Behavioural Brain Research 197 (2009) 323–330

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Differential effects of 5-HT$_{2C}$ receptor activation by WAY 161503 on nicotine-induced place conditioning and locomotor activity in rats

Dave J. Hayes[a,*], Tera M. Mosher[a], Andrew J. Greenshaw[b,1]

[a] Centre for Neuroscience, 513 HMRC, University of Alberta, Edmonton, AB T6G 2S2, Canada
[b] Centre for Neuroscience and W.G. Dewhurst Laboratory, Department of Psychiatry, 1E7.44 WMHSC, University of Alberta, Edmonton, AB T6G 2R7, Canada

ARTICLE INFO

Article history:
Received 3 August 2008
Received in revised form 21 August 2008
Accepted 26 August 2008
Available online 2 September 2008

Keywords:
5-HT$_{2C}$ receptor
WAY 161503
SB 242084
Nicotine
Place conditioning
Locomotor activity
Serotonin
Mesolimbic

ABSTRACT

Rationale: Numerous studies indicate a role for both the serotonin 2C receptor (5-HT$_{2C}$) and the nicotinic acetylcholine receptor in locomotion, reinforcement and motivated behaviours. Nicotine, a potent nicotinic acetylcholine receptor agonist, interacts with the dopaminergic and serotonergic systems and is known to positively affect reward-related behaviours.
Objectives: The current study examined the effects of 5-HT$_{2C}$ receptor activation on nicotine-induced (0.6 mg/kg) place conditioning and spontaneous locomotion.
Methods: Using Sprague–Dawley rats, the effects of the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (0–1.0 mg/kg) and the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg) alone, in combination, and on nicotine-induced (0.6 mg/kg) spontaneous locomotor activity were assessed. The effects of WAY 161503 (1.0, 3.0 mg/kg) were also investigated in nicotine-induced place conditioning using a two-compartment biased design; amphetamine (1.0 mg/kg) served as a positive control. As differential effects were observed between place conditioning and locomotor activity, the subjects used in the place conditioning experiments were also tested for effects on locomotor activity.
Results: WAY 161503 decreased baseline and nicotine-induced locomotor activity at the highest dose tested (1.0 mg/kg) and these effects were attenuated by SB 242084. Amphetamine and nicotine both induced robust place preferences and WAY 161503 did not have any effects in the context of place conditioning. In contrast, WAY 161503 (1.0 mg/kg) blocked nicotine-induced locomotor activity.
Conclusions: These results suggest that 5-HT$_{2C}$ receptors may play an inhibitory role in nicotine-induced locomotor activity, but do not appear to influence place conditioning under the current conditions.

1. Introduction

The mesocorticolimbic dopamine system plays an important role in mediating motivated and reward-related behaviours [1,2] and the reinforcing properties of many drugs of abuse [3–5]. Although the precise role of dopaminergic cells has not been established, they may carry reward-related signals involved in reward valuation, prediction, incentive salience and conditioning [6–11]. The midbrain raphe serotonergic system shows extensive connectivity with dopamine-containing areas suggesting a role of serotonin (5-HT) in the control of these cells [12–14]. There is evidence for many distinct structural and pharmacological subtypes of 5-HT receptors with subtype-dependent excitatory or inhibitory effects [15]. Serotonin 2C (5-HT$_{2C}$) receptor activation may inhibit the release of mesolimbic dopamine [16,17].

Nicotine, a potent nicotinic acetylcholine receptor agonist, may facilitate dopamine-related behaviours including drug self-administration, place conditioning, intracranial self-stimulation and locomotion [18–20]. Many studies have demonstrated nicotinic–serotonergic interactions, generally finding increased 5-HT release following nicotinic receptor stimulation [21]. In particular, 5-HT$_{2C}$ receptor activation may attenuate nicotine-induced mesolimbic dopamine release [22,23]. Given the evidence for functional relationships between 5-HT$_{2C}$ receptors and the cholinergic and dopaminergic systems in the context of reward-related behaviours, it is possible that the 5-HT$_{2C}$ receptor may play a role in nicotine-mediated behaviours—an idea that has been previously suggested from studies involving locomotion and drug self-administration [24–26].

Few studies have investigated the reinforcing effects of 5-HT$_{2C}$ receptor ligands in place conditioning. The mixed 5-HT$_{1B/2C}$ receptor agonist mCPP did not induce place conditioning on its own,

* Corresponding author. Tel.: +1 780 492 6550; fax: +1 780 492 6841.
E-mail addresses: dave.hayes@ualberta.ca (D.J. Hayes), andy.greenshaw@ualberta.ca (A.J. Greenshaw).
[1] Neurochemical Research Unit, Department of Psychiatry, 1E7.44 WMHSC Centre, University of Alberta, Edmonton, AB T6G 2R7, Canada. Tel.: +1 780 492 6550; fax: +1 780 492 6841.

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.08.034

but was able to block the conditioned place aversion induced by mianserin (a mixed 5-HT_2 antagonist) and eltoprazine (a mixed 5-HT_{1B} receptor agonist and 5-HT_{2C} receptor antagonist) [27]. Recently, Mosher et al. (2005) showed that systemic administration of the mixed 5-$HT_{1A/1B/2C}$ receptor agonist TFMPP and the selective 5-HT_{2C} receptor agonist WAY 161503 did not induce place conditioning. Nevertheless, in a separate study, WAY 161503 did produce a state-dependent place aversion [28]. Under these conditions, both compounds reduced spontaneous locomotor activity [29]; these findings are in agreement with previous locomotion studies demonstrating an inhibitory role for the 5-HT_{2C} receptor [30–35].

A recent paper has suggested that nicotine-induced place conditioning could be inhibited through the indirect activation of the 5-HT_{2C} receptor [36]. Though there is still some debate [37], there is evidence that nicotine's rewarding effects may be related to direct activation of dopamine cells in the ventral tegmental area (VTA) as well as its desensitization effects on GABA cells [38–42]. Dopaminergic cell lesions within the mesolimbic system and dopamine antagonists both attenuate nicotine-induced locomotor activity and self-administration [43–46]. Though many authors have reported conflicting results regarding the peripheral administration of nicotine, a recent review of the literature and subsequent systematic study has clearly demonstrated that nicotine may induce robust place preference conditioning, over a range of doses, under well-defined parameters [47].

