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mCPP-induced hyperactivity in 5-HT_{2C} receptor mutant mice is mediated by activation of multiple 5-HT receptor subtypes☆

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Abstract

The serotonin receptor agonist mCPP induces hyperlocomotion in 5-HT_{2C} receptor knockout (KO) mice or in the presence of a 5-HT_{2C} receptor antagonist. In the present group of experiments, we evaluate the role of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A} receptors in mCPP-induced hyperactivity in 5-HT_{2C} KO mice. We also assess the ability of agonists at these receptors to induce hyperactivity in wildtype (WT) mice pre-treated with a selective 5-HT_{2C} receptor antagonist. As previously reported, mCPP (3 mg/kg) induced hyperactivity in 5-HT_{2C} KO mice. A combination of the 5-HT_{1B} receptor agonist CP-94,253 (20 mg/kg) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.5 mg/kg) induced marked hyperactivity in WT but not in 5-HT_{2C} KO mice, nor in mice treated with the selective 5-HT_{2C} receptor antagonist, SB 242084 (1.5 mg/kg). Neither CP-94,253 nor 8-OH-DPAT had any intrinsic effect on locomotion in WTs. mCPP-induced hyperactivity was attenuated in 5-HT_{2C} KO mice by the 5-HT_{1B} receptor antagonist SB 224289 (2.5 mg/kg), and the 5-HT_{2A} receptor antagonists ketanserin (0.3 mg/kg) and M100907 (0.01 mg/kg) but not by the 5-HT_{1A} receptor antagonist WAY 100635 (1 mg/kg). The 5-$HT_{2A/2B/2C}$ receptor agonist, Ro 60-0175 (3 mg/kg), induced a modest increase in locomotor activity in WT mice pre-treated with SB 242084. However, the combination of Ro 60-0175 with CP-94,253 induced a substantial increase in activity in 5-HT_{2C} KO mice, an effect comparable to mCPP-induced hyperactivity. Thus, joint activation of 5-HT_{1A} and 5-HT_{1B} receptors stimulates locomotion in WT mice but this response is dependent on a functional 5-HT_{2C} receptor population and hence is absent in 5-HT_{2C} KO mice. By contrast, mCPP-induced hyperactivity depends on the inactivation of a separate 5-HT_{2C} receptor population and is mediated by 5-HT_{2A} and 5-HT_{1B} receptor activation.

Keywords: 5-HT_{2C} mutant; 5-HT_{2C} antagonist; mCPP; Locomotor activity; 5-HT_{2A}; 5-HT_{1B}

1. Introduction

The non-selective serotonin agonist 1-(3-chlorophenyl)piperazine (mCPP) induces hypophagia (Kennett and Curzon, 1988a; Kitchener and Dourish, 1994), hypolocomotion (Kennett and Curzon, 1988b; Lucki et al., 1989) and anxiogenic-like behavioural responses (Kennett et al., 1989) in rodents. As many of these effects can be suppressed by antagonists selective for the 5-HT_{2C} subtype, mCPP has been used as a pharmacological tool for evaluating 5-HT_{2C} receptor function (Curzon and Kennett, 1990). However, in vitro studies demonstrate that mCPP also has affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2B} receptors (Hoyer, 1988; Hamik and Peroutka, 1989), and may release serotonin (Baumann et al., 1993). This suggests that the behavioural effects of mCPP could result from interactions with multiple 5-HT receptors (Fiorella et al., 1995; Callahan and Cunningham, 1994). Indeed, in drug discrimination trials, mCPP has been shown to partially substitute for the 5-HT_1 receptor agonist RU24969 (Gardner, 1989).

More recently, it has been reported that mCPP induces an apparently paradoxical *hyper*locomotion when administered to 5-HT_{2C} receptor null mutant (KO)

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mice (Heisler and Tecott, 2000). The observation that this effect in KO mice was abolished by pre-treatment with the 5-$HT_{1B/1D}$ antagonist GR 127935 led Heisler and Tecott to propose that the hyperlocomotion is mediated through an 'unmasking' of 5-HT_{1B} receptor activation. This hypothesis is consistent with the finding that mCPP-induced hyperlocomotion in mice pre-treated with a non-selective 5-HT_2 receptor antagonist can be attenuated by pre-treatment with a 5-HT_{1B} receptor antagonist (Gleason and Shannon, 1998). However, the additional finding that a 5-HT_{1A} receptor antagonist can also block this response suggests that multiple 5-HT receptors may be responsible (Gleason and Shannon, 1998). Reports that 5-HT_{2A} receptor antagonists can attenuate the hyperactivity induced by either cocaine or a combination of the 5-$HT_{2A/2B/2C}$ receptor agonist Ro 60-0175 and the 5-HT_{2C} receptor antagonist SB 242084 (Higgins et al., 2001) also suggests a potential role for 5-HT_{2A} receptor in mCPP-induced hyperlocomotion.

