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ORIGINAL INVESTIGATION

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Serotonin 1B and 2C receptor interactions in the modulation of feeding behaviour in the mouse

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Abstract *Rationale:* This study was conducted to examine the functional relationship between 5-HT_{1B} receptors (5-HT_{1B}-R) and 5-HT_{2C} receptors (5-HT_{2C}-R) in the control of food intake. *Objectives:* The aim of this study was to compare the hypophagic effect of the 5-$HT_{2C/1B}$-R agonist *m*-chlorophenylpiperazine (mCPP) with that of the selective 5-HT_{1B}-R agonist CP-94,253 in both wild-type (WT) and 5-HT_{2C} knockout (KO) mice. *Methods:* The hypophagic effects of mCPP (1, 3 and 5.6 mg/kg) and CP-94,253 (5, 10 and 20 mg/kg) were assessed in WT and 5-HT_{2C} KO mice using the behavioural satiety sequence paradigm. The effects of pretreatment with the selective 5-HT_{2C}-R antagonist SB 242,084 (0.5 and 1.5 mg/kg) were assessed in WT mice given mCPP or CP-94,253. *Results:* The 5-$HT_{2C/1B}$ receptor agonist mCPP and the selective 5-HT_{1B} receptor agonist CP-94,253 both suppressed food intake in WT mice. 5-HT_{2C} KO mice were insensitive to the hypophagic effects of mCPP but were more sensitive to CP-94,253-induced hypophagia than WT controls. mCPP induced a significant increase in postprandial activity in 5-HT_{2C} KO mice but this effect was absent in 5-HT_{2C} KO mice given CP-94,253. Data from WT mice pretreated with the 5-HT_{2C} receptor antagonist SB 242,084 and then challenged with either mCPP or CP-94,253 were similar to those obtained from 5-HT_{2C} KO mice. *Conclusions:* 5-HT_{2C}-R and 5-HT_{1B}-R activation are each sufficient to induce a hypophagic response. However, concurrent 5-HT_{2C}-R inactivation can potentiate the hypophagic response to 5-HT_{1B}-R activation, consistent with an inhibitory role for the 5-HT_{2C}-R in behaviour mediated by activation of other 5-HT receptors. These results also confirm that 5-HT_{1B}-R activation alone cannot account for the hyperactive response of 5-HT_{2C} KO mice to mCPP.

Keywords 5-HT_{2C} receptor · 5-HT_{1B} receptor · mCPP · CP-94,253 · Feeding · Hyperactivity

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Introduction

It has long been established that activation of the serotonergic system results in the suppression of feeding behaviour (Blundell 1977). The appetite suppressant *d*-fenfluramine, withdrawn from clinical use in 1997, causes the release of 5-HT from nerve terminals while also functioning as a potent inhibitor of the 5-HT reuptake transporter (Garattini et al. 1986). Studies using both selective and non-selective 5-HT receptor antagonists have suggested that the 5-HT_{1B} and 5-HT_{2C} receptor subtypes are primary candidates for mediating the inhibitory action of serotonin on feeding behaviour (Kennett and Curzon 1988; Dourish et al. 1989). However, the question of whether these two receptors influence food intake in a cooperative or in an independent manner remains to be clarified.

Evidence in support of a role for the 5-HT_{1B} receptor in central modulation of food intake and satiety comes from both pharmacological and anatomical data. Administration of the selective 5-HT_{1B} receptor agonist, CP-94,253, reduces food intake and advances the behavioural satiety sequence in rats (Halford and Blundell 1996) and mice (Lee et al. 2004a) as well as producing a dose-related decrease in intake of both food pellets and sucrose solutions in rats (Lee and Simansky 1997). In addition, the mixed

5-$HT_{1A/1B}$ receptor agonist RU24969 reduces feeding in mice (Lucas et al. 1998) and rats (Simansky and Vaidya 1990; Kitchener and Dourish 1994), as well as increasing c-fos immunoreactivity expression in the paraventricular nucleus of the hypothalamus, the central amygdaloid nucleus and in the bed nucleus of the stria terminalis (Lucas et al. 1998). Finally, activation of 5-HT_{1B} receptors in the lateral parabrachial nucleus of the pons has been shown to underlie the hypophagic effects of the selective 5-HT_{1B} receptor agonist CP-93,129 (Lee et al. 1998) as well as *d*-fenfluramine (Simansky and Nicklous 2002).

There is equally compelling evidence in support of a role for the 5-HT_{2C} receptor in central control of food intake. For example, the nonselective 5-HT_2 receptor agonist Ro 60-0175 suppresses feeding in rats and mice across a number of behavioural paradigms, including meal pattern analysis (Clifton et al. 2000), the behavioural satiety sequence (Hewitt et al. 2002) and chronic infusions studies (Vickers et al. 2000). The 5-$HT_{2C/2A}$ receptor agonist ORG 37,684 also inhibits food intake in both free-feeding (Schreiber and De Vry 2002) and operant paradigms (De Vry et al. 2003). The fact that the hypophagic effects of both Ro 60-0175 and ORG 37,684 can be suppressed by pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242,084 suggests that these compounds induce hypophagia as the result of their affinity for 5-HT_{2C} receptors. The more recently described selective 5-HT_{2C} receptor agonist YM348 also potently suppresses food intake in rats following a 20-h food deprivation (Hayashi et al. 2004).

It has been suggested that the mixed 5-$HT_{1B/2C}$ receptor agonist, *m*-chlorophenylpiperazine (mCPP), induces a more potent hypophagic response in rodents than other serotonergic compounds as the result of its affinity for both 5-HT_{1B} and 5-HT_{2C} receptors (Kennett and Curzon 1988; Kitchener and Dourish 1994). However, more recent studies suggest that the hypophagic effect of mCPP is mediated primarily by its affinity for, and agonist activity at, the 5-HT_{2C} receptor. There have been a number of reports of a significant attenuation of mCPP-induced hypophagia in mice pretreated with the selective 5-HT_{2C} receptor antagonist SB 242,084, with little attenuation apparent following pretreatment with the selective 5-HT_{1B} receptor antagonist GR 127,935 (Hewitt et al. 2002; Clifton et al. 2003). Reports that the hypolocomotor (Kennett et al. 1994, 1997; Jones et al. 2002), discriminative stimulus (Gommans et al. 1998) and anxiogenic-like (Jones et al. 2002) behavioural effects of mCPP can be blocked by pretreatment with 5-HT_{2C} receptor antagonists lend further support to assertions that mCPP acts in vivo as a preferential 5-HT_{2C} receptor agonist. However, in direct contrast to this conclusion, Schreiber and De Vry (2002) report that the selective 5-HT_{2C} receptor antagonist SB 242,084 failed to attenuate mCPP-induced hypophagia in female rats, whereas the non-selective 5-HT antagonist metergoline successfully attenuated this effect in the same study. These authors suggested that given the relatively high affinity of metergoline for both 5-HT_{2C} and 5-HT_{1B} receptors (Boess and Martin 1994), mCPP-induced hypophagia is mediated by 5-HT_{1B} receptor activation.

The apparent inconsistency between these reports could be explained by a more recent report that mCPP induces a hyperactive response in mice lacking functional 5-HT_{2C} receptors [5-HT_{2C} knockout (KO) mice; Heisler and Tecott 2000]. The observation that this hyperactive response to mCPP was blocked in 5-HT_{2C} KO mice by pretreatment with the selective 5-HT_{1B} receptor antagonist GR127935 led the authors to propose that in wild-type (WT) animals, mCPP acts as a preferential 5-HT_{2C} receptor agonist and that, in the absence of functional 5-HT_{2C} receptors, the affinity of mCPP for 5-HT_{1B} receptors is expressed in the form of increased locomotion. Interestingly, in a separate study, Heisler et al. (1998) report that mCPP (5 mg/kg) failed to suppress food intake in 5-HT_{2C} KO mice. The absence of a 5-HT_{1B}-mediated hypophagic effect of mCPP in 5-HT_{2C} KO mice in that study could be explained by the existence of a common hypophagic pathway where the presence of a population of functional 5-HT_{2C} receptors is required for 5-HT_{1B}-mediated hypophagia to be expressed. This hypothesis predicts that a selective 5-HT_{1B} receptor agonist would fail to suppress food intake in 5-HT_{2C} KO mice.