The present study investigated the role of the 5-HT_{2C} receptor in nicotine-induced place conditioning and locomotor activity. Nicotine-induced (0.6 mg/kg) place conditioning was compared to the well-established effects of amphetamine [13,48]. In addition to place conditioning, nicotine was also used as a locomotor stimulant, as many studies have demonstrated that repeated exposure to nicotine produces locomotor sensitization [44,49,50] and at least one study has found that the 5-HT_{2C} receptor may be involved in attenuating nicotine-induced locomotion in rats [26]. Given that 5-HT_{2C} receptor activation has been shown to attenuate nicotine-induced mesolimbic dopamine release, and both spontaneous locomotor activity and place conditioning are affected by mesolimbic dopamine transmission, the authors hypothesized that 5-HT_{2C} receptor activation by WAY 161503 would attenuate the behavioural effects of nicotine.

2. Materials and methods

2.1. Subjects

One hundred and thirty four male Sprague–Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200–250 g were housed individually in standard Plexiglas laboratory cages at 20 °C and 50% humidity, with a 12-h light/dark cycle (lights from 07:00 h to 19:00 h) with food and water freely available. All testing took place under red light during the light phase of the light/dark cycle. All apparatus were cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

2.2. Drugs

The 5-HT_{2C} receptor agonist WAY 161503·HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one hydrochloride] was purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) α-Methylphenethylamine (amphetamine) sulphate was purchased from Health and Welfare Canada. (−)-Nicotine hydrogen tartrate salt (nicotine) and the 5-HT_{2C} receptor antagonist SB 242084·HCl [6-chloro-5-methyl-1-[[2-(2methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] were purchased from Sigma–Aldrich (Oakville, Ontario, Canada). Nicotine was dissolved in saline; all other compounds were dissolved in double-distilled water (ddH_2O). SB 242084 was injected intraperitoneally (i.p.) and all other drugs were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg. Nicotine was given immediately before testing; amphetamine and WAY 161503 were both given 10 min before testing; SB 242084 was given 30 min before testing. All drug doses are expressed as free-base.

2.3. Place conditioning

The place conditioning apparatus (I. Halvorsen System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two compartments (30 cm L × 30 cm W × 25 cm H). The compartments differed only in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

The procedure consisted of three phases. *Phase 1* (pre-conditioning): animals were habituated to the place conditioning apparatus for three consecutive days, during which animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded. Animals were assigned to drug groups such that each animal was conditioned to the compartment in which it spent the least time, as determined on pre-conditioning day three (biased design). *Phase 2* (conditioning): on alternate days, animals received drug and vehicle treatments and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals were conditioned for eight consecutive days during which they received four drug treatments. *Phase 3* (post-conditioning): during retention testing, animals were placed in the apparatus in a drug-free state and allowed free access to both compartments for 15 min. The amount of time spent in each compartment was recorded. Each dose of WAY 161503 (1.0, 3.0 mg/kg) was tested in a separate experiment to allow for a replication of the nicotine-induced place preference.

2.4. Spontaneous locomotor activity

2.4.1. Apparatus

Spontaneous locomotor activity was measured using computer-monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L × 43 cm W × 30 cm H) with a 12 × 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical activity was measured using 12 additional photobeams located 12 cm above the floor.

2.4.2. Procedure

For the initial locomotor experiments, animals were habituated to the locomotor activity boxes for 14 days, during which, they were injected daily with nicotine (0.6 mg/kg; to establish behavioural sensitization) or saline vehicle. Following the 14-day sensitization period, the animals received randomized counterbalanced injections with the compound of interest. Three days were allowed between each treatment; during these days, animals continued to receive respective nicotine or vehicle injections and locomotor activity was measured. Locomotor activity was measured over a 60 min time course.

To ensure that the differential effects seen with WAY 161503 on nicotine-related locomotor activity and place conditioning behaviour were not due to subject or design variability, animals from the completed place conditioning experiments described above were randomly assigned to one of four treatment groups: vehicle + vehicle; vehicle + nicotine; WAY 161503 + vehicle; WAY 161503 + nicotine. The dose of the nicotine challenge was 0.6 mg/kg, while the WAY 161503 dose was 1.0 mg/kg. Only animals with prior nicotine exposure (i.e. sensitized to nicotine) in place conditioning were assigned to the locomotor groups containing nicotine. Locomotor activity was monitored over 30 min in order to explore the time course of drug effects.

2.5. Statistical analysis

Paired samples *t*-tests were used to analyze place conditioning effects, comparing time spent in the drug-paired side on pre- vs. post-conditioning days; to compare initial preferences for the bar vs. grate compartments for all animals ($p \leq 0.05$). Experimental effects on the initial spontaneous locomotor activity experiments were determined using three-way (WAY 161503 × time × group) or four-way (agonist × antagonist × time × group) repeated measures analysis of variance (ANOVA) with drug treatment group – defined as animals who have received nicotine injections vs. those who have not – as a between subjects factor. For the single-day locomotor activity experiment, following place conditioning, a three-way (nicotine × WAY 161503 × time) ANOVA with repeated measures on one factor (time) was conducted. Where appropriate, analysis of time course data was performed using one-way ANOVA across treatments for each 5 min time interval. A significant *F* ratio ($p \leq 0.05$) on a 5 min interval was followed by Newman–Keuls post hoc tests ($\alpha = 0.05$). As the results of the analyses of consecutive and vertical activity paralleled those for horizontal locomotor activity counts, only the latter results are reported. For experiments involving repeated measures, Greenhouse–Geisser corrected degrees of freedom are used as a conservative estimate of the *F*-ratio. All statistical analyses were completed using SPSS statistical software (SPSS 14.0, SPSS Inc., Chicago, U.S.A.).

3. Results

3.1. Effects of systemic WAY 161503 on basal and nicotine-induced locomotor activity

WAY 161503 (1.0 mg/kg) significantly decreased spontaneous locomotor activity as well as nicotine-induced hyperactivity [Fig. 1A, $F(1.53, 21.35) = 39.73$, $p < 0.05$]. There were significant effects of time [$F(5.51, 77.08) = 50.01$, $p < 0.05$] and group [$F(1, 14) = 50.59$, $p < 0.05$]; interactions of WAY 161503 × group [$F(1.53, 21.35) = 25.23$, $p < 0.05$], time × group [$F(5.51, 77.08) = 15.57$, $p < 0.05$] and WAY 161503 × time [$F(5.54, 77.58) = 4.97$, $p < 0.05$]. Local time course analysis revealed that WAY 161503 (1.0 mg/kg) reduced locomotor activity in vehicle-treated animals during the first 10 min of testing (Fig. 1B) and during the first 30 min, and again at 40 and 45 min, of testing for the nicotine-sensitized animals (Fig. 1C).