In the present paper, we directly assess the role of 5-HT_{1B} and 5-HT_{2C} receptors in the hyperlocomotion evoked by mCPP by comparing its effects with those of the selective 5-HT_{1B} receptor agonist, CP-94,253 alone or in combination with the 5-HT_{1A} receptor agonist 8-OH-DPAT in wildtype (WT) and 5-HT_{2C} receptor null mutant (KO) mice. In addition, we investigate the ability of the 5-HT_{2A} receptor antagonists M100907 and ketanserin to attenuate mCPP-induced hyperactivity in KO mice and measure locomotion in WT mice treated with a combination of the selective 5-HT_{2C} antagonist SB 242084 and the 5-$HT_{2A/2B/2C}$ receptor agonist Ro 60-0175 or the 5-$HT_{2A/2C}$ receptor agonist DOI. Finally, we investigate the ability of concurrent activation of 5-HT_{1B} and 5-HT_{2A} receptors to induce hyperactivity in KO mice in a manner comparable to that seen following mCPP administration.

2. Materials and methods

2.1. Animals

Initial breeding stocks of adult WT and 5-HT_{2C} KO mice of the C57BL/6J strain were kindly donated by L.H. Tecott UCSF, USA. WT males were crossed with females heterozygous for the 5-HT_{2C} mutation allowing WT and KO littermates to be used in each experiment. Genotyping was achieved by taking a 2 mm sample from the tail tip under local anaesthesia, with identification of WT or KO mice attained using a PCR-based strategy in Vernalis Research Ltd, Wokingham, UK. After weaning, male progeny were held in groups of 2–4 until required. The animals used in these studies were taken from group-housed stock immediately before each experiment. Animals were maintained in a controlled environment held at $21 \pm 1\ ^{\circ}C$ and $50 \pm 15\%$ relative humidity with 12:12 h photoperiod (lights on: 05:30 h). The experiments were licensed under the UK Animals (Scientific Procedures) Act 1986 (Project License 70/5033) following approval by the University of Sussex, Local Ethical Review Committee. All efforts were made to minimise animal suffering and to reduce the number of animals used.

2.2. Drugs

3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine (CP-94,253), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635), *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-flourophenylethyl)]-4-piperadine methanol (M100907), (S)-2-(6-Chloro-5-fluoroindol-1-yl)-1-methylethylamine (Ro 60-0175), 6-chloro-5-methyl-1-[2(2methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242084) and 6,7-dihydro-1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-3-yl)[1.1′-biphenyl]-4-yl]carconyl]-spiro-[2H-furo[2,3-f]indole-3(5H)hyrochloride (SB 224289) were synthesised in the Chemistry Department of Vernalis Research Ltd (Wokingham). 1-(3-Chlorophenyl)piperazine (mCPP), 8-hydroxy2-(di-*N*-propylamino)tetralin (8-OH-DPAT) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) were purchased from Sigma-Aldrich (Poole, UK) and 3-[2-[4-(4-flurobenzoyl)-1-piperidinyl]ethyl]-2,4[1H,3H]-quinazolinedione (ketanserin tartrate) was purchased from Tocris (Bristol, UK).

CP-94,253, ketanserin tartrate, WAY 100635 and DOI were dissolved in distilled water. mCPP, 8-OH-DPAT, M100907 and Ro 60-0175 were dissolved in 0.9% NaCl. SB 242084 was initially dissolved in PEG400 at 20% of the final required volume which was then made up with 10% (w/v) hydroxypropyl-β-cyclodextrin (Fluka, Poole, UK). SB 224289 was moistened with 150 μl 10% lactic acid (v/v in distilled water) and brought to final volume using 10% (w/v) hydroxypropyl-β-cyclodextrin.

mCPP, CP-94,253, 8-OH-DPAT, Ro 60-0175 and DOI were administered i.p. at a volume of 10 ml/kg. SB 242084, WAY 100635, SB 224289, M100907 and ketanserin were administered s.c. at the nape of the neck in a volume of 4 ml/kg.

Doses for mCPP and Ro 60-0175 were chosen with reference to studies previously reported by Hewitt et al. (2002). The dose for CP-94,253 was chosen with reference to Clifton et al. (2003). The doses of WAY 100635, DOI and ketanserin were chosen with reference to the studies reported by Gleason and Shannon (1997, 1998) and Darmani et al. (1996), respectively. SB 242084, 8-OH-DPAT, SB 224289 and M100907 doses were determined following preliminary dose response studies.

2.3. Locomotor activity

Mice were placed in circular runways (24 cm diameter) containing eight infrared light beams at 45° separation. Forward locomotor activity was assessed by counting instances of beam breaks in a consistent direction that occurred following three consecutive beam breaks in that direction. Spontaneous forward locomotor activity was recorded for a 60 min habituation period. For all agonist studies, a between subjects design was used. For studies using antagonists, a mixed design was used. Experiments attempting to *block* mCPP-induced hyperactivity had antagonist as a between subjects factor and agonist a within subjects factor. In experiments attempting to *induce* hyperactivity in WT mice, the 5-HT_{2C} antagonist SB 242084 was administered as a within subjects factor with agonists being a between subjects factor. Testing was conducted allowing at least two drug free days between treatments. Statistical significance was assessed by a three-way analysis of variance (genotype or antagonist× agonist × time) followed by paired comparisons using either Dunnett's procedure (within subjects design) or *t*-tests with Bonferroni correction (between subjects design).