The aim of the present group of experiments was to investigate the functional relationship between 5-HT_{1B} and 5-HT_{2C} receptors in feeding behaviour and satiety and, in particular, to investigate whether the hypothesis that the hypophagic effects of 5-HT_{1B}-R and 5-HT_{2C}-R activation are mutually dependent is correct. We initially determined whether a high dose of mCPP would be capable of suppressing food intake in the absence of functional 5-HT_{2C} receptors. Thus, in our first experiment we established a dose–response effect of mCPP in WT and 5-HT_{2C} KO mice in the behavioural satiety sequence, a rodent model of satiety (Antin et al. 1975; Halford et al. 1998). Previous data suggest that mCPP-induced hyperactivity in 5-HT_{2C} KO mice arises through the ability of mCPP to activate *both* 5-HT_{1B} and 5-HT_{2A} receptors (Dalton et al. 2004). Therefore, it is possible that the agonist activity of mCPP at the 5-HT_{1B} receptor is not sufficient in itself to induce a hypophagic effect. In light of the hypothesis outlined above, a second group of experiments was conducted in order to determine if a more selective 5-HT_{1B} receptor agonist would exert a hypophagic effect in 5-HT_{2C} KO mice. Thus, using the behavioural satiety sequence, the dose–response relationship of the selective 5-HT_{1B} receptor agonist CP-94,253 (Koe et al. 1992) was established in WT and 5-HT_{2C} KO mice.

Although the use of genetically modified mice has several advantages in pharmacological research, developmental compensation for gene knockout is an issue that must always be considered (e.g. Clifton et al. 2003). Evidence indicating the extent of developmental compensation can be obtained by a comparison of the consequences of receptor inactivation with acute and selective pharmacological blockade of that receptor. Thus, in a group of parallel experiments, we address the possibility of developmental compensation in 5-HT_{2C} KO mice by comparing behaviours induced in these animals by mCPP

and CP-94,253 to those in WT mice pretreated with the selective 5-HT_{2C} receptor antagonist, SB 242,084.

Materials and methods

Subjects

Initial breeding stocks of adult WT and 5-HT_{2C} KO mice of the C57BL/6J strain were provided by L.H. Tecott (UCSF, USA). WT males were crossed with females heterozygous for the 5-HT_{2C} mutation, allowing WT and 5-HT_{2C} 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 5-HT_{2C} KO mice attained using a PCR-based strategy in the Molecular Biology Department, Vernalis Research Ltd, Wokingham, UK. After weaning, male progeny were held in groups of two to four until required.

Animals were maintained in a controlled environment held at 21±1°C and 50±15% relative humidity with 12:12-h photoperiod (lights on 0530 hours). There were 15–20 air changes per hour. Animals were allowed free access to standard laboratory chow (BeeKay Foods, UK) and tap water. Mice were checked and handled daily and home cages were cleaned out once a week.

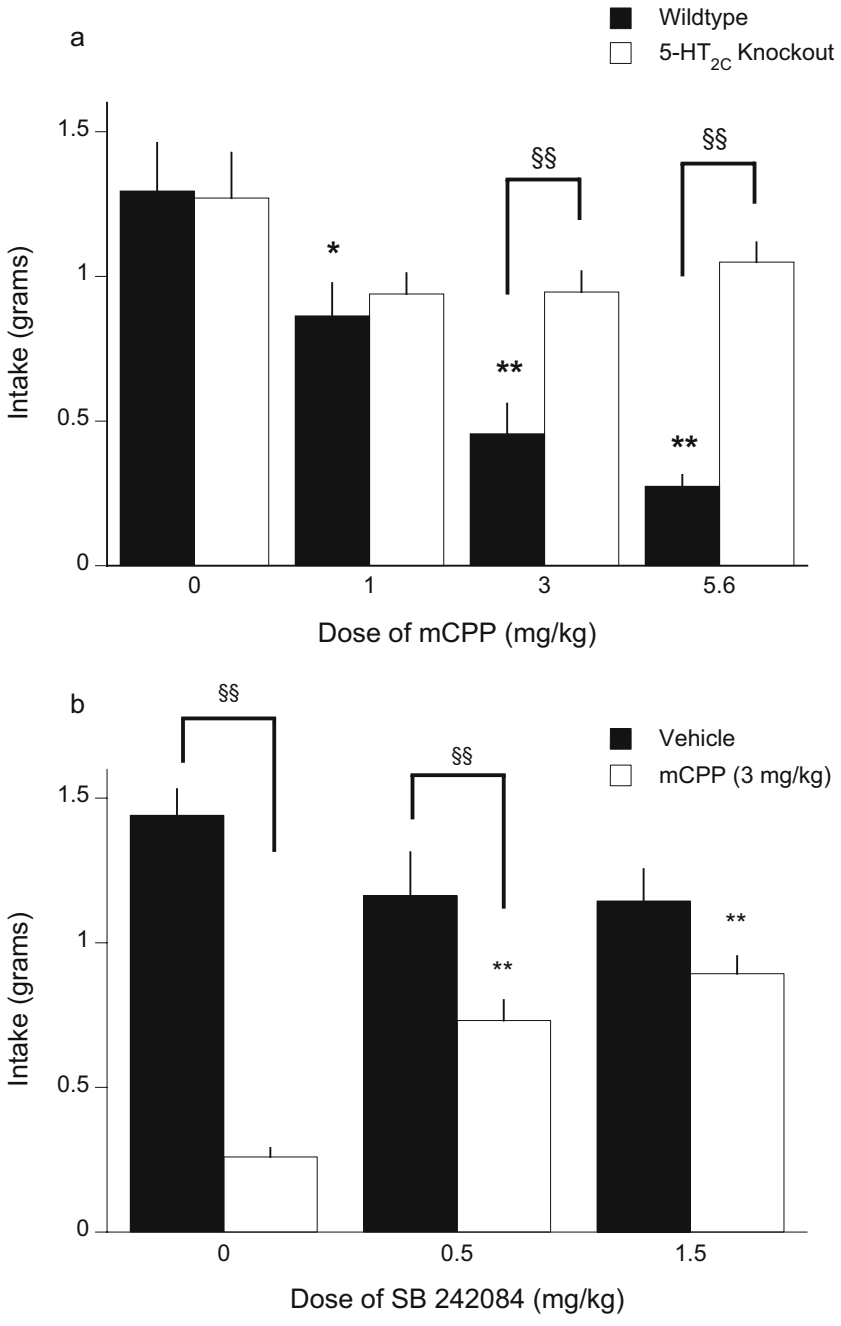

Fig. 1 **a** Dose-related effects of mCPP administration on wet mash intake in WT and 5-HT_{2C} KO mice over a 40-min time period. Values are expressed as mean±SEM. $^{§§}p<0.01$ across genotype comparison; $*p<0.05$, $**p<0.01$ to relevant control. **b** Effects of mCPP (3 mg/kg) alone or in combination with SB 242084 (0.5 and 1.5 mg/kg) on wet mash intake in WT mice over a 40-min period. Values expressed as mean±SEM. Significant differences represented as $**p<0.01$ compared to mCPP treatment alone and $^{§§}p<0.01$ across agonist comparison

Four weeks prior to the testing phase, male mice (30–45 g) were singly housed in solid-bottomed cages (North Kent Plastics, type M2) and moved to the experimental room. All animals were allowed a 4-day period to acclimatize to the novel environment before habituation began.

Twelve WT and 12 5-HT_{2C} KO mice were used for each of experiments 1 and 3. Twelve WT mice were used for each of experiments 2 and 4. All 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.

Drugs

Experiment 1 mCPP was dissolved in 0.9% NaCl and administered i.p. at an injection volume of 10 ml/kg. Vehicle (0.9% NaCl, 10 ml/kg) was used for the control. The doses of mCPP (0, 1, 3 and 5.6 mg/kg) were chosen with reference to studies previously reported by Hewitt et al. (2002).

Experiment 2 SB 242,084 was initially dissolved in PEG400 at 20% of the final required volume, which was then made up with 10% (w/v) hydroxypropyl-β-cyclodextrin. The vehicle used for SB 242,084 conditions was 20% of the final volume PEG400 and 80% of the final volume 10% (w/v) hydroxypropyl-β-cyclodextrin. SB 242,084 was administered s.c. at the nape of the neck at a volume of 4 ml/kg. Doses of SB 242,084 were 0, 0.5 and 1.5 mg/kg and were also chosen with reference to Hewitt et al. (2002). mCPP (0 and 3 mg/kg) was dissolved and administered as described above.

