Psychopharmacology (2014) 231:787–800
DOI 10.1007/s00213-013-3295-2

ORIGINAL INVESTIGATION

On the behavioural specificity of hypophagia induced in male rats by *m*CPP, naltrexone, and their combination

F. L. Wright · R. J. Rodgers

Received: 21 June 2013 / Accepted: 16 September 2013 / Published online: 11 October 2013

Abstract
Rationale Serotonergic (5-hydroxytryptamine, 5-HT) and opioidergic mechanisms are intimately involved in appetite regulation.
Objectives In view of recent evidence of positive anorectic interactions between opioid and various non-opioid substrates, our aim was to assess the behavioural specificity of anorectic responses to the opioid receptor antagonist naltrexone, the 5-$HT_{2C/1B}$ receptor agonist *m*CPP and their combination.
Methods Behavioural profiling techniques, including the behavioural satiety sequence (BSS), were used to examine acute drug effects in non-deprived male rats tested with palatable mash. Experiment 1 characterised the dose–response profile of *m*CPP (0.1–3.0 mg/kg), while experiment 2 assessed the effects of combined treatment with a sub-anorectic dose of *m*CPP (0.1 mg/kg) and one of two low doses of naltrexone (0.1 and 1.0 mg/kg).
Results Experiment 1 confirmed the dose-dependent anorectic efficacy of *m*CPP, with robust effects on intake and feeding-related measures observed at 3.0 mg/kg. However, that dose was also associated with other behavioural alterations including increased grooming, reductions in locomotion and sniffing, and disruption of the BSS. In experiment 2, naltrexone dose-dependently reduced food intake and time spent feeding, effects accompanied by a behaviourally selective acceleration in the BSS. However, the addition of 0.1 mg/kg *m*CPP did not significantly alter the behavioural changes observed in response to either dose of naltrexone given alone.
Conclusions In contrast to recently reported positive anorectic interactions involving low-dose combinations of opioid receptor antagonists or *m*CPP with cannabinoid CB1 receptor antagonists, present results would not appear to provide any support for potentially clinically relevant anorectic interactions between opioid and 5-$HT_{2C/1B}$ receptor mechanisms.

Keywords Naltrexone · *m*CPP · Co-treatment · Drug interaction · Food intake · Ingestive and non-ingestive behaviour · Behavioural specificity · Behavioural satiety sequence

F. L. Wright · R. J. Rodgers (✉)
Behavioural Neuroscience Laboratory, Institute of Psychological Sciences, University of Leeds, Leeds LS2 9JT, UK
e-mail: r.j.rodgers@leeds.ac.uk

Introduction

Over the past 15 years, significant advances have been made in our understanding of the neurobiology of appetite (Adan 2013; Halford et al. 2010; Harrold et al. 2012; Heal et al. 2012; Kennett and Clifton 2010; Rodgers et al. 2012; Vickers et al. 2011). Thus, despite the clinical disappointments of even the very recent past (i.e. rimonabant, sibutramine), there are reasons to be optimistic regarding the feasibility of therapeutic innovation in the field of anti-obesity medication. Such optimism has been reinforced by FDA (http://www.fda.gov/NewsEvents/NewsRoom/PressAnnouncements/ucm309993.htm, http://www.fda.gov/NewsEvents/NewsRoom/PressAnnouncements/ucm312468.htm) approval in 2012 of two new anti-obesity agents, Belviq® (lorcaserin; a 5-hydroxytryptamine $(5\text{-HT})_{2C}$ receptor agonist; O'Neill et al. 2012) and Qsymia® (a polytherapeutic combination of phentermine, a sympathomimetic, and topiramate, an anticonvulsant; Garvey et al. 2012). In this context, pharmacological polytherapies are attracting renewed interest in view of the potential advantages of concurrently targeting multiple mechanisms, e.g. use of lower drug doses, possible additive or synergistic interactions and reductions in toxic risk as well as the likelihood of counter-regulation (Adan 2013; Gadde and Allison 2009; Padwal 2009; Roth et al. 2010; Young 2012). At least two other (initially rejected) drug combinations are likely

to be re-filed with the FDA in the near future: Contrave® (a combination of the atypical antidepressant bupropion and the opioid receptor antagonist naltrexone; http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/endocrinologicandmetabolicdrugsadvisorycommittee/ucm235671.pdf; Greenway et al. 2009) and Empatic® (a combination of bupropion and the anticonvulsant zonisamide; Gadde et al. 2007).

Four research strategies can be identified in the polytherapy literature (for review, see Roth et al. 2010): (a) combinations of satiety peptides, e.g. CCK, glucagon and bombesin (Hinton et al. 1986), amylin with CCK or $PYY_{3–36}$ (Bhavsar et al. 1998; Roth et al. 2007), exendin-4 with calcitonin or $PYY_{3–36}$ (Bello et al. 2010; Reidelberger et al. 2011; Talsania et al. 2005) and GLP-1 with glucagon or $PYY_{3–36}$ (Day et al. 2009; Paulik et al. 2011); (b) combinations of an adiposity signal and a satiety peptide, e.g. leptin with amylin (Roth et al. 2008a; Ravussin et al. 2009) or exendin-4 (Bojanowska and Nowak 2007); (c) a small molecule agent with either a satiety peptide or an adiposity signal, e.g. sibutramine with amylin (Roth et al. 2008b) or leptin (Boozer et al. 2001), phentermine with amylin (Roth et al. 2008b) and the cannabinoid CB1 receptor antagonist/inverse agonist AM-251 with exendin-4 (Bojanowska and Radziszewska 2011) and (d) combinations of small molecule agents, e.g. phentermine with fenfluramine (Weintraub et al. 1992), and rimonabant with either *d*-fenfluramine (Rowland et al. 2001) or the preferential $5\text{-}HT_{2C}$ receptor agonist *m*CPP (Ward et al. 2008). All of these combinations have produced at least additive anorectic/weight loss effects in rodents with some showing similar effects in early phase clinical trials.

