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Genetic and pharmacological evidence that 5-HT_{2C} receptor activation, but not inhibition, affects motivation to feed under a progressive ratio schedule of reinforcement

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ABSTRACT

Previous work showed that 5-HT_{2C} receptor agonists reduce cocaine self-administration on a progressive ratio (PR) schedule of reinforcement, whereas a 5-HT_{2C} receptor antagonist enhances responding for cocaine. The present experiments examined the effects of Ro60-0175 (5-HT_{2C} agonist) and SB242084 (5-HT_{2C} receptor antagonist) in rats on responding for food on a PR schedule; responding was also determined in mice lacking functional 5-HT_{2C} receptors. In food-restricted rats, lever pressing reinforced by regular food pellets or sucrose pellets was reduced by Ro60-0175. This effect was blocked by SB242084, and was absent in mice lacking functional 5-HT_{2C} receptors. A number of studies examined the effects of SB242084 on responding for food under a variety of conditions. These included manipulation of food type (regular pellets versus sucrose pellets), nutritional status of the animals (food restriction versus no restriction), and rate of progression of the increase in ratio requirements on the PR schedule. In all cases there was no evidence of enhanced responding for food by SB242084. Mice lacking functional 5-HT_{2C} receptors did not differ from wildtype mice in responding for food in either food-restricted or non-restricted states. The effects of Ro60-0175 are consistent with its effects on food consumption and motivation to self-administer cocaine. Unlike their effects on cocaine self-administration, pharmacological blockade of 5-HT_{2C} receptors, and genetic disruption of 5-HT_{2C} receptor function do not alter the motivation to respond for food. Because the 5-HT_{2C} receptor exerts a modulatory effect on dopamine function, the differential effects of reduced 5-HT_{2C} receptor mediated transmission on responding for food versus cocaine may relate to a differential role of this neurotransmitter in mediating these two behaviours.

1. Introduction

Although dopamine is the neurotransmitter most closely linked to drug reward and reinforcement, serotonin (5-HT) can also modulate these behavioural processes. This is likely achieved through serotonergic modulation of dopamine function. The interaction between serotonin and dopamine at the neurochemical and behavioural level is complex, and is determined by where in the brain these interactions occur and the specific receptor subtypes involved (Alex and Pehek, 2007; Di Giovanni et al., 2008; Di Matteo et al., 2008). Several studies have shown that general widespread elevations in brain 5-HT levels resulting from systemically administered treatments such as fenfluramine, fluoxetine or L-tryptophan, reduced self-administration of a variety of substances including alcohol, cocaine, amphetamine and heroin (Carroll et al., 1990; Fletcher et al., 1999; Higgins et al., 1994; Howell and Byrd, 1995; Peltier and Schenk, 1993; Porrino et al., 1989; Richardson and Roberts, 1991). In contrast, wide-spread reductions in 5-HT levels enhanced responding for alcohol, amphetamine and cocaine in some (Loh and Roberts, 1990; Lyness et al., 1980) though not all studies (Fletcher et al., 1999). In behavioural tests of reinstatement fluoxetine and fenfluramine attenuated reinstatement of cocaine (Burmeister et al., 2003), or alcohol-seeking (Le et al., 2006).

Some of the effects on drug self-administration induced by fenfluramine and fluoxetine are reproduced by ligands that have a more restricted pharmacological action on serotonergic transmission. The 5-HT_{2C} receptor agonists Ro60-1075 and MK212 reduced cocaine self-administration, and reinstatement of drug-seeking induced by

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cocaine, and by drug associated cues and contexts (Burbassi and Cervo, 2008; Grottick et al., 2000; Neisewander and Acosta, 2007). The 5-HT_{2C} receptor antagonist SB242084 (Kennett et al., 1997) increased responding for cocaine on a progressive ratio schedule (Fletcher et al., 2002), an effect that was also observed in mice lacking functional 5-HT_{2C} receptors (Rocha et al., 2002). SB242084 also enhanced responding for alcohol (Tomkins et al., 2002). Taken together, these findings suggest that serotonergic signalling through 5-HT_{2C} receptors alters motivation to seek drug reinforcers, with blockade of 5-HT_{2C} mediated transmission enhancing this process.

Manipulation of 5-HT activity also affects feeding behaviour and food intake. Fenfluramine, the 5-HT precursors tryptophan and 5-hydoxytryptophan, and fluoxetine reduce feeding (Blundell and Latham, 1979; Blundell et al., 1973; Goudie et al., 1976; Halford et al., 2007; Latham and Blundell, 1979). In the case of fenfluramine 5-HT_{2C} receptors play a prominent role since its effects are attenuated by 5-HT_{2C} receptor antagonists, and blunted in 5-HT_{2C} receptor null mutant mice (Clifton et al., 2000; Vickers et al., 1999; Vickers et al., 2001). Additionally, selective 5-HT_{2C} receptor agonists including Ro60-0175 and MK212 also reduce feeding behaviour (Clifton et al., 2000; Fletcher et al., 2009; Halford et al., 1997; Hayashi et al., 2005; Hewitt et al., 2002; Somerville et al., 2007; Vickers et al., 2000). Several non-selective 5-HT receptor antagonists, including metergoline, methysergide and methiothepin, enhance food intake under certain conditions (Dourish et al., 1989; Fletcher, 1988), and there is one report of increased food intake in rats following treatment with the 5-HT_{2C} receptor antagonist RS100011 (Bonhaus et al., 1997). Superficially, it appears that 5-HT_{2C} receptor ligands have similar effects to alter consumption of drugs and food. However, the most commonly used procedures used to measure drug and food consumption are somewhat different. Generally, drug self-administration studies are conducted using operant conditioning procedures that allow for precise manipulation of factors such as work requirements and reinforcer quality. Although such operant conditioning procedures have been used on occasion to study the impact of serotonergic drugs on feeding motivation (De Vry et al., 2003; Ebenezer, 1992; Sanabria et al., 2008) most studies in this area have relied on measures of consummatory and ancillary behaviours.

