and Balfour, 1992; O'Neill et al., 1988). Dopamine receptor agonists increase dopaminergic function in the nucleus accumbens and this is claimed to model a similar pathophysiological change in the brains of schizophrenics (Kerwin, 1992; Swerdlow et al., 1994). Indeed, amphetamine impairs pre-pulse inhibition and induces psychotic-like effects in human volunteers (Snyder, 1972; Swerdlow et al., 1992). Therefore, one explanation for the deficit in pre-pulse inhibition after chronic administration of nicotine may be excess dopaminergic activity in the mesolimbic dopamine system. An up-regulation of high affinity nicotinic binding sites has been implicated in both increased dopamine release and increased locomotor activity after chronic treatment with nicotine, and may therefore also mediate the deficit in pre-pulse inhibition we observed (Ksir et al., 1985; Benwell and Balfour, 1992; Marks et al., 1983). This implies that by smoking, schizophrenics may exacerbate their symptoms. However, curiously, the up-regulation of [^{3}H]nicotine binding sites seen in normal smokers compared to non-smokers is not observed when comparing smoking and non-smoking schizophrenic populations (Breese et al., 1997).

Our findings with nicotine are interesting in light of the effects of dopamine receptor agonists on pre-pulse inhibition. Kinney et al. (1999) showed that when administered acutely apomorphine and amphetamine increased locomotor activity in both Wistar and Sprague–Dawley rats, whereas they only attenuated pre-pulse inhibition in Wistar rats. Furthermore, chronic administration of amphetamine or apomorphine induces a sensitized locomotor response, but not a sensitized disruption of pre-pulse inhibition (Druhan et al., 1998). By contrast, we have demonstrated that nicotine has no effect acutely but impairs pre-pulse inhibition after chronic treatment, i.e. when a sensitized locomotor response develops. Therefore, unlike apomorphine and amphetamine, the pre-pulse inhibition deficit we observed with nicotine may reflect a gross behavioural activating effect of the drug due to increased dopamine activity in the nucleus accumbens. However, nicotine can increase locomotor activity after acute administration in rats habituated to the test environment (O'Neill et al., 1988). Moreover, Bakshi et al. (1995) have elegantly demonstrated that drug-induced increases in locomotor activity and/or startle amplitude can be dissociated from deficits in pre-pulse inhibition or latent inhibition.

Alternatively, the deficits in pre-pulse inhibition we have observed after chronic treatment with nicotine may

be mediated by the α_7 nicotinic receptor. It has been shown that schizophrenics may have fewer α_7 nicotinic receptors compared to matched controls, and this has been linked to deficits in gating (Freedman et al., 1997; Leonard et al., 1996). Moreover, α_7 nicotinic receptor antagonists block the P50 auditory evoked potential in rats, and this is reversed by nicotinic agonists acting at the α_7 receptor (Leonard et al., 1996; Luntz-Leybman et al., 1992; Stevens and Wear, 1997; Stevens et al., 1998). Since the α_7 nicotinic receptor is rapidly desensitized, this may explain the transient improvements in gating seen after smoking and nicotine gum in schizophrenics and their relatives, respectively (Adler et al., 1992, 1993). If the α_7 nicotinic receptor is important in normal gating, then chronic treatment with nicotine as in study 2 would be expected to desensitize the receptor and lead to the deficits in pre-pulse inhibition we observed. However, the α_7 nicotinic receptor comprises the low affinity nicotinic binding site (i.e. the α Bungaratoxin sensitive site) and nicotine desensitises the receptor in milliseconds, raising doubts as to whether nicotine would activate the receptor in-vivo or whether it has any functional relevance (Wonnacott, 1990; but see Fujii et al., 2000).

In conclusion, nicotine administered acutely had no effect on either pre-pulse inhibition or baseline startle. After chronic administration of nicotine, there was a deficit in pre-pulse inhibition with no change in baseline startle. Rats injected with nicotine chronically also showed an increase in locomotor activity and an increase in the number of [^{3}H]nicotine binding sites in brain. Two explanations are proposed to explain the deficit in pre-pulse inhibition after chronic treatment with nicotine. Firstly, that an increase in locomotor activity after chronic treatment with nicotine resulted in a non-specific deficit in pre-pulse inhibition. The second possibility is that rapid desensitization of the α_7 nicotinic receptor subtype, which may be important for normal information gating, impairs pre-pulse inhibition independently of nicotine's locomotor activating effects. Finally, the results of this study suggest that schizophrenics smoke excessively for reasons other than to alleviate deficits in information processing as measured by pre-pulse inhibition.

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