It has been known for at least 40 years that both serotonin (5-hydroxytryptamine) and the endogenous opioids are intimately involved in appetite regulation. Stemming from early work on precursors, releasers and reuptake inhibitors, the anorectic/weight loss effects of 5-HT are currently thought to be mediated via $5\text{-}HT_{1B}$ and $5\text{-}HT_{2C}$ (formerly $5\text{-}HT_{1C}$) receptors expressed, respectively, on arcuate NPY/AgRP and POMC neurons which, in turn, influence downstream signalling in the melanocortin system (Adan 2013; Dourish 1995; Halford et al. 2007; Heisler et al. 2002, 2003, 2006). Although this avenue of research has resulted in the recent clinical introduction of Belviq®, a selective $5\text{-}HT_{2C}$ receptor agonist (see above), almost all the pioneering work in this field has employed the $5\text{-}HT_{2C/1B}$ receptor agonist, *m*CPP (Barnes and Sharp 1999; Hoyer et al. 1994). Numerous studies have shown that this agent dose-dependently suppresses food intake and weight gain both in rodents (Hewitt et al. 2002; Kennett et al. 1987; Kennett and Curzon 1988a, b; Kitchener and Dourish 1994; Lee et al. 2004; Samanin et al. 1979; Simansky and Vaidya 1990; Ward et al. 2008) and humans (Cowen et al. 1995; Sargent et al. 1997; Walsh et al. 1994). However, this same database also raises questions about the selectivity of the anorectic response in that *m*CPP is reported to concomitantly induce excessive grooming, nausea and hypoactivity. Similar behavioural profiles have been reported for more recently developed $5\text{-}HT_{2C}$ receptor agonists, such as CP-809101, lorcaserin, Ro 60-0175 and VER 23779 (Clifton et al. 2000; Kennett et al. 2000; Hewitt et al. 2002; Higgins et al. 2012, 2013; Somerville et al. 2007). *m*CPP has additionally been found to enhance anxiety in rodents (Benjamin et al. 1990; Griebel et al. 1991; Kennett et al. 1989; Rodgers et al. 1992) and humans (e.g. Cowen et al. 1995; Westenberg and den Boer 1994).

In parallel to the 5-HT story, research on the role of endogenous opioids in appetite has repeatedly demonstrated that opioid receptor antagonists (e.g. naloxone, naltrexone) inhibit feeding in numerous species and test situations and that such effects are stereospecific, μ-receptor-dependent and largely centrally mediated (Berridge 2009; Bodnar 2004; Cooper et al. 1988; Giuliano et al. 2012). More recent studies strongly suggest that endogenous opioids are predominantly involved in the hedonics of feeding, a proposal supported by the discovery of 'hot-spots' for μ-opioid enhancement of taste hedonics in the ventral forebrain (Berridge 2009; Nathan and Bullmore 2009; Berridge et al. 2010). Significantly, in the present context, opioid antagonist-induced anorexia is behaviourally selective in that it occurs without disrupting the normal structure of feeding behaviour, i.e. the behavioural satiety sequence (BSS) (Cooper and Turkish 1989: Kirkham and Blundell 1986, 1987; Tallett et al. 2008a; Wright and Rodgers 2013). For these reasons, several research groups have explored the appetite-suppressant potential of low-dose combinations of opioid receptor antagonists and other appetite-modulating agents. For example, dose-dependent additive and/or synergistic anorectic interactions have been reported for naloxone or naltrexone in combination with cannabinoid CB1 receptor antagonist/inverse agonists such as rimonabant and AM-251 (e.g. Kirkham and Williams 2001; Pietras and Rowland 2002; Rowland et al. 2001; Tallett et al. 2008b, 2009a) and, more recently, with the atypical antidepressant bupropion (e.g. Greenway et al. 2009; Wright and Rodgers 2013).

In this context, opioid-5-HT interactions have been well documented in the literature, with pain inhibition perhaps the best known example (e.g. Basbaum and Fields 1984). Of more direct relevance to the present study, it is known that the medial hypothalamus is especially responsive to the appetite-inhibiting effects both of opioid receptor antagonists (for review, see Bodnar 2004) and the SSRI fluoxetine (e.g. Weiss et al. 1991). Furthermore, although we have previously failed to observe a positive anorectic interaction between naloxone and the dual 5-HT/noradrenaline reuptake blocker sibutramine (Tallett et al. 2010), additive anorectic effects have in fact been reported for naloxone given in combination with fluoxetine (Hagan et al. 1997) and 5-hydroxytryptophan (Fernandez-Tome et al. 1988). On the basis of these findings and the potential advantages of concurrently targeting hedonic

and satiety signalling, the present study employed BSS methodology (Halford et al. 1998; Rodgers et al. 2010; Vickers and Clifton 2012) to assess the anorectic efficacy and behavioural specificity of combined low-dose treatment with the opioid receptor antagonist naltrexone and the 5-$HT_{2C/1B}$ receptor agonist *m*CPP. *m*CPP was selected for these studies in view of the involvement of both 5-HT_{2C} and 5-HT_{1B} receptor mechanisms in serotonergic regulation of appetite and a very much more comprehensive published literature on this compound relative to other more recently developed agents. As relevant dose–response data on naltrexone were already available (Wright and Rodgers 2013), experiment 1 characterises the dose–response profile of *m*CPP under local test conditions while experiment 2 explores the effects of combined low-dose treatment with these agents.

Methods

Subjects

Adult male Lister hooded rats (experiment 1, 200.5±2.3 g; experiment 2, 216.3±1.4 g) were pair-housed (46×26.5×26 cm) for 1 week following arrival from Charles River, UK. They were then transferred to individual cages (45×20×20 cm), each containing a polycarbonate rat tunnel (Datesand Ltd, Manchester, UK), for the remainder of the study. Single housing facilitated initial familiarisation with the test diet as well as daily bodyweight tracking. Animals were maintained on a 12-h normal light cycle (lights on, 0700 h) in a temperature- (21±1 °C) and humidity (50±2 %)-controlled environment. A normal light cycle was employed as a much clearer BSS is obtained when rats are tested during the light phase of the cycle (Tallett et al. 2009b). Rats were handled regularly during routine husbandry and were fully habituated to all experimental procedures prior to drug testing (see below). With the exception of the injection-test interval, chow pellets (BK No. 1 Rodent Breeder and Grower, Special Diets Services, UK; digestible energy value=13.62 kJ/g) and tap water were available ad libitum in the home cages. Bodyweights were recorded at the same time daily throughout the experiment. All procedures were conducted under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

Drugs

1-(3-Chlorophenyl) piperazine hydrochloride (*m*CPP; Tocris Bioscience, UK) and naltrexone hydrochloride (NTX; Sigma-Aldrich, UK) were dissolved to required concentrations in physiological saline (0.9 %) which, alone, served for control injections. In experiment 1 (*m*CPP dose–response), drug doses (0.1, 1.0 and 3.0 mg/kg) were selected from published research (e.g. Hewitt et al. 2002; Kennett and Curzon 1988a, b; Kitchener and Dourish 1994; Lee et al. 2004; Simansky and Vaidya 1990; Ward et al. 2008). In experiment 2, a low dose of *m*CPP (0.1 mg/kg) was used in combination with one of two doses of naltrexone (0.1 mg/kg=NL, 1.0 mg/kg=NH) chosen on the basis of BSS profiles recently reported by our research group (Wright and Rodgers 2013). In both studies, treatments were administered intraperitoneally in a volume of 1 ml/kg. In experiment 1, injections were given 30 min prior to testing while, in experiment 2, the first injection (vehicle (VV) or *m*CPP) was given 30 min prior to testing with the second (vehicle, NL or NH) given 15 min prior to testing.