3.2. WAY 161503 and SB 242084 on basal and nicotine-induced locomotor activity

WAY 161503 (1.0 mg/kg) significantly decreased locomotor activity in vehicle- and nicotine-treated animals [Fig. 2A, $F(1, 14) = 39.93$, $p < 0.05$]. There was an effect of SB 242084 (1.0 mg/kg) [$F(1, 14) = 93.31$, $p < 0.05$], time [$F(4.05, 56.68) = 35.43$, $p < 0.05$] and group [$F(1, 14) = 80.98$, $p < 0.05$]; interactions of WAY 161503 × SB 242084 [$F(1, 14) = 19.13$, $p < 0.05$], WAY 161503 × group [$F(1, 14) = 36.23$, $p < 0.05$], SB 242084 × time [$F(1, 14) = 33.31$, $p < 0.05$], time × group [$F(4.05, 56.68) = 7.17$, $p < 0.05$], WAY 161503 × time [$F(4.77, 66.75) = 8.51$, $p < 0.05$], SB 242084 × time [$F(5.56, 77.77) = 2.16$, $p < 0.05$] and WAY 161503 × SB 242084 × time [$F(4.45, 62.28) = 2.57$, $p < 0.05$] (Fig. 2A). Local time course analysis revealed that WAY 161503 decreased locomotor activity in vehicle-treated animals during the first 15 min of testing (Fig. 2B) and during the first 35 min, and again at 45 and 55 min, of testing for nicotine-treated animals (Fig. 2C). SB 242084 (1.0 mg/kg) attenuated the reduction in locomotor activity seen with vehicle-treated animals (Fig. 2B; with the exception of the first 5 min) and nicotine-treated animals (Fig. 2C; with the exception of the 15, 20 and 30 min test points). SB 242084 did not significantly affect locomotor activity when administered alone.

3.3. Verification of nicotine-induced place conditioning

Using a biased place conditioning design, both 0.6 mg/kg nicotine [$t(7) = 4.47$, $p < 0.05$] and 1.0 mg/kg amphetamine [$t(7) = 4.54$, $p < 0.05$] produced conditioned place preferences, indicating a significant difference in time spent in the conditioned compartment on post- over pre-conditioning days (Fig. 3). Though each animal was conditioned to the compartment in which it spent the least time on pre-conditioning day 3 (i.e. biased design), as a group, animals showed no initial preference for either compartment (which differed only in bar vs. grate flooring) [395 ± 14 s; 391 ± 13 s; $t(89) = 0.15$, $p > 0.05$].

3.4. WAY 161503 and nicotine in place conditioning

Nicotine (0.6 mg/kg) induced a place preference [Fig. 4A, $t(11) = 2.36$, $p < 0.05$; Fig. 4B, $t(9) = 3.36$, $p < 0.05$]; WAY 161503 (1.0, 3.0 mg/kg) did not induce place conditioning [Fig. 4A, $t(11) = 1.77$, $p > 0.05$; Fig. 4B, $t(9) = 2.13$, $p > 0.05$] and did not influence nicotine-induced place conditioning [Fig. 4A, $t(11) = 4.73$, $p < 0.05$; Fig. 4B, $t(9) = 4.59$, $p < 0.05$].

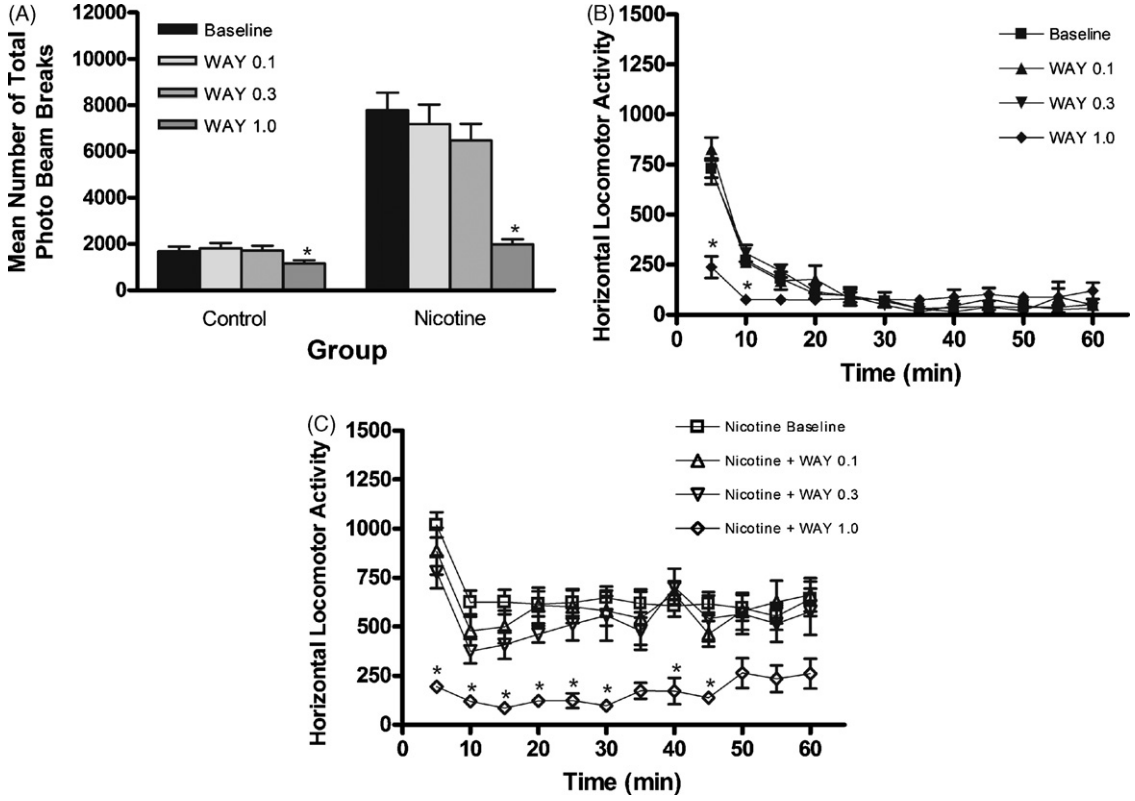

Fig. 1. (A) Locomotor effects of WAY 161503 (WAY; 0–1.0 mg/kg) in vehicle- and nicotine-treated (0.6 mg/kg) rats ($n = 16$). Time course activity over 60 min in vehicle-treated (B) and nicotine-treated (C) rats. The term 'baseline' refers to recorded activity measured in vehicle-treated animals in the control group and nicotine-treated animals in the nicotine group. Data shown are means ± S.E.M. *Significant at $p < 0.05$ following Newman–Keuls post hoc tests.