The present experiments examined the effects of 5-HT_{2C} receptor ligands that have been shown to alter self-administration of cocaine, on feeding motivation measured by responding on a progressive ratio schedule of reinforcement. Based on the fact that 5-HT_{2C} receptor agonists reduced cocaine self-administration on a PR schedule (Fletcher et al., 2008; Grottick et al., 2000) we predicted that the 5-HT_{2C} receptor agonist Ro60-0175, which reduces food consumption, would reduce responding for food. Blocking 5-HT_{2C} receptors with SB242084 enhances responding for cocaine on a PR schedule (Fletcher et al., 2002), and also increases cocaine-induced reinstatement of responding (Grottick et al., 2000). Since some 5-HT receptor antagonists may increase food consumption we were especially interested in whether the 5-HT_{2C} receptor antagonist SB242084 increased responding on the PR schedule. We also examined responding on the PR schedule in 5-HT_{2C} receptor knockout mice, in the presence and absence of Ro60-0175. Both food palatability and the state of hunger or satiety are major determinants of the motivation to eat. Therefore these variables were manipulated in some of the experiments to determine whether there were any interactions between these factors and the 5-HT_{2C} receptor ligands.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Charles River, Quebec) weighing 280–320 g at the beginning of each study were used for Experiments 1–4. They were housed singly in clear plastic, rectangular, solid-bottomed cages. The housing room was maintained on a 12 h light/dark cycle (lights on at 08:00 h) and at a temperature of 22 ± 2 °C. During testing rats either had free access to food, or had restricted access as detailed below. All training and testing was conducted during the light phase. Experiment 5 involved the use of 5-HT_{2C} receptor mutant mice generated from a breeding pair (The Jackson Laboratory, Bar Harbor, Maine, USA) derived from a 129 ES cell line bearing a targeted disruption of the X-linked 5-HT_{2C} receptor gene (Tecott et al., 1995). The original pair of mice had been back-crossed for at least five generations. Mice used in these studies were derived by crossing wild-type C57BL/6 males with females heterozygous for the 5-HT_{2C} receptor mutation for an additional 5–8 generations. Genotyping was performed by PCR analysis using a protocol (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype=protocol&protocol_id=214) described by The Jackson Laboratory. Mice were 10–12 weeks old at the start of the experiments. They were singly housed and reduced to 85% of their free-feeding body weight by feeding a measured amount of food per day.

Experimental procedures conformed to the guidelines laid down by the Canadian Council on Animal Care, and were approved by the CAMH Animal Care Committee.

2.2. Apparatus

For experiments involving rats testing was conducted in standard operant conditioning chambers (Med. Associates Inc., St Albans, VT, USA). Each chamber contained two response levers located either side of a central food magazine; only the left response lever was active. In Experiment 5 operant conditioning chambers for mice were used (Med. Associates Inc., St Albans, VT, USA). Each box was equipped with a removable ultra-sensitive response lever located to the left of a central magazine. The rear wall of the chamber was curved and contained 5 apertures; this device was not used for any experimental purpose in the present studies. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. The rat and mouse systems were located in two different laboratories, and each was controlled by a separate computer.

2.3. Behavioural training

All rats were initially food- restricted, by giving them approximately 18 g food per day; this results in animals reaching approximately 85–90% of their free-feeding weight. Rats were then trained to press the left lever of the test chamber for food (standard 45 mg Noyes Precision pellets) according to a fixed ratio (FR) 1 schedule. Rats were allowed a maximum of 100 pellets during daily 30 min sessions. Any rats failing to obtain 100 pellets by the third day of training were placed in the operant boxes overnight and allowed 300 food pellets delivered according to the FR1 schedule. A stainless steel dish filled with water was also placed inside the operant chamber during this session. Thereafter, rats were placed in the chamber only during the 30 min daytime session. Once rats had earned 100 pellets on 3 consecutive days they were considered lever-trained. A progressive ratio (PR) schedule was then implemented in which the number of responses required to obtain a food pellet increased for successive reinforcers. The progression was derived from the equation: response ratio $= [5 \times e^{(0.2 \times \text{reinforcer number})} - 5]$, and yielded ratios of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118 etc. after rounding to the closest integer. Sessions lasted until a period of 20 min had elapsed without earning a food pellet. The number of food pellets earned before this break-point, and the total number of responses made, were recorded. Testing began when break-points did not vary by more than 15% on 3 consecutive days. For mice, the exact same procedure was followed except that lever pressing was reinforced by 14 mg food pellets (Bioserv).

2.4. Experiment 1. Effects of Ro60-0175 on responding for food in food-restricted rats

Two groups ($n=8$ each) of food-restricted rats were trained to respond for regular 45 mg Noyes food pellets (Formula A/I) or 45 mg Noyes sucrose pellets (Formula F) on the standard PR schedule (exponential value of 0.2). Each rat was tested 4 times at 72 h intervals following injection with 0.1, 0.3 and 1 mg/kg Ro60-0175 and saline vehicle. Injection order was determined from a Latin square. On days between injections rats responded for food on the PR schedule as during training.

