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Mecamylamine but not the α7 receptor antagonist α-bungarotoxin blocks sensitization to the locomotor stimulant effects of nicotine

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1 The involvement of α7 receptors in the locomotor stimulant effects of nicotine has been examined by determining the ability of intracerebroventricular (i.c.v.) administration of the α7 receptor antagonist α-bungarotoxin (α-bgt) to modify sensitization to the locomotor activating effects of chronic nicotine.

2 Intracerebroventricular administration of α-bgt (0.02–8 nmoles) produced a dose dependent increase in convulsive behaviour. At doses less than 1.0 nmole, minimal convulsive behaviour occurred but larger doses evoked convulsions in all rats which displayed a more rapid onset time as the dose increased.

3 The binding distribution of α7 receptors 20 min and 3 h following an i.c.v. administration of [^{125}I]-α-bgt (0.02 nmoles) revealed clear binding in the hippocampus, cingulate cortex and hypothalamus which was more intense after 3 h.

4 Rats chronically treated with nicotine (0.4 mg kg^{-1}) and exposed to the locomotor activity apparatus daily acquired an increase in locomotor activity relative to the control group after 3 days of treatment which reached a maximum after 7 days of treatment and was maintained for the 2 week treatment period.

5 Pre-treatment with mecamylamine (1 mg kg^{-1}) prevented the expression of the locomotor stimulant effects of nicotine but pre-treatment with i.c.v. α-bgt (0.02 nmoles) did not affect nicotine-induced changes in locomotor activity.

6 The results of this study support the conclusion that nicotinic receptors of the α4β2 subtype rather than the α7 subtype are important in mediating the expression of the locomotor stimulant effects of nicotine.

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Abbreviations: α-bgt, α-bungarotoxin

Introduction

The mesolimbic dopamine system is thought to be critically involved in the processes that lead to addiction to psychostimulant drugs (Robinson & Berridge 1993). In particular, the ability of repeated exposure to cocaine or amphetamine to produce sensitization to the locomotor stimulant effects and to enhance mesolimbic dopamine secretion are thought to be key events in the addictive process. Nicotine also shares properties with psychostimulant drugs of abuse. Thus the mesolimbic dopamine system is thought to be involved in the locomotor stimulant and reinforcing effects of this drug (Clarke & Kumar, 1983; Corrigall *et al*., 1992; Clarke 1990; Reavill & Stolerman, 1990; Benwell & Balfour, 1992). Moreover, sensitization to enhanced mesolimbic dopamine secretion after repeated nicotine administration has been associated with locomotor sensitization (Benwell & Balfour, 1992). In addition, nicotine-induced sensitization of nucleus accumbens dopamine secretion appears to depend upon co-stimulation of NMDA receptors although the role of glutamate receptors in the expression of locomotor sensitization appears more complex (Shoaib *et al*., 1994; Balfour *et al*., 1998).

Neuronal nicotinic acetylcholine receptors are pentameric structures composed of different α and β subunits. In the mammalian brain there are at least nine different α subunit members of the nicotinic acetylcholine receptor group and four β subunits, suggesting greater heterogeneity than originally anticipated (Sargent, 1993). Of these, several subunits produce functional receptors when co-expressed such as the α4β2 receptor which is thought to be the most prevalent receptor type in the rat brain. The α7 subunit of the nAChR, originally identified as the α-bungarotoxin (α-bgt) binding site also appears to be widely expressed in rat brain (Del Toro *et al*., 1994; Seguela *et al*., 1993) and a body of evidence is accumulating to suggest that an α7 containing receptor is involved in modifying fast excitatory neurotransmission in the CNS (McGehee *et al*., 1995; Gray *et al*., 1996; Radcliff & Dani, 1998). However, the role of α7 containing receptors in the psychopharmacological properties of nicotine are largely unexplored.

Previous studies have attempted to elucidate the receptor subtypes involved in tolerance to the locomotor depressant effects of nicotine. Thus tolerance to these effects is paralleled by an upregulation of nicotine and α-bgt binding although the time course of these changes correlated most with changes in nicotine binding (Marks *et al*., 1983; 1985; Collins *et al*., 1990).