3. Results

3.1. 5-HT_{1B} receptor stimulation in 5-HT_{2C} KO mice

mCPP (3 mg/kg) induced a significant and substantial (4.5-fold) increase in locomotor activity in 5-HT_{2C} KO mice (Fig. 1a) which was not apparent in WT mice given mCPP (genotype × drug interaction, $F_{1,12}= 24.65$; $p< 0.001$). Post-hoc analysis revealed that KO mice given mCPP were significantly more active than any other group 15 min following mCPP administration, a difference that remained significant throughout the testing period. In contrast, administration of the selective 5-HT_{1B} receptor agonist CP-94,253 (20 mg/kg) failed to increase locomotor activity in either genotype (Fig. 1b).

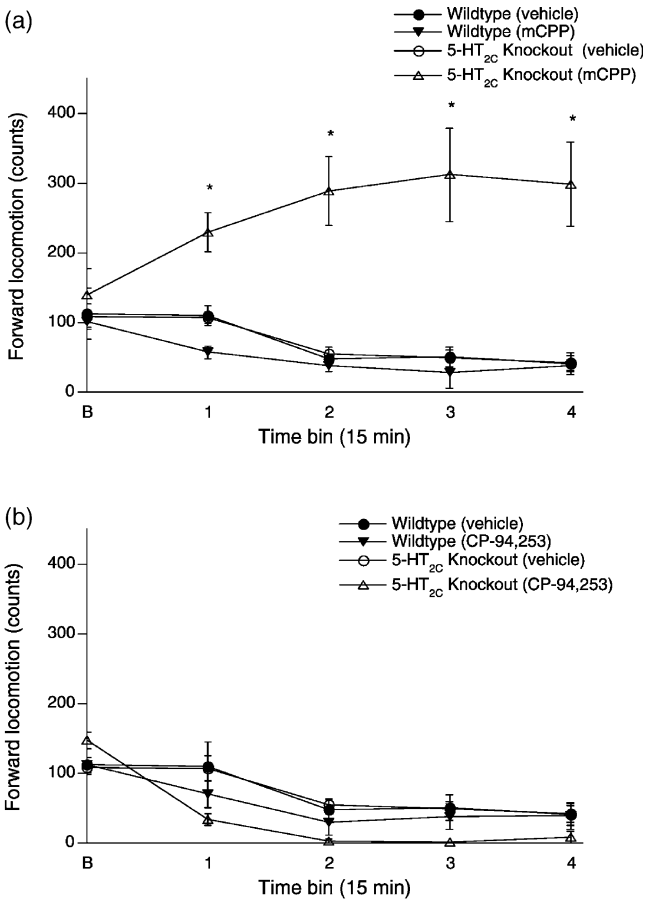

Fig. 1. Comparison of the effects of mCPP (a: top panel) and CP-94,253 (b: lower panel) on 5-HT_{2C} receptor null mutant (KO) and wildtype (WT) mice. Activity is expressed in 15 min time bins over a 60 min time period following administration of vehicle (0.9% saline) mCPP (3 mg/kg) or CP-94,253 (20 mg/kg). 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values are expressed as mean ± SEM, $^{*}p< 0.001$, $n= 7$.

3.2. Combined 5-HT_{1A} + 5-HT_{1B} receptor stimulation in WT and 5-HT_{2C} KO mice

When the 5-HT_{1B} receptor agonist CP-94,253 or the 5-HT_{1A} receptor agonist 8-OH-DPAT were given alone they had no effect on locomotor activity in either genotype (Fig. 2a,b). However, the combination of CP-94,253 and 8-OH-DPAT produced a significant increase in locomotor activity of wildtype mice ($F_{3,30}= 16.88$; $p< 0.001$, Fig. 2a) with total activity counts for the 1 h period being almost 8-times higher for combination treated mice than for vehicle treated counterparts. Post-hoc analysis confirmed that this increase was significant across all time bins. In contrast, the combination of CP-94,253 and 8-OH-DPAT had no effect on locomotor activity in KO mice (Fig. 2b; genotype × drug interaction, $F_{3,30}= 10.63$; $p< 0.001$). The same combination of drugs also had no effect on activity in WT mice pre-treated with the selective 5-HT_{2C} receptor antagonist SB 242084 (Fig. 2c).