Apparatus

Feeding studies were conducted in a glass vivarium (60×30×45 cm), large enough for animals to freely engage in a range of ingestive and non-ingestive behaviours (e.g. Ishii et al. 2003a, b; Tallett et al. 2009a, b; Wright and Rodgers 2013). The arena floor was covered with wood shavings, a water bottle was suspended from one of the end-walls and a preweighed glass food pot was secured (by Velcro™ and an annular metal mounting) to centre of the floor. The test diet (mash) was prepared freshly each morning by simply hydrating a powdered form of the maintenance diet (BK No. 1 Rodent Breeder and Grower, Special Diets Services, UK; 1 g dry=3.125 g mash; digestible energy value=4.36 kJ/g). Portions of mash were disbursed to individual pots, covered and kept cool until shortly before use. Mash has the advantage of high palatability (e.g. Ishii et al. 2003b), while its consistency minimises spillage and hoarding (e.g. Halford et al. 1998). Two videocameras, one positioned above the arena and the other horizontal to the front wall, recorded test sessions for subsequent behavioural analysis. As a split-screen view greatly facilitates scoring accuracy, camera signals were fed via an image merger to a nearby monitor and DVD recorder.

Procedure

All feeding tests (habituation and test) were conducted during the light phase of the light/dark cycle (0700–1900 hours) under normal laboratory illumination (265 lux). During each session, two control food pots (positioned adjacent to the test arena) confirmed minimal (<0.22 %) loss of food mass through evaporation alone.

Habituation phase

After 10-day acclimatisation to local conditions, rats were home cage-familiarised with mash for 3 h on two consecutive days. The following week, they were habituated daily for 5 days to all aspects of the experimental procedure. For experiment 1, this involved the removal of home cage food

and environmental enrichment (rat tunnel), IP injection of saline (1 ml/kg) and return to the home cage for 30 min. For experiment 2 (interaction study), animals were given two saline injections spaced 15 min apart and returned to their home cages after each injection. After a total of 30 min, subjects in both studies were individually placed in the test arena for 1 h with pre-weighed mash and ad libitum tap water. Mash consumption (controlling for any spillage) was accurately measured on each of these trials, with subjects immediately returned to their home cages (chow and enrichment reinstated). This habituation phase served both to familiarise animals with all procedures and to facilitate the development of stable mash consumption prior to the experimental phase.

Experimental phase

In both experiments, drug testing commenced within 3 days of the final habituation trial. In a within-subjects (crossover) design, a Latin square was used to determine treatment order with a 7-day washout period between treatments. On test days, rats were individually transported to a preparation room where they received IP drug treatment and then immediately returned to their home cages from which chow and enrichment had been removed. After the drug-appropriate injection-test interval, they were transferred to an adjacent laboratory, individually placed in the test arena with preweighed mash and ad libitum tap water and left undisturbed for the 1-h DVD-recorded test session. At the end of the test, any spillage was carefully retrieved, food pots accurately reweighed and animals returned to their home cages (chow and enrichment reinstated). Experiment 1 assessed the dose–response profile of *m*CPP, while experiment 2 assessed the effects of a single sub-anorectic dose of *m*CPP in the presence or absence of one of two doses of NTX.

Behavioural analysis

Test DVDs were scored blind by a highly trained observer (intra-rater reliability≥0.8), using ethological analysis software ('Hindsight'; Weiss 1995) that permits real-time scoring of behaviour by direct keyboard entry to a PC. A continuous observation method was employed due to its advantages over time-sampling techniques (Halford et al. 1998). Based on previous research (e.g. Ishii et al. 2003a, b; Tallett et al. 2009a, b; Wright and Rodgers 2013), measures recorded from DVD were *latency to locate food source* (time in seconds between the start of testing and first contact with the food pot) and *latency to feed* (time in seconds between first contact with the food source and the first feeding episode), together with frequency and duration of the following mutually exclusive behavioural categories: *feeding* (biting, gnawing or swallowing food from food pot or from forepaws), *drinking* (licking the spout of the water bottle), *grooming* (licking of the body, feet and genitals; stroking of face and whiskers with forepaws, biting the tail), *scratching* (repetitive ipsilateral hind paw scratching of flanks, neck and head), *sniffing* (rapid wrinkling of the nose/twitching of vibrissae at an aspect of the environment, head movements with rear limbs immobile), *locomotion* (walking around the cage or circling; movements involving all four limbs), *rearing* (forepaws raised from the cage floor, either supported against a wall or free standing) and *resting* (sitting or lying in a relaxed position with head curled to body or resting on the floor; animal inactive). Two further measures of feeding behaviour were derived from the recorded parameters: *average duration of feeding bouts* (total feeding duration in seconds divided by total feeding frequency) and *average feeding rate* (total food intake in grams divided by total feeding duration in minutes).

In addition to examining treatment effects on total behavioural scores, each 60-min test period was divided into 12×5-min timebins thereby permitting analysis of treatment effects over time. Within these time-course analyses, specific attention was paid to the BSS, i.e. the temporal relationship between eating, grooming and resting (Rodgers et al. 2010).

Test-day bodyweight and post-treatment bodyweight gain

Bodyweights were recorded at the same time daily from day 1 of individual housing until 7 days post-dosing. This procedure was used both to confirm the equivalence of test-day bodyweights across the different treatment conditions and to check for possible carry-over effects of acute drug treatment on weight gain. In addition to analysing treatment effects on 7-day absolute weight gain, finer-grain analysis was conducted by expressing bodyweights for each post-treatment day as a percentage of test-day bodyweight (where test day=100 %).

Statistical analysis

For experiment 1, data for food intake (habituation and test), test day bodyweight, total behavioural scores and 7-day absolute weight gain were analysed by one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni comparisons. Effects on behavioural change over time within the test session, as well as on percentage bodyweight gain over the 7-day post-dosing, were analysed by two-way repeated measures ANOVA (drug by timebin; drug by day). Significant interactions were initially explored using one-way ANOVA for each time period/day followed, where significant, by Bonferroni tests. For experiment 2, habituation data were analysed by one-way repeated measures ANOVA, whereas data for test intake, behaviour totals and 7-day absolute weight gain were analysed by 2-way repeated measures ANOVA (*m*CPP×NTX). Effects on behavioural change over time, as well as on percentage bodyweight gain over the 7-day post-dosing, were analysed by three-way repeated measures

ANOVA (*m*CPP×NTX×by timebin or day). Significant interactions were initially explored using two-way repeated measures ANOVA for each time period/day followed, where significant, by Bonferroni tests. In all cases, where datasets failed Mauchly's test of sphericity, Greenhouse–Geisser significance levels are reported. Findings were accepted as significant when $p \leq 0.05$.

Results

Experiment 1: mCPP dose–response

Habituation

Over the course of the study, mean bodyweight for the sample increased from 200.5±2.3 to 470.6±9.0 g. Mash consumption differed significantly during habituation (trial 1 (T1), 13.14± 1.42 g; T2, 15.86±1.80 g; T3, 17.62±1.64 g; T4, 18.75± 1.58 g; T5, 19.00±1.86 g; $F(4,36)=12.70$, $p<0.001$), with intake on T1 lower than on T3, T4 and T5 ($p \leq 0.01$). However, the development of stable intake was confirmed both by the lack of significant variation across habituation T2–5 and the close similarity in scores between habituation T5 and vehicle control in the subsequent experiment (19.26±1.20 g).