Experiment 3 CP-94,253 was dissolved in distilled water and administered i.p. at an injection volume of 10 ml/kg. Vehicle (dH_2O, 10 ml/kg) was used for the control condition. Doses of CP-94,253 used were 0, 5, 10 and

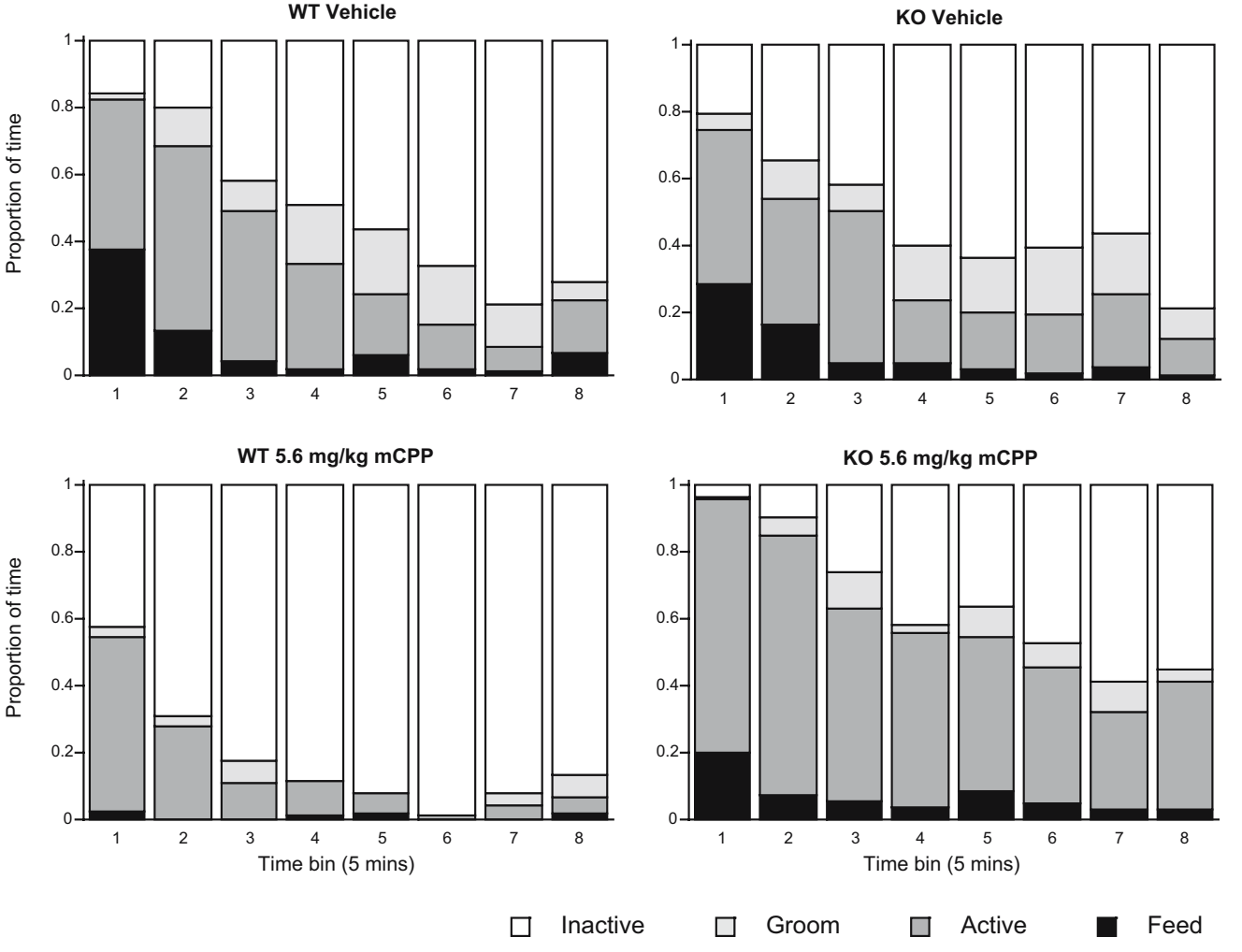

Fig. 2 Behavioural effects of mCPP in WT (*left*) and 5-HT_{2C} KO (*right*) mice as measured using the behavioural satiety sequence paradigm. Results are expressed as the proportion of behavioural observations in 5-min time bins classified as either *feed*, *active*, *groom* or *inactive*. Each animal (n=12) was observed on 10 occasions (i.e. every 30 s) during each time bin

20 mg/kg and were chosen with reference to the doses reported by Clifton et al. (2003).

Experiment 4 SB 242,084 (0, 0.5 and 1.5 mg/kg) and CP-94,253 (0 and 10 mg/kg) were dissolved and administered as described above.

Method and design

Habituation Methods used were similar to those described by Vickers et al. (1999). Animals were habituated to a 40-min daily presentation of wet mash (1 part powdered laboratory diet mixed with 2.4 parts tap water by weight) until daily consumption reached a consistent level (approximately 14 days). During this time, mice were given three habituation satiety sequence sessions separated by 72 h. All mice received injections of 0.9% NaCl (10 ml/kg i.p.) during the last two of these sessions. Mice used in experiments 2 and 4 received an additional injection of 0.9% NaCl (4 ml/kg s.c.) 30 min prior to i.p. injection. Thirty minutes after i.p. injection, wet mash was presented and the behavioural satiety sequence was recorded.

Experimental procedure In experiments 1 and 3, animals were injected with either vehicle or drug in a within-subjects Latin square design, 30 min prior to presentation of wet mash. Thus, each animal was tested on four occasions. Animals were then observed for abnormal or stereotyped behaviour and scored on a scale of 0 (absent)

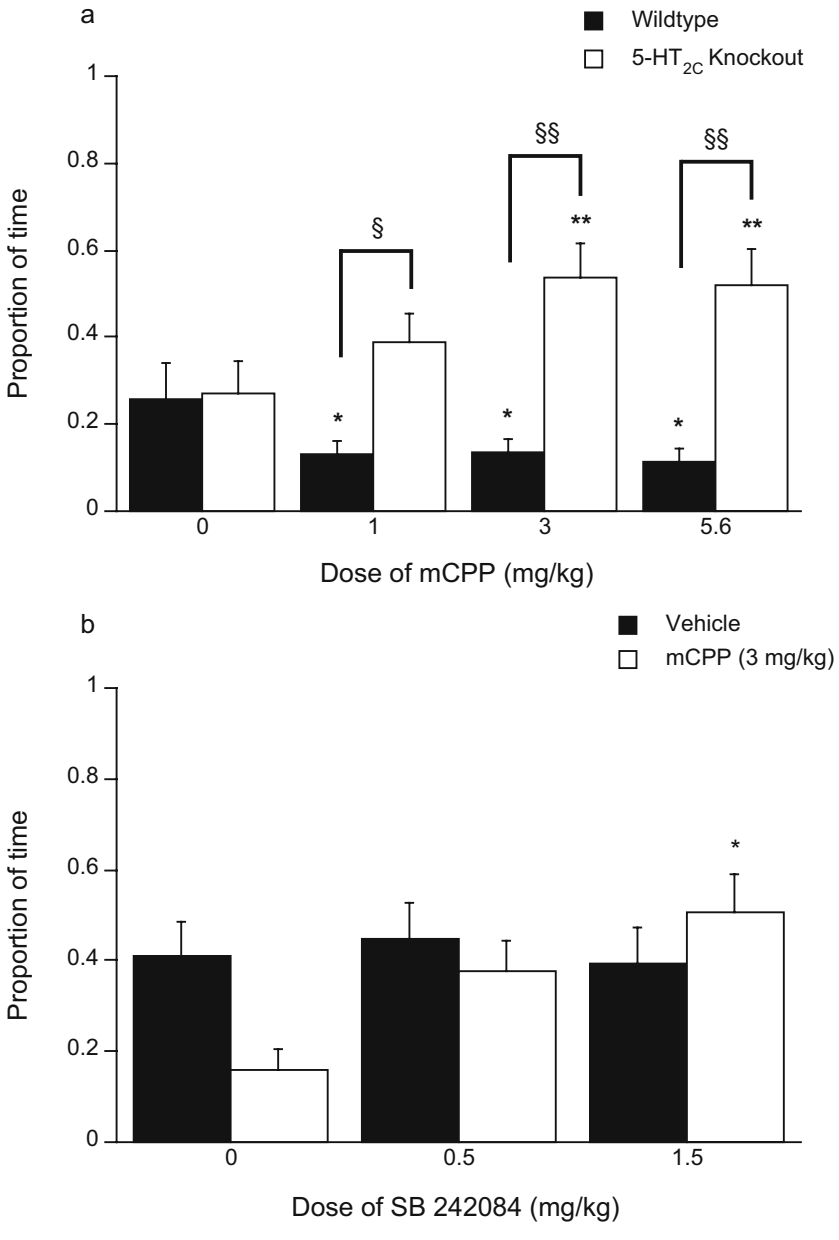

Fig. 3 Dose response effect of mCPP, alone in WT and 5-HT_{2C} KO mice (**a**) or in combination with SB242084 in WT mice (**b**) on proportion of the behavioural satiety sequence engaged in postprandial active behaviour over a 40-min period. Values expressed as mean±SEM. $^{\S}p<0.05$, $^{\S\S}p<0.01$ across genotype comparison; $^{*}p<0.05$, $^{**}p<0.01$ to relevant control