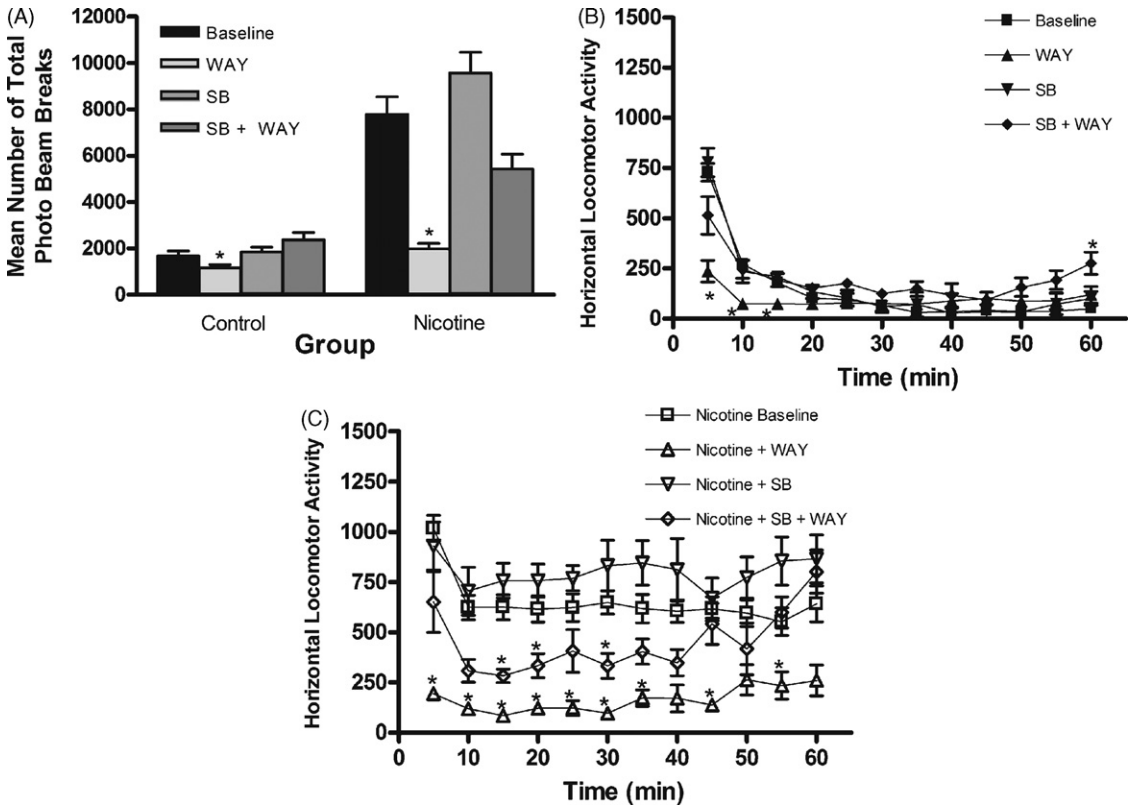

Fig. 2. (A) Locomotor effects of WAY 161503 (WAY; 1.0 mg/kg) and SB 242084 (1.0 mg/kg) alone and in combination in vehicle- and nicotine-treated (0.6 mg/kg) rats ($n = 16$). Time course activity over 60 min in vehicle-treated (B) and nicotine-treated (C) rats. The term 'baseline' refers to recorded activity measured in vehicle-treated animals in the control group and nicotine-treated animals in the nicotine group. Data shown are means ± S.E.M. *Significant at $p < 0.05$ following Newman–Keuls post hoc tests.

3.5. WAY 161503 and nicotine in locomotor activity following place conditioning

Following the nicotine-induced place preference experiments above, the effects of WAY 161503 on spontaneous and nicotine-induced locomotor activity of those animals was examined. Testing took place over 30 min as the initial locomotor experiments indicated that the effects of WAY 161503 on basal locomotor activity occur within this time period. Three-way ANOVA revealed a significant interaction for nicotine (0.6 mg/kg) × WAY 161503 (1.0 mg/kg) × time [Fig. 5A, $F(21.12) = 6.43$, $p < 0.05$]. To investigate this interaction, one-way ANOVA was performed for each 5 min interval; following significant one-way ANOVA, Newman–Keuls post hoc tests ($\alpha = 0.05$) showed that 1.0 mg/kg WAY 161503 reduced locomotor activity during the first 20 min of testing, as did the nicotine and WAY 161503 combination. Nicotine significantly increased locomotor activity, over saline-treated animals, from 15 to 30 min of testing (Fig. 5B). Three-way ANOVA revealed an interaction for nicotine (0.6 mg/kg) × WAY 161503 (3.0 mg/kg) × time [Fig. 5C, $F(9.53) = 17.01$, $p < 0.05$]. Locomotor activity was reduced for the group receiving 3.0 mg/kg WAY 161503 during the first 10 to 25 min of testing and for the group receiving WAY 161503 and nicotine for the first 20 min. The group that received nicotine showed increased activity during the first 10 min of testing (Fig. 5D).

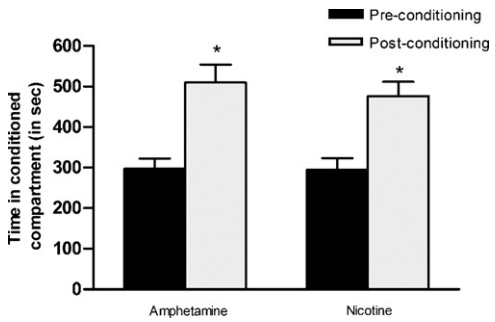

Fig. 3. Verification of the biased place conditioning design using (+) amphetamine (1.0 mg/kg) as a positive control compared to nicotine (0.6 mg/kg) ($n = 16$). Data shown are means ± S.E.M. *Significant at $p < 0.05$ following paired samples t-test.