Effects of m CPP

Test day bodyweight and food intake Test-day bodyweights were equivalent across the four treatment conditions (V, 402.4± 12.2 g; 0.1*m*CPP, 407.4±11.6 g; 1.0*m*CPP, 413.2±15.1 g; 3.0*m*CPP, 403.2±9.3 g ($F(3,27)=0.18$, $p>0.05$). The effects of *m*CPP on mash consumption are shown in Table 1. Treatment with the 5-HT_{2C} receptor agonist significantly influenced food intake ($F(3,27)=33.77$, $p<0.001$), with Bonferroni comparisons confirming significant suppression relative to vehicle control at 1.0 mg/kg (38.6 % decrease; $p<0.01$) and 3.0 mg/kg (57.4 % decrease; $p<0.001$). The intermediate ($p=0.051$) and highest ($p<0.001$) dose levels both differed significantly from 0.1 mg/kg but not from each other.

Total behavioural scores Effects of *m*CPP on feeding-related parameters (latencies, average duration of eating bouts and average rate of eating) are summarised in Table 1, while effects on the total frequency and duration of ingestive and non-ingestive behaviours are shown in Fig. 1. As animals did not show appreciable amounts of drinking during the 1-h test, these data are not reported. ANOVA revealed significant effects of *m*CPP on: latency to locate the food ($F(1.22, 10.94)=18.95$, $p=0.001$), the average duration of eating bouts ($F(1.29, 11.60)=6.43$, $p<0.01$) and eating rate ($F(3,27)=40.15$, $p<0.001$), as well as the frequency of eating ($F(3,27)=11.60$, $p<0.001$), sniffing ($F(3,27)=5.83$, $p<0.01$) and resting ($F(3,27)=3.13$, $p<0.05$), the frequency and duration of locomotion ($F(3,27)\geq 7.22$, $p \leq 0.001$) and the duration of grooming ($F(3,27)=9.82$, $p<0.001$). No other variables showed a significant effect of drug ($F(3,27)\leq 2.89$, $p>0.05$).

As summarised in Table 1 and Fig. 1, the lowest dose of *m*CPP (0.1 mg/kg) had no significant effects on behaviour. The intermediate dose of 1.0 mg/kg significantly reduced the rate of eating ($p<0.001$) while the reduction in eat frequency and the increase in eat bout duration at this dose level closely approached significance ($p \leq 0.06$). Most treatment effects were observed at the highest dose tested (3.0 mg/kg), which, relative to vehicle control, increased the time taken to locate the food source at the beginning of the test ($p<0.01$) and time spent grooming ($p<0.001$), while reducing the rate of eating ($p<0.001$), as well as the frequency of eating, locomotion and sniffing ($p \leq 0.02$). It is worth noting that this dose also produced effects on eat bout duration (increase) and locomotion frequency (decrease) that only just failed to reach significance ($p \leq 0.06$).

Behavioural time courses and behavioural satiety sequence With the exception of grooming and scratching ($F \leq 1.78$, $p>0.05$), two-way ANOVA revealed significant main effects of time for the frequency ($F(11, 99)\geq 11.29$, $p \leq 0.001$) and duration ($F(11, 99)\geq 3.13$, $p \leq 0.001$) of all behavioural measures. This result confirms the typical pattern of behaviour during these 1-h feeding tests which, as the session progresses, comprises a gradual reduction in active behaviours and an increase in resting (e.g. Ishii et al. 2003a, b; Rodgers et al. 2001; Tallett et al. 2009a, b; Wright and Rodgers 2013). Significant drug×time interactions were found for the frequency and duration of eating ($F(33, 297)\geq 1.80$, $p \leq 0.01$), rearing ($F(33, 297)\geq 1.77$, $p \leq 0.01$) and locomotion ($F(33,$

Table 1 Dose–response effects of *m*CPP hydrochloride (0.1–3.0 mg/kg, IP) on feeding-related parameters in male rats tested for 1 h with palatable mash

Measure	Vehicle	*m*CPP 0.1	*m*CPP 1.0	*m*CPP 3.0
Mash intake (g)	19.26±1.20	16.29±1.24	11.82+1.45*	8.20±1.00**
Latency to find food (s)	5.75±1.82	11.13±3.78	9.29±2.25	52.04±8.90*
Latency to eat (s)	16.64±2.61	15.94±6.71	34.34±14.70	48.50±22.36
Eat bout (s)	8.23±0.73	10.18±1.33	13.31±1.73***	20.35±4.23***
Eat rate (g/min)	1.75±0.08	1.52±0.13	1.04±0.07**	0.67±0.08**

Data are mean values (± SEM)