to 4 (pronounced) (Vickers et al. 1999). Normal laboratory chow was then removed and a dish containing a pre-weighed amount of wet mash was immediately presented. The animals were observed for a 40-min period to record a behavioural satiety sequence and scored for each of four behaviours (inactive, feed, groom and active; Vickers et al. 1999). Following the test session, wet mash was removed and reweighed and standard chow was replaced. Experimental days were separated by 72 h. Experiments took place between 1200 and 1400 hours.

For experiments 2 and 4, the antagonist was administered 30 min prior to agonist administration and testing continued as described above.

Statistical analysis

For experiments 1 and 3, food intake was analysed using a two-factor ANOVA with genotype (between subjects) and dose (within subjects) as factors. For experiments 2 and 4, food intake was analysed using a two-factor ANOVA with antagonist and dose (both within subjects) as factors. For all experiments, significant effects were further explored using Dunnett's *t* test. The behavioural satiety sequence data were divided into eight 5-min time bins for each of the four behaviours and subjected to a three-way ANOVA with time as the third factor. All four behavioural categories were analysed separately.

Results

Experiment 1: dose–response curve of mCPP, effects on feeding and the behavioural satiety sequence in WT and 5-HT$_{2C}$ KO mice

mCPP administration induced a dose-dependant reduction in wet mash intake in WT mice with 1, 3 and 5.6 mg/kg reducing intake by 40, 69 and 81%, respectively, (Fig. 1a). WT mice treated with vehicle showed the expected sequence of behaviours in the behavioural satiety sequence with the majority of feeding occurring early in the sequence, leading to increased activity followed by bouts of grooming, leading ultimately to inactivity and resting (Fig. 2, top).

All doses of mCPP induced a leftward shift in the temporal pattern of these behaviours while keeping the sequence intact (Fig. 2, bottom). This is evident since feeding terminated at an earlier time point in drug-treated animals (time × dose interaction, $F_{21,420}$=5.63, p<0.001), with time spent feeding decreasing by 48, 81 and 90% following 1, 3 and 5.6 mg/kg mCPP, respectively. Postprandial activity was also significantly decreased in WT mice given mCPP with decreases in activity of 49, 48 and 56% respective of dose (Fig. 3a); this effect is mirrored by a significant increase in time spent inactive in the same animals (Fig. 2, bottom).

In contrast to WTs, 5-HT$_{2C}$ KO mice were insensitive to the hypophagic effects of mCPP with no significant effect

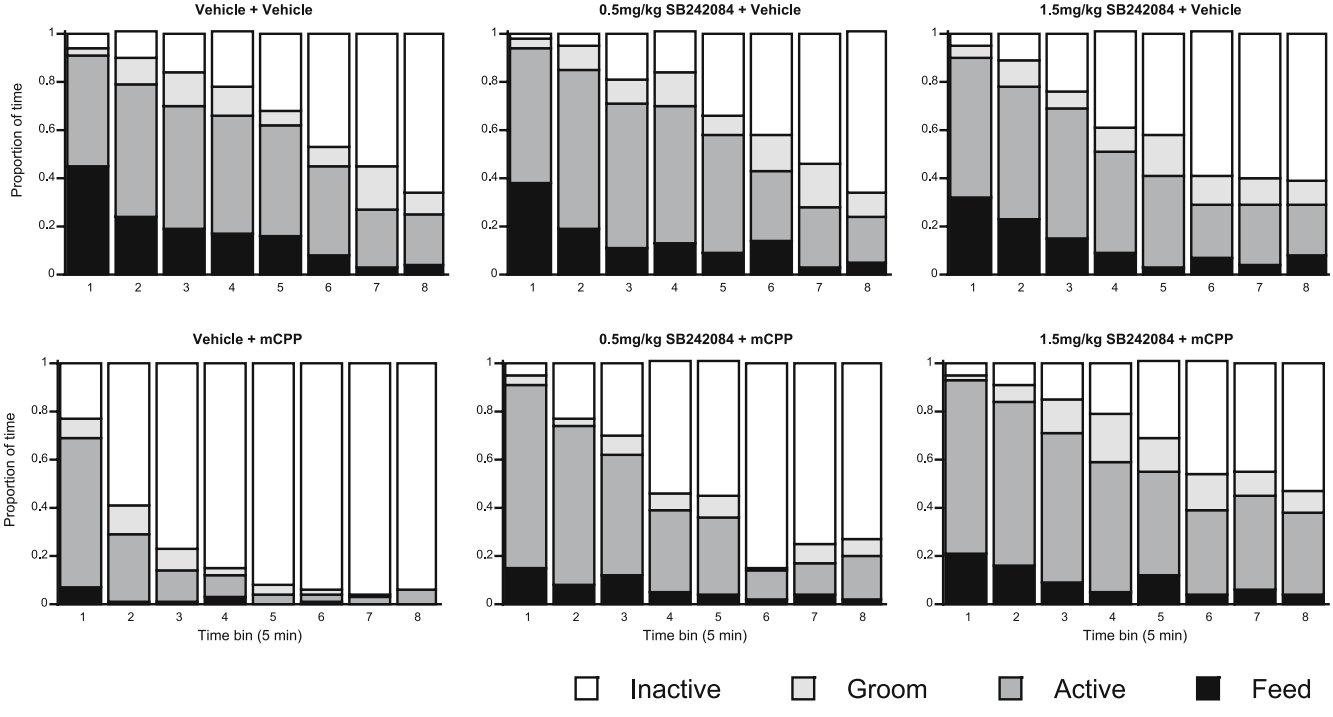

Fig. 4 Behavioural effects of 0, 0.5, and 1.5 mg/kg SB 242084 and 0 (*top*) or 3 mg/kg mCPP (*bottom*) in WT mice as measured over a 40-min period using the behavioural satiety sequence paradigm. Results expressed as in Fig. 2

of any dose of mCPP on wet mash intake in these mice (genotype × drug interaction, $F_{3,66}$=6.60, p<0.001; Fig. 1a). While vehicle treated 5-HT_{2C} KO mice did not differ from their WT counterparts in the behavioural satiety sequence, mCPP administration produced a very different pattern of behaviours in these animals (Fig. 2, bottom). mCPP administration did not significantly affect feeding behaviour in 5-HT_{2C} KO mice. However, all doses of mCPP induced a significant and substantial increase in postprandial activity in these animals, such that 5-HT_{2C} KO mice were significantly more active than WT counterparts at all doses tested (Fig. 3a; genotype × dose interaction, $F_{3,60}$=16.20, p<0.001). This increased activity in 5-HT_{2C} KO mice was dose dependant, in that 1 and 3 mg/kg induced increases of 44% and 100%, respectively, while 5.6 mg/kg induced an increase of 93%.

Grooming behaviour was significantly suppressed following administration of mCPP (main effect of drug, $F_{3,60}$=10.93, p<0.001), but this effect did not differ between genotypes (genotype × drug interaction, NS).