4. Discussion

This study investigated the effects of 5-HT_{2C} receptor activation on spontaneous locomotor activity and nicotine-induced place conditioning. Consistent with prior data [26], 5-HT_{2C} receptor activation decreased locomotion when administered alone and in combination with nicotine (0.6 mg/kg; Fig. 1A–C) and these effects were attenuated by the selective 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg; Fig. 2A–C). These data further support an inhibitory role for the 5-HT_{2C} receptor in basal and nicotine-induced locomotor activity. A floor effect may be responsible for the fact that the selective 5-HT_{2C} receptor agonist WAY 161503 (1.0 mg/kg) only decreased basal locomotor activity in the first 10–15 min of testing (Figs. 1B and 2B), given that this same dose is effective in attenuating nicotine-induced activity over at least a 45 min time period (Figs. 1C and 2C). As all relevant drug effects are noted within the first 30 min, subsequent locomotor testing focused on this time period.

Nicotine (0.6 mg/kg) induced a conditioned place preference comparable to the well-established effects of amphetamine (Figs. 3 and 4A and B) [13,48]. These data are consistent with a

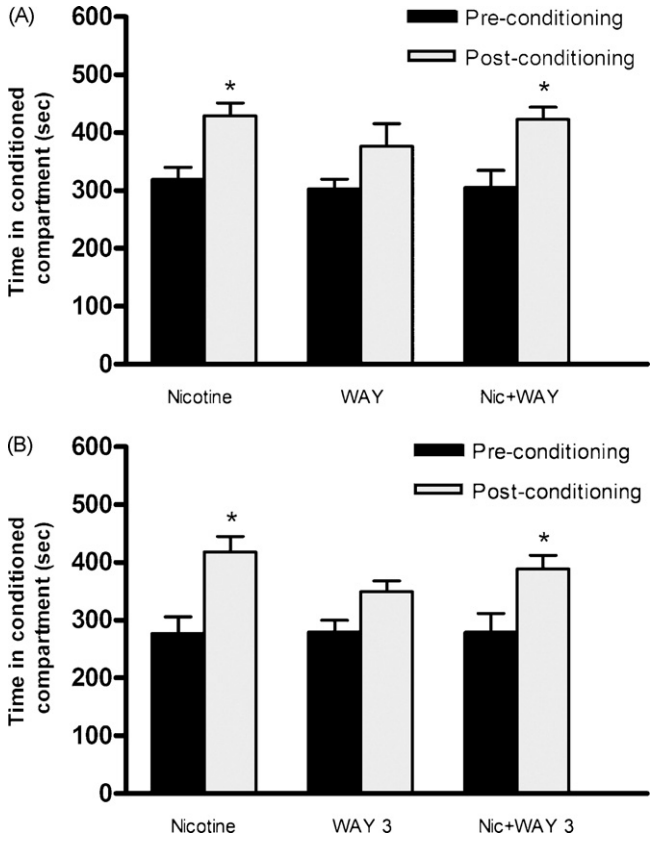

Fig. 4. Place conditioning effects of (A) nicotine (0.6 mg/kg) and WAY 161503 (WAY; 1.0 mg/kg) ($n = 24$); (B) nicotine (0.6 mg/kg) and WAY 161503 (WAY; 3.0 mg/kg) ($n = 30$). Data shown are means ± S.E.M. *Significant at $p < 0.05$ following paired samples t-test.

number of studies indicating that systemic nicotine-induced place preference is reliable over a range of doses, under well-defined parameters, and with the use of a biased place conditioning design (for review see Le Foll and Goldberg [47]). Some researchers have suggested that factors such as age, strain, timing and route of administration may contribute to the varying results seen across studies [47,51–53], as some groups have reported a place preference [52,54–56], place aversion [57,58] or absence of place conditioning [59–61] following systemic nicotine administration using an unbiased design. Nonetheless, the vast majority of studies demonstrating a nicotine-induced place preference used a biased design [47].

It is a possibility that the place preference induced by nicotine in the biased design is not a reward-related effect, but rather the result of a reduction of an aversive state related to the initially non-preferred compartment—suggesting a potentially anxiolytic effect of nicotine. This is a rather unlikely hypothesis as nicotine's effects on anxiety vary largely based on route and timing of administration, the behavioural model used, and subject variability [62] and a number of behavioural studies have demonstrated the affects of 5-HT_{2C} receptor compounds on anxiety, yet none of these compounds have been shown to induce place conditioning [30,32,63]. Another possible concern involves the definition of a biased design [64,65]. The term 'biased design' often incorrectly elicits the notion of a biased apparatus (e.g. rats naturally prefer darker compartments), though an apparatus which produces a general bias for one compartment over others may be a detriment to the investigation of reward-related effects [2,47,64,66]. The biased design (or 'biased compartment assignment') involves the assignment of individual animals to the compartment in which they initially spent the least, or most, amount of time. As a group there is no compartment preference; as such, the biased design may allow for greater sensitivity in detecting the reinforcing effects of drugs [64,65]. The apparatus used in the present study differed only in floor texture (bar vs. grate) and as a group, animals showed no preference for either side.

We are aware of one report that 5-HT_{2C} receptor activity may inhibit nicotine-induced place preference [36]. These authors pro-

