Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmacology xx (2005) xxx – xxx

www.elsevier.com/locate/ejphar

Differential effects of 5-HT_{2C} receptor ligands on place conditioning and locomotor activity in rats

Tera Mosher, David Hayes, Andrew Greenshaw*

W.G. Dewhurst Laboratory, Neurochemical Research Unit, Department of Psychiatry and Centre for Neuroscience, 1E7.44 WMC, University of Alberta, Edmonton, AB, Canada T6G 2R7

Received 23 March 2005; accepted 30 March 2005

Abstract

Effects of the 5-hydroxytryptamine (5-HT)$_{1A/1B/2C}$ receptor agonist *N*-[3-(trifluoromethyl)phenyl] piperazine (TFMPP, 0–3.0 mg/kg s.c.) and the 5-HT_{2C} receptor agonist 8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one (WAY 161503, 0–3.0 mg/kg s.c.) in place conditioning were measured in male Sprague–Dawley rats. Effects of TFMPP, alone and with the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamine (WAY 100635), the 5-HT_{1B} receptor antagonist *N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide (GR 127935) or the 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline (SB 242084) and of WAY 161503 alone and with SB 242084 on locomotor activity were also assessed. Neither TFMPP nor WAY 161503 induced place conditioning. WAY 161503 (1.0 and 3.0 mg/kg s.c.) decreased locomotor activity; SB 242084 (1.0 mg/kg i.p.) blocked this effect. Reduced locomotor activity following TFMPP was blocked by SB 242084 but not WAY 100635 (0.1 mg/kg s.c.) or GR 127935 (3.0 mg/kg s.c.). Behaviourally relevant levels of 5-HT_{2C} receptor stimulation may not exert reinforcing effects, although other studies indicate that such manipulations alter reinforcing effects of drugs of abuse.

Keywords: 5-HT_{2C} receptor; Place conditioning; Locomotor activity; (Rat)

1. Introduction

Central 5-hydroxytryptamine (5-HT) containing neurons originate from the midbrain raphé nuclei and project to the ventral tegmental area, the substantia nigra and the nucleus accumbens (Azmitia and Segal, 1978; Hervé et al., 1987; Moukhles et al., 1997). The actions of synaptically released 5-HT are mediated by actions among at least 14 distinct structural and pharmacological subtypes of 5-HT receptors (Barnes and Sharp, 1999). Activation of these receptor subtypes may regulate a variety of behavioural responses including: sleeping, feeding, memory, pain, sexual activity, locomotion and positive and negative reinforcement (Barnes and Sharp, 1999; Jacobs and Fornal, 1999; Jouvet, 1999). In particular, activation of the 5-HT_{2C} receptor has been associated with decreased locomotor activity, hypophagia, anxiety, penile erections and hyperthermia (Barnes and Sharp, 1999; Koek et al., 1992).

Currently, it is hypothesized that the mesocorticolimbic dopamine system plays a role in reward mediated behaviour (Tzschentke, 1998; Wise and Rompre, 1989). Within the mesocorticolimbic system, dopamine has significant interactions with 5-HT; neurons containing 5-HT may play an inhibitory role with respect to dopamine (Di Giovanni et al., 2000; Di Matteo et al., 2000b; Tzschentke, 1998). 5-HT receptor subtypes may play a functional role in the regulation of neural processes underlying motivation and reward. Systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT may alter lateral hypothalamic reward thresholds, with lower doses decreasing and higher doses increasing thresholds, respectively in two studies (Harrison and Markou, 2001; Montgomery et al., 1991). Ahn et al. (2004) have reported a monotonic increase in reward sites

* Corresponding author. Tel.: +1 780 492 6550; fax: +1 780 492 6841.
E-mail address: andy.greenshaw@ualberta.ca (A. Greenshaw).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.03.041

for self-stimulation at ventral tegmental electrode sites over a wide systemic range of doses of 8-OH-DPAT. Systemically administered 8-OH-DPAT may also decrease self-administration of cocaine and ethanol (Burmeister et al., 2004; Peltier and Schenk, 1993; Parsons et al., 1998; Roberts et al., 1998). Nevertheless, when this drug was administered directly into the dorsal or median raphé nucleus, respectively, a decrease in brain stimulation reward thresholds was observed (Ahn et al., 2004; Harrison and Markou, 2001; Fletcher et al., 1995). In agreement with these findings, stimulation of the 5-HT_{1A} receptor may result in a conditioned place preference (Papp and Willner, 1991; Fletcher et al., 1993; Neisewander et al., 1990). These results have generally been interpreted in terms of an action of 8-OH-DPAT at somatodendritic 5-HT_{1A} receptors on 5-HT neurons (reward increasing effects) or post-synaptic 5-HT_{1A} receptors (reward decreasing effects, see Ahn et al., 2004). 5-HT_{1B} receptor activation may also be relevant to motivation and reward. Activation of the 5-HT_{1B} receptor may induce a conditioned place preference when administered with a sub-threshold dose of cocaine (Cervo et al., 2002) and has been observed to facilitate sub-threshold levels of cocaine self-administration (Parsons et al., 1998). By contrast, activation of that receptor may reduce both responding for amphetamine self-administration (Fletcher et al., 2002a,b) and brain stimulation reward thresholds (Harrison et al., 1999) as well as induce a conditioned place aversion (Cervo et al., 2002).

With regard to the 5-HT_2 family of receptors, few studies have examined the effects of specific ligands in reward paradigms. It has been shown that 5-HT_{2A} receptor blockade does not alter the effects of amphetamine on brain stimulation reward thresholds (Moser et al., 1996), but may attenuate cocaine reinstatement in self-administration paradigm (Fletcher et al., 2002a,b). Furthermore, unpublished data from our laboratory have shown that 5-HT_{2C} receptor stimulation decreases brain stimulation reward thresholds whereas the 5-HT_{2C} receptor antagonist SB 242084 may facilitate brain stimulation reward (Clements RLH, personal communication). In agreement with these results, 5-HT_{2C} receptor blockade has been demonstrated to increase cocaine breaking points in self-administration (Fletcher et al., 2002b) and increase responding for low ethanol drinking rats (Tomkins et al., 2002).

The 5-HT_{2C} receptor is a G-protein coupled receptor which activates phospholipase C and increases phosphotidyl inositol hydrolysis (Boess and Martin, 1994; Barnes and Sharp, 1999). Available data indicate that 5-HT_{2C} receptors are located on γ-aminobutyric acid-containing neurons but not on dopamine neurons (Di Giovanni et al., 2001; Eberle-Wang et al., 1997; Serrats et al., 2005). In vivo, 5-HT_{2C} receptor activation may reduce activity in the mesocorticolimbic dopamine system (Di Giovanni et al., 2000; Di Matteo et al., 1999, 2000a,b, 2001, 2002; Millan et al., 1998; Pozzi et al., 2002). As 5-HT_{2C} receptors may not be expressed on dopamine neurons, the decrease in dopamine release observed after activation of the 5-HT_{2C} receptor is thought to be mediated by activation of γ-aminobutyric acid interneurons in the ventral tegmental area (Di Giovanni et al., 2001; Di Matteo et al., 2001, 2002). Few studies have examined the positive or negative reinforcing effects of 5-HT_{2C} receptor ligands in place conditioning. Rocha et al. (1993) found that the 5-$HT_{1B/2C}$ agonist 1-(3-chlorophenyl) piperazine (mCPP) did not induce place conditioning on its own, but was able to block the conditioned place aversion induced by 1,2,3,4,10,14b-Hexahydro-2-methyldibenzo [c,f]pyrazino[1,2-a]azepine [mianserin] (mixed 5-HT_2 antagonist) and 1-(2,3-dihydro-1, 4-benzodioxin-5-yl)-piperazine [eltoprazine] (mixed 5-HT_{1B} receptor agonist/5-HT_{2C} receptor antagonist). Selective 5-HT_{2C} receptor ligands have recently been developed, allowing for the behavioural characterization of these receptors in place conditioning.