Experiment 2: effects of mCPP in WT mice pretreated with the selective 5-HT_{2C} receptor antagonist SB 242,084 on food intake and the behavioural satiety sequence

mCPP administration (3 mg/kg) significantly reduced food intake in WT mice by 81.9% (main effect of agonist, $F_{1,11}$=47.18, p<0.001; Fig. 1b). Pretreatment with SB 242,084 significantly attenuated the hypophagic effect of mCPP in a dose-dependant manner. Thus, 0.5 and

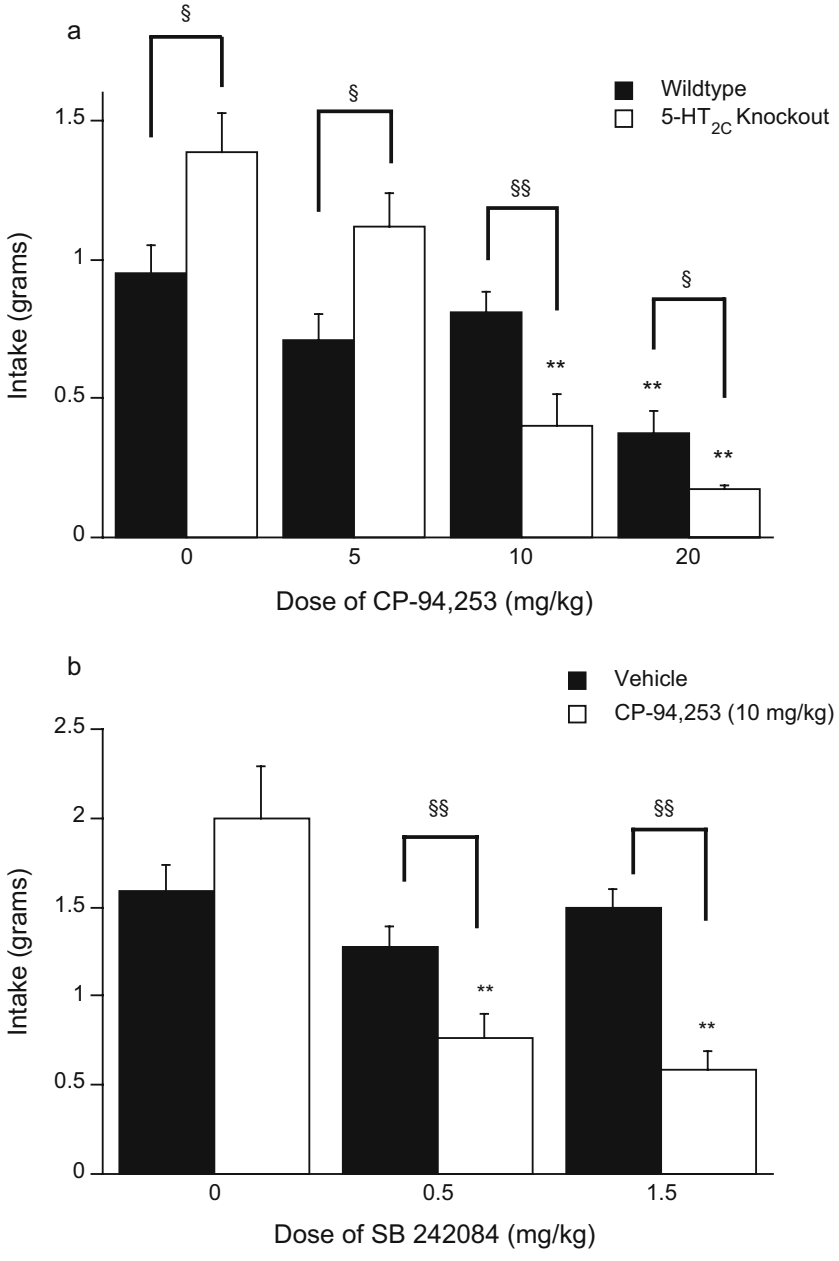

Fig. 5 **a** Effects of CP 94,253 administration on wet mash intake in WT and 5-HT_{2C} KO mice over a 40-min time period. Values are expressed as mean± SEM. Significant differences are denoted by *p<0.05, **p<0.01 compared to relevant control and by $^{§§}p$<0.01 across genotype comparison. **b** Effects of CP-94,253 (10 mg/kg) alone or in combination with SB 242084 (0.5 and 1.5 mg/kg) on wet mash intake in WT mice over a 40-min period. Values expressed as mean±SEM. Significant differences represented as *p<0.05, **p<0.01 compared to relevant control and $^{§§}p$<0.01 across agonist comparison

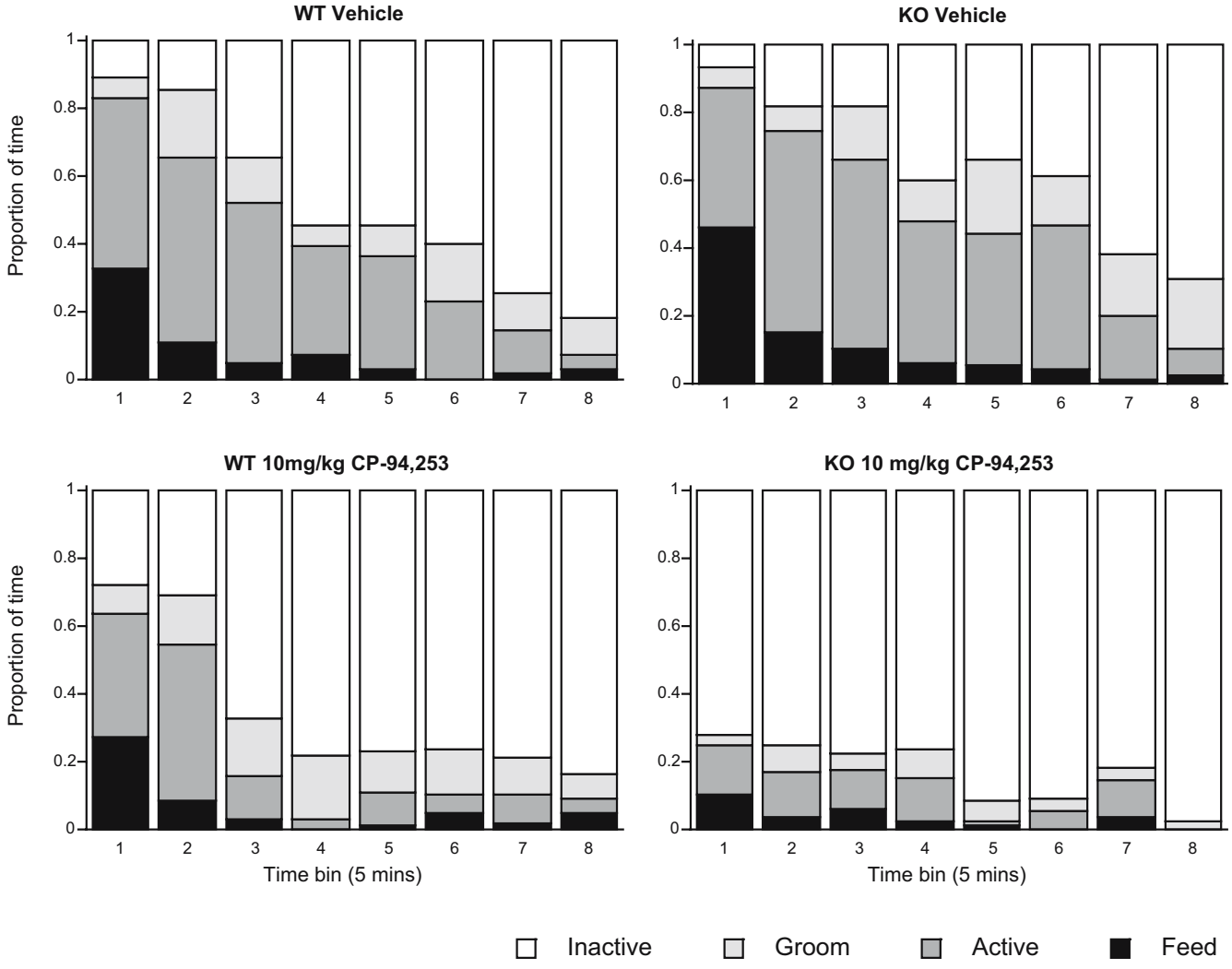

Fig. 6 Behavioural effects of CP-94,253 in WT (*left*) and 5-HT_{2C} KO (*right*) mice as measured using the behavioural satiety sequence paradigm. Results expressed as in Fig. 2

1.5 mg/kg SB 242,084 attenuated mCPP-induced hypophagia by 32.68 and 43.96%, respectively, and (SB 242,084 + mCPP)-treated animals consumed significantly more mash than mice treated with mCPP alone (agonist × antagonist interaction, $F_{2,22}$=23.17, p<0.001; Fig. 1b).