2.5. Experiment 2a. Effects of SB242084 on responding for food in food-restricted rats

Two groups of rats were trained to respond for regular food pellets ($n=10$), or sucrose pellets ($n=10$) on the PR schedule. Once responding was stable drug testing began. Each rat was tested 3 times following injection with 0.5 and 1 mg/kg SB242084, and its vehicle. The order of treatments was determined from a Latin square, and at least 72 h intervened between tests, with rats run as usual on these intervening days.

2.6. Experiment 2b. Effects of SB242084 on responding for food in non-food-restricted rats

After completion of experiment 2a all rats were allowed free access to regular lab chow in the home cage. One week later the PR reinforcement sessions were resumed with non-restricted rats continuing to be reinforced with either regular food pellets or sucrose pellets. When responding had re-stabilised drug testing was begun. Each rat was tested 3 times following injection with 0.5 and 1 mg/kg SB242084, and its vehicle. The order of treatments was determined from a Latin square. At least 72 h intervened between each test day, with rats run as usual on these intervening days.

2.7. Experiment 3. Effects of combined treatment with SB242084 and Ro60-0175 on responding for food in food deprived rats

Eight rats were food-restricted and trained to respond for regular food pellets on the PR schedule as described above. When responding was stable each rat was tested four times under every combination of 0.5 mg/kg SB242084 and its vehicle, and 1 mg/kg Ro60-0175 and its vehicle. The order of treatments was determined from a Latin square, and at least 72 h intervened between each test day, with rats run as usual on these intervening days. SB242084 or its vehicle was administered 15 min before the injection of Ro60-0175 or its vehicle. The same animals were used to repeat the experiment using sucrose pellets as the reinforcer.

2.8. Experiment 4. Effects of SB242084 on PR responding for food with different rates of progression

Nine rats were initially food-restricted and trained to respond for food on a FR1 schedule. The schedule of reinforcement was then changed to a PR schedule using the formula response ratio $=[5\times e^{(0.2\times \text{reinforcer number})}-5]$. Once responding had stabilised rats were tested for responding on this schedule following injection of vehicle or 0.5 mg/kg SB242084 30 min before the session started. The two tests were separated by 72 h and administered in a counterbalanced order. Next, the rate of progression of the PR schedule was reduced, using the formula: response ratio $=[5\times e^{(0.1\times \text{reinforcer number})}-5]$. Rats were again tested for responding following injection of saline or 0.5 mg/kg SB242084 administered in a counterbalanced fashion 72 h apart. The whole process, including drug treatments, was then repeated with a further adjustment to the rate of progression of the ratios in the schedule, achieved by changing the formula to: response ratio $=[5\times e^{(0.08\times \text{reinforcer number})}-5]$.

2.9. Experiment 5. Responding for food on a PR schedule in 5-HT_{2C} receptor knockout mice

Nine wildtype and seven 5-HT_{2C} null mutant mice were habituated to food restriction for 7 days, and then trained to lever press for 14 mg food pellets according to a FR1 schedule. Mice received a maximum of 60 pellets during daily 30 min sessions. Any mice failing to obtain 60 pellets by the third day of training were placed in the chambers overnight and allowed a maximum of 200 food pellets delivered according to the FR1 schedule. A stainless steel dish filled with water was also placed inside the operant chamber during this session. Thereafter, mice were placed in the chamber only during the 30 min daytime session. Once they had earned 60 pellets on each of 3 consecutive days they were considered lever-trained. A progressive ratio schedule was then implemented using the equation: response ratio $=[5\times e^{(0.2\times \text{reinforcer number})}-5]$. Sessions lasted until a period of 20 min had elapsed without earning a food pellet. Responding on this schedule was monitored for 5 consecutive days. Responding was then determined after mice had been treated with saline 15 min prior to the start of one session, and 1 mg/kg Ro60-0175 prior to another session. The drug tests were administered in counterbalanced order, 72 h apart. After the second test food was freely available in the home cage and responding on the PR schedule was measured for a further 5 consecutive days.

2.10. Drugs and injections

Ro60-0175 ((S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$) was synthesised in the PRPN Chemistry department at F. Hoffmann-La Roche Ltd., Basel. Ro60-0175 was dissolved in 0.9% saline and injected SC 15 min before testing. SB242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl] indoline) was synthesised in the Department of Chemistry, Vernalis Research Ltd, Wokingham, UK., and prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid and injected by the IP route 30 min before testing.

2.11. Statistical analysis

The main dependent measures were the number of reinforcers earned, and the total number of responses made per session. The data were analysed using two way analysis of variance followed by post-hoc comparisons with Tukey's' HSD test.

3. Results

3.1. Experiment 1. Effects of Ro60-0175 on responding for food in food-restricted rats

Fig. 1 shows that Ro60-0175 reduced the number of reinforcers (food pellets) earned and the total number of responses in rats responding for regular and sucrose pellets. Significant main effects of Ro60-0175 confirmed that the number or reinforcers and responses were both significantly reduced [$F(2,28)=17.04$ and 9.71, respectively, $p<0.001$]. The main effect of food type and the interaction between food type and Ro60-0175 were not significant ($p>0.2$].