The effects of the 5-HT_{2C} receptor agonist 8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one (WAY 161503) on locomotor activity per se have not been reported, but WAY 161503 may decrease immobility and increase swimming in the forced swim test (Cryan and Lucki, 2000). These effects were attenuated by pretreatment with the 5-HT_{2C} receptor antagonist 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f] indole (SB 206533) and the non-specific 5-HT_2 receptor antagonist mianserin. Administration of 5-HT_{2C} receptor antagonists such as 6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)-pyrid-5-yl] carbamoyl] indoline (SB 242084) does not result in significant changes in locomotor activity (Kennett et al., 1997; Martin et al., 2002).

The mixed 5-$HT_{1B/2C}$ receptor agonist mCPP reduces locomotor activity, an effect that is blocked by pretreatment with SB 242084 (Gleason et al., 2001; Kennett et al., 1997; Martin et al., 2002). In addition, a dose-dependent reduction in locomotor activity has been reported following administration of the mixed 5-HT_2 receptor agonist (a*S*)-6-chloro-5-fluoro-a-methyl-1*H*-indole-1-ethanamine fumarate (Ro 60-0175), acting at the 5-$HT_{2A/2B/2C}$ receptor subtype, an effect that was reversed by pre-treatment with SB 242084 (Higgins et al., 2001; Kennett et al., 2000). SB 242084 also potentiated the increase in locomotor activity induced by the indirect 5-HT releaser/reuptake inhibitor (+) fenfluramine (Higgins et al., 2001).

Lucki et al. (1989) reported a dose-dependent reduction in locomotor activity following *N*-[3-(trifluoromethyl)-phenyl] piperazine (TFMPP) (0.31–5.0 mg/kg) that was not blocked by 1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl) amino]-2-propanol (pindolol) and (*RS*)-1-[(1-methylethyl) amino]-3-(1-naphthalenyloxy)-2-propanol (propranolol): compounds which are potent α-adreneoceptor blockers but also act as potent antagonists of the 5-$HT_{1A/1B}$ and 5-HT_{1B} receptors, for pindolol and propranolol, respectively. These effects were attenuated by the non-selective 5-HT antagonists [(8â)-1,6-dimethylergolin-8-yl)methyl]carbamic acid phenylmethyl ester (metergoline), [8*b*(*S*)]-9,10-didehydro-*N*-[1-(hydroxymethyl)propyl]-1,6-dimethylergoline-8-car-

boxamide maleate (methysergide) and mianserin. These authors hypothesized that these locomotor inhibitory effects of TFMPP may be due to 5-HT_{2C} receptor activation (Lucki et al., 1989), in accordance with the above studies that indicate a possible role for 5-HT_{2C} receptors in the regulation of exploratory motor behaviour.

To explore the potential role of 5-HT_{2C} receptors in place conditioning, WAY 161503 and TFMPP were assessed using this paradigm. It was hypothesized that these compounds may induce a conditioned place aversion, as activation of 5-HT_{2C} receptors by Ro 60-0175 and 6-chloro-2-(1-piperazinyl) pyrazine hydrochloride (MK 212) may decrease mesocorticolimbic dopamine release from areas such as the frontal cortex, nucleus accumbens and ventral tegmental area (Di Giovanni et al., 2000; Di Matteo et al., 2000b, 2001; Millan et al., 1998). The present study also attempted to clarify the role of 5-HT_{2C} receptors in the regulation of locomotor activity. It was hypothesized that the systemic effects of the selective 5-HT_{2C} receptor agonist WAY 161503 and of the less selective 5-HT agonist TFMPP on spontaneous locomotor activity would be attenuated or blocked by administration of the 5-HT_{2C} antagonist SB242084 but not by administration of a 5-HT_{1A} antagonist [(*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamine maleate, WAY 100635] or a 5-HT_{1B} antagonist [*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride, GR 127935]. Based on previous studies, it was hypothesized that (a) SB 242084 alone would not alter locomotor activity and (b) TFMPP and WAY 161503 would decrease locomotor activity and that these effects may be attenuated by pre-treatment with SB 242084.

2. Materials and methods

2.1. Animals

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 0700 h–1900 h) with food and water freely available. 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. Place conditioning

2.2.1. Apparatus

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 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.

2.2.2. Procedure

The procedure consisted of three phases. *Phase 1* (Pre-conditioning): Animals ($n=8$) 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. Independent experiments were conducted according to an unbiased and a biased design. For the unbiased design, animals were randomly assigned to each drug group and adjusted if necessary to equalize baseline compartment preference between and within each group.

In the biased design, because induction of place aversion was expected following administration of the 5-HT_{2C} receptor agonist, animals were randomly assigned to drug groups such that they were each conditioned to the most preferred compartment, as determined on pre-conditioning day three. In the biased design, the (+) α-methylphenylethylamine (amphetamine) sulphate positive control group was conditioned to the least preferred side, as determined on pre-conditioning day three. This was arranged because induction of a place preference was expected in that group. *Phase 2* (Conditioning): On alternating days, animals received drug and vehicle treatment and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals were conditioned for six consecutive days (Bilsky and Reid, 1991; Kankaanpaa et al., 2002; Shippenberg, 1991). *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 to the nearest second.

All testing took place under red light during the light phase of the light/dark cycle. The place conditioning apparatus was cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia).

2.3. Spontaneous locomotor activity

2.3.1. 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.

2.3.2. Procedure

Animals ($n=8$) were habituated to the locomotor activity boxes for two consecutive days (60 min per day) to establish baseline exploratory activity. Animals then received four randomized counterbalanced injections with three drug-free days between injections. Locomotor activity was monitored for 60 min during the WAY 161503 and SB 242084 dose–response experiments to explore the time course of drug effects. Locomotor activity was monitored for 30 min during TFMPP experiments, as TFMPP may alter activity during the first 30 min of testing (Waddock, 1997). All testing took place under red light during the light phase of the light/dark cycle. The photobeam boxes were cleaned between animals with ammonia-based window cleaner (No Name® Glass Cleaner with ammonia) diluted with water (1:6).