The different profile of action of nicotinic receptor agonists, upon locomotor activity may suggest a potential involvement of α7 containing receptors. For example, isoarecolone, only weakly cross sensitizes to chronic nicotine induced locomotor sensitization and fails to enhance mesolimbic dopamine secretion (Whiteaker *et al*., 1995; Mirza *et al*., 1996). Since

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isoarecolone, unlike nicotine, fails to compete with [I^{125}]-α-bgt (Whiteaker *et al*., 1995) it is plausible that activity at α7 binding sites is necessary to confer nicotine-induced locomotor sensitization and enhanced dopamine secretion.

Furthermore, the presence of large concentrations of the α7 receptor subtype in the hippocampus together with the known glutamatergic input from the hippocampus to the nucleus accumbens, suggests that receptors containing the α7 subunit have the potential to be involved in modifying mesolimbic dopamine activity and hence to be involved in locomotor sensitization. This is supported by recent studies showing that intra hippocampal injections of nicotine result in an increase in the extracellular concentrations of glutamate in the hippocampus (Toth, 1996) and that stimulation of nAChRs enhances glutamatergic synaptic transmission in hippocampal neurons through a presynaptic α7-type receptor (Gray *et al*., 1996; Radcliffe & Dani, 1998).

The aim of this study was therefore to investigate the involvement of an α7 containing receptor in the locomotor stimulant effects of nicotine in the rat. A preliminary account of some of this work has appeared in abstract form (Kempsill & Pratt 1996).

Methods

Animals

Male Sprague Dawley rats (bred at University of Strathclyde) weighing 250–300 g at the beginning of the experiment were used. All animals had free access to food and water and were housed in groups of two or three. Those, which underwent surgery, were housed individually following surgery. In order to habituate animals to the handling and injection procedure they were gently handled for a few minutes daily for 4 days and injected with saline i.p. 2 days prior to the beginning of each experiment. All procedures were carried out in accordance with current guidelines of the Home Office.

Drugs

Drugs were obtained from Sigma (Poole, Dorset U.K.). All doses of drugs were calculated from those of the bases. In these experiments the agonist, (−) nicotine and the antagonist, mecamylamine were made up in 0.9% saline whereas α-bgt was made up in 150 mM NaCl. The pH of the nicotine solution was adjusted to seven using sodium hydroxide. Except for α-bgt the doses of the drugs used in the experiments were based on previously published work (Benwell & Balfour, 1992) and were administered in mg kg^{-1} from ml kg^{-1} solutions. α-bgt does not penetrate the blood brain barrier and so the drug was administered intracerebroventricularly. Prior to conducting the experiments it was necessary to conduct a pilot study in order to establish suitable doses for subsequent experiments. For *in vivo* autoradiography 50 μl of [^{125}I]-α-bgt (2000 Ci $mmol^{-1}$; Sigma, Poole, Dorset, U.K.) was diluted with 5 μl cold toxin diluted in 150 mM NaCl (0.168 mg ml^{-1}) in order to ensure that a corresponding concentration of toxin was administered as in the behavioural experiments.

Intracerebroventricular bilateral cannulae placement

Rats were anaesthetized by inhalation using a combination of 1% halothane, 30% oxygen and 70% nitrous oxide. Bilateral 22 guage guide cannulae were implanted into the two lateral ventricles using co-ordinates from Paxinos & Watson, 1986 (AP: −0.06 mm from Bregma; ML: +0.15 mm, DV: −3.2 mm) and were secured in place by dental cement. Rats were allowed to recover from surgery for at least 3 days before commencement of experiments.