3.2. Experiment 2a. Effects of SB242084 on responding for food in food-restricted rats

Fig. 2A shows that rats reinforced with sucrose pellets tended to respond more, and earn more reinforcers than rats responding for regular pellets. However, a two way analysis of variance showed that

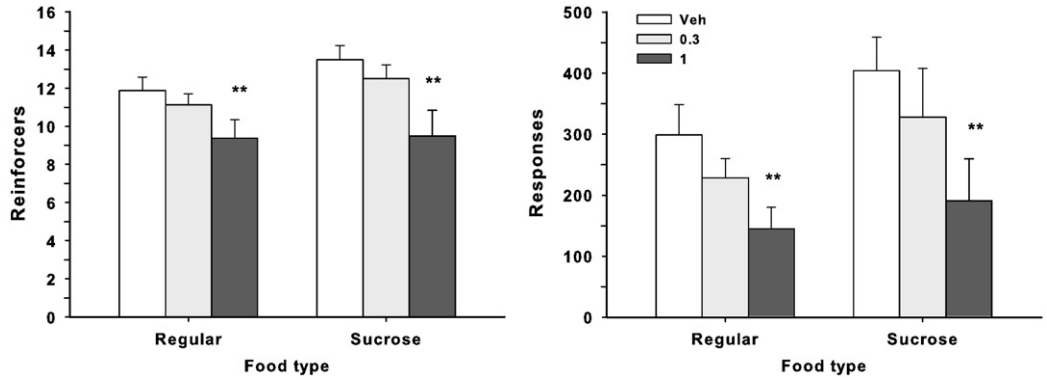

Fig. 1. This figure shows the effects of treatment with Ro60-0175 (vehicle, 0.3 and 1 mg/kg) on performance on a progressive ratio (PR) schedule of reinforcement in food deprived rats responding for either regular food ($n=8$) or sucrose ($n=8$) pellets. The separate panels show the number of reinforcers earned and the total number of responses made during the session. ** $p<0.01$ compared to Veh treatment.

the main effect of food type was not significant for reinforcers and responses [$F(1,18)=2.77$, and 3.21, $p>0.09$, respectively]. Responding was not altered by SB242084, since neither the main effect of SB242084 or the interaction between SB242084 and Food type were not significant (all $p>0.1$).

3.3. Experiment 2b. Effects of SB242084 on responding for food in non-food-restricted rats

Fig. 2B shows that non-deprived rats working for sucrose pellets made more responses [$F(1,18)=7.43$, $p<0.01$] and earned more reinforcers [$F(1,18)=8.47$, $p<0.01$] than rats working for regular pellets. A main effect of SB242084 was found for the measure of responses [$F(2,36)=3.41$, $p<0.05$] but not the number of reinforcers earned ($p>0.1$). There were no interactions between food type and SB242084 ($p>0.5$).

3.4. Experiment 3. Effects of combined treatment with SB242084 and Ro60-0175 on responding for food in food deprived rats

Data for this experiment are shown in Fig. 3. Analysis of the data for number of reinforcers earned found a significant main effect of Ro60-0175 treatment [$F(1,9)=7.28$, $p<0.02$], but not SB242084 ($p>0.4$). The interaction between Ro60-1075 and SB242084 was significant [$F(1,9)=10.1$, $p<0.01$]. For number of responses only the interaction term was significant [$F(1,9)=8.11$, $p<0.02$]. On both measures post-hoc testing confirmed that the effect of Ro60-0175 to reduce responding and the number of reinforcers earned was