2.4. Drugs

The 5-$HT_{1A/1B/2C}$ receptor agonist TFMPP HCl [*N*-[3-(trifluoromethyl)phenyl] piperazine hydrochloride], the 5-HT_{2C} antagonist SB 242084 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] and the 5-HT_{1A} antagonist WAY 100635 maleate [*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamine maleate] were purchased from Sigma Chemical Company (St. Louis, MO, USA). The 5-HT_{1B} antagonist GR 127935 HCl [*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride] and the 5-HT_{2C} agonist WAY 161503 HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5 (6*H*)-one hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) α-methylphenylethylamine (amphetamine) sulphate was purchased from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). Amphetamine, TFMPP and WAY 100635 were dissolved in 0.9% saline. SB 242084, GR 127935 and WAY 161503 were dissolved in doubled distilled water. SB 242084 was injected intraperitoneally (i.p.) and all other drugs were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg. Drug doses are expressed as free-base.

2.5. Drug treatment

2.5.1. Experiment 1: The effects of systemic TFMPP or WAY 161503 on place conditioning

The effects of TFMPP (3.0 mg/kg, s.c., 10 min prior) on place conditioning were examined. Animals received three injections of TFMPP and vehicle on alternating days. The effects of TFMPP were tested in both an unbiased and biased place conditioning paradigm.

The effects of WAY 161503 (1.0 or 3.0 mg/kg, s.c., 10 min prior) on place conditioning in an unbiased design were examined. Animals received three injections of WAY 161503 and vehicle on alternating days. In addition, the effects of WAY 161503 (3.0 mg/kg, s.c., 10 min prior) on place conditioning in a biased design ($n=8$) were also assessed. Animals received three injections of WAY 161503 and vehicle on alternating days. The doses of TFMPP and WAY 161503 were chosen based on previous work in this laboratory and Cryan and Lucki (2000).

For all treatments, a positive control group was included. These animals received amphetamine (1.0 mg/kg, s.c., 10 min prior) and vehicle on alternating days.

2.5.2. Experiment 2: Effect of systemic SB 242084 on locomotor activity

Eight animals received a randomized, counterbalanced series of four injections of either saline or SB 242084 (0.3, 1.0 or 3.0 mg/kg, i.p., 30 min prior to testing) and activity was measured for 60 min. Three days of vehicle treatment test days intervened between drug injection days.

2.5.3. Experiment 3: Effect of systemic WAY 161503 and SB 242084 on locomotor activity

Eight animals received a randomized, counterbalanced series of four injections of either saline or WAY 161503 (0.3, 1.0 or 3.0 mg/kg, s.c., 10 min prior to testing) and activity was measured for 60 min. Following completion of that experiment, each animal received an injection of SB 242084 (1.0 mg/kg, i.p., 30 min prior) followed by an injection of WAY 161503 (3.0 mg/kg, s.c., 10 min prior) on a single test day. This dose of SB 242084 was chosen based on prior studies (Martin et al., 2002). Three days of vehicle treatment test days intervened between drug injection days.

2.5.4. Experiment 4: Effects of 5-HT receptor antagonists on TFMPP induced changes in locomotor activity

Separate groups of animals ($n=8$) received four randomized counterbalanced injections as follows: TFMPP (3.0 mg/kg, s.c., 10 min prior) and SB 242084 (1.0 mg/kg, i.p., 30 min prior) alone, in combination and with control; TFMPP (3.0 mg/kg, s.c., 10 min prior) and WAY100635 (0.1 mg/kg, s.c., 15 min prior) alone, in combination and with control; TFMPP (3.0 mg/kg, s.c., 10 min prior) and GR 127935 (3.0 mg/kg, s.c., 30 min prior) alone, in combination and with control. The dose of TFMPP was chosen based on previous work in this laboratory (Waddock, 1997). Three days of vehicle treatment test days intervened between drug injection days.

2.6. Statistical analysis

Experimental effects in the place conditioning experiments were determined by paired sample *t*-tests to compare time spent in the drug-paired side on pre-conditioning day 3 and post conditioning day 1 ($P \leq 0.05$, $n=8$ per group). Experimental effects on spontaneous locomotor activity were

determined using a one-way repeated measures analysis of variance (ANOVA). WAY 161503+SB 242084 and SB 242084 dose–response data were analyzed using two-way repeated measures ANOVA (drug × time). Drug interaction data for experiments involving TFMPP were analyzed using three-way repeated measures ANOVA [TFMPP × antagonist × time]. Where appropriate, analysis of time course data using one-way repeated measures ANOVA across all drug groups at each 5 min interval was conducted. A significant F ratio ($P \leq 0.05$) on a 5 min interval was followed by comparison of each drug condition to vehicle using Newman–Keul's 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. All statistical analyses were completed using SPSS statistical software (SPSS 11.5, SPSS Inc., Chicago, U.S.A.).

3. Results

3.1. The effect of systemic TFMPP or WAY 161503 on place conditioning

TFMPP did not induce place conditioning in either an unbiased [$t(7) = 1.51$, $P > 0.05$] or biased design [$t(7) = 1.41$, $P > 0.05$] (Fig. 1A,B), under these conditions amphetamine induced a significant place preference [$t(7) = 4.987$, $P < 0.05$, unbiased; $t(7) = 4.845$, $P < 0.05$, biased]. The 5-HT_{2C} agonist WAY 161503 did not induce place conditioning in an unbiased [1.0 mg/kg $t(7) = 0.46$, $P > 0.05$; 3.0 mg/kg $t(7) = 0.12$, $P > 0.05$] (Fig. 1C) or biased [3.0 mg/kg $t(7) = 0.25$, $P > 0.05$] design (Fig. 1D). Under these conditions, amphetamine induced a significant place preference [$t(7) = 3.281$, $P < 0.05$; $t(7) = 4.878$, $P < 0.05$, respectively].

3.2. Effect of systemic SB 242084 on locomotor activity

Two-way repeated measures ANOVA (dose × time) revealed that there was no significant effect of the 5-HT_{2C} receptor antagonist SB 242084 (0.3–3.0 mg/kg) [$F(2.2, 15.6) = 2.262$, $P > 0.05$], there was a significant effect of time [$F(3.1,21.3) = 87.582$, $P < 0.05$], but no significant (SB 242084 × time) interaction [$F(5.3,36.9) = 0.843$, $P > 0.05$] (Fig. 2A). The lack of effect of SB 242084 on locomotor activity over the 60 min test period is illustrated in Fig. 2B.