3.3. Attenuation of mCPP-induced hyperactivity in 5-HT_{2C} knockout mice using 5-HT receptor antagonists

Pre-treatment with the 5-HT_{2A} receptor antagonists M100907 (0.01 mg/kg) and ketanserin (Fig. 3) and the selective 5-HT_{1B} receptor antagonist SB 224289 (Fig. 4) significantly attenuated hyperactivity in KO mice given mCPP by 46%, 44% and 46%, respectively (agonist× antagonist interaction, $F_{5,48}= 5.07$; $p< 0.001$). Pre-treatment with WAY 100635 (Fig. 4) and the lower dose of M100907 (0.003 mg/kg) (Fig. 3) had no signifi-

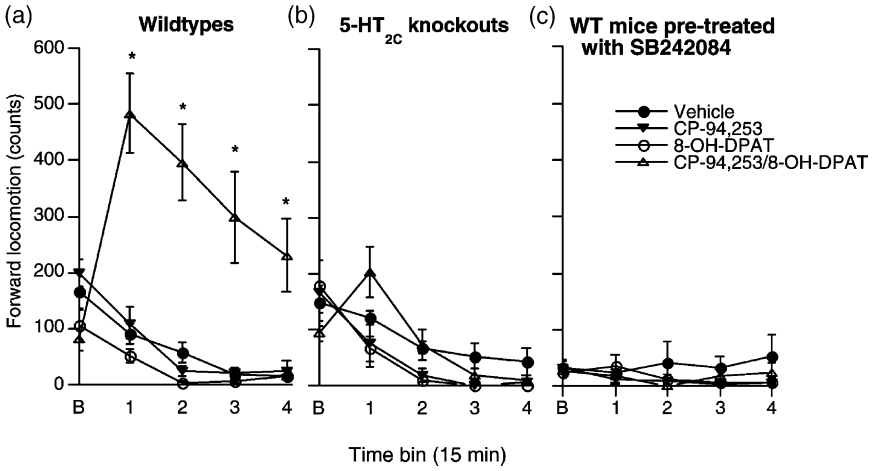

Fig. 2. Forward locomotor activity for WT mice (a: left panel), KO mice (b: middle panel) and WT mice pre-treated with 1.5 mg/kg SB 242084 (c: right panel) following administration of CP-94,253 (20 mg/kg) and 8-OH-DPAT (0.5 mg/kg) alone or in combination. Activity is expressed in 15 min time bins over a 60 min time period following administration of drugs. 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values expressed as mean ± SEM, $^{*}p< 0.001$, $n= 6$.

cant effect on mCPP-induced hyperactivity in KO mice. None of the antagonists had any intrinsic affect on locomotor behaviour ($n= 6$, data not shown). In a separate experiment, pre-treatment with WAY 100635 (1 mg/kg) successfully attenuated CP-94, 253 + 8-OH-DPAT-induced hyperactivity in WT mice, with average locomotor counts per 15 min time period falling by 71% in WAY 100635 pre-treated conditions ($p< 0.01$).

3.4. *Effects of 5-HT$_{2C/2A}$ receptor agonists on locomotor activity in wildtype mice pre-treated with the selective 5-HT$_{2C}$ receptor antagonist SB 242084*

The 5-HT$_{2A/2B/2C}$ receptor agonist Ro 60-0175 when given in combination with the selective 5-HT$_{2C}$ receptor antagonist SB 242084 significantly increased (2.6-fold) locomotor activity (agonist× antagonist interaction, $F_{5,50}= 4.02$; $p< 0.01$; Fig. 5). While DOI, in the presence of SB 242084, tended to increase locomotor activity, this effect did not reach significance at the dose used. Neither Ro 60-0175 nor DOI had an intrinsic effect on locomotor activity (mean count per 15 min time bin being 8.38, 10.92 and 21.46 for vehicle/vehicle, vehicle/Ro 60-0175 and vehicle/DOI treated mice, respectively; main agonist effect $F_{5,50}= 1.47$, ns).

3.5. *Effects of simultaneous activation of* 5-HT$_{1B}$ + 5-HT$_{2A/2B/2C}$ *receptors on locomotor activity in WT and 5-HT$_{2C}$ KO mice*

Co-administration of the 5-HT$_{2A/2B/2C}$ receptor agonist, Ro 60-0175 with the 5-HT$_{1B}$ receptor agonist, CP-

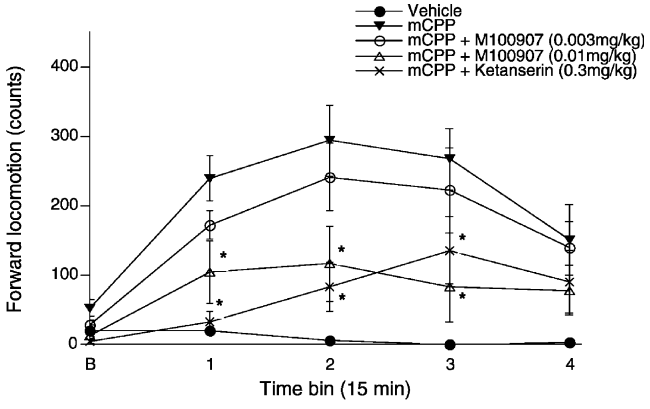

Fig. 3. Forward locomotor activity for KO mice given 3 mg/kg mCPP and pre-treated with WAY 100635 (1 mg/kg) or SB 224289 (2.5 mg/kg). Activity is expressed in 15 min time bins over a 60 min time period following administration of mCPP. 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values expressed as mean ± SEM, $^{*}p< 0.001$, $n= 6$.