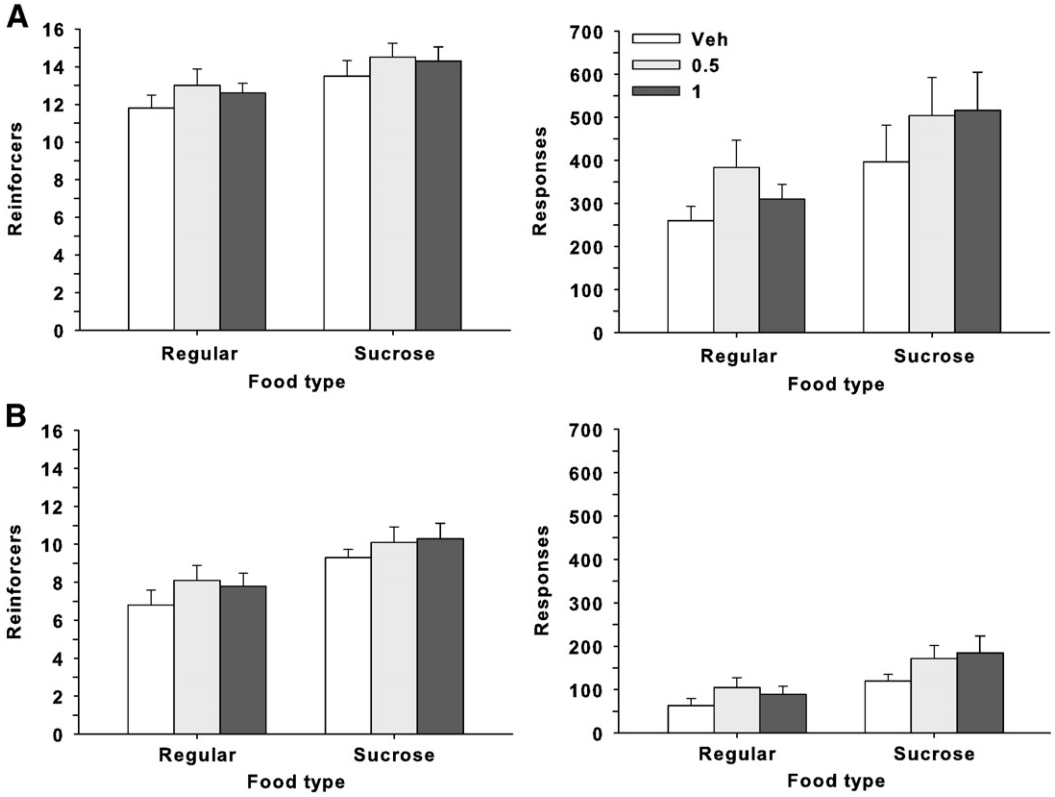

Fig. 2. This figure shows the effects of treatment with SB242084 (0.5 and 1 mg/kg) or its vehicle on performance on a progressive ratio (PR) schedule of reinforcement under conditions of food restriction (A) and no food restriction (B). The separate panels show the number of reinforcers earned and the total number of responses made during the sessions. Two different groups of rats responded for regular food pellets ($n=10$) or for sucrose food pellets ($n=10$). Rats were first tested under conditions of food restriction and then with no feeding restrictions.

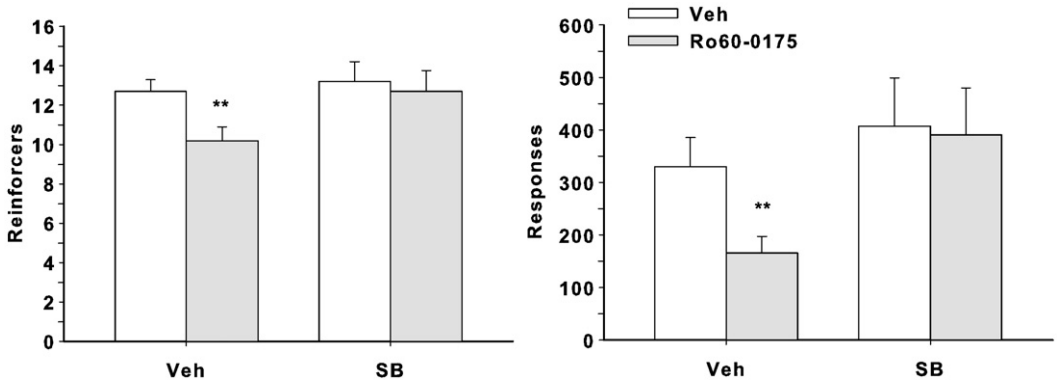

Fig. 3. The figure shows the effects on performance on the PR schedule of combining SB242084 (0.5 mg/kg), or its vehicle, with Ro60-0175 (1 mg/kg), or its vehicle. Rats were tested in a food-restricted state and responses were reinforced with regular food pellets. The separate panels show the number of reinforcers earned and the total number of responses made during the session. ** $p<0.01$ – compared to Veh-Veh condition.

reversed by SB242084. An identical pattern of responding was observed using sucrose as the reinforcer (data not shown).

3.5. Experiment 4. Effects of SB242084 on PR responding for food with different rates of progression

Response number and the number of reinforcers earned varied across the different PR schedules of reinforcement (Fig. 4). The main effects of schedule were significant for reinforcers [$F(2,16)=56.0$, $p<0.001$] and responses [$F(2,16)=5.77$, $p<0.01$]. SB242084 did not significantly affect the number of reinforcers earned or responses ($p>0.5$). The interaction between SB242084 and schedule was significant for the measure of reinforcers [$F(2,16)=4.05$, $p<0.04$] but not responses. Post-hoc analysis of this effect revealed that SB242084 slightly but significantly reduced the number of reinforcers earned on the schedule with the shallowest progression of ratios.

3.6. Experiment 5. Responding for food on a PR schedule in 5-HT$_{2C}$ receptor knockout mice

Fig. 5 shows performance of mice on the PR schedule. Analysis of data obtained under conditions of food-restriction found no significant effects of genotype on number of responses or number of reinforcers earned ($p>0.1$). Significant main effects of session for responses and reinforcers [$F(4,48)=2.74$ and 2.95, $p<0.05$] respectively, were found. The interactions between session and genotype were not significant. Post-hoc analyses on the main effect of session found that responses and reinforcers were lower on session 1 than on all other sessions.

Treatment with Ro60-0175 reduced responses and reinforcers earned [$F(1,12)=13.6$ and 23.3, respectively, $p<0.001$]. Significant interactions between genotype and Ro60-0175 treatment for responses [$F(4,48)=5.89$ $p<0.03$] and reinforcers [$F(4,48)=23.3$ $p<0.01$] were found. Ro60-0175 reduced both measures in WT mice, but not in 5-HT$_{2C}$ receptor null mutant mice.

Fig. 5 also shows that responses and reinforcers were reduced when mice were tested under free-feeding conditions. For the measure of reinforcers there was a significant main effect of genotype [$F(1,12)=5.19$, $p<0.05$]. Although the interaction between genotype and session was not significant ($p>0.1$) post-hoc testing showed that 5-HT$_{2C}$ receptor KO mice earned more reinforcers than WT mice on the first session, but not thereafter. A similar pattern of effects was observed for response number, where both the main effect of genotype [$F(1,12)=6.13$, $p<0.03$] and the genotype × session interaction were significant [$F(4,48)=3.59$, $p<0.02$].