3.3. Effect of systemic WAY 161503 and SB 242084 on locomotor activity

WAY 161503 decreased spontaneous locomotor activity [$F(1.9,13.2) = 28.435$, $P < 0.05$]. There was a significant

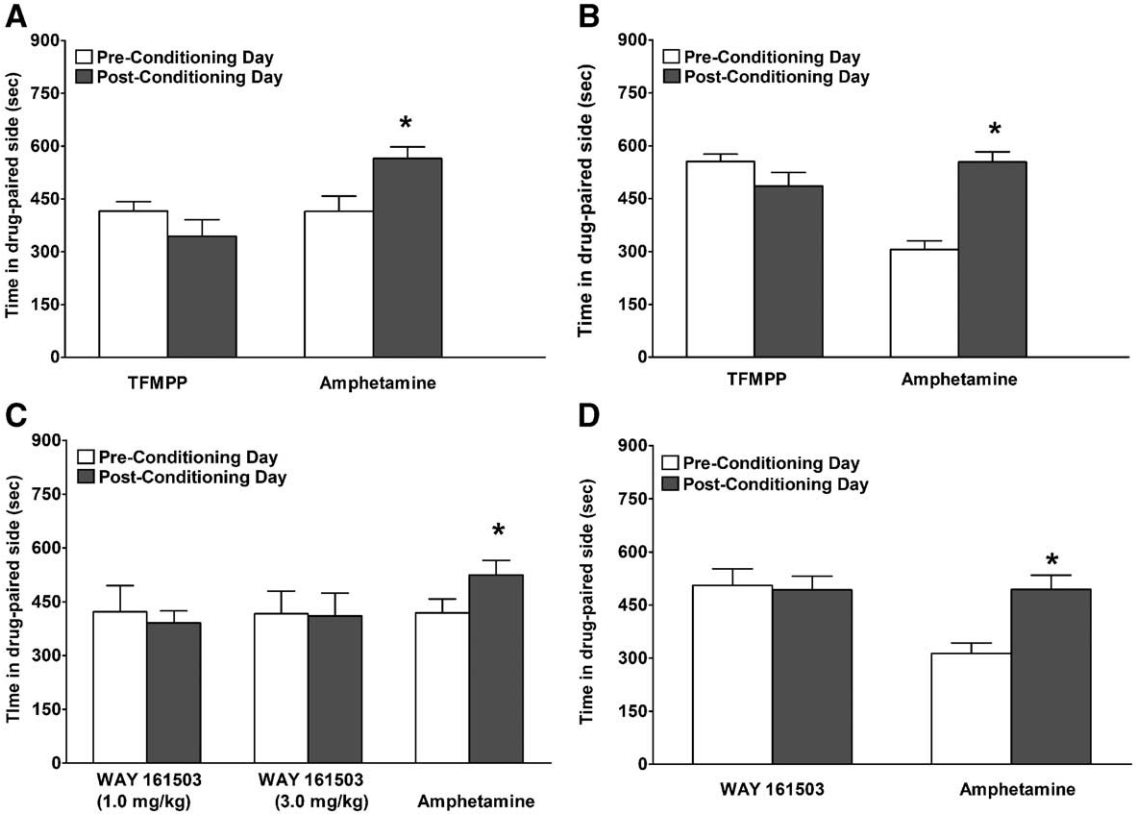

Fig. 1. Effects of TFMPP (3.0 mg/kg) and (+) amphetamine (1.0 mg/kg) with unbiased (A) and biased (B) place conditioning procedures. The effects of WAY 161503 (1.0 and 3.0 mg/kg) and (+) amphetamine (1.0 mg/kg) with unbiased (C) and biased (D) place conditioning procedures. Data shown are means±S.E.M. *Significant $P < 0.05$.

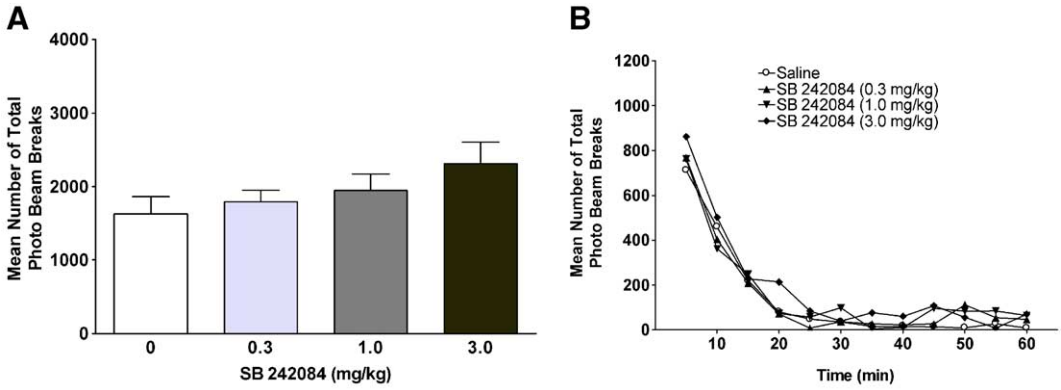

Fig. 2. Effects of SB 242084 (0.3–3.0 mg/kg) on total locomotor activity (A) and time course of changes in locomotor activity over 60 min (B). Data shown are means±S.E.M.

effect of time [$F(4.4,30.8)=34.931$, $P<0.05$] and a significant (WAY 161503 × time) interaction [$F(5.4,38.1)=3.787$, $P<0.05$] (Fig. 3A).

Since there was a significant interaction, one-way repeated measures ANOVA was performed for each time period. Following a significant one-way ANOVA at a time period, a Newman–Keul's post hoc test ($\alpha=0.05$) was performed. This time course analysis revealed that, at 1.0 mg/kg, WAY 161503 reduced locomotor activity during the first 15 min of testing. At 3.0 mg/kg, WAY 161503 reduced locomotor activity during the first 25 min of testing. SB 242084 blocked the effects of WAY 161503 (3.0 mg/kg; see Fig. 3B).

3.4. Effects of 5-HT receptor antagonists on TFMPP hypoactivity

3.4.1. TFMPP and the 5-HT$_{2C}$ receptor antagonist SB 242084

TFMPP decreased spontaneous locomotor activity [$F(1,7)=38.178$, $P<0.05$]. There was also a significant effect of the 5-HT$_{2C}$ receptor antagonist SB 242084 [$F(1,7)=14.652$, $P<0.05$], and time [$F(2.1,15)=126.742$, $P<0.05$]. Significant interactions of TFMPP × time [$F(2.3,6.5)=3.979$, $P<0.05$], SB 242084 × time [$F(2.7,19)=13.366$, $P<0.05$], SB 242084 × TFMPP [$F(1,7)=17.199$, $P<0.05$] and SB 242084 × TFMPP × time time [$F(3.4,23.8)=7.267$, $P<0.05$] were observed (Fig. 4A).

TFMPP significantly reduced locomotor activity during the first 10 min. Pre-treatment with SB 242084 significantly blocked the decrease in locomotor activity produced by TFMPP for the duration of the test period (Fig. 4B). Although the two-way repeated measures ANOVA revealed a significant SB 242084 × time interaction, Newman–Keul's post hoc tests failed to reveal any effects of SB 242084 on the time course of changes in locomotor activity measures.