*$p<0.01$; **$p<0.001$ vs vehicle; ***$p<0.06$

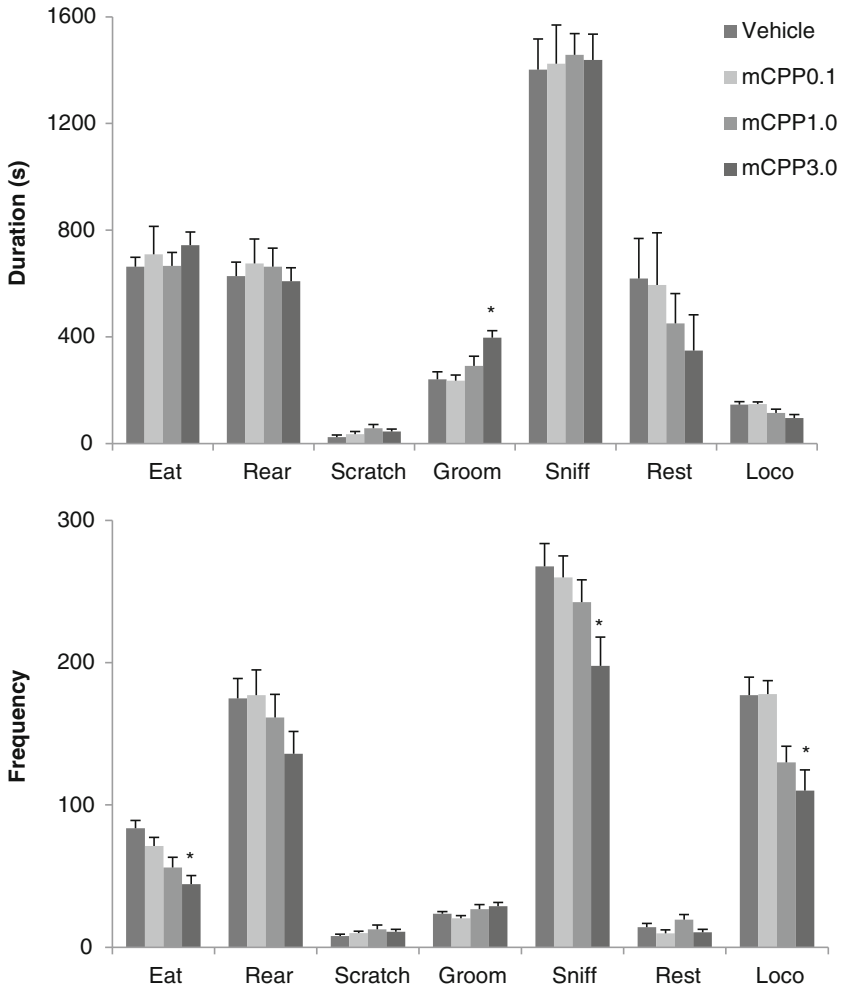

Fig. 1 Effects of acute *m*CPP HCl (0.1–3.0 mg/kg, IP) on behaviours displayed by male rats during a 1-h test with palatable mash. *Upper panel* total duration scores. *Lower panel* total frequency scores. Data are expressed as mean values±SEM. *$P \leq 0.05$ versus vehicle control. See Table 1 for complementary data and text for details

297)≥1.82, $p \leq 0.01$), as well as the frequency of sniffing and scratching ($F(33, 297) \geq 1.74$, $p \leq 0.01$) and the duration of grooming ($F(33, 297) = 1.90$, $p < 0.01$).

A series of one-way ANOVAs (and Bonferroni post hocs) within each timebin indicated that 1.0 and 3.0 mg/kg *m*CPP reduced the frequency of feeding during timebins 1–3 ($p \leq 0.01$). Furthermore, over the same early timeframe, the 3.0-mg/kg dose additionally increased time spent grooming ($p < 0.05$) and reduced the frequency of rearing and sniffing, as well as the frequency and duration of locomotion (all $p < 0.05$). Figure 2 illustrates the temporal effects of *m*CPP for the frequency of eating, locomotion, rearing and sniffing.

Treatment effects on the BSS are summarised in Fig. 3. The vehicle control profile shows a clear peak feeding response during the first 15–20 min of the test. Over time, feeding is seen to decline while time spent resting increases, with an eat-to-rest transition occurring just over half-way through the session (timebin 7). This profile is fully consistent with findings in our laboratory over a number of years (e.g. Ishii et al. 2003a, b; Rodgers et al. 2001; Tallett et al. 2009a, b; Wright and Rodgers 2013). Although very similar normal behaviour patterns were evident with both the low and intermediate doses of *m*CPP, the highest dose of the compound tended to disrupt the BSS. Although an eat-to-rest transition is discernible (timebin 8), 3 mg/kg *m*CPP not only suppressed the peak feeding response but also induced an unusual behaviour pattern characterised by periodic feeding and higher-than-normal levels of grooming throughout the test session (see Fig. 3).

Post-treatment bodyweight gain (data not shown) ANOVA failed to reveal any significant effect of acute *m*CPP treatment on 7-day absolute weight gain ($F(3, 27) = 0.11$, $p > 0.05$). Although analysis of percent daily weight gain confirmed normal growth over time ($F(6, 54) = 228.27$, $p < 0.001$), this analysis also failed to reveal a main effect for drug treatment ($F(3, 27) = 0.20$, $p > 0.05$) or a drug×time interaction ($F(18, 162) = 0.78$, $p > 0.05$).

Experiment 2: *m*CPP/naltrexone co-treatment

Habituation

Mean bodyweight on arrival was 216.3±1.4 and 532.8±9.6 g on completion of the study. Intake differed significantly during

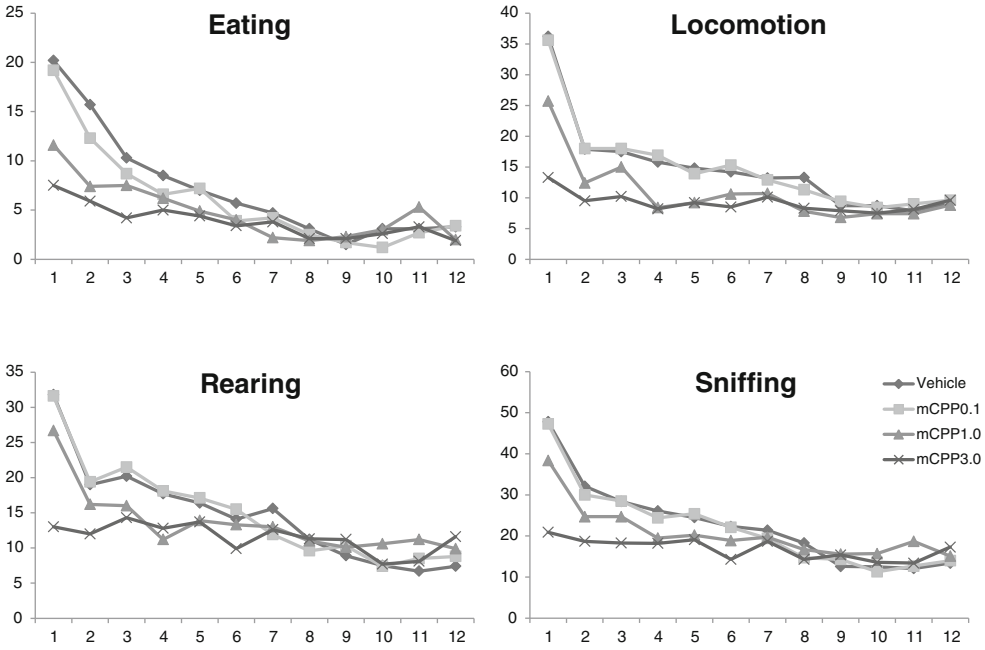

Fig. 2 Effects of acute *m*CPP HCl (0.1–3.0 mg/kg, IP) on the frequency of eating, locomotion, rearing and sniffing in male rats during a 1-h test with palatable mash. Data are expressed as the mean frequency of each behaviour in 12×5-min timebins. Dose-dependent suppression of behaviour is apparent during the early part of the test session. See Fig. 3 for complementary data and text for details

habituation week ($F(4, 36)=32.75$, $p<0.001$), with intake on T1 significantly lower ($p \leq 0.05$) than on T2, T3 and T5 and intake on T2 significantly different from that on T3, T4 and T5 ($p \leq 0.05$): T1 = 9.61 ± 1.82 g, T2 = 16.17 ± 1.79 g, T3=20.22±1.51 g, T4=21.94±1.24 g and T5=22.18±1.73 g. However, the lack of significant difference across T3–5 indicated stabilisation of intake toward the end of the habituation period, a conclusion confirmed by the similarity in scores between habituation T5 and the VV condition in the main experiment (22.63±1.28 g).