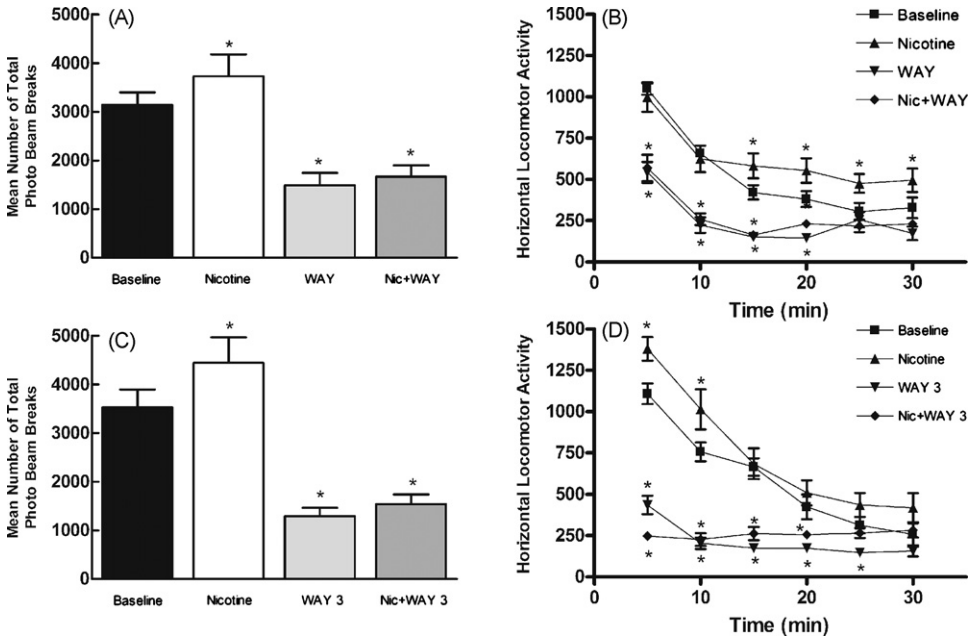

Fig. 5. Spontaneous locomotor activity, following place conditioning experiments, for (A) nicotine (Nic; 0.6 mg/kg) and WAY 161503 (WAY; 1.0 mg/kg) ($n = 10$/group); (C) nicotine (Nic; 0.6 mg/kg) and WAY 161503 (WAY 3; 3.0 mg/kg) ($n = 9$/group). Time course activity over 30 min for rats receiving (B) nicotine and WAY 161503 (1.0 mg/kg) (D) and nicotine and WAY 161503 (3.0 mg/kg). Data shown are means ± S.E.M. *Significant at $p < 0.05$ following Newman–Keuls post hoc tests.

posed that the tumor suppressor molecule, PTEN, complexes with the 5-HT_{2C} receptor and tonically inhibits its activity in vivo. The putative PTEN:5-HT_{2C} receptor complex was disrupted using a Tat-conjugated interfering peptide and it was suggested that this may mimic the effects of 5-HT_{2C} receptor activation. It is difficult to assess the effects of 5-HT_{2C} receptor activity on nicotine-induced place preference from this study because it is not clear to what extent the PTEN molecule affects 5-HT_{2C} receptor activity. Though the authors used a relatively selective 5-HT_{2C} receptor agonist (RO 600175) to attenuate tetrahydrocannibanol-induced place conditioning, they did not use the same compound to directly assess nicotine-induced place conditioning.

The present study is, to the author's knowledge, the first to investigate the effects of a specific 5-HT_{2C} receptor agonist (WAY 161503) on nicotine-induced place conditioning (Fig. 4A and B). No place conditioning was seen under the present conditions using systemic doses of WAY 161503 (1.0, 3.0 mg/kg) that are behaviourally active in locomotor activity [29]; this observation is consistent with previous results indicating that compounds with 5-HT_{2C} receptor activity do not induce place conditioning when testing occurs in a drug-free state [27,29]. Contrary to the initial hypothesis, WAY 161503 did not attenuate nicotine's ability to induce a place preference. This was unexpected because 5-HT_{2C} receptor activation attenuates nicotine-induced mesolimbic dopamine release [22,23], locomotor activity and self-administration [26]. While the current place conditioning results do not support the notion that 5-HT_{2C} receptor activation attenuates all nicotine-induced behaviours, it must be noted that testing in the current place conditioning study took place in a drug-free state, while the attenuation of nicotine's effects by 5-HT_{2C} receptor stimulation in locomotion and self-administration were drug-dependent. It is also important to note that the present study investigated the effects of a single dose of nicotine (0.6 mg/kg) that was above the previously reported threshold dose of 0.1 mg/kg for producing a CPP [47]. While it is possible that 5-HT_{2C} receptor stimulation may have attenuated the effects of a lower dose of nicotine, the fact that other nicotine-induced activities (using comparable or higher doses of nicotine) are attenuated by 5-HT_{2C} receptor stimulation remains [26].

Previous studies have demonstrated 5-HT_{2C} receptor-related decreases in both basal [29,30,33,35] and nicotine-induced [24–26] locomotor activity. Because of the differential effects observed between the place conditioning and locomotor activity experiments, the subjects used in the current place conditioning experiments were subsequently tested in the locomotor apparatus over 30 min (Fig. 5A–D). The results of these locomotor experiments were consistent with all previous demonstrations showing that nicotine-induced increases in locomotor activity are attenuated through 5-HT_{2C} receptor activation. Based on the current results, it is possible that this 5-HT_{2C} receptor agonist-induced effect may not be influenced by the duration of nicotine exposure (14 treatments in the initial locomotor studies vs. 4 treatments during the place conditioning studies; Fig. 1A–C and Fig. 5A–D, respectively). In addition, future studies should address the possibility that the effects of WAY 161503 on nicotine-induced locomotor activity may be non-specific, as subthreshold doses of WAY 161503 failed to attenuate nicotine-induced locomotion and 5-HT_{2C} receptor activity has been shown to affect both basal locomotion and drug-induced increases in locomotor activity [25,33,34,67,68].

While 5-HT_{2C} receptor activation may inhibit other dopamine-related behaviours such as cocaine and ethanol self-administration [69–71], nicotine-induced locomotion and self-administration [26], we are not aware of any other studies regarding 5-HT_{2C} receptor activation on nicotine-induced place conditioning. It is of interest that these results are consistent with reports suggesting dopamine-independence of the place preference-inducing effects of intra-ventral tegmental nicotine [37]. Indeed, nicotinic receptor-stimulated increases in nucleus accumbens dopamine levels and locomotor activity do not always correspond with the establishment of place preference conditioning [72,73] and some direct manipulations of dopamine signalling may differentially affect nicotine-induced place preference and locomotor activity [74]. 5-HT_{2C} receptor stimulation may differentially affect nicotine-induced increases in dopamine release from the nigrostriatal vs. mesolimbic systems [22,23]. Also, other studies investigating the effects of dopaminergic or serotonergic manipulations on the effects of drugs of abuse show that the dissociation between place conditioning and locomotor effects are not unique to 5-HT_{2C} receptor activation and nicotine [75–81]. The current results suggest that the 5-HT_{2C} receptor may play an inhibitory role in nicotine-induced locomotor activity without having effects on nicotine-induced place conditioning under the present experimental conditions. The complex role of the 5-HT_{2C} receptor in nicotine-mediated and dopamine-related reward may be currently underappreciated and future studies will be needed to determine the precise roles of dopamine, acetylcholine and 5-HT in this regard.