3.4.2. TFMPP and the 5-HT$_{1A}$ receptor antagonist WAY 100635

TFMPP significantly reduced locomotor activity [$F(1,7)=39.336$, $P<0.05$] (Fig. 4C). There was also a significant effect of time [$F(3.1,21.9)=44.433$, $P<0.05$] and a significant TFMPP × time interaction [$F(3.1,21.6)=27.907$, $P<0.05$]. The interaction of TFMPP and the 5-HT$_{1A}$ antagonist WAY 100635 [$F(1,7)=0.527$, $P>0.05$] was not significant. WAY 100635 did not block the effects of TFMPP during the testing period. TFMPP

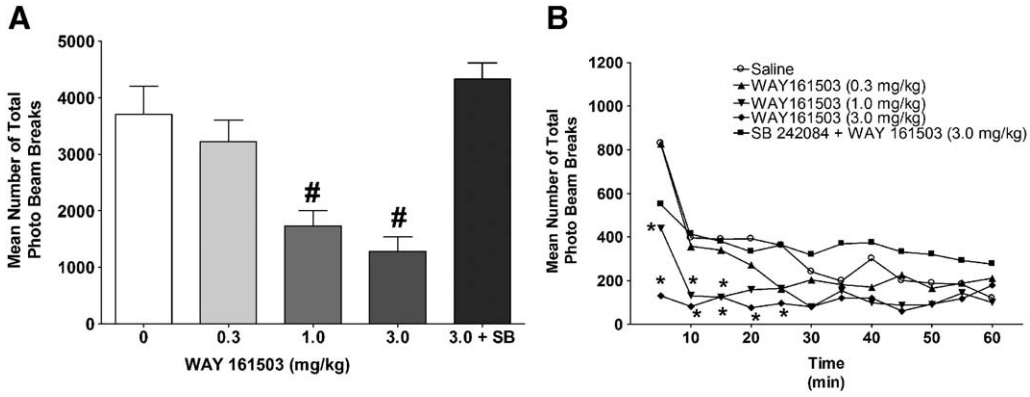

Fig. 3. Effects of the 5-HT$_{2C}$ receptor agonist WAY 161503 (0.3–3.0 mg/kg) and WAY 161503+the 5-HT$_{2C}$ receptor antagonist SB 242084 on total locomotor activity (A) and time course changes in locomotor activity over 60 min (B). Data shown are means±S.E.M. #Significant effect of WAY 161503, $P<0.05$. *Significant $P<0.05$, relative to control.

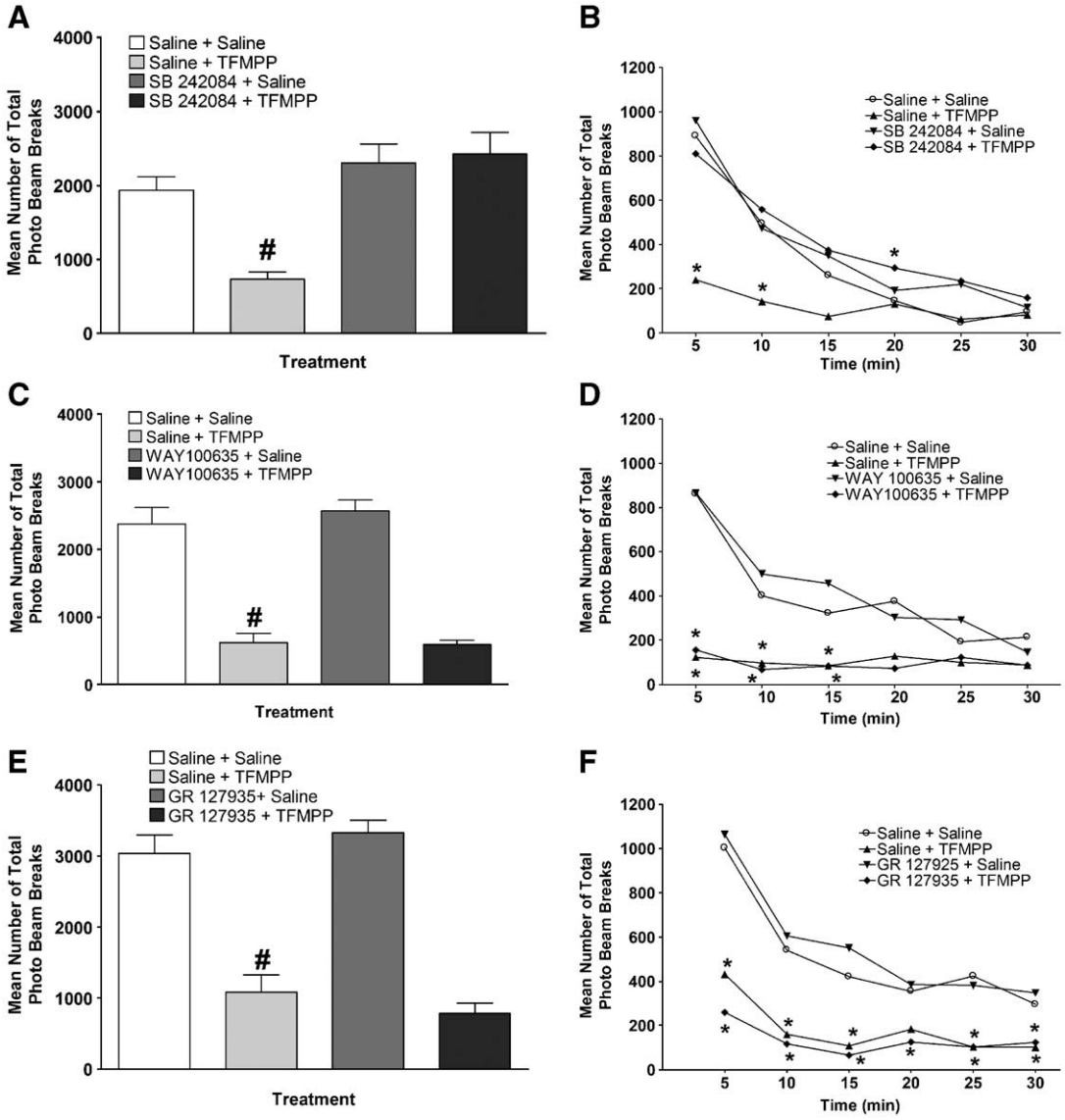

Fig. 4. Effects of TFMPP (3.0 mg/kg) and SB 242084 on total locomotor activity (A) and time course changes in locomotor activity (B) over 30 min. Effects of TFMPP (3.0 mg/kg) and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg) on total locomotor activity (C) and time course changes in locomotor activity (D) over 30 min. Effects of TFMPP (3.0 mg/kg) and the 5-HT_{1B} receptor antagonist GR 127935 (3.0 mg/kg) on total locomotor activity (E) and time course changes in locomotor activity (F) over 30 min. Data shown are means ± S.E.M. #Significant effect of TFMPP, $P<0.05$. *Significant $P<0.05$, relative to control.

and TFMPP + WAY 100635 were significantly different from control during the first 15 min of testing (Fig. 4D).

3.4.3. TFMPP and the 5-HT_{1B} receptor antagonist GR 127935

TFMPP significantly reduced locomotor activity [$F(1,7)=263.244$, $P<0.05$] (Fig. 4E). There was also a significant effect of time [$F(2.3,15.9)=52.950$, $P<0.05$] and a significant TFMPP × time interaction [$F(2.8,19.9)=13.035$, $P<0.05$]. The interaction of TFMPP and the 5-HT_{1B} antagonist GR 127935 was not significant [$F(1,7)=2.465$, $P>0.05$]. TFMPP significantly reduced locomotor activity for the first 15 min and the last 10 min of testing compared to control. GR 127935 did not block the effects of TFMPP during the 30 min test period (Fig. 4F).