The proportion of time spent feeding was significantly suppressed by mCPP administration. This effect was significantly attenuated in a dose-dependant manner by pretreatment with SB 242,084 (Fig. 4; agonist × antagonist interaction, $F_{2,22}$=17.36, p<0.001). mCPP administration also caused a decrease of 61% in postprandial activity in WT mice. Consistent with 5-HT_{2C} KO data from the previous experiment, the combination of SB 242,084 (1.5 mg/kg) with mCPP induced a significant increase (28%) in activity compared to vehicle-treated counterparts (Fig. 3b). This increase was dose dependant, with the lower dose of SB 242,084 (0.5 mg/kg) reversing the hypoactive effect of mCPP (agonist × antagonist interaction, $F_{2,22}$=5.53, p<0.05) without causing an increase above control levels (main antagonist effect, $F_{2,22}$=0.23, NS).

Although higher doses of mCPP significantly suppressed grooming behaviour in experiment 1, 3 mg/kg mCPP had no significant effect on grooming behaviour in experiment 2. SB 242,084 alone had no significant effect on time spent inactive in WT mice. mCPP alone induced a significant increase in time spent inactive throughout the 40-min observation period. Pretreatment with SB 242,084 attenuated this effect in a dose-dependant manner (agonist × antagonist interaction, $F_{2,22}$=9.83, p<0.001).

Experiment 3: dose response effect of the selective 5-HT_{1B} receptor agonist CP-94,253 on food intake and the behavioural satiety sequence in WT and 5-HT_{2C} KO mice

Administration of CP-94,253 induced a significant decrease in wet mash intake in WT mice only at the highest dose tested (20 mg/kg), with 5, 10 and 20 mg/kg reducing intake by 30, 20 and 60%, respectively (Fig. 5a). This

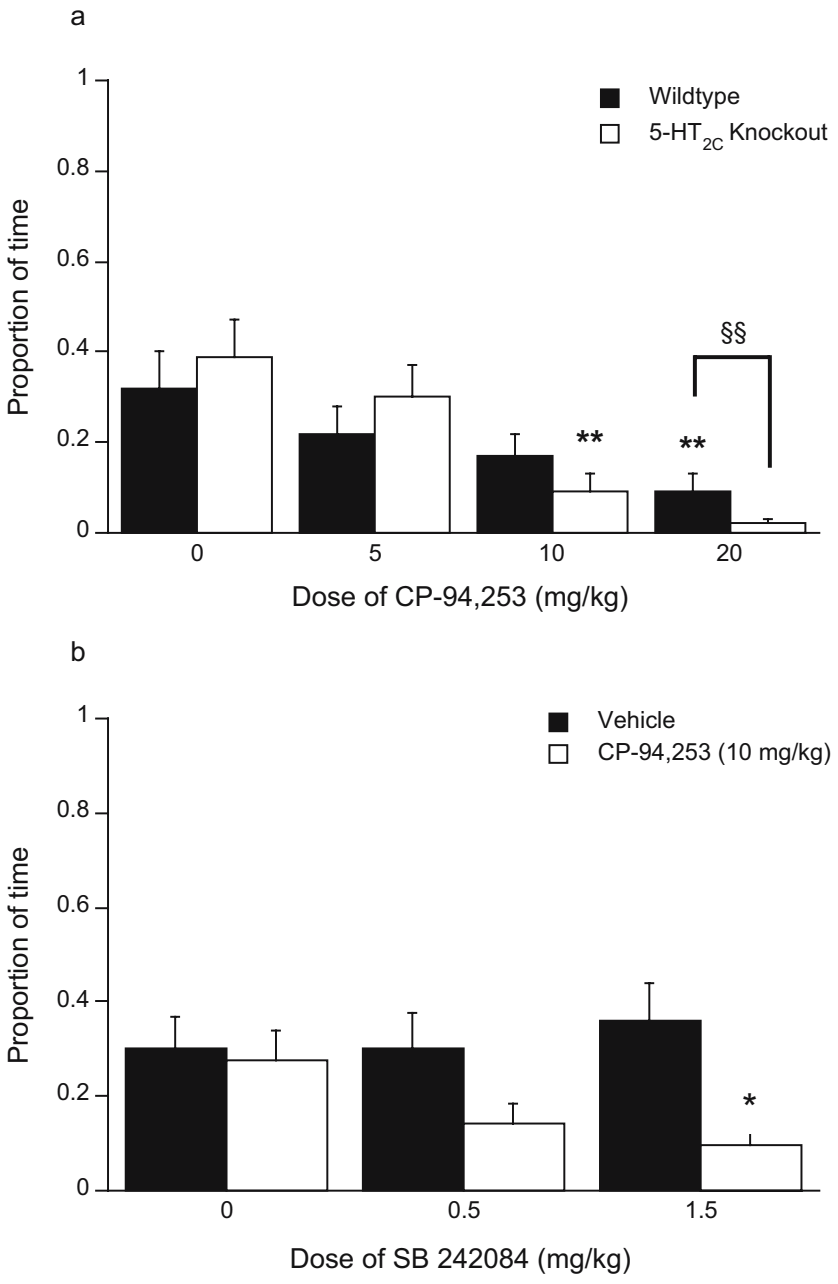

Fig. 7 Dose–response effect of CP-94,253, alone in WT and 5-HT_{2C} KO mice (*top*) or in combination with SB242084 in WT mice (*bottom*), on proportion of the behavioural satiety sequence engaged in postprandial active behaviour over a 40-min period. Values expressed as mean±SEM. $^{§§}p<0.01$ across genotype comparison; $^{**}p<0.01$ to relevant control

hypophagic effect is mirrored in the behavioural satiety sequence (Fig. 6, left) with number of feeding observations decreasing by 42, 20 and 70%, respectively. Postprandial activity was also significantly decreased in WT mice given CP-94,253, with exploration of the home cage decreasing by 40, 51 and 73%, respectively (Fig. 7a). CP-94,253 induced a significant hypophagic effect in KO mice at both 10 and 20 mg/kg, with intake decreasing by 71 and 86%, respectively. This hypophagic effect was more pronounced in 5-HT_{2C} KO mice, such that at both 10 and 20 mg/kg, 5-HT_{2C} KO mice ate significantly less than WT counterparts given the same dose (genotype × dose interaction, $F_{3,63}=8.79$, $p<0.001$; Fig. 5a). This hypophagic effect arose as a result of 5-HT_{2C} KO mice eating significantly less than WT counterparts in early time periods (genotype × dose× time interaction, $F_{21,\ 420}=2.33$, $p<0.001$; Fig. 6, bottom).

CP-94,253 administration (10 and 20 mg/kg) also induced a significant decrease in postprandial activity in 5-HT_{2C} KO mice of 77 and 95%, respectively (Fig. 7a). This effect was also most prominent in early periods when 5-HT_{2C} KO mice were significantly less active than WT-treated counterparts (genotype × dose × time interaction, $F_{21,\ 420}=1.97$, $p<0.01$). Grooming behaviour was significantly suppressed following CP-94,253 administration (main dose effect, $F_{3,60}=8.63$, $p<0.001$) but there was no significant difference between genotypes (genotype × dose interaction: NS).