Acknowledgements

This work was funded by the Canadian Institutes of Health Research (CIHR) (A.J.G.). D.J.H. was the recipient of a postgraduate scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

[1] Ikemoto S, Wise RA. Mapping of chemical trigger zones for reward. Neuropharmacology 2004;47(Suppl. 1):190–201.
[2] Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Progress in Neurobiology 1998;56:613–72.
[3] Self DW. Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system. Neuropharmacology 2004;47(Suppl. 1):242–55.
[4] Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Molecular Psychiatry 2004;9:557–69.
[5] Wise RA. Addictive drugs and brain stimulation reward. Annual Review of Neuroscience 1996;19:319–40.
[6] Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Brain Research Reviews 1998;28:309–69.
[7] Schultz W. Predictive reward signal of dopamine neurons. Journal of Neurophysiology 1998;80:1–27.
[8] Tobler PN, Fiorillo CD, Schultz W. Adaptive coding of reward value by dopamine neurons. Science (New York, NY) 2005;307:1642–5.
[9] Schultz W. Behavioral theories and the neurophysiology of reward. Annual Review of Psychology 2006;57:87–115.
[10] Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. Nature 2006;442:1042–5.
[11] Stefani MR, Moghaddam B. Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. Journal of Neuroscience 2006;26:8810–8.
[12] Van Bockstaele EJ, Cestari DM, Pickel VM. Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. Brain Research 1994;647:307–22.
[13] McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behavioural Brain Research 1999;101:129–52.
[14] Van Bockstaele EJ, Biswas A, Pickel VM. Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. Brain Research 1993;624:188–98.
[15] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083–152.
[16] Di Giovanni G, Di Matteo V, Di Mascio M, Esposito E. Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. Synapse (New York, NY) 2000;35:53–61.

[17] Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E. SB 242084, a selective serotonin2C receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology 1999;38:1195–205.
[18] Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. European Journal of Pharmacology 2000;393:295–314.
[19] Wonnacott S, Sidhpura N, Balfour DJ. Nicotine: from molecular mechanisms to behaviour. Current Opinion in Pharmacology 2005;5:53–9.
[20] Ivanova S, Greenshaw AJ. Nicotine-induced decreases in VTA electrical self-stimulation thresholds: blockade by haloperidol and mecamylamine but not scopolamine or ondansetron. Psychopharmacology 1997;134:187–92.
[21] Seth P, Cheeta S, Tucci S, File SE. Nicotinic–serotonergic interactions in brain and behaviour. Pharmacology, Biochemistry, and Behavior 2002;71:795–805.
[22] Di Matteo V, Pierucci M, Esposito E. Selective stimulation of serotonin2c receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. Journal of Neurochemistry 2004;89:418–29.
[23] Pierucci M, Di Matteo V, Esposito E. Stimulation of serotonin2C receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. The Journal of Pharmacology and Experimental Therapeutics 2004;309:109–18.
[24] Batman AM, Munzar P, Beardsley PM. Attenuation of nicotine's discriminative stimulus effects in rats and its locomotor activity effects in mice by serotonergic 5-HT2A/2C receptor agonists. Psychopharmacology 2005;179:393–401.
[25] Fletcher PJ, Sinyard J, Higgins GA. The effects of the 5-HT(2C) receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists. Psychopharmacology 2006;187:515–25.
[26] Grottick AJ, Corrigall WA, Higgins GA. Activation of 5-HT(2C) receptors reduces the locomotor and rewarding effects of nicotine. Psychopharmacology 2001;157:292–8.
[27] Rocha B, Di Scala G, Jenck F, Moreau JL, Sandner G. Conditioned place aversion induced by 5-HT(1C) receptor antagonists. Behavioural Pharmacology 1993;4:101–6.
[28] Mosher TM, Smith JG, Greenshaw AJ. Aversive stimulus properties of the 5-HT(2C) receptor agonist WAY 161503 in rats. Neuropharmacology 2006;51:641–50.
[29] Mosher T, Hayes D, Greenshaw A. Differential effects of 5-HT2C receptor ligands on place conditioning and locomotor activity in rats. European Journal of Pharmacology 2005;515:107–16.
[30] Martin JR, Ballard TM, Higgins GA. Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety. Pharmacology, Biochemistry, and Behavior 2002;71:615–25.
[31] Gleason SD, Lucaites VL, Shannon HE, Nelson DL, Leander JD. *m*-CPP hypolocomotion is selectively antagonized by compounds with high affinity for 5-HT(2C) receptors but not 5-HT(2A) or 5-HT(2B) receptors. Behavioural Pharmacology 2001;12:613–20.
[32] Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, et al. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 1997;36:609–20.
[33] Higgins GA, Ouagazzal AM, Grottick AJ. Influence of the 5-HT(2C) receptor antagonist SB242 084 on behaviour produced by the 5-HT(2) agonist Ro60-0175 and the indirect 5-HT agonist dexfenfluramine. British Journal of Pharmacology 2001;133:459–66.
[34] Kennett G, Lightowler S, Trail B, Bright F, Bromidge S. Effects of RO 60 0175, a 5-HT(2C) receptor agonist, in three animal models of anxiety. European Journal of Pharmacology 2000;387:197–204.
[35] Lucki I, Ward HR, Frazer A. Effect of 1-(*m*-chlorophenyl)piperazine and 1-(*m*-trifluoromethylphenyl)piperazine on locomotor activity. The Journal of Pharmacology and Experimental Therapeutics 1989;249:155–64.
[36] Ji SP, Zhang Y, Van Cleemput J, Jiang W, Liao M, Li L, et al. Disruption of PTEN coupling with 5-HT2C receptors suppresses behavioral responses induced by drugs of abuse. Nature Medicine 2006;12:324–9.
[37] Laviolette SR, van der Kooy D. Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. Molecular Psychiatry 2003;8:50–9, 9.
[38] Pontieri FE, Tanda G, Orzi F, Di Chiara G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 1996;382:255–7.
[39] Sziraki I, Sershen H, Hashim A, Lajtha A. Receptors in the ventral tegmental area mediating nicotine-induced dopamine release in the nucleus accumbens. Neurochemical Research 2002;27:253–61.
[40] Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. Neuron 2002;33:905–19.
[41] Ferrari R, Le Novere N, Picciotto MR, Changeux JP, Zoli M. Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. The European Journal of Neuroscience 2002;15:1810–8.
[42] Nisell M, Nomikos GG, Svensson TH. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse (New York, NY) 1994;16:36–44.
[43] Singer G, Wallace M, Hall R. Effects of dopaminergic nucleus accumbens lesions on the acquisition of schedule induced self injection of nicotine in the rat. Pharmacology, Biochemistry, and Behavior 1982;17:579–81.
[44] Clarke PB, Fu DS, Jakubovic A, Fibiger HC. Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. The Journal of Pharmacology and Experimental Therapeutics 1988;246:701–8.
[45] Corrigall WA, Coen KM. Nicotine self-administration and locomotor activity are not modified by the 5-HT3 antagonists ICS 205-930 and MDL 72222. Pharmacology, Biochemistry, and Behavior 1994;49:67–71.
[46] Corrigall WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology 1992;107:285–9.
[47] Le Foll B, Goldberg SR. Nicotine induces conditioned place preferences over a large range of doses in rats. Psychopharmacology 2005;178:481–92.
[48] Spyraki C, Fibiger HC, Phillips AG. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research 1982;253:185–93.
[49] Arnold B, Allison K, Ivanova S, Paetsch PR, Paslawski T, Greenshaw AJ. 5HT3 receptor antagonists do not block nicotine induced hyperactivity in rats. Psychopharmacology 1995;119:213–21.
[50] Imperato A, Mulas A, Di Chiara G. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. European Journal of Pharmacology 1986;132:337–8.
[51] Wilkinson JL, Bevins RA. Intravenous nicotine conditions a place preference in rats using an unbiased design. Pharmacology, Biochemistry, and Behavior 2008;88:256–64.
[52] Horan B, Smith M, Gardner EL, Lepore M, Ashby Jr CR. (−)-Nicotine produces conditioned place preference in Lewis, but not Fischer 344 rats. Synapse (New York, NY) 1997;26:93–4.
[53] Philibin SD, Vann RE, Varvel SA, Covington III HE, Rosecrans JA, James JR, et al. Differential behavioral responses to nicotine in Lewis and Fischer-344 rats. Pharmacology, Biochemistry, and Behavior 2005;80:87–92.
[54] Forget B, Barthelemy S, Saurini F, Hamon M, Thiebot MH. Differential involvement of the endocannabinoid system in short- and long-term expression of incentive learning supported by nicotine in rats. Psychopharmacology 2006;189:59–69.
[55] Dewey SL, Brodie JD, Gerasimov M, Horan B, Gardner EL, Ashby Jr CR. A pharmacologic strategy for the treatment of nicotine addiction. Synapse (New York, NY) 1999;31:76–86.
[56] Ashby Jr CR, Paul M, Gardner EL, Gerasimov MR, Dewey SL, Lennon IC, et al. Systemic administration of 1*R*,4*S*-4-amino-cyclopent-2-ene-carboxylic acid, a reversible inhibitor of GABA transaminase, blocks expression of conditioned place preference to cocaine and nicotine in rats. Synapse (New York, NY) 2002;44:61–3.
[57] Fudala PJ, Iwamoto ET. Conditioned aversion after delay place conditioning with nicotine. Psychopharmacology 1987;92:376–81.
[58] Jorenby DE, Steinpreis RE, Sherman JE, Baker TB. Aversion instead of preference learning indicated by nicotine place conditioning in rats. Psychopharmacology 1990;101:533–8.
[59] Shram MJ, Funk D, Li Z, Le AD. Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. Psychopharmacology 2006;186:201–8.
[60] Carboni E, Acquas E, Leone P, Di Chiara G. 5HT3 receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward. Psychopharmacology 1989;97:175–8.
[61] Acquas E, Carboni E, Leone P, Di Chiara G. SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology 1989;99:151–5.
[62] Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport 2002;13:1097–106.
[63] Kennett GA, Whitton P, Shah K, Curzon G. Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. European Journal of Pharmacology 1989;164:445–54.
[64] Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology 2003;170:409–22.
[65] Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addiction Biology 2007;12:227–462.
[66] Roma PG, Riley AL. Apparatus bias and the use of light and texture in place conditioning. Pharmacology, Biochemistry, and Behavior 2005;82:163–9.
[67] McMahon LR, Filip M, Cunningham KA. Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. Journal of Neuroscience 2001;21:7781–7.
[68] Filip M, Cunningham KA. Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin(2C) (5-HT(2C)) receptors in rat prefrontal cortex. The Journal of Pharmacology and Experimental Therapeutics 2003;306:734–43.
[69] Fletcher PJ, Chintoh AF, Sinyard J, Higgins GA. Injection of the 5-HT2C receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. Neuropsychopharmacology 2004;29:308–18.
[70] Tomkins DM, Joharchi N, Tampakeras M, Martin JR, Wichmann J, Higgins GA. An investigation of the role of 5-HT(2C) receptors in modifying ethanol self-administration behaviour. Pharmacology, Biochemistry, and Behavior 2002;71:735–44.
[71] Rocha BA, Goulding EH, O'Dell LE, Mead AN, Coufal NG, Parsons LH, et al. Enhanced locomotor, reinforcing, and neurochemical effects of cocaine in serotonin 5-hydroxytryptamine 2C receptor mutant mice. Journal of Neuroscience 2002;22:10039–45.