4. Discussion

At doses that are behaviourally active (see Figs. 3 and 4), neither TFMPP nor WAY 161503 induced place conditioning; however, these drugs were shown to be effective during this time frame as shown during locomotor activity. This result is consistent with the observation that the 5-

$HT_{1B/2C}$ agonist mCPP failed to produce place conditioning (Rocha et al., 1993). Rocha et al. (1993) have reported that the mixed 5-HT receptor antagonists with some affinity for the 5-HT_{2C} receptor (mianserin and eltoprazine) induced a conditioned place aversion. That result is difficult to reconcile with the observation that 5-HT_{2C} receptor blockade increases dopamine release (Di Matteo et al., 1999, 2000a, 2001; Millan et al., 1998; Pozzi et al., 2002). However, mianserin and eltoprazine also have high affinities for other receptors (such as the 5-HT_{1B} and α_2 adrenergic receptors). The conditioned place aversion induced by mianserin and eltoprazine may have been due to actions on multiple 5-HT receptors (Rocha et al., 1993). In contrast, Risinger and Oakes (1996) found that mianserin did not produce a place aversion/preference alone, but did enhance ethanol induced conditioned place preference in mice. Although mianserin produced differing results in the place conditioning paradigm (Risinger and Oakes, 1996; Rocha et al., 1993), these results may be explained by methodological differences. Risinger and Oakes (1996) used male Swiss-Webster mice, which underwent four drug conditioning trials for a 60 min period. In contrast, Rocha et al. (1993) used male Long-Evans rats, which were conditioned to the drug paired compartment for four trials (30 min each). In addition, Rocha et al. (1993) used a place conditioning apparatus with both visual and tactile cues, whereas Risinger and Oakes (1996) only had tactile cues. The compartments in the place conditioning apparatus used in the present study only differed in floor texture. Nevertheless, the inclusion of the (+) amphetamine (positive control) in these experiments confirms the validity of the experiments.

The putative 5-HT_{2C} agonist Ro 60-0175 injected systemically or centrally into the ventral tegmental area, dose-dependently reduced responding for ethanol and for cocaine self-administration (Fletcher et al., 2004; Tomkins et al., 2002). These effects were blocked by pretreatment with the 5-HT_{2C} antagonist SB 242084 (0.5 mg/kg) (Fletcher et al., 2004; Tomkins et al., 2002). This observation is consistent with our present results indicating that 5-HT_{2C} agonists may not exhibit intrinsic rewarding properties, but suggests that activity at these receptors may serve to alter the reinforcing properties of other drugs.

The selective 5-HT_{2C} receptor agonist WAY 161503 induced a dose-dependent decrease in locomotor activity, which was blocked (at the 3.0 mg/kg dose of WAY 161503) by pretreatment with the 5-HT_{2C} receptor antagonist SB 242084. The 5-HT_{2C} receptor antagonist SB 242084 did not significantly alter locomotor activity, although there appeared to be a slight increase in activity seen as dose increases, which may also be apparent from data presented in other studies (see Martin et al., 2002, Fig. 1). TFMPP also induced a marked decrease in locomotor activity, consistent with the earlier findings of Lucki et al. (1989). This effect of TFMPP was attenuated by co-administration of SB 242084, but not the specific 5-HT_{1A} receptor antagonist WAY 100635 nor the specific 5-HT_{1B} receptor antagonist GR 127935. These results indicate the decrease in locomotor activity induced by TFMPP is mediated by the 5-HT_{2C} receptor, results which are consistent with the actions of other putative 5-HT_{2C} receptor agonists such as Ro 60-0175 (0.5–10 mg/kg), MK 212 (0.31 and 0.62 mg/kg) and mCPP (2.5–7.0 mg/kg) (Higgins et al., 2001, Kennett et al., 1994, 1997, 2001; Lucki et al., 1989; Martin et al., 2002). The present results represent the first specific evidence that the effects of TFMPP on locomotor activity are mediated by the 5-HT_{2C} receptor as suggested by Lucki et al. (1989).

The results of these locomotor activity experiments are also consistent with recent cellular and molecular data that associate 5-HT with mesocorticolimbic dopamine systems (Di Matteo et al., 2001, 2002). 5-HT_{2C} receptor blockade by SB 242084 may cause an increase in dopamine release and basal cell firing in the nucleus accumbens and ventral tegmental area, respectively (Di Matteo et al., 1999, 2000a; Pozzi et al., 2002), an effect that may be due to inhibition of γ-aminobutyric acid-containing interneurons in the ventral tegmental area (Di Matteo et al., 2001). This is supported by work demonstrating that administration of the 5-HT_{2C} agonists Ro 60-0175, mCPP and MK 212 may decrease dopamine cell firing in the ventral tegmental area and decrease dopamine release in the nucleus accumbens (Di Giovanni et al., 2000; Di Matteo et al., 2000b). As it is hypothesized that increased mesocorticolimbic dopamine release is reflected by increased locomotion (Dunnett and Robbins, 1992; Mathé et al., 1996), 5-HT_{2C} receptors may regulate or influence dopamine mediated locomotor activity (Di Matteo et al., 1999).

Further evidence that 5-HT_{2C} receptors affect dopamine activity comes from studies concerning cocaine and nicotine induced changes in locomotor activity. Hyperactivity induced by either cocaine or nicotine may be mediated by the mesocorticolimbic dopamine system (Fung and Lau, 1988; Koob, 1992; Neisewander et al., 1995; Nisell et al., 1996). It has been demonstrated that Ro 60-0175 injected systemically (0.1–3.0 mg/kg) or centrally (3.0 and 10.0 μg/kg) into the ventral tegmental area may reduce cocaine induced hyperactivity (Fletcher et al., 2004; Grottick et al., 2000). The reduction in cocaine-induced hyperactivity by Ro 60-0175 was blocked by SB 242084 (0.5 mg/kg see Grottick et al., 2000). In addition, Ro 60-0175 dose-dependently blocked nicotine (0.4 mg/kg) induced hyperactivity. The reduction in nicotine-induced hyperactivity by Ro 60-0175 (1.0 mg/kg) was reversed by SB 242084 (0.5 mg/kg, see Grottick et al., 2001). These reports add further evidence for the hypothesis that 5-HT_{2C} receptor activation/blockade may regulate or influence behaviours, such as locomotor activity, which are regulated by the mesocorticolimbic dopamine system. In addition to systemic effects of 5-HT_{2C} receptor agonists on locomotor activity, central effects have also been examined. The 5-$HT_{2A/2B/2C}$ receptor agonist Ro 60-0175 and 5-$HT_{2B/2C}$ receptor agonist MK 212 injected into the shell of the

nucleus accumbens, were devoid of effects on locomotor activity, but were able to increase the hyperactivity induced by cocaine (Filip and Cunningham, 2002). In addition, Ro 60-0175 was shown to have no effect on locomotor activity when injected into the ventral tegmental area (Fletcher et al., 2004). However, when administered systemically, these compounds decrease locomotor activity (Higgins et al., 2001; Lucki et al., 1989).