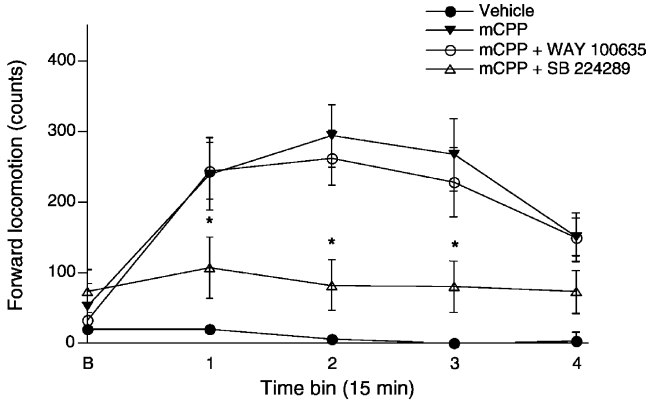

Fig. 4. Effect of the 5-HT$_{2A}$ receptor antagonists M100907 (0.03 and 0.01 mg/kg) and ketanserin (0.3 mg/kg) on mCPP-induced hyperactivity in KO mice. Activity is expressed in 15 min time bins over a 60 min time period following administration of mCPP. 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values expressed as mean ± SEM, $^{*}p< 0.001$, $n= 6$.

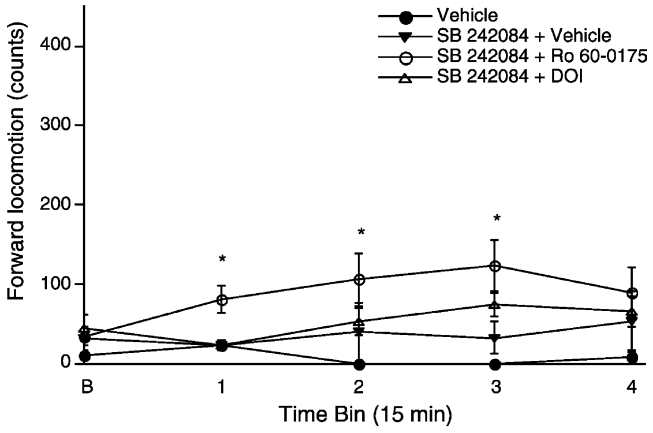

Fig. 5. Forward locomotor activity for WT mice pre-treated with 1.5 mg/kg SB 242084 and given either Ro 60-0175 (3 mg/kg) or DOI (0.3 mg/kg). Activity is expressed in 15 min time bins over a 60 min time period following administration of mCPP. 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values expressed as mean ± SEM, $^{*}p< 0.001$, $n= 6$.

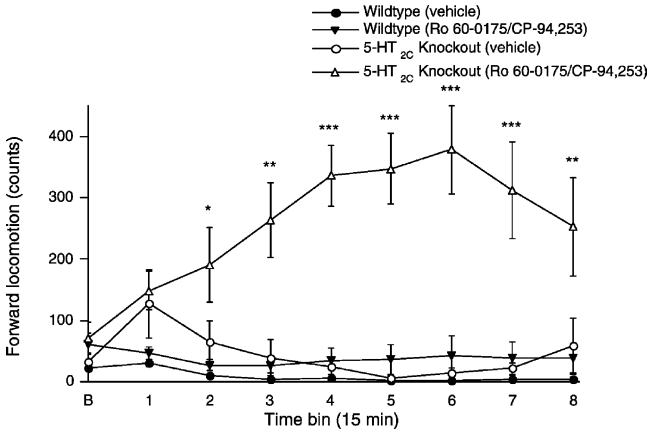

Fig. 6. Forward locomotor activity for WT and KO mice following administration of Ro 60-0175 (3 mg/kg) and CP-94,253 (20 mg/kg) in combination. Activity is expressed in 15 min time bins over a 120 min time period following administration of drugs. 'B' on the *X*-axis refers to baseline activity measured during the last 15 min of habituation. Values expressed as mean ± SEM, $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$, $n= 6$.

94,253 significantly and substantially increased locomotor activity in 5-HT_{2C} KO mice (10.7-fold), an effect that was not apparent in WT mice given the same treatment (genotype × drug interaction, $F_{1,10}= 11.54$; $p< 0.01$). Post-hoc analysis revealed that KO mice given Ro 60-0175 and CP-94,253 in combination were significantly more active than any other group 30 min following drug administration, a difference that remained significant throughout the testing period (see Fig. 6).