α-bungarotoxin autoradiography

In order to ensure that the α-bgt had penetrated into the brain tissue within the time in which LMA was being measured, [^{125}I]-labelled α-bgt was administered i.c.v. to drug naïve, experimentally naïve rats and autoradiograms prepared. [^{125}I]-α-bungarotoxin (90 Ci $mmol^{-1}$, 0.02 nmoles administered as 0.01 nmoles in 5 μl/ventricle) or saline (5 μl/ventricle) was administered. Five rats received i.c.v. α-bungarotoxin and were killed after 20 mins ($n=3$) or 3 h ($n=2$). The 20 min time point corresponds to the beginning of locomotor activity scoring. A later time point of 3 h was also selected in order to gain some insight into the extent of drug binding over time. Three control rats received i.c.v. saline injections and were killed after 20 mins ($n=2$) and 3 h ($n=1$). Brains were removed and frozen in ice-cold (−42°C) isopentane and stored at −70°C for 2 days before being sectioned for autoradiography.

Fifteen μm brain sections were cut and collected at five levels through the brain using a cryostat (Bright Ltd) maintained at −20°C. These levels were (i) the nucleus accumbens, (ii) the lateral ventricles (injection sites), (iii) anterior hippocampus, (iv) posterior hippocampus and (v) inferior colliculus. Sections were thaw mounted onto 1% gelatin coated slides at −20°C and were allowed to dry for 2 h at room temperature before being washed. The sections were washed according to the protocol of Fuchs & Hoppens (1987), followed by a 5 min wash in distilled water to remove contaminating salts. Sections were then drained and dried in a stream of cool air and exposed to Hyperfilm (Amersham) in a light tight cassette for 10 days. The films were then developed according to the manufacturer's instructions.

Locomotor activity

Locomotor activity was measured using a wooden activity box. The floor of the box (40 × 40 cm) was divided into nine equal areas and was enclosed by a 25 cm wooden wall. Rats were placed in the centre of the box and their locomotor activity measured for 15 min, 15 min after the injection of nicotine, by recording the number of times the rat moved between squares. The number of rears was also recorded. The behaviour was monitored using a video camera, thus allowing an observer to monitor behaviour in an adjacent room.

The acquisition of locomotor sensitization was established using doses of nicotine previously employed by Reavill *et al*. (1990) and Benwell & Balfour (1992). Two experiments were conducted. In the first experiment ($n=16$ rats) the ability of the non selective antagonist mecamylamine to block nicotine-induced changes in locomotor activity was examined. In the second experiment ($n=20$ rats) the ability of the selective α7 antagonist α-bgt to modify nicotine behaviours was examined. In each experiment, animals were allocated to one of two pre-treatment groups. One group received injections of saline (0.9% s.c.) and the other injections of nicotine (0.4 mg kg^{-1} s.c.) daily for 14 days. The former are subsequently referred to as the chronic-saline group and the latter as the chronic-nicotine group. Each day, 15 min after the injection, animals were placed in the activity box and their locomotor activity recorded for 15 min. Following the assessment of locomotor sensitization to a challenge dose of nicotine (0.4 mg kg^{-1} s.c.)

the antagonist studies were performed. In experiment one, involving the reversible antagonist drug mecamylamine, the experiments were carried out over two test days according to a crossover counterbalanced design. Thus half the rats in each group received mecamylamine (1 mg kg^{-1} s.c.) 15 mins prior to their nicotine or saline treatment and the remainder received saline pretreatment on test day one. Fifteen minutes after the last injection, locomotor activity was recorded for 15 min. This injection schedule was reversed on the next test day, 2 days later.

In experiment two, rats underwent intracerebroventricular surgery for bilateral cannulae placement 15 days after their chronic treatment regime. Chronic nicotine or saline treatment was resumed in the rats on day 19. Each day the animals were exposed to the activity box for 15 min. On day 23 the bungarotoxin antagonist test commenced. A crossover-counterbalance design was not used in these experiments since α-bgt binds covalently and may have not cleared the brain completely within 48 h. Thus in this experiment all rats received i.c.v. saline (10 μl) on test day 1 and i.c.v. α-bgt (0.02 nmoles) on test day 2. Intercerebroventricular treatments of saline and α-bgt were administered 5 min prior to systemic nicotine or saline injections. Therefore the locomotor activity measurements began 20 min after the injection of α-bgt. The locomotor activity of these animals was then recorded as before.