[72] Janhunen S, Linnervuo A, Svensk M, Ahtee L. Effects of nicotine and epibatidine on locomotor activity and conditioned place preference in rats. Pharmacology, Biochemistry, and Behavior 2005;82:758–65.
[73] Janhunen S, Ahtee L. Comparison of the effects of nicotine and epibatidine on the striatal extracellular dopamine. European Journal of Pharmacology 2004;494:167–77.
[74] Le Foll B, Sokoloff P, Stark H, Goldberg SR. Dopamine D3 receptor ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects. Neuropsychopharmacology 2005;30:720–30.
[75] Suzuki T, Shiozaki Y, Masukawa Y, Misawa M. 5-HT3 receptor antagonists block cocaine- and methamphetamine-induced place preference. Yakubutsu Seishin Kodo 1992;12:33–8.
[76] Baker DA, Khroyan TV, O'Dell LE, Fuchs RA, Neisewander JL. Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. The Journal of Pharmacology and Experimental Therapeutics 1996;279:392–401.
[77] Khroyan TV, Baker DA, Fuchs RA, Manders N, Neisewander JL. Differential effects of 7-OH-DPAT on amphetamine-induced stereotypy and conditioned place preference. Psychopharmacology 1998;139:332–41.
[78] Baker DA, Fuchs RA, Specio SE, Khroyan TV, Neisewander JL. Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. Synapse (New York, NY) 1998;30:181–93.
[79] Le AD, Tomkins D, Higgins G, Quan B, Sellers EM. Effects of 5-HT3, D1 and D2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacology, Biochemistry, and Behavior 1997;57:325–32.
[80] Suzuki T, Shiozaki Y, Moriizumi T, Misawa M. Establishment of the ethanol-induced place preference in rats. Arukoru Kenkyuto Yakubutsu Ison 1992;27:111–23.
[81] Ferguson SM, Mitchell ES, Neumaier JF. Increased expression of 5-HT6 receptors in the nucleus accumbens blocks the rewarding but not psychomotor activating properties of cocaine. Biological Psychiatry 2008;63:207–13.