4. Discussion

Our data confirm reports that mCPP induces hyperactivity in 5-HT_{2C} receptor mutant mice (Heisler and Tecott, 2000). We have also demonstrated that mCPP induces a similar hyperlocomotor response in wildtype mice pre-treated with a selective 5-HT_{2C} receptor antagonist. In agreement with previous studies (Gleason and Shannon, 1998), joint activation of 5-HT_{1A} and 5-HT_{1B} receptors induced a substantial hyperlocomotion in WT mice but unexpectedly, in the present study, this effect was absent in both 5-HT_{2C} KO mice and in mice pre-treated with the selective 5-HT_{2C} receptor antagonist SB 242084. In addition, neither the 5-HT_{1A} nor the 5-HT_{1B} receptor agonists had any intrinsic effect on locomotor activity in either genotype. Furthermore, mCPP-induced hyperactivity in 5-HT_{2C} KO mice was attenuated in a dose dependent manner by the selective 5-HT_{1B} receptor antagonist SB 224289, by the selective 5-HT_{2A} receptor antagonist M100907 and the less selective 5-HT_{2A} receptor antagonist ketanserin. Data from WT mice pre-treated with the selective 5-HT_{2C} receptor antagonist, SB 242084, demonstrated increased locomotion following administration of the mixed 5-$HT_{2A/2B/2C}$ agonist, Ro 60-0175, while DOI induced a non-significant increase in activity in the same animals. These data suggest that mCPP-induced hyperactivity in 5-HT_{2C} KO mice is not attributable solely to its agonist activity at the 5-HT_{1B} receptor but that the 5-HT_{2A} or the 5-HT_{2B} receptors also may play a pivotal role. Thus, in a final experiment, 5-HT_{2C} KO mice demonstrated a substantial hyperlocomotor response following concurrent stimulation of 5-HT_{1B} and 5-$HT_{2A/2B}$ receptors by CP-94,253 and Ro 60-0175 administration.

These data suggest that hyperactivity induced in WT mice following joint activation of 5-HT_{1A} and 5-HT_{1B} receptors is dependent on a population of operative 5-HT_{2C} receptors. They also suggest that mCPP-induced hyperactivity in 5-HT_{2C} KO mice occurs as the result of concurrent stimulation of 5-HT_{1B} and 5-$HT_{2A/2B}$ receptors.

Activation of the mesolimbic dopamine (DA) system has long been implicated in stimulating locomotor activity (Mogenson et al., 1993). With respect to the present study, the ability of mCPP to induce hyperlocomotion in KO mice is consistent with facilitation of mesolimbic dopamine activity in these animals. In keeping with this idea, Rocha et al. (2002) have shown that 5-HT_{2C} receptor null mutant mice show an increased sensitivity to the behavioural effects of cocaine as well as enhanced levels of DA in the nucleus accumbens following cocaine administration. These results suggested that loss of the 5-HT_{2C} receptor disinhibits mesolimbic DA efflux following cocaine administration. Accordingly, recent neurochemical evidence

has implicated 5-HT_{2C} receptors in the modulation of mesoaccumbens DA projections (Di Matteo et al., 2001). This modulation may occur either at the level of the pre-frontal cortex (Filip and Cunningham, 2003) or the ventral tegmental area (VTA) (Eberle-Wang et al., 1997; Gobert et al., 2000; Di Giovanni et al., 2001).

It has been proposed that mCPP induces hyperlocomotion in 5-HT_{2C} KO mice through activation of 5-HT_{1B} receptors (Heisler and Tecott, 2000). This hypothesis is consistent with evidence that 5-HT_{1B} receptor activation can potentiate the locomotor and reinforcing effects of cocaine and enhances cocaine-induced increases in mesolimbic DA transmission (Cameron and Williams, 1994; Parsons et al., 1998). However, results from the present group of experiments demand a more complex explanation. Although the 5-HT_{1B} receptor antagonist SB 224289 significantly attenuated mCPP-induced hyperactivity in KO mice, the selective 5-HT_{1B} receptor agonist CP-94,253, at a dose higher than has previously been demonstrated to be behaviourally effective (Fish et al., 1999; Przegalinski et al., 2001; Clifton et al., 2003), had no effect on locomotion in the same mice. This suggests that 5-HT_{1B} receptor activation alone is insufficient to induce hyperactivity, and that mCPP may act on other 5-HT receptor subtypes. Indeed, the finding that mCPP-induced hyperlocomotion in WT mice pre-treated with the non-selective 5-HT_2 antagonist, LY53857, can also be attenuated with the 5-HT_{1A} antagonist WAY 100635 (Gleason and Shannon, 1998) is consistent with this idea. Further evidence comes from a report that the 5-$HT_{1A/1B}$ receptor agonist RU24969 induces marked hyperactivity in rats (O'Neill and Parameswaran, 1997) and that mCPP can partially substitute for RU24969 in drug discrimination trials (Gardner, 1989). The actions of 5-HT_{1A} receptors on locomotor activity may also be mediated by its interactions with the mesolimbic DA system. Evidence for this hypothesis comes from findings that extracellular DA levels in the VTA and nucleus accumbens are increased following systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Chen and Reith, 1995; Boulenguez et al., 1996).