Analysis of data

The results obtained from the experiments studying the chronic effects of treatments on LMA and rears over time were analysed using two-way analysis of variance with repeated measures. Counterbalance designed experiments were analysed using two-way analysis of variance (ANOVA) with repeated measures followed by a one-way ANOVA if a significant effect was found. Acceptable statistical significance was established as $P<0.05$.

Results

Dose response assessment of α-bgt in drug naïve rats

The aim of these experiments was to determine a subconvulsive dose of α-bgt that could be used in subsequent experiments. In drug naive rats i.c.v. α-bgt administration induced convulsions in all rats tested in the dose range 1.0–8.0 nmoles. At the largest dose of α-bgt (8 nmoles) administered the mean time to convulsion was 5.7 ± 1.9 min. Convulsions did not occur in rats receiving doses in the range 0.02–0.20 nmoles α-bgt with the exception of one of the four rats administered 0.10 nmole α-bgt which experienced convulsions 45 min after α-bgt. Doses of α-bgt ranging from 0.10–8 nmoles induced ataxia during the 20 min scoring period immediately after i.c.v. injection of the toxin. At the smallest dose of α-bgt tested (0.02 nmoles) there was a non significant decrease in locomotor activity ($P>0.05$) and a significant reduction in rears ($P<0.05$)(Table 1). Based upon these results a dose of 0.02 nmoles of α-bgt was selected for the subsequent locomotor experiments.

Analysis of [^{125}I]-α-bgt binding in rat brain in vivo

In order to determine whether the dose of α-bgt selected had penetrated into brain tissue, receptor autoradiography was performed using [^{125}I]-α-bgt. The distribution pattern of [^{125}I]-α-bgt binding after an i.c.v. injection of α-bgt (0.02 nmoles) is shown in a representative autoradiogram (Figure 1). Thus binding was clearly present in the region of the cingulate cortex, hippocampus and hypothalamus. There was a small amount of binding in the central periaqueductal gray and the superior colliculus, whereas binding was not visible above background in the nucleus accumbens. The toxin binding distribution pattern was similar 3 h after α-bgt but appeared qualitatively more intense than that after 20 min.

Locomotor stimulant effects of nicotine: antagonism by mecamylamine

Rats chronically treated with nicotine (0.4 mg kg^{-1}) and exposed to the test apparatus daily acquired an increase in locomotor activity significantly different to that of saline control rats ($F_{(5,70)}=5.9$, $P<0.0001$). Figure 2 represents the effects of chronic saline and chronic nicotine treatment over this 13 day period. The nicotine-induced increase in locomotor activity became significant from day 3 ($F_{(1,14)}=8.52$, $P<0.01$) and appeared to peak at day 7. Rearing was also increased in the rats which had been chronically treated with nicotine compared to the saline control group ($F_{(1,14)}=14.2$, $P<0.01$). This effect was significant from chronic treatment day 5 ($F_{(1,14)}=10.80$ $P<0.01$) but did not increase further over subsequent treatment days (Figure 2).

Following 2 weeks of chronic treatment, the rats were challenged with nicotine and saline treatments in a crossover-counterbalanced design experiment and their locomotor activity was scored for 15 min. This experiment was carried out over 2 days (days 15 and 17). As expected, nicotine treatment increased locomotor activity in rats chronically treated with nicotine. ($F_{(1,14)}=7.50$, $P<0.05$). Analysis of the

Table 1 The effect of i.c.v α-bungarotoxin upon locomotor activity and rearing behaviour

Treatment (i.c.v.)	*Number of line crosses*	*Number of rears*
Saline	100 ± 18	26 ± 4
α-bgt (0.02 nmoles)	56 ± 19	2 ± 1*

Measurements are represented as the mean ± s.e.mean number of line crosses and rears made over a 15 min period ($n=5$ per group). Data was analysed using an unpaired *t*-test (*$P<0.05$ compared to saline control group).