5-HT_{2C} receptors may play a role in the regulation of mesocorticolimbic dopamine release, effects that may be related to changes in reward/motivational behaviour. The present experiments indicate that selective stimulation of the 5-HT_{2C} receptor may not result in place conditioning. However, 5-HT_{2C} receptor activation did decrease locomotor activity, an effect that was blocked by a selective 5-HT_{2C} receptor antagonist. The present locomotor activity experiments confirm the efficacy of the current drug treatments in the behavioural context. 5-HT_{2C} receptor agonists may modify reinforcing effects of electrical brain stimulation and of self-administered drugs in laboratory animals (Clements et al., submitted for publication ; Fletcher et al., 2004; Tomkins et al., 2002). The lack of a direct reinforcing effect of 5-HT_{2C} receptor stimulation is illustrated by the current failure of 5-HT_{2C} receptor related drugs to induce place conditioning. This pattern of results indicates that 5-HT_{2C} receptor stimulation may play a modulatory rather than a direct role in the regulation of reinforcement.

Acknowledgements

The authors would like to thank RLH Clements for his assistance on this paper. Work supported by CIHR (Institute of Neuroscience Mental Health and Addiction).

References

Ahn, K.C., Pazderka-Robinson, H., Clements, R., Ashcroft, R., Ali, T., Morse, C., Greenshaw, A.J., 2004. Differential effects of intra-midbrain raphe and systemic 8-OH-DPAT on VTA self-stimulation thresholds in rats. Psychopharmacology (Berl).

Azmitia, E.C., Segal, M., 1978. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. 179, 641–668.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Bilsky, E., Reid, J., 1991. MDL72222, a serotonin 5-HT3 receptor antagonist, blocks MDMA's ability to establish a conditioned place preference. Pharmacol. Biochem. Behav. 39, 509–512.

Boess, F.G., Martin, I.L., 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33, 275–317.

Burmeister, J.J., Lungren, E.M., Kirschner, K.F., Neisewander, J.L., 2004. Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. Neuropsychopharmacology 29, 660–668.

Cervo, L., Rozio, M., Ekalle-Soppo, C.B., Carnovali, F., Santangelo, E., Samanin, R., 2002. Stimulation of $serotonin_{1B}$ receptors induces conditioned place aversion and facilitates cocaine place conditioning in rats. Psychopharmacology 163, 142–150.

Clements, R.L.H., Ahn, K.C., Ashcroft, R., Greenshaw, A.J., submitted for publication. 5-HT_{2C} receptor-related changes in VTA electrical self-stimulation thresholds in rats.

Cryan, J.F., Lucki, I., 2000. Antidepressant-like behavioural effects mediated by 5-hydroxytryptamine2C receptors. J. Pharmacol. Exp. Ther. 295, 1120–1126.

Di Giovanni, G., Di Matteo, V., Di Mascio, M., Esposito, E., 2000. Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin2C/2B receptor agonists: a combined in vivo electrophysiological and microdialysis study. Synapse 35, 53–61.

Di Giovanni, G., Di Matteo, V., La Grutta, V., Esposito, E., 2001. m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. Neuroscience 103, 111–116.

Di Matteo, V., Di Giovanni, G., Di Mascio, M., Esposito, E., 1999. SB 242084, a selective $serotonin_{2C}$ receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology 38, 1195–1205.

Di Matteo, V., Di Giovanni, G., Esposito, E., 2000a. SB 242084: a selective 5-HT_{2C} receptor antagonist. CNS Drug Rev. 6, 195–205.

Di Matteo, V., Di Giovanni, G., Di Mascio, M., Esposito, E., 2000b. Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through $serotonin_{2C}$ receptors. Brain Res. 865, 85–90.

Di Matteo, V., De Blasi, A., Di Giulio, C., Esposito, E., 2001. Role of the 5-HT_{2C} receptors in the control of central dopamine function. Trends Pharmacol. Sci. 22, 29–32.

Di Matteo, V., Cacchio, M., Di Giulio, C., Esposito, E., 2002. Role of $serotonin_{2C}$ receptors in the control of brain dopaminergic function. Pharmacol. Biochem. Behav. 71, 727–734.

Dunnett, S.B., Robbins, T.W., 1992. The functional role of mesotelencephalic dopamine systems. Biol. Rev. Camb. Philos. Soc. 67, 491–518.

Eberle-Wang, K., Mikeladze, Z., Uryu, K., Chesselet, M.-F., 1997. Pattern of expression of the $sertonin_{2C}$ receptor messenger RNA in the basal ganglia of adult rats. J. Comp. Neurol. 384, 233–247.

Filip, M., Cunningham, K.A., 2002. Serotonin 5-HT_{2C} receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. Pharmacol. Biochem. Behav. 71, 45–56.

Fletcher, P.J., Ming, Z.H., Higgins, G.A., 1993. Conditioned place preference induced by microinjection of 8-OH-DPAT into the dorsal or median raphe nucleus. Psychopharmacology 113, 31–36.

Fletcher, P.J., Tampakeras, M., Yeomans, J.S., 1995. Median raphe injections of 8-OH-DPAT lower frequency thresholds for lateral hypothalamic self-stimulation. Pharmacol. Biochem. Behav. 52, 65–71.

Fletcher, P.J., Azampanahc, A., Korth, K.M., 2002a. Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. Pharmacol. Biochem. Behav. 71, 717–725.

Fletcher, P.J., Grottick, A.J., Higgins, G.A., 2002b. Differential effects of the 5-HT_{2A} receptor antagonist M100,907 and the 5-HT_{2C} receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. Neurospsychopharmacology 27, 578–586.

Fletcher, P.J., Chintoh, A.F., Sinyard, J., Higgins, G.A., 2004. Injection of the 5-HT_{2C} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. Neuropsychopharmacology 29, 308–318.

Fung, Y.K., Lau, Y.S., 1988. Receptor mechanisms of nicotine-induced locomotor hyperactivity in chronic nicotine treated rats. Eur. J. Pharamacol. 2, 263–271.

Gleason, S.D., Lucaites, V.L., Shannon, H.E., Nelson, D.L., Leander, J.D., 2001. m-CPP hypolocomotion is selectively antagonized by compounds with high affinity for 5-HT_{2C} receptors but not 5-HT_{2A}, or 5-HT2B receptors. Behav. Pharmacol. 12, 613–620.

Grottick, A.J., Fletcher, P.J., Higgins, G.A., 2000. Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. J. Pharm. Exp. Ther. 295, 1183–1191.

Grottick, A.J., Corrigall, W.A., Higgins, G.A., 2001. Activation of 5-HT_{2C} receptors reduces the locomotor and rewarding effects of nicotine. Psychopharmacology 157, 292–298.

Harrison, A.A., Markou, A., 2001. Serotonergic manipulations both potentiate and reduce brain stimulation reward in rats: involvement of serotonin-1A receptors. J. Pharmacol. Exp. Ther. 297, 316–325.