4. Discussion

The results of the present experiments show that the 5-HT$_{2C}$ receptor agonist Ro60-0175 reduces responding for food on a progressive ratio schedule of reinforcement. This effect is mediated by 5-HT$_{2C}$ receptors

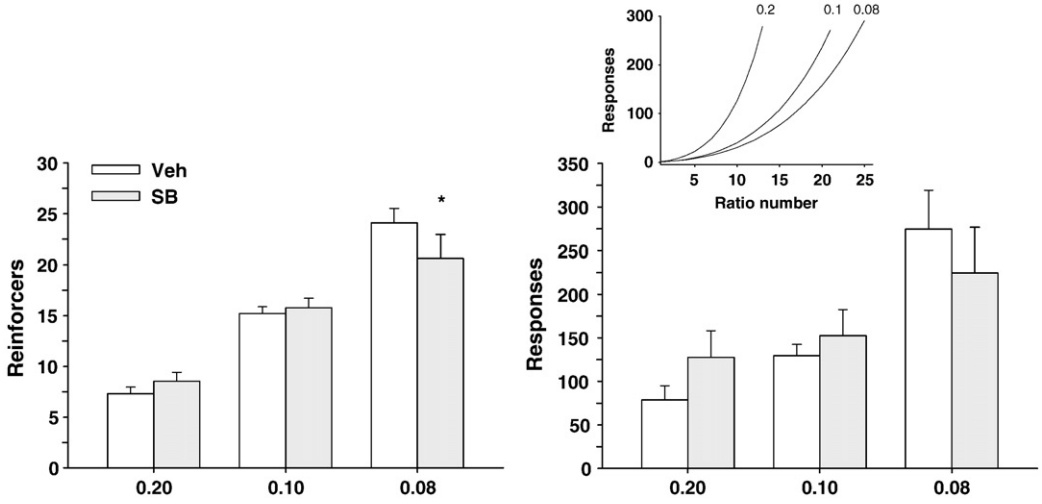

Fig. 4. The figure shows the effects of SB242084 (0.5 mg/kg) and vehicle on responding on 3 different progressive ratio schedules of reinforcement. The three schedule of reinforcement differed in terms of the rate of progression through successive ratios. The formula used to generate the progression was response ratio = $[5\times e^{(Y\times \text{reinforcer number})}-5]$, where $Y=0.2$, 0.1 or 0.08 for the three schedules. Rats were food-restricted and responded for regular food pellets. The same group of 9 rats was tested under both Vehicle and SB242084 on each of the three schedules after a 7-day period of stable responding on each schedule. The inset panel shows cumulative responses as a function of ratio number for the 3 schedules. * $p<0.05$ compared to Vehicle.

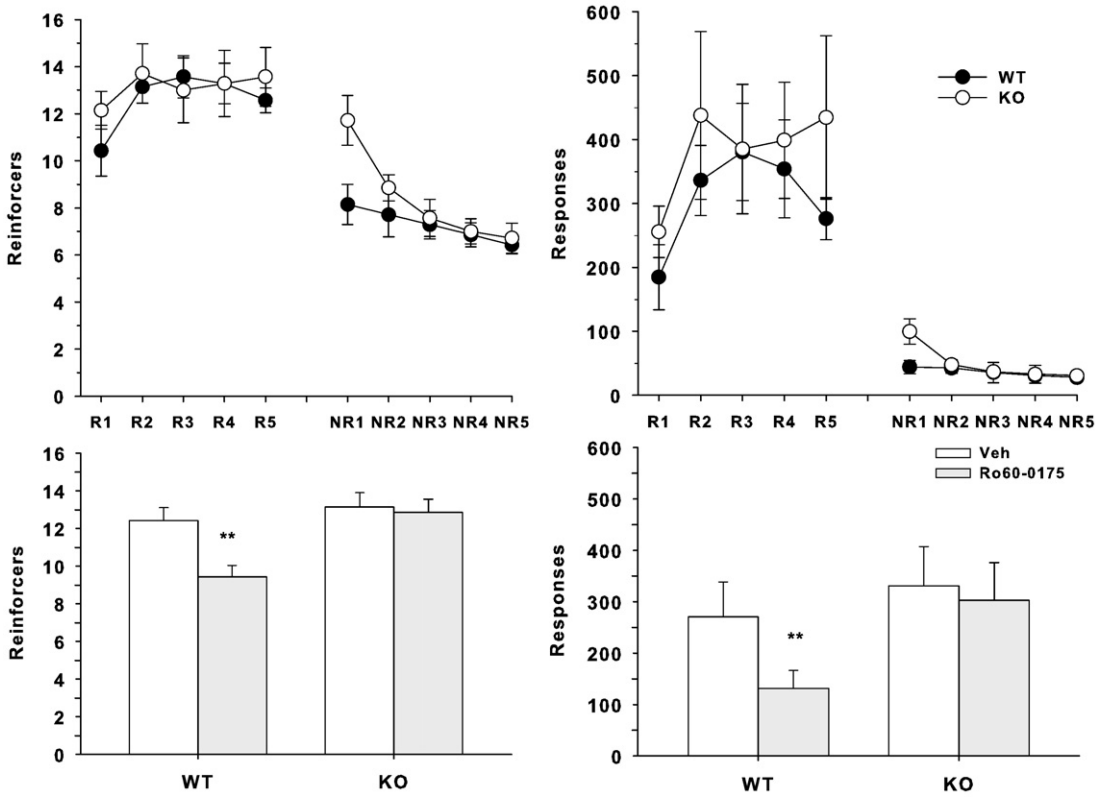

Fig. 5. The upper panels show performance on the PR schedule of 5-HT_{2C} receptor mutant mice (KO) and their wild type littermates (WT). The line graphs show the number of reinforcers earned and number of responses made on 5 consecutive sessions when mice were food-restricted (R), and 5 sessions with no food restriction (NR). The two phases were spaced 2 weeks apart during which time mice were food-restricted and tested for performance following treatment with 1 mg/kg Ro60-0175 and its vehicle. Data from this experiment are shown in the two lower panels. ** $p<0.01$ compared to WT-Veh treatment.