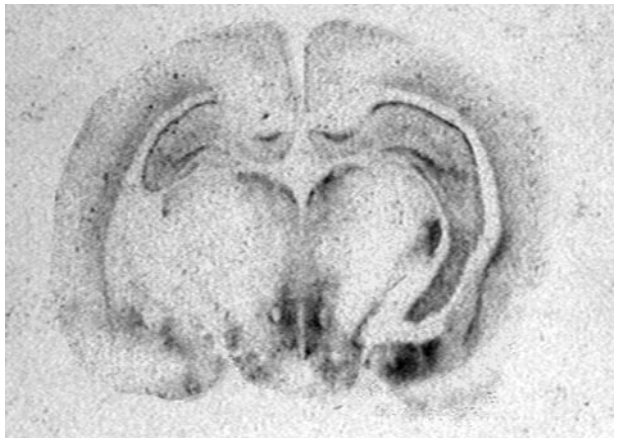

Figure 1 Representative autoradiogram at the level of the hippocampus showing the distribution of [^{125}I]-α-bungarotoxin binding following an i.c.v. injection of α-bungarotoxin (0.02 n moles).

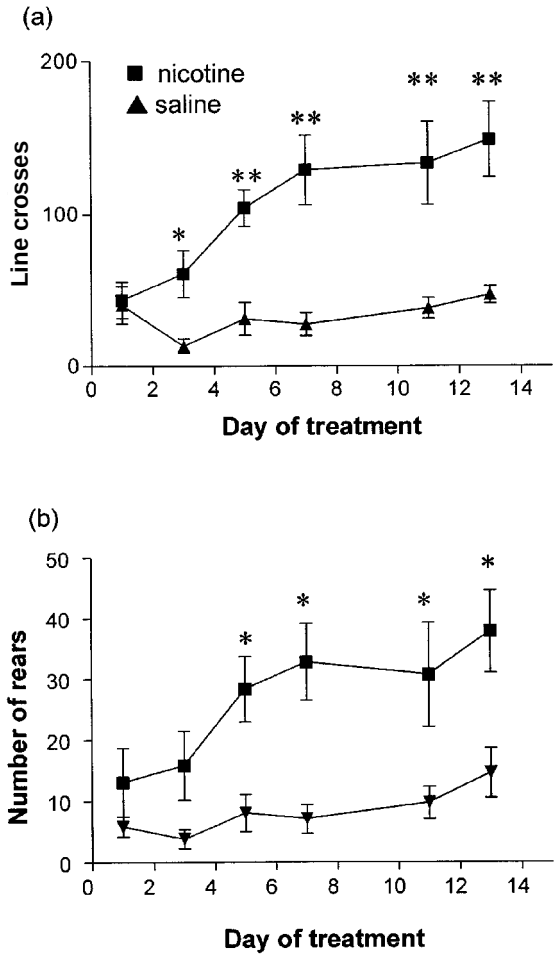

Figure 2 Acquisition of nicotine-induced locomotor sensitization and enhanced rearing. Time course effect of chronic saline and chronic nicotine treatment (0.4 mg kg^{-1}) on the locomotor activity (a) and rears (b) of rats in experiment 1. Results shown are the mean ± s.e.mean number of line crosses and rears made by eight rats in a 15 min period. Locomotor activity and rearing was significantly increased from day 3 and 5 respectively in the rats treated with chronic nicotine (*$P<0.05$, **$P<0.01$ compared to chronic saline group). Data was analysed using two-way ANOVA with repeated measures. Further individual comparisons were made using a one-way ANOVA.