In light of these considerations, we sought to determine if mCPP exerted its effects in KO mice as a result of dual activation of 5-HT_{1A} and 5-HT_{1B} receptors. Co-administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT with CP-94,253 induced a significant increase in locomotor activity in WT mice. These data suggest that activation of both 5-HT_{1A} and 5-HT_{1B} receptors is necessary for hyperlocomotion and is consistent with data from Gleason and Shannon (1998) for WT mice. While 8-OH-DPAT administration alone has been demonstrated to increase ambulation scores in rats (Tricklebank et al., 1984), this effect was not observed in the present studies. This difference can be accounted for in that, in the previous study, ambulation was scored as number of quadrants entered which scores small movements and does not reflect true forward locomotion. The absence of any hyperactivity following 8-OH-DPAT administration is also in agreement with observations that the same compound (0.03–1 mg/kg) suppresses exploratory behaviours in mice (Tsuji et al., 2000), while doses as low as 0.1 mg/kg (Evenden and Angeby-Moller, 1990) and as high as 5 mg/kg (Chojnacka-Wojcik, 1992) have been demonstrated to suppress spontaneous locomotor activity in mice. Surprisingly, while dual activation of 5-HT_{1A} and 5-HT_{1B} receptors greatly enhanced locomotor activity in WT mice, this effect was completely absent in KO mice. The fact that the hyperlocomotion evident in WT mice given 8-OH-DPAT and CP-94,253 in combination, was not apparent in WT mice pre-treated with the selective 5-HT_{2C} receptor antagonist SB 242084, suggests that this lack of effect in KO mice is not attributable to developmental abnormalities arising from gene knockout. Taken together, these results suggest that hyperactivity induced by co-stimulation of 5-HT_{1A} and 5-HT_{1B} receptors is dependent on the presence of a population of functional 5-HT_{2C} receptors.

The above observations are consistent with those of McMahon et al. (2001) who showed that infusion of the 5-HT_{2C} receptor antagonist RS 102221 into the nucleus accumbens shell blocks cocaine-induced hyperactivity, whereas, the 5-$HT_{2A/2B/2C}$ receptor agonist Ro 60-0175 enhances this effect when administered to the same site (Filip and Cunningham, 2002). However, it is of interest to note that administration of 5-HT_{2C} receptor antagonists either systemically (McCreary and Cunningham, 1999) or directly into the pre-frontal cortex (Filip and Cunningham, 2003) can have the diametrically opposite effect and can potentiate cocaine-induced hyperactivity. In addition, administration of the 5-$HT_{2C/2B}$ antagonist SB 206553 into the VTA has been demonstrated to increase basal firing activity of mesolimbic DA projections (Di Giovanni et al., 1999). In light of these considerations, it seems plausible that there exist two distinct populations of 5-HT_{2C} receptors that are anatomically distinct and have opposing roles in the modulation of mesolimbic dopamine. As such, the present data suggests that hyperactivity induced by 5-$HT_{1A/1B}$ receptor activation is dependent to some extent on tonic 5-HT_{2C} receptor tone, and that in the absence of functional 5-HT_{2C} receptors in the nucleus accumbens shell, activation of additional receptors is necessary. Thus, in the present study, the observation that the selective 5-HT_{1A} receptor antagonist, WAY 100635, failed to attenuate mCPP-induced hyperactivity in KO mice, at a dose that reverses CP-94, 253 + 8-OH-DPAT-induced hyperactivity in WT mice, suggests that activation of 5-HT_{1A} receptors is not involved in mCPP-induced hyperactivity in KO

mice. Given the in vitro profile of mCPP, it is possible that the behavioural effects of this compound in KO mice arise as a result of its actions at multiple 5-HT receptors. As such, we propose that mCPP induces hyperlocomotion in KO's through an additional mechanism beyond 5-$HT_{1A/1B}$ receptor activation.

mCPP has been reported to have an additional affinity for the 5-HT_{2A} receptor subtype. Vickers et al. (2001) reported a robust mCPP-induced head-twitch response in rats pre-treated with the selective 5-HT_{2C} receptor antagonist SB 242084, an effect that was abolished upon administration of the selective 5-HT_{2A} receptor antagonist M100907. Given the low efficacy of mCPP at the rat 5-HT_{2A} receptor (Vickers et al., 2001) this may indicate the presence of a population of 5-HT_{2A} receptors with a high receptor reserve or could suggest indirect activation of 5-HT_{2A} receptors via enhanced 5-HT release (Baumann et al., 1993). Thus, we hypothesised that mCPP may induce hyperlocomotion in KO mice through activation of 5-HT_{2A} receptors.