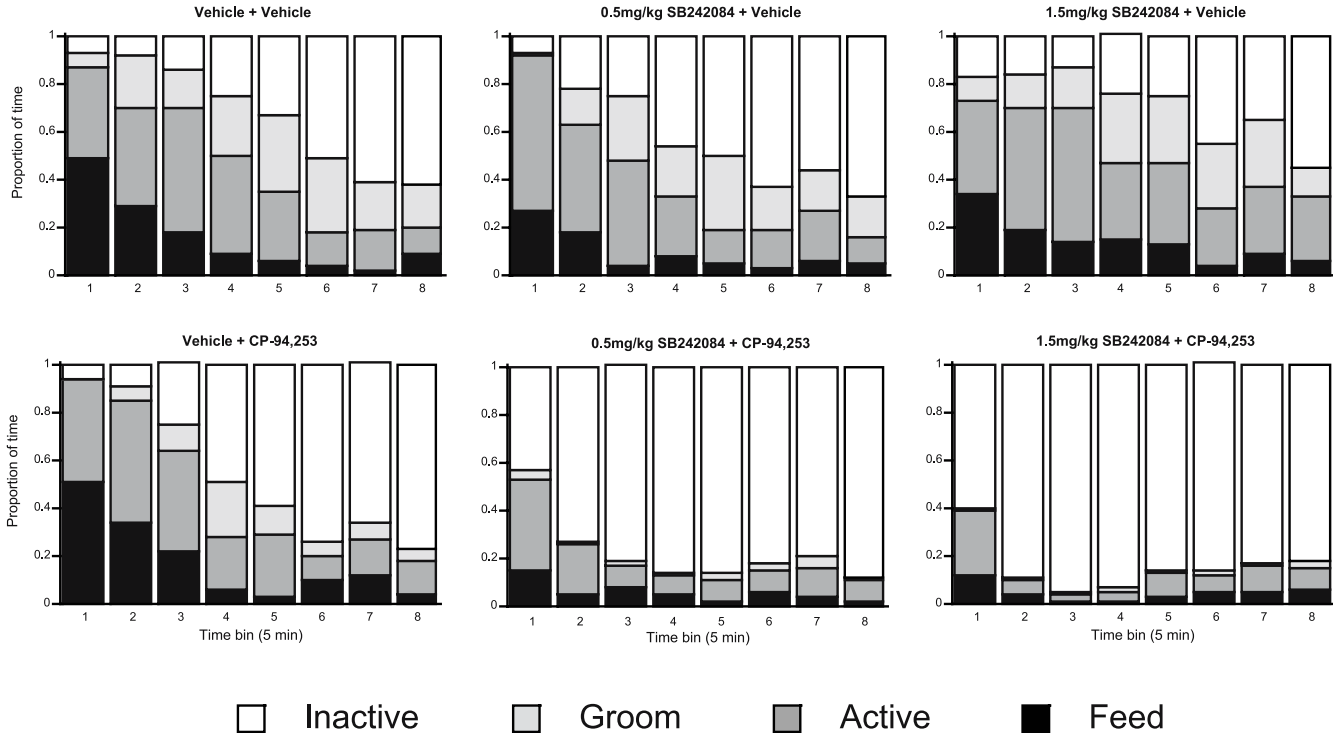

Fig. 8 Behavioural effects of 0, 0.5, and 1.5 mg/kg SB 242084 and 0 (*top*) or 10 mg/kg CP-94,253 (*bottom*) in WT mice as measured over a 40-min period using the behavioural satiety sequence paradigm. Results expressed as in Fig. 2

Experiment 4: effects of CP-94,253 in WT mice pretreated with the selective 5-HT_{2C} receptor antagonist SB 242,084 on food intake and the behavioural satiety sequence

Neither CP-94,253 (10 mg/kg) nor SB 242,084 (0.5 or 1.5 mg/kg) when given alone had any effect on wet mash intake in WT mice. However, the combination of SB 242,084 and CP-94,253 caused a substantial and significant reduction in food intake (Fig. 4b; agonist × antagonist interaction, $F_{2,22}$=9.75, p<0.001). This hypophagic effect was dose dependant for SB 242,084 such that 0.5 and 1.5 mg/kg caused decreases of 41 and 61%, respectively. Feeding behaviour was similarly affected, with the combination of SB 242,084 and CP-94,253 causing a significant decrease in time spent feeding (Fig. 8; agonist × antagonist interaction, $F_{2,22}$=5.5, p<0.05).

In a similar manner, WT mice given the combination of SB 242,084 and CP-94,253 spent significantly less time engaged in postprandial active behaviours than did both vehicle-treated animals and those given either CP-94,253 or SB 242,084 alone (Fig. 7b; agonist × antagonist interaction, $F_{2,22}$=7.33, p<0.01). SB 242,084 treatment alone had no effect on postprandial activity (main antagonist effect, $F_{2,22}$=2.72; NS). Administration of CP-94,253 caused a reduction in grooming behaviour (main agonist effect, $F_{1,11}$=31.14, p<0.01), this was not affected, however, by pretreatment with SB 242,084 (agonist × antagonist interaction, $F_{2,22}$=1.04; NS). Resting or inactive behaviour was not affected by either CP-94,253 or SB 242,084 administration, while the combination of SB 242,084 and CP-94,253 induced a significant and substantial increase in time spent inactive (agonist × antagonist interaction, $F_{2,22}$=13.75, p<0.001).

Discussion

Data derived from the present group of experiments demonstrate that mice lacking functional 5-HT_{2C} receptors are less sensitive than WT mice to the hypophagic effects of mCPP and are consistent with previous reports (Heisler and Tecott 2000) that mCPP induces a hyperactive response in 5-HT_{2C} KO mice in a novel environment. Our data demonstrate that the response is seen even in the home cage and therefore cannot be explained in terms of a heightened response to an unfamiliar test situation. These data demonstrate for the first time that 5-HT_{2C} KO mice are more sensitive than their WT counterparts to the hypophagic effects of the selective 5-HT_{1B} receptor agonist CP-94,253, while also confirming our previous report (Dalton et al. 2004) that CP-94,253, when administered alone, does not induce a hyperactive response in 5-HT_{2C} KO mice. The data also suggest that the behavioural effects of mCPP and CP-94,253 in 5-HT_{2C} KO mice (experiments 1 and 3) do not arise as a result of developmental compensation for gene knockout. Thus, very similar effects on feeding and postprandial activity can be induced in WT mice that have been pretreated with the selective 5-HT_{2C} receptor antagonist, SB 242,084 (experiments 2 and 4). It should also be noted that 5-HT_{2C} KO mice given vehicle ate significantly more wet mash in experiment 3 than their

age-matched WT counterparts. The mice used in this particular experiment were slightly older (by 6–8 weeks) than the mice used in experiment 1, where there was no difference between genotypes in the vehicle condition. It has previously been demonstrated that 5-HT_{2C} KO mice display a hyperphagia that intensifies with age, and it is with this developing hyperphagia that obesity develops in this genotype (Nonogaki et al. 1998). However, it seems unlikely that body weight is a significant confound in these experiments since similar results were obtained in WT animals pretreated with the 5-HT_{2C} antagonist SB242084.

Despite a convincing array of evidence demonstrating a central role for both the 5-HT_{1B} and 5-HT_{2C} receptors in serotonergic mediation of ingestive behaviour, little is known about the manner in which these two receptor subtypes interact. Some researchers have suggested that the hypophagia induced following activation of either of these receptors is mediated by separate systems (Kennett and Curzon 1988). In contrast, Simansky (1996) and Clifton (1994) have suggested that 5-HT_{1B} and 5-HT_{2C} receptors are associated with qualitatively different aspects of satiation. Finally, Schreiber and De Vry (2002) suggest that concurrent activation of both 5-HT_{2C} and 5-HT_{1B} receptors is necessary for a serotonergically mediated reduction in food intake. However, data from the present group of experiments would suggest that the relationship between 5-HT_{1B} and 5-HT_{2C} receptor activation in feeding behaviour is more complex than any of these theories would imply.

Data presented in experiment 1 demonstrate that 5-HT_{2C} KO mice are less sensitive than WT counterparts to the hypophagic effects of mCPP (Fig. 1a). This effect was replicated in WT mice pretreated with the selective 5-HT_{2C} receptor antagonist, SB 242,084 (Fig. 1b). This suggests that hypophagia induced by low doses of mCPP is mediated in WT mice primarily by the affinity of this compound for 5-HT_{2C} receptors, indicating that the affinity of mCPP for 5-HT_{1B} receptors can play only a minor role in the hypophagic profile of this compound in mice. This is consistent with previous reports from both our laboratory (Hewitt et al. 2002; Clifton et al. 2003) and others (Heisler and Tecott 2000) showing that pretreatment with 5-HT_{1B} receptor antagonists has minimal impact on mCPP-induced hypophagia.