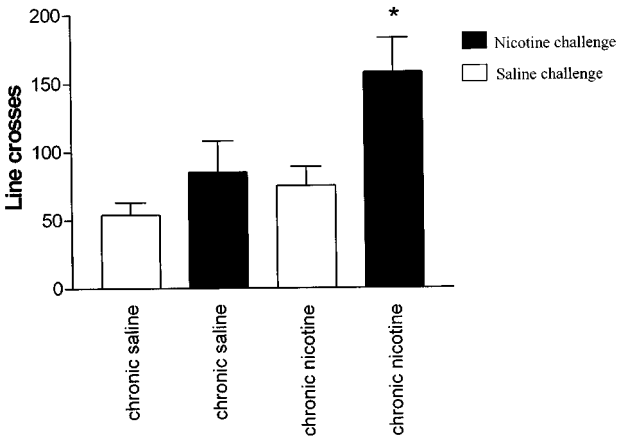

Figure 3 The influence of a nicotine challenge (0.4 mg kg^{-1}) upon the locomotor activity of rats that had previously received chronic nicotine (0.4 mg kg^{-1}) or chronic saline treatment. Data are represented as mean ± s.e.mean ($n=8$ per group). *$P<0.01$ versus saline/saline control.

effect of test day treatments on the chronic-nicotine group revealed that nicotine treatment increased line crosses in the rats chronically treated with nicotine ($F_{(1,7)}=7.26$ $P<0.05$) as compared to the group that received nicotine after chronic saline. Saline treatment did not increase locomotor activity in these rats ($F_{(1,7)}=1.01$, $P>0.05$) (Figure 3). There were also no significant effects upon rears.

Subsequent to the acquisition of the locomotor stimulant effects of nicotine, rats were administered mecamylamine in a crossover-counterbalanced design. Two-way ANOVA showed that there was a significant effect of mecamylamine on LMA ($F_{(1,14)}=25.7$, $P<0.01$). Mecamylamine (1 mg kg^{-1}) completely blocked the expression of locomotor sensitization in the chronic-nicotine group (Table 2). This dose of mecamylamine also modestly affected locomotor activity in the chronic-saline rats as compared to the same rats administered a saline pretreatment

Two-way ANOVA also revealed that rearing was affected by mecamylamine ($F_{(1,14)}=8.53$, $P<0.01$). Mecamylamine reduced rearing in the chronic nicotine group but not the chronic-saline group (Table 2).

Locomotor stimulant effects of nicotine: influence of α-bungarotoxin

In accordance with the acquisition data from experiment one, rats chronically treated with nicotine acquired a significant increase in locomotor activity ($F_{(1,18)}=11.6$, $P<0.01$) within three days of the chronic treatment. Animals chronically treated with nicotine also acquired an increase in the mean number of rears which became significant after day 5 ($F_{(1,18)}=6.54$, $P<0.05$). As expected, animals treated repeatedly with saline did not show any changes in locomotor activity and rears with time. Similarly, a nicotine challenge injection significantly increased the number of cage crosses and rears (data not shown).

The i.c.v. administration of α-bgt (0.02 nmoles) 5 min before saline or nicotine treatment did not affect nicotine-induced increases in locomotor activity (Table 3). Thus, two-way ANOVA revealed that there was no interaction between the administration of nicotine or saline and the test day treatments of α-bgt or saline ($F_{(1,13)}=0.42$, $P>0.05$). As well as having no effect on cage crosses, the α-bgt had no significant effect on rearing behaviour in either the rats chronically treated with saline or those chronically treated with nicotine (Table 3).

Table 2 Reversal of the expression of nicotine-induced locomotor senstizitation by mecamylamine

Chronic treatment	*Test day treatment*	*Number of line crosses*	*Number of rears*
Saline	saline/saline	10 ± 2	5 ± 2
Saline	mecamylamine/saline	38 ± 10*	4 ± 2
Nicotine	saline/nicotine	134 ± 26#	28 ± 7
Nicotine	mecamylamine/nicotine	24 ± 9**	4 ± 1*

Locomotor activity was measured over a 15 min period in rats that received mecamylamine (1 mg kg^{-1}) or saline following chronic treatment with saline or nicotine (0.4 mg kg^{-1}) ($n=8$ per group). Measurements are represented as the mean ± s.e.mean number of a line crosses and rears. Data was analysed using a two-way ANOVA and further individual comparisons were made using a one-way ANOVA (*$P<0.05$ or **$P<0.01$ compared to the respective saline pretreated control; #$P<0.05$ versus saline/saline control).