Evidence that $5HT_{2A}$ receptors may play a role in modulation of behaviours mediated by the mesolimbic DA system comes from ultrastructural anatomical studies which have shown that $5HT_{2A}$ receptors are localised on DA neurons in the ventral tegmental area (Doherty and Pickel, 2000). Moreover, both MDMA-induced DA release and cocaine-induced hyperactivity are attenuated by administration of the 5-HT_{2A} receptor antagonist M100907 (Schmidt et al., 1995; O'Neill et al., 1999; McMahon and Cunningham, 2001). In light of these considerations, it was of interest to assess whether blockade of 5-HT_{2A} receptors with ketanserin or M100907 could attenuate mCPP-induced hyperlocomotion in KOs. The fact that both antagonists attenuated mCPP-induced hyperactivity in KOs suggests that the 5-HT_{2A} receptor may indeed play a pivotal role in mCPP-induced hyperactivity in the absence of 5-HT_{2C} receptor function. As such, in contrast to previous studies excluding a role for the 5-HT_{2A} receptor in some mCPP-induced behaviours (Kennett and Curzon, 1988b; Kennett et al., 1989; Callahan and Cunningham, 1994; Gleason et al., 2001), these data provide evidence that mCPP can elicit appreciable stimulation of this receptor subtype in vivo.

As our antagonist challenge studies suggested a role for the 5-HT_{2A} receptor in mediating mCPP-induced hyperactivity, we sought to determine if stimulation of 5-HT_{2A} receptors alone was sufficient to induce hyperactivity. Wildtype animals were therefore pre-treated with the selective 5-HT_{2C} receptor antagonist, SB 242084, and were subsequently administered Ro 60-0175, a drug shown previously to have affinity for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (Higgins et al., 2001; Vickers et al., 2001). In keeping with the above mentioned findings, administration of this 5-$HT_{2A/2B/2C}$ agonist, in the presence of 5-HT_{2C} receptor blockade significantly increased locomotor activity in WT mice. While this effect was statistically significant, locomotor counts did not approach the levels seen in WT mice given mCPP in the presence of SB 242084. Although Ro 60-0175 demonstrates an appreciable affinity for the 5-HT_{2B} receptor (Vickers et al., 2001), administration of the 5-HT_{2B} receptor agonist BW723C86 does not affect locomotor activity in either the rat (Kennett et al., 1996) or the mouse (Nic Dhonnchadha et al., 2003). In addition, the absence of 5-HT_{2B} receptor expression in the striatum of the mouse brain (Choi and Maroteaux, 1996) further suggests that these effects of Ro 60-0175 are mediated by 5-HT_{2A} receptor activation.

While there remains an array of evidence suggesting that under basal conditions 5-HT_{2A} antagonism has little effect on mesolimbic DA transmission, recent evidence suggests that under conditions of increased DA activity, the 5-HT_{2A} receptor can facilitate DA release (see McMahon and Cunningham, 2001 for discussion). Given the known ability of 5-HT_{1B} agonists to increase DA efflux and the observation that mCPP-induced hyperactivity can also be attenuated by pre-treatment with the 5-HT_{1B} antagonists GR 127935 and SB 224289 we hypothesise that mCPP-induced hyperactivity occurs as the result of a 5-HT_{2A} receptor mediated potentiation of 5-HT_{1B} induced increases in mesolimbic DA efflux. The substantial increase in activity demonstrated here in 5-HT_{2C} KO mice following concurrent stimulation of 5-HT_{1B} and 5-HT_{2A} receptors by a CP-94,253/Ro 60-0175 cocktail provides initial pharmacological evidence in support of this hypothesis.

The complex interactions between 5-HT_{2C} receptor antagonists and drugs that interact with other serotonin receptor subtypes may have important clinical significance. Many atypical antipsychotic drugs, including clozapine, olanzapine and risperidone are antagonists at 5-HT_{2C} receptors (Bergqvist et al., 1999; Bymaster et al., 1999). The effects of 5-HT_{2C} receptor antagonism, in combination with other aspects of the pharmacology of these drugs may be of importance in their generally more favourable impact on the negative symptoms of schizophrenia. Selective 5-HT_{2C} receptor antagonists are also under consideration as potential treatments for anxiety (Kennett et al., 1997; Martin et al., 2002) and depression (Loo et al., 2002). It will be of particular importance to explore the potential interactions of these compounds with other drugs that may be co-administered in such situations.

In summary, we have demonstrated that mCPP-induced hyperactivity in 5-HT_{2C} receptor mutant mice is dependent upon *both* 5-HT_{1B} and 5-HT_{2A} receptor stimulation and that the absence of an inhibitory influence of 5-HT_{2C} receptors is central to this effect. We have also shown that while 5-HT_{1B} receptor stimulation is essential for mCPP-induced hyperactivity in

KO mice it is not sufficient in itself to increase locomotor activity in these animals. In addition, our data demonstrates that the 5-HT_{1A} receptor does not play a role in mCPP-induced hyperactivity in KO mice. In contrast, we have also shown that dual activation of 5-HT_{1A} and 5-HT_{1B} receptors results in a substantial increase in locomotor activity, but that this effect *is* dependent on a population of functional 5-HT_{2C} receptors.

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