*m*CPP/naltrexone interaction

Treatment effects on test day bodyweight and food intake Test-day bodyweights did not differ significantly across treatment conditions: V/V 451.0±12.2 g, V/NL 481.1±14.6 g, V/NH 457.2±16.3 g, *m*CPP/V 465.1±16.4 g, *m*CPP/NL 474.5±17.4 g and *m*CPP/NH 465.5±16.5 g (main effect *m*CPP: $F(1, 9)=0.14$, $p>0.05$; main effect NTX: $F(2, 18)=2.24$, $p>0.05$; interaction: $F(2, 18)=0.21$, $p>0.05$). Treatment effects on food intake are summarised in Table 2.

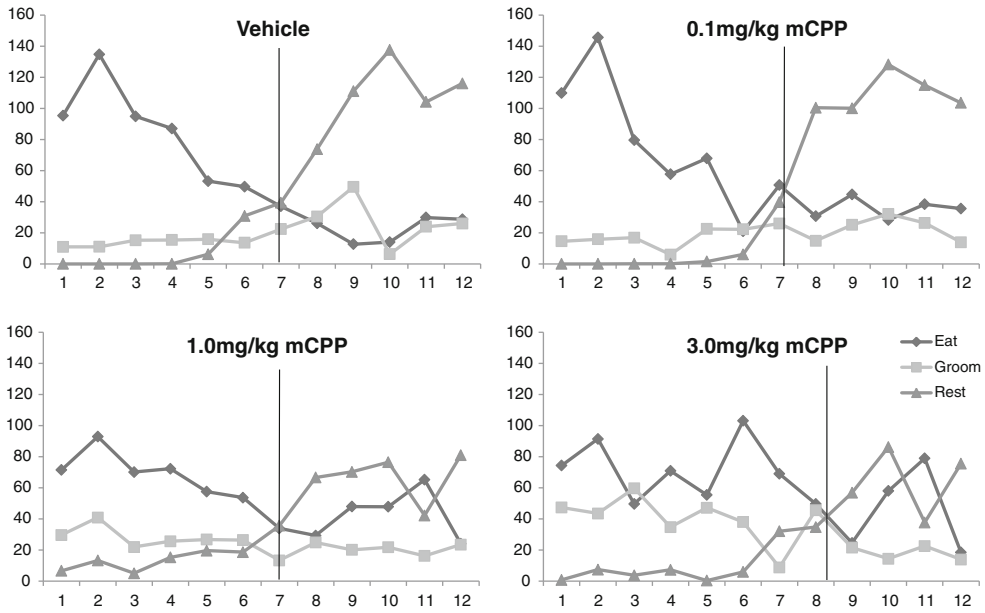

Fig. 3 Effects of acute *m*CPP HCl (0.1–3.0 mg/kg, IP) on the behavioural satiety sequence in male rats tested for 1 h with palatable mash. Data are expressed as mean duration scores in each of 12×5 min timebins comprising the 1-h test period. The *vertical line* bisecting the *x*-axis is merely an aid to visualisation of the transition between eating and resting. There was no evidence that *m*CPP accelerated the BSS; indeed, the highest dose increased grooming and periodic feeding throughout the session resulting in a *delayed* eat-to-rest transition. See text for details

Table 2 Effects of *m*CPP hydrochloride (0 or 0.1 mg/kg, IP) and naltrexone hydrochloride (0, 0.1 or 1.0 mg/kg IP), alone and in combination, on mash intake and feeding-related parameters in male rats exposed for 1 h to palatable mash

Measure	Vehicle/vehicle	Vehicle/NL	Vehicle/NH	mCPP/vehicle	mCPP/NL	mCPP/NH
Mash intake (g)	22.63±1.28	16.81±1.80*	13.63±1.20**	19.48±1.41	12.35±1.88**·***	13.11±0.99**·***
Latency to locate food (s)	3.38±0.45	4.01±1.04	3.12±0.54	2.99±0.34	4.28±0.99	3.81±1.04
Latency to eat (s)	9.23±1.92	7.83±1.24	14.19±4.28	18.54±8.27	9.82±2.66	9.27±3.87
Eat bout (s)	11.09±1.18	9.67±1.37	8.69±0.70	9.59±0.80	11.51±1.48	11.16±1.48
Eat rate (g/min)	1.78±0.06	1.72±0.11	1.60±0.13	1.70±0.08	1.43±0.11	1.48±0.07

Data are presented as mean values (± SEM). See text for full details. NL=0.1 mg/kg naltrexone, NH=1.0 mg/kg naltrexone

*$p<0.05$; **$p<0.001$ vs vehicle/vehicle; ***$p\leq0.01$ vs *m*CPP/vehicle

ANOVA confirmed significant main effects for *m*CPP ($F(1, 9)=24.21$, $p<0.001$) and NTX ($F(2, 18)=29.30$, $p<0.001$), but no significant interaction ($F(2, 18)=1.56$, $p>0.05$). Post hoc comparisons revealed that, relative to vehicle control (V/V), mash intake was significantly suppressed by NL ($p<0.05$) and NH ($p<0.001$) when given alone and when each was given in combination with *m*CPP ($p\leq0.001$). By contrast, *m*CPP per se had no significant effect on mash consumption. Importantly, appetite suppression under neither drug combination differed significantly from that seen with the opioid antagonist given alone (i.e. *m*CPP/NL vs VNL or *m*CPP/NH vs VNH). This observation, combined with the significant differences between *m*CPP given alone and when administered with either dose of NTX ($p\leq0.01$), would be consistent with a lack of meaningful anorectic interaction between the two compounds.

Treatment effects on total behavioural scores Figure 4 shows treatment effects on the total frequency and duration of ingestive and non-ingestive elements, while Table 2 summarises effects on feeding-related measures. Significant *m*CPP×NTX interactions were found only for the frequency and duration of