Table 3 Inhability of α-bungarotoxin to reverse the expression of nicotine-induced locomotor senstizitation

Chronic treatment	*Test day treatment*	*Number of line crosses*	*Number of rears*
Saline	Saline/saline	46±8	8±3
Saline	α-bgt/saline	40±12	4±1
Nicotine	Saline/nicotine	186±38*	26±7
Nicotine	α-bgt/nicotine	165±27*	13±6

Locomotor activity was measured over a 15 min period after administration of i.c.v. α-bgt (0.02 nmoles) or saline in rats chronically treated with saline ($n=7$) or nicotine (0.4 mg kg^{-1}, $n=8$) and repeatedly exposed to the test apparatus. Measurements are represented as the mean± s.e.mean number of line crosses and rears. Data was analysed using a two-way ANOVA and further individual comparisons were made using a one-way ANOVA *$P<0.05$ saline/saline control).

Discussion

The main aim of the present study was to evaluate a potential role of the α7 nicotinic receptor subtype in nicotine-induced locomotor sensitization. The finding that mecamylamine but not α-bgt blocks the expression of this behavioural response supports the conclusion that nicotinic receptors of the α4β2 subtype rather than the α7 subtype are important in this effect. However, since mecamylamine is a non-selective antagonist other receptor subtypes e.g. α3β4 cannot be ruled out at this stage.

Alpha-bungarotoxin was selected as the α7 receptor antagonist ligand over methyllcaconitine (MLA) in the present study for a number of reasons. Firstly, it displays greater specificity for the α7 subunit as compared to MLA which shows some affinity, albeit low, for the α3β4 and α4β2 subunits (Drasdo *et al*., 1992). Secondly, as both drugs do not readily penetrate the CNS they have to be administered i.c.v. and so it is difficult to estimate the exact concentrations that reach the brain. In light of this it could be predicted that MLA might reach the brain in concentrations that would act on other receptor subtypes. Finally, it has been demonstrated that MLA binds to an additional unidentified site (Yum *et al*., 1996).

The lack of effect of α-bgt on nicotine-induced locomotor sensitization cannot be attributed to a lack of penetration into the brain. Thus when the same concentration employed in the behavioural studies (0.02 nmoles i.c.v.) was administered in the form of [^{125}I]-α-bgt, a clear binding distribution pattern was evident throughout the brain (Figure 1). This is the first demonstration of the distribution of [^{125}I]-α-bgt binding after i.c.v. administration. The pattern of binding showed qualitative similarities with results from *in vitro* autoradiography and immunocytochemical localization studies. Thus high levels of binding were present in the hippocampus and the superior colliculus (Clarke *et al*., 1985; Del Toro *et al*., 1994).

Whilst the dose of α-bgt employed in the present study (0.02 nmoles i.c.v.) did show an effect on rearing behaviour (but not line crosses) in the initial dose response studies, this change in rears is unlikely to have influenced the pattern of results obtained for chronic nicotine-induced changes in locomotor activity. In the behaviour antagonism studies there were in fact no differences in rears or line crosses when the animals treated with chronic saline received α-bgt as compared to when they received saline (see Table 3). Similarly, α-bgt did not influence rearing behaviour in rats treated with chronic nicotine. It is likely that the α-bgt induced change in rearing observed in the initial dose response studies were related to the fact that animals were naïve to the test apparatus when the behavioural measures were made. In contrast, in the locomotor sensitization studies the rats had been repeatedly exposed to the apparatus. Whatever the reason, it would appear that a dose of α-bgt that penetrates into the brain does not influence rearing behaviour in rats chronically treated with either saline or nicotine. Similarly the locomotor stimulant effects of nicotine, as measured by increases in forward locomotion (line crosses) were completely unaffected by α-bgt. Whilst we did not control for a treatment order effect in the α-bgt experiment the fact that the chronic nicotine controls induced similar degrees of locomotor stimulation in the mecamylamine and the α-bgt experiments would argue against this. Overall, these data therefore suggest a lack of involvement of the α7 receptor subtype in the expression of nicotine-induced locomotor sensitization.