Harrison, A.A., Parsons, L.H., Koob, G.F., Markou, A., 1999. RU 24969, a 5-HT1A/1B agonist, elevates brain stimulation reward thresholds: an effect reversed by GR 127935, a 5-HT1B/1D antagonist. J. Pharmacol. Exp. Ther. 297, 316–325.

Hervé, D., Pickel, V.M., Tong, H.J., Beaudet, A., 1987. Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res. 435, 71–83.

Higgins, G.A., Ouagazzal, A.M., Grottick, A.J., 2001. Influence of the 5-HT_{2C} receptor antagonist SB 242084 on behaviour produced by the 5-HT_2 agonist Ro 60-0175 and the indirect 5-HT agonist dexfenfluramine. Br. J. Pharmacol. 133, 459–466.

Jacobs, B.L., Fornal, C.A., 1999. Activity of serotonergic neurons in behaving animals. Neuropsychopharmacology 21, 9S–15S (Suppl).

Jouvet, M., 1999. Sleep and serotonin: an unfinished story. Neuropsychopharmacology 21, 24S–27S (Suppl).

Kankaanpaa, A., Meririnne, E., Seppala, T., 2002. 5-HT3 receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. Psychopharmacology 159, 341–350.

Kennett, G.A., Wood, M.D., Glen, A., Grewal, S., Forbes, I., Gadre, A., Blackburn, T.P., 1994. In vivo properties of SB 200646A, a 5-$HT_{2C/2B}$ receptor antagonist. Br. J. Pharmacol. 111, 797–802.

Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, V., Avenell, K.Y., Stean, T., Upton, N., Bromidge, S., Forbes, I.T., Brown, A.M., Middlemiss, D.N., Blackburn, T.P., 1997. SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. Neuropharmacology 36, 609–620.

Kennett, G.A., Lightowler, S., Trial, B., Bright, F., Bromidge, S., 2000. Effects of RO 60 0175, a 5-HT_{2C} receptor agonist, in three animal models of anxiety. Eur. J. Pharmacol. 387, 197–204.

Koek, W., Jackson, A., Colpaert, F.C., 1992. Behavioural pharmacology of antagonists at 5-HT_2/5-HT_{1C} receptors. Neurosci. Biobehav. Rev. 16, 95–105.

Koob, G.F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13, 177–184.

Lucki, I., Ward, H.R., Frazer, A., 1989. Effect of 1-(m-cholorophenyl)piperazine and 1-(m-trifluoromethylyphenyl)piperazine on locomotor activity. J. Pharmacol. Exp. Ther. 249, 155–164.

Martin, J.R., Ballard, T.M., Higgins, G.A., 2002. Influence of the 5-HT_{2C} receptor antagonist SB 242084, in tests of anxiety. Pharmacol. Biochem. Behav. 71, 615–625.

Mathé, J.M., Nomikos, F.F., Hildebrand, N.E., Hertel, P., Svensson, T.H., 1996. Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. Eur. J. Pharmacol. 309, 1–11.

Millan, M.J., Dekeyne, A., Gobert, A., 1998. Serotonin (5-HT)$_{2C}$ receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 37, 953–955.

Montgomery, A.M., Rose, I.C., Herberg, L.J., 1991. 5-$HT1_A$ agonists and dopamine: the effects of 8-OH-DPAT and buspirone on brain-stimulation reward. J. Neural. Transm. Gen. Sect. 83, 139–148.

Moser, P.C., Moran, P.M., Frank, R.A., Kehne, J.H., 1996. Reversal of amphetamine-induced behaviours by MDL 100,907, a selective 5-HT_{2A} antagonist. Behav. Brain Res. 73, 163–167.

Moukhles, H., Bosles, O., Bolam, J.P., Vallée, A., Umbriaco, D., Geffard, M., Doucet, G., 1997. Quantitative and morphometric data indicate precise cellular interactions between serotonin and postsysnaptic targets in rat substania nigra. Neuroscience 76, 1159–1171.

Neisewander, J.L., McDougall, S.A., Bowling, S.L., Bardo, M.T., 1990. Conditioned taste aversion and place preference with buspirone and gepirone. Psychopharmacology 100, 485–490.

Neisewander, J.L., O'Dell, L.E., Redmond, J.C., 1995. Localization of dopamine receptor subtypes occupied by intra-accumbens antagonists that reverse cocaine-induced locomotion. Brain Res. 671, 201–212.

Nisell, M., Nomikos, G.G., Hertel, P., Panagis, G., Svensson, T.H., 1996. Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. Synapse 22, 369–381.

Papp, M., Willner, P., 1991. 8-OH-DPAT-induced place preference and place aversion: effects of PCPA and dopamine antagonists. Psychopharmacology 103, 99–102.

Parsons, L.H., Weiss, F., Koob, G.F., 1998. Serotonin1B receptor stimulation enhances cocaine reinforcement. J. Neurosci. 18, 10078–10089.

Peltier, R., Schenk, S., 1993. Effects of serotonergic manipulations on cocaine self-administration in rats. Psychopharmacology 110, 390–394.

Pozzi, L., Acconcia, S., Ceglia, I., Invernizzi, R.W., Samanin, R., 2002. Stimulation of 5-hydroxytryptamine (5-HT_{2C}) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. J. Neurochem. 82, 93–100.

Risinger, F.O., Oakes, R.A., 1996. Mianserin enhancement of ethanol-induced conditioned place preference. Behav. Pharmacol. 7, 294–298.

Roberts, A.J., McArthur, R.A., Hull, E.E., Post, C., Koob, G.F., 1998. Effects of amperozide, 8-OH-DPAT, and FG 5974 on operant responding for ethanol. Psychopharmacology 137, 25–32.

Rocha, B., Di Scala, G., Jenck, F., Moreau, J.L., Sandner, G., 1993. Conditioned place aversion induced by 5-HT_{1C} receptor antagonists. Behav. Pharmacol. 4, 101–106.

Serrats, J., Mengod, G., Cortes, R., 2005. Expression of serotonin 5-HT2C receptors in GABAergic cells of the anterior raphe nuclei. J. Chem. Neuroanat. 29, 83–91.

Shippenberg, T.S., 1991. Conditioned reinforcing effects of 8-hydroxy-2-(di-*N*-propylamino) tetralin: involvement of 5-hydroxytryptamine 1A and D1 dopamine receptors. Neurosci. Lett. 121, 136–138.

Tomkins, D.M., Joharchi, N., Tampakeras, M., Martin, J.R., Wichmann, J., Higgins, G.A., 2002. An investigation of the role of 5-HT (2C) receptors in modifying ethanol self-administration behaviour. Pharmacol. Biochem. Behav. 71, 735–744.

Tzschentke, T.M., 1998. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog. Neurobiol. 56, 613–672.

Waddock, S.L., 1997. Behavioral and neurochemical effects of 5HT-related drugs in a model of mesolimbic dopamine hyperactivity. University of Alberta, Edmonton, Canada. Thesis.

Wise, R.A., Rompre, P.P., 1989. Brain dopamine and reward. Annu. Rev. Psychol. 40, 191–225.