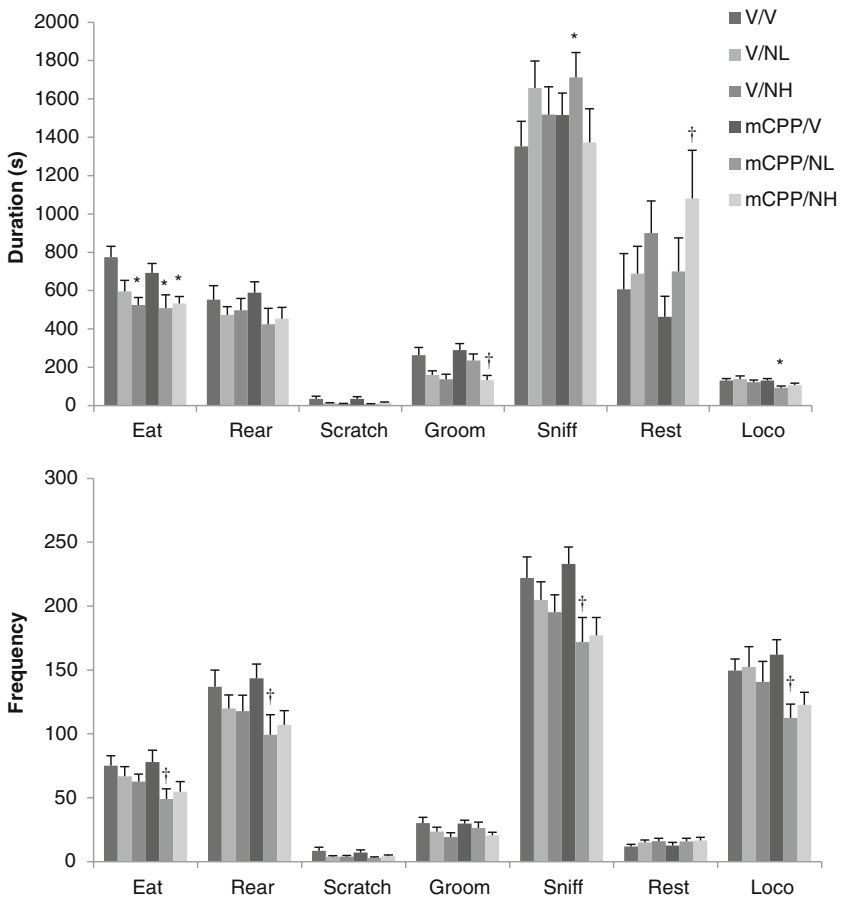

Fig. 4 Effects of *m*CPP HCl and naltrexone HCl, alone and in combination, on behaviours expressed by male rats during a 1-h test with palatable mash. *Upper panel* total duration scores. *Lower panel* total frequency scores. *V* vehicle; *m*CPP= 0.1 mg/kg *m*CPP; NL=0.1 mg/kg naltrexone; NH=1.0 mg/kg naltrexone. Data are expressed as mean values±SEM. *$p\leq0.05$ versus vehicle control; †$p\leq0.05$ versus mCPP/V. See Table 2 for complementary data and text for details

locomotion ($F(2, 18)\geq3.93$, $p\leq0.05$), while a significant main effect of *m*CPP was found only for the rate of eating ($F(1, 9)=6.70$, $p<0.05$). By contrast, many variables demonstrated significant main effects for NTX: eating rate ($F(2, 18)=5.59$, $p<0.05$); the frequency and duration of eating ($F(2, 18)\geq5.81$, $p\leq0.05$), grooming ($F(2, 18)\geq4.73$, $p\leq0.05$), scratching ($F(2, 18)\geq6.87$, $p\leq0.01$) and sniffing ($F(2, 18)\geq7.18$, $p\leq0.01$); the frequency of rearing ($F(2, 18)=5.36$, $p<0.05$) and the duration of resting ($F(2, 18)=6.46$, $p<0.01$). No other interactions or main effects were significant.

As summarised in Fig. 4, post hoc analyses actually revealed relatively few treatment effects compared to V/V control. This outcome suggests that the ANOVA pattern of drug main effects (see above) reflects relatively weak responses that reach significance only as a result of the increased statistical power of larger sample sizes. Nevertheless, eat duration was significantly reduced by the higher dose of NTX given alone and by both doses of NTX in combination with *m*CPP ($p\leq0.05$), while the combination of *m*CPP and the lower (but not higher) dose of NTX significantly increased the duration of sniffing and decreased the duration of locomotion ($p\leq0.05$). However, in only one of these instances (locomotion duration) was there any significant difference between the drug combination and either drug given alone (*m*CPP/NL vs VNL, $p<0.02$). All other significant pairwise comparisons concerned differences between *m*CPP given alone and when given in combination with NTX. Thus, relative to the 5-HT_{2C} receptor agonist given alone (*m*CPP/V), the low-dose combination (*m*CPP/NL) reduced the frequency of eating, rearing, sniffing and locomotion ($p\leq0.05$) while the high-dose combination (*m*CPP/NH) significantly reduced the duration of grooming and increased the duration of resting ($p\leq0.05$). However, as shown in Fig. 5, none of these *m*CPP/NTX dose combinations differed significantly from the corresponding NTX only treatment conditions. This overall pattern confirms the large number of main effects for NTX and the minimal impact of its combination with *m*CPP.

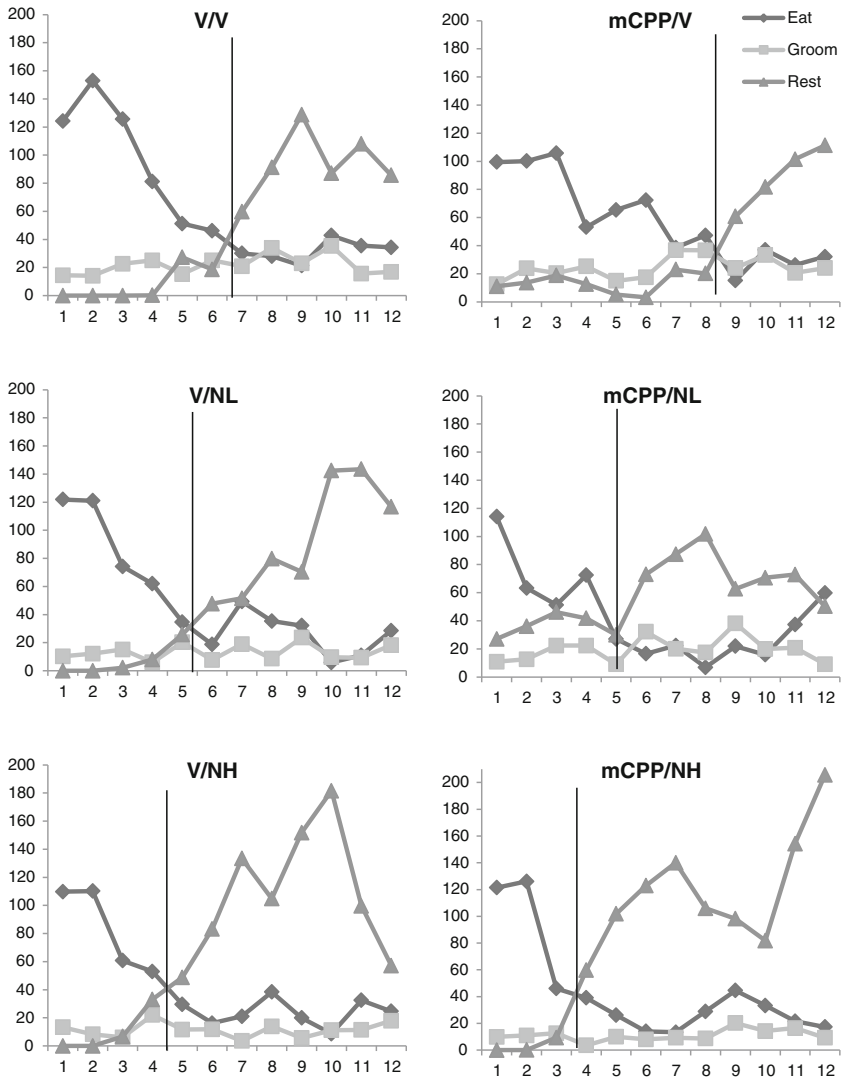

Fig. 5 Effects of *m*CPP HCl and naltrexone HCl, alone and in combination, on the behavioural satiety sequence in male rats tested for 1 h with palatable mash. Data are expressed as mean duration scores (in seconds) in each of 12×5 min timebins comprising the 1-h test period. The *vertical line* bisecting the *x*-axis is merely an aid to visualisation of the transition between eating and resting. 12×5-min timebins. *V* vehicle; *m*CPP=0.1 mg/kg *m*CPP; NL=0.1 mg/kg naltrexone; NH=1.0 mg/kg naltrexone. See text for details