since it was blocked by the 5-HT_{2C} receptor antagonist SB242084, and was absent in mice lacking functional 5-HT_{2C} receptors. The effect of Ro60-0175 is consistent with observations that 5-HT_{2C} receptor stimulation reduces cocaine (Fletcher et al., 2008; Grottick et al., 2000), nicotine (Grottick et al., 2001) and alcohol (Tomkins et al., 2002) self-administration. The 5-HT_{2C} receptor antagonist SB242084 did not significantly increase responding for food under a range of testing conditions; similarly, mice lacking functional 5-HT_{2C} receptors did not differ from wildtype controls in their responding for food on the PR schedule. These results contrast with what has been reported for responding for cocaine on a PR schedule, where both SB242084 (Fletcher et al., 2002) and a 5-HT_{2C} receptor null mutation (Rocha et al., 2002) resulted in apparent enhanced motivation to self-administer cocaine.

It is well documented that 5-HT_{2C} receptor agonists reduce the consummatory component of feeding behaviour as shown by reductions in food intake (Clifton et al., 2000; Fletcher et al., 2009; Halford et al., 1997; Hayashi et al., 2005; Hewitt et al., 2002; Somerville et al., 2007; Vickers et al., 2000). The reduction in food intake induced by 5-HT_{2C} receptor agonists is associated with an advancement of the normal sequence of behaviours that characterise satiety (Clifton et al., 2000; Hewitt et al., 2002; Somerville et al., 2007). Therefore, one way in which 5-HT_{2C} receptor activation reduces feeding is by enhancing the process of satiation. On the PR schedule used in most of the current experiments rats typically earned on average fewer than 14 food pellets per session. This is a relatively small amount of food, especially considering that during the early part of training rats were readily consuming 100 pellets in less than 30 min, on a FR1 schedule. It seems unlikely then that the reduction in responding induced by Ro60-0175 on the PR schedule reflects on action on satiety processes, which likely were not even engaged by this small amount of food eaten. Rather the lower breaking point observed after treatment with Ro60-0175 suggests a reduction in appetitive aspects of feeding behaviour. This is consistent with the effects of a different 5-HT_{2C} receptor agonist, VER23779, that also reduced operant responding on a second-order schedule of food reinforcement (Somerville et al., 2007).

The effect of Ro60-0175 to reduce responding for food was similar regardless of whether responding was reinforced by regular or sucrose pellets. At first sight this could suggest that the effects of Ro60-0175 are independent of food palatability. However, it should be noted that the break-points for responding reinforced by regular and sucrose pellets were not significantly different, and so the test situation may not have been sensitive enough to detect palatability related changes in rate of responding. It seems likely that the food restriction regimen was sufficient to overcome any palatability-related differences in the motivation to respond for regular versus sucrose pellets since non-restricted animals in subsequent experiments responded to higher breaking points for sucrose compared to regular food. Regardless, the data still demonstrate a strong inhibitory effect of 5-HT_{2C} receptor stimulation on food-reinforced operant behaviour.

In addition to acting as an agonist at 5-HT_{2C} receptors Ro60-0175 also has appreciable affinity for 5-HT_{2A} and 5-HT_{2B} receptors (Porter et al., 1999). However, many of the behavioural effects of Ro60-0175 are mediated via 5-HT_{2C} receptors. This was confirmed in the context of the present experiments where in rats a 5-HT_{2C} receptor antagonist blocked the effect of Ro60-0175, and the drug was not effective in mice lacking functional 5-HT_{2C} receptors. A previous report showed that m-CPP reduced responding for food on a PR schedule (Ward et al., 2008). While m-CPP activates 5-HT_{2C} receptors it also has affinity for 5-HT_{1B} receptors and this contributes to its effects on feeding behaviour (Dalton et al., 2004, 2006; Kennett and Curzon, 1988). The complete reversal of the effects of Ro60-0175 by SB242084 indicate that 5-HT_{2C} receptor activation alone is sufficient to reduce responding for food on the PR schedule.

We previously reported that SB242084 (0.25–1 mg/kg) increased breaking points for intravenous infusions of cocaine on a progressive ratio schedule of reinforcement (Fletcher et al., 2002). Here, SB242084 at doses of 0.5 mg/kg and 1 mg/kg had no effect on breaking points in animals responding for food reinforcement. These doses were sufficient to induce functional blockade of 5-HT_{2C} receptors since SB242084 prevented the effect of Ro60-0175 to reduce responding. The effects of SB242084 were tested under a variety of test conditions. Under food restriction conditions animals responded to a similar degree for regular food and sucrose reinforcers, while under free-feeding conditions they reached higher breakpoints for sucrose pellets, suggesting an increased reinforcer efficacy for sucrose under these conditions. However, neither food palatability nor basic motivational state of the animals proved to be significant factors in determining the effects of SB24084 on responding. Similarly, it cannot be argued that a ceiling effect masked an effect of SB242084 to alter responding. Manipulation of food type and motivational state of the animal produced clear separations among the various conditions in terms of breaking point (or number of reinforcers earned) and response rate. Similarly, manipulation of the formula used to generate the rate of progression of the ratios was used to explicitly induce baseline differences in breaking points. Across all of these variations in basal response rate SB242084 showed no evidence of enhancing the motivation to respond for food. Consistent with this, the 5-HT_{2C} receptor null mutant mouse also was not different from control animals under conditions of food restriction or free-feeding.