However, it is still somewhat surprising that 5-HT_{2C} KO mice failed to decrease feeding at higher doses of mCPP. Activation of 5-HT_{1B} receptors has repeatedly been shown to suppress feeding in WT animals (Lee and Simansky 1997; Hewitt et al. 2002; Schreiber and De Vry 2002; Clifton et al. 2003), and the hypophagic effects of mCPP have been shown to be partially attenuated by non-selective 5-HT_{1B} receptor antagonists such as cyanopindolol (Bendotti and Samanin, 1987; Kennett and Curzon, 1988). It is interesting to note that even at a dose higher than that required for a maximal hyperactive response (see Figs. 2 and 3) 5-HT_{2C} KO mice demonstrated no suppression of feeding. This would suggest two things. First, in the absence of functional 5-HT_{2C} receptors, the incentive motivation for rewarding stimuli (in this case, food) can overcome the competitive influence of other behaviours that may be stimulated by mCPP. This suggests that the hypophagic effect of mCPP in WT animals arises as the result of a genuine advancement in the process of satiation brought about by activation of 5-HT_{2C} receptors and that despite the lack of selectivity of this compound in vitro, feeding behaviour is not suppressed through a disruption in normal behavioural profiles. In addition, these data are consistent with the hypothesis that any activation of 5-HT_{1B} receptors by higher doses of mCPP is not sufficient to induce hypophagia in the absence of 5-HT_{2C} receptor stimulation. In fact, such doses are likely to have non-specific effects on serotonergic function as a result of the serotonin-releasing properties of this compound (Baumann et al. 1993).

The absence of a hypophagic response in 5-HT_{2C} KO mice given mCPP also raises the possibility of the existence of a common hypophagic pathway, where activation of a population of 5-HT_{2C} receptors, located downstream from 5-HT_{1B} receptors, is critical for the behavioural expression of 5-HT_{1B}-induced hypophagia. In light of this, experiment 3 investigated whether activation of 5-HT_{1B} receptors by the selective 5-HT_{1B} receptor agonist CP-94,253 was capable of suppressing food intake in 5-HT_{2C} KO mice.

CP-94,253 displays a 45-fold higher selectivity for 5-HT_{1B} over 5-HT_{1A} receptors (Koe et al. 1992). It has been demonstrated to reduce food intake in both rats and mice across a number of behavioural paradigms, including behavioural satiety sequences (Halford and Blundell 1996; Lee and Simansky 1997), operant responding (De Vry et al. 1999) and free-feeding paradigms (Lee et al. 2002; Schreiber and De Vry 1999, 2002; Fletcher and Davies 1990). In the present study, CP-94,253 induced a significant hypophagic effect in WT mice only at the highest dose tested (20 mg/kg; Fig. 5a). This is consistent with previous reports from studies using both mice (Clifton et al. 2003) and rats (Lee and Simansky 1997; Schreiber and De Vry 2002). Surprisingly, 5-HT_{2C} KO mice (Fig. 5a) and SB 242,084-treated mice (Fig. 5b) demonstrated an *increased* sensitivity to the hypophagic effects of this compound, with intake being significantly suppressed at both 10 and 20 mg/kg. The significant and substantial decrease in food consumption observed in 5-HT_{2C} KO mice and in SB 242,084 treated mice given 10 mg/kg CP-94,253 was accompanied by an advancement in the behavioural profile of the satiety sequence, with a significant decrease in the number of feeding observations in the early time periods as well as an early termination of postprandial activity and an early onset of inactivity (see Figs. 6 and 8). Such a behavioural profile indicates a genuine advancement in the process of satiation and demonstrates that the increased sensitivity of 5-HT_{2C} KO mice to the hypophagic effects of CP-94,253 does not occur as the result of behavioural competition.

The ability of CP-94,253 to induce a significant hypophagic effect in 5-HT_{2C} KO mice indicates that activation, or even presence, of 5-HT_{2C} receptors is not necessary for 5-HT_{1B}-induced hypophagia and is incon-

sistent with the hypothesis of a common hypophagic pathway in which activation of 5-HT_{2C} receptors, subsequent to 5-HT_{1B} receptor stimulation, is required for satiety to be expressed. Furthermore, the observation that 5-HT_{2C} KO mice were *more* sensitive to the hypophagic effects of CP-94,253 suggests that in a WT mouse, 5-HT_{1B}-induced hypophagia may be limited by endogenous 5-HT acting at 5-HT_{2C} receptors. This lends further support to the suggestion that 5-HT_{2C} receptor activation can function to suppress the behavioural consequences of activation of other 5-HT receptors (Heisler and Tecott 2000; Higgins et al. 2001; Vickers et al. 2001; Dalton et al. 2004).

It could be argued that the hypersensitivity of 5-HT_{2C} KO mice to the hypophagic effects of CP-94,253 indicates either an up-regulation of 5-HT_{1B} receptor function or an increase in 5-HT_{1B} receptor sensitivity arising due to developmental compensation for gene knockout. There are two pieces of evidence that argue against this hypothesis. First, Lopez-Gimenez et al. (2002) report, from autoradiographical studies, that there is no evidence of up- or down-regulation in the expression of other 5-HT receptors in 5-HT_{2C} KO mice. Second, and more directly, data presented in the present series of experiments show that WT mice pretreated with the selective 5-HT_{2C} receptor antagonist SB 242,084 demonstrate a dose-related increase in the hypophagic response, as well as behavioural similarities to those observed in 5-HT_{2C} KO mice, following CP-94,253 administration. Therefore, both results are likely to reflect genuine short-term receptor interactions and hence provide additional evidence that the 5-HT_{2C} receptor plays a modulatory role in the behavioural expression of 5-HT_{1B} receptor stimulation. They also demonstrate that the slightly greater age and food intake of 5-HT_{2C} KO mice in experiment 3 is unlikely to be an explanation of this enhanced sensitivity to CP-94,253. Taken together, data from experiments 3 and 4 are consistent with an inhibitory role of 5-HT_{2C} receptors over the behavioural expression of other 5-HT receptor subtypes. The fact that 5-HT_{2C} KO mice were more sensitive to the hypophagic effects of CP-94,253 not only provides strong evidence in support of this claim, but also indicates that 5-HT_{1B}-induced hypophagia is *not* dependant on tonic 5-HT_{2C} receptor activation.

Thus, the present studies, when taken together with data from earlier investigations of the effects of 5-HT_{2C} agonists following disruption of either 5-HT_{1B} receptor function or pharmacological blockade of 5-HT_{1B} receptors (Clifton et al. 2003, Lee et al. 2004a), clarify the relationship between 5-HT_{1B} and 5-HT_{2C} receptor stimulation in the modulation of feeding behaviour in mice. 5-HT_{2C} receptor activation induces hypophagia in mice that has the broad characteristic of enhanced satiation rather than being the result of behavioural disruption (Hewitt et al. 2002). Although 5-HT_{1B} KO mice show reduced sensitivity to 5-HT_{2C} receptor agonists (Clifton et al. 2003), it is clear that this results from a longer term compensatory response since the reduced sensitivity is not observed following pharmacological blockade of 5-HT_{1B} receptors (Clifton et al. 2003, Lee et al. 2004b). Thus, 5-HT_{2C} receptor-mediated hypophagia is *not* dependant on the presence of functional 5-HT_{1B} receptors In a similar way, the present studies confirm that the 5-HT_{1B} receptor agonist CP-94,253 reduces feeding behaviour in mice in a behaviourally selective manner (see also Lee et al. 2004a), and that this effect does *not* require intact 5-HT_{2C} receptor signalling. Indeed, 5-HT_{2C} receptor inactivation actually serves to enhance the 5-HT_{1B}-mediated hypophagic response. Although unexpected, this relationship is identical to the one already described in the modulation of locomotor activity in rodents. In that case, joint activation of 5-HT_{1B} and 5-HT_{2A} receptors induces hyperactivity, which is opposed by concurrent activation of 5-HT_{2C} receptors (Dalton et al. 2004). This effect is likely to be mediated by 5-HT_{2C} receptors in the ventral tegmental area (Fletcher et al. 2004), whereas the direct hypophagic affect of 5-HT_{2C} receptor agonists is more likely to be mediated by receptors located in the brainstem or hypothalamus.

In conclusion, in mice at least, it appears that 5-HT_{1B}- and 5-HT_{2C}-mediated hypophagia occur through independent mechanisms rather than through a common pathway giving rise to mutual dependence. The only pharmacological interaction between the two receptor subtypes appears to be one that might have been predicted from earlier studies of locomotor activity in which a separate population of 5-HT_{2C} receptors limits the hypophagic response to 5-HT_{1B} receptor activation.

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