An alternative explanation for the lack of effect of α-bgt on nicotine-induced locomotor sensitization is that the drug is not penetrating into a brain region that could modify mesoaccumbens dopamine transmission.

It was argued in the introduction that modification of accumbens dopamine transmission could have potentially occurred through hippocampal α7 receptors modifying the activity of glutamatergic inputs to the accumbens. Despite the demonstration of binding of i.c.v. administered [^{125}I]-α-bgt in the hippocampus, the present results do not support a role for a hippocampal α7 type receptor in the locomotor stimulant effects of nicotine. The potential role of this receptor in other brain regions therefore cannot be excluded. For example, recent studies have implicated α7 nicotinic receptors in the ventral tegmental area in the nicotine withdrawal syndrome (Nomikos *et al*., 1999). However, in the present study, in accord with earlier *in vitro* studies (Clarke *et al*., 1985; Del Toro *et al*., 1994), α-bgt binding sites were not apparent in the region. Similarly binding in other potentially relevant regions such as the nucleus accumbens was absent in the present study.

Larger doses of α-bgt could not be tested because of their propensity to cause convulsions. In our initial dose response studies with α-bgt, doses of 1.0 nmole and above caused convulsions in all animals tested. These convulsions exhibited a dose dependent reduction in their latency of onset. Previous studies have also noted seizures following α-bgt administration to rodents. The seizures are thought to be mediated at the level of the CA3 field of the hippocampus where α-bgt sites are located on GABA-containing interneurones (Miner & Collins, 1989; Marks *et al*., 1989; McCormick *et al*., 1993; Freedman *et al*., 1993).

The finding that mecamlyamine blocks nicotine-induced locomotor sensitization concurs with previous results (Benwell *et al*., 1995). Previous studies have also shown that many of the psychopharmacological effects of nicotine including drug discrimination and enhanced mesolimbic dopamine secretion are blocked by mecamylamine but not the peripherally acting antagonist hexamethonium. (Benwell *et al*., 1995; Stolerman *et al*., 1983). Such studies generally use photobeam crossings to monitor locomotor activity which presumably measures both forward locomotion and rears. In the present study these parameters were measured separately and it was noted that sensitization was apparent in both forward locomotion (line crosses) and rears following chronic nicotine. However, the forward locomotion effects were more pronounced and robust than the enhanced rearing. The latter effect was not sufficiently robust to show a significant effect in the crossover challenge tests.

There are been relatively few studies which have aimed to dissociate the receptors involved in nicotine-induced locomo-

tor changes. Previous studies reported that tolerance to the depressant effect of chronic nicotine upon rotarod performance in mice was accompanied by widespread regional increases in [^{3}H]-nicotine binding whereas changes in [^{125}I]-α-bgt binding showed more limited changes (Marks *et al*., 1983). Subsequent studies showed that the development and loss of tolerance correlated more closely with changes in nicotine binding than changes in α-bgt binding (Marks *et al*., 1985) although this correlation was less clear in rats (Collins *et al*., 1988). Assuming that tolerance to the depressant effects of nicotine and nicotine-induced locomotor sensitization involve similar nicotinic receptors, then the present results of a lack of participation of α7 containing nicotinic receptors in nicotine-induced sensitization are consistent with the findings of Marks and colleagues. In addition, the discriminative stimulus properties of nicotine also does not appear to involve alpha 7 containing receptors (Brioni *et al*., 1996).

In conclusion, the present data suggest that α7 containing nicotinic receptors (particularly those in the hippocampus) are unlikely to participate in nicotine-induced locomotor sensitization. Since this sensitized response appears to involve the mesolimbic dopamine system it is unlikely that α7 receptors are targets for the treatment of nicotine-induced drug craving. Furthermore, the data may suggest that α7 drugs being developed for disorders such as schizophrenia (see Wonnacott & Marks 1999) may lack abuse liability.

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