The non-selective 5-HT receptor antagonists metergoline, methysergide, ritanserin and mesulergine have all been shown to induce short-term acute increases in consumption of food (Dourish et al., 1989; Fletcher, 1988). It was suggested that these effects might be mediated by 5-HT_{1C} (renamed 5-HT_{2C}) receptors (Dourish et al., 1989; Fletcher, 1988). However, this has not been borne out by subsequent studies with 5-HT_{2C} receptor antagonists. A number of studies have shown that SB242084, at doses that block the effects of 5-HT_{2C} receptor agonists on food intake, does not alter basal levels of intake (Clifton et al., 2000; Dalton et al., 2006; Hewitt et al., 2002; Kennett et al., 1997). One exception to this is the 5-HT_{2C} receptor antagonist RS-102221, that induced an increase in food intake (measured over periods of 72 h) and body weight gain (Bonhaus et al., 1997). However, the contribution of 5-HT_{2C} receptor blockade to this effect is questionable since RS-102221 did not alter hypolocomotion induced by m-CPP (Bonhaus et al., 1997), an effect that is blocked by SB242084 (Kennett et al., 1997; Martin et al., 2002) and altered in mice lacking functional 5-HT_{2C} receptors (Dalton et al., 2004; Fletcher et al., 2009; Heisler and Tecott, 2000). The 5-HT_{2C} receptor null mutant mouse consumes more food than wildtype animals, and develops a late-onset obesity. However, numerous reports show that this mutant mouse does not consistently show an increase in short-term food intake when food, including palatable items, is presented for a brief period of time (1 or 2 h). Therefore the lack of effect of SB242084 or genetically reduced 5-HT_{2C} function on progressive ratio responding for food is consistent with the majority of studies showing that these manipulations generally do not alter short-term consummatory feeding behaviour. In turn, this suggests that the previously described effects of 5-HT receptor antagonists to increase food intake result from non-5-HT_{2C} receptor mechanisms, and may even be mediated by the mixed pharmacological effects of these non-selective compounds at several different receptor subtypes.

Both 5-HT_{2C} receptor agonists and antagonists alter the activity of mesolimbic dopamine (DA) neurons. Agonists, including Ro60-0175, inhibit the firing rate of DA neurons leading to reduced extracellular levels of DA in the nucleus accumbens (Di Giovanni et al., 2000; Di Matteo et al., 2000). Conversely, antagonists including SB242084 increase DA neuronal firing rate and increase extracellular levels of DA (Di Giovanni et al., 1999; Di Matteo et al., 1998, 1999). Both DA depletion from the nucleus accumbens (Aberman et al., 1998) and DA receptor antagonists (Barbano et al., 2009; Sharf et al., 2005) reduce responding for food on PR schedules, an effect that is likely due in part to motivational factors. While these results imply a role for DA in mediating operant responding for food on the PR schedule it has proven far more difficult to demonstrate that responding can be enhanced by increasing DA release. For example, amphetamine increased break-points in one report (Poncelet et al., 1983), but had no effect or even reduced break-points in several more (Blokland et al., 2005; Mobini et al., 2000; Poncelet et al., 1983; Smith et al., 1997; Wiley and Compton, 2004). Therefore, the effects of Ro60-0175 to apparently reduce feeding motivation and the lack of effect of SB242084, and disruption of the 5-HT_{2C} receptor on feeding motivation are generally consistent with their known effects on DA function, and the role of DA in mediating responding on a PR schedule. The increase in responding for cocaine on a PR schedule induced by SB242084 is also consistent with the fact that responding for psychomotor stimulants is directly related to the extent to which the stimulant increase DA levels, and with the fact that SB242084 (Navailles et al., 2004) and the 5-HT_{2C} receptor null mutation (Rocha et al., 2002) both increase the capacity of cocaine to elevate extracellular levels of DA.

Another potential explanation for the apparent differential impact of SB242084 on responding maintained by food reinforcers and by cocaine may relate to the impact food and cocaine have on 5-HT systems. Cocaine blocks the serotonin transporter, and self-administered cocaine increases extracellular levels of 5-HT in areas such as the nucleus accumbens (Parsons et al., 1995; Reith et al., 1997). Food ingestion has been shown to induce small increases in 5-HT release in hypothalamic areas (Schwartz et al., 1989) but not in the nucleus accumbens (Verhagen et al., 2009). Thus, there may be reinforcer-induced differences in endogenous 5-HT tone, which is likely higher in animals engaged in responding for cocaine than in those responding for food pellets. Accordingly, blockade of this higher degree of tone by SB242084 may facilitate enhanced responding for cocaine but not food.

In conclusion, while we confirm that 5-HT_{2C} receptor activation reduces responding for foods available under a PR schedule, 5-HT_{2C} receptor blockade does not increase responding. The effect of Ro 60-0175 is consistent with the clinical evidence that another 5-HT_{2C} receptor agonist, lorcaserin, reduces body weight and meal size in obese individuals (Smith et al., 2009). Conversely, the failure of a 5-HT_{2C} antagonist to increase feeding behaviour when tested under a variety of relatively short-term experimental conditions may indicate that these conditions were not appropriate, or optimal, for detecting such an effect. Recently, it was shown that a sustained period of access to highly palatable foods leads to compulsive eating and down-regulation of striatal DA D_2 receptors (Johnson and Kenny, 2010), suggesting possible adaptive changes to central DA signalling. Extending the period of access to palatable foods may also represent a valid strategy for testing 5-HT_{2C} receptor antagonists on feeding behaviour.

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