Treatment effects on temporal patterns and behavioural satiety sequence With the exceptions of the frequency and duration of grooming and scratching ($F(11, 99) \leq 1.85$, $p > 0.05$), significant main effects of time were found for the frequency ($F(11, 99) \geq 7.30$, $p \leq 0.001$) and duration ($F(11, 99) \geq 2.31$, $p \leq 0.05$) of all behavioural measures. As seen in experiment 1, this profile reflects the typical pattern of behavioural change over the course of the test session. Significant three-way interactions (*m*CPP×NTX×time) were found for four measures: eat frequency, rest duration and both the frequency and duration of sniffing ($F(22, 198) \geq 1.83$, $p \leq 0.05$). Additional two-way interactions were found for eat duration and rest frequency (NTX×time: $F(22, 198) \geq 2.34$, $p \leq 0.001$), as well as the frequency and duration of locomotion (*m*CPP×time: $F(22, 198) \geq 2.08$, $p \leq 0.05$).

Significant interactions involving time were further explored by a series of two-way ANOVAs within each timebin. These analyses revealed significant drug main effects or interactions for eat frequency and/or duration in timebins T1, T2, T3, T5, T6, T8 and T10 ($F(1, 9) \geq 5.15$, $p \leq 0.05$; $F(2, 18) \geq 3.94$, $p \leq 0.05$); locomotion frequency and/or duration in timebins T3, T6, T10 and T12 ($F(1, 9) \geq 5.20$, $p \leq 0.05$; $F(2, 18) \geq 3.60$, $p \leq 0.05$); rest frequency and/or duration in timebins T3, T5, T6, T7 and T12 ($F(1, 9) = 5.09$, $p < 0.05$; $F(2, 18) \geq 4.93$, $p \leq 0.05$) and sniff frequency and/or duration in timebins T2, T3, T5, T6, T7 and T12 ($F(1, 9) = 5.09$, $p = 0.05$; $F(2, 18) \geq 3.65$, $p \leq 0.05$). Although followed up by a series of within-timebin Bonferroni comparisons, such fine-grain analyses were associated with higher variance around each datapoint and, as such, produced few significant contrasts. However, it is worth noting that (relative to V/V control) eat frequency was significantly reduced by *m*CPP/NL in timebins 1 and 2 ($p \leq 0.05$); eat duration was decreased in timebin 2 by *m*CPP/V and *m*CPP/NL ($p \leq 0.05$) and, in timebin 3, by V/NH, *m*CPP/V and *m*CPP/NH ($p \leq 0.02$).

Figure 5 illustrates the BSS profiles for each of the treatment conditions. The control BSS profile (V/V; top left panel) indicates the typical peak feeding response in the first 15–20 min of the test. Feeding gradually gives way to grooming and resting as time progressed, with an eat-to-rest transition occurring circa half-way through the test session. Although neither dose of NTX given alone interfered with normal behavioural structure (centre and bottom left panels), there is a clear dose-dependent acceleration (shift to the left) of the entire sequence. *m*CPP given alone (top right panel) maintained the BSS but actually produced a modest shift to the right (delay in the eat-rest transition), whereas its combination with either dose of NTX (centre and bottom right) produced effects indistinguishable from those of the opioid receptor antagonist alone (centre and bottom left).

Post-treatment bodyweight gain (data not shown) No significant main effects or interactions were found for 7-day absolute weight gain—animals typically gained 21–25 g irrespective of treatment condition (main effect *m*CPP: $F(1, 9) = 0.49$, $p > 0.05$; main effect NTX: $F(2, 18) = 0.79$, $p > 0.05$; interaction: $F(2, 18) = 0.39$, $p > 0.05$). Analysis of percent bodyweight change over days following treatment confirmed normal growth patterns (main effect DAY: $F(2, 18) = 122.54$, $p < 0.001$), but it too failed to reveal any significant drug main effects, drug interactions or drug×time interactions.

Discussion

The present study was designed to assess the behavioural effects of low-dose combined treatment with the opioid receptor antagonist NTX and the preferential 5-$HT_{2C/1B}$ receptor agonist *m*CPP. Although several studies have previously examined the effects of *m*CPP on the BSS in rats (Kitchener and Dourish 1994) and mice (Hewitt et al. 2002; Lee et al. 2004), inter-laboratory variation in pharmacological sensitivity, species and strain led us to initially characterise the dose–response effects of *m*CPP under local test conditions. This was particularly important in view of the design-led need to identify a sub-threshold anorectic dose of the compound for the interaction experiment with NTX. Experiment 1 confirmed that acute treatment with *m*CPP dose-dependently reduced food intake and the frequency (but not duration) of feeding behaviour (Kennett et al. 1987; Kennett and Curzon 1988a, b; Samanin et al. 1979). *m*CPP also dose-dependently increased the time taken to find food and to commence feeding and reduced the rate of eating (see also Clifton et al. 1993; Simansky and Viadya 1990). Notably, these effects were accompanied by dose-dependent reductions in the frequency (but not duration) of sniffing and locomotion and by a significant increase in time spent grooming. Dose-dependent hypoactivity is fully consistent with earlier reports both in rats (Kennett and Curzon 1988a, b; Kitchener and Dourish 1994; Samanin et al. 1979) and mice (Hewitt et al. 2002; Lee et al. 2004). However, there would appear to be a species difference in the effects of *m*CPP on grooming, with increases typically observed in rats (e.g. Bagdy et al. 1992; Bagdy and Makara 1995; Kitchener and Dourish 1994; this study) but decreases in mice (Hewitt et al. 2002; Lee et al. 2004; see also Somerville et al. 2007). While this species difference in grooming would argue against a non-specific explanation for *m*CPP-induced anorexia, there are other reasons to be somewhat skeptical about the behavioural selectivity of the anorectic response, especially that seen at 3.0 mg/kg.

As already noted, the alterations in ingestive behaviour at the highest dose of *m*CPP were associated with reductions in the frequency (but not duration) of other active behaviours such as locomotion and sniffing. Although such changes would not necessarily be inconsistent with a behaviourally

selective anorectic action (i.e. enhanced satiety), the time-course effects of *m*CPP on eating and other active behaviours are virtually identical (see Fig. 2). In other words, the frequency of all of these non-ingestive elements was suppressed from the very start of the test session and not, as would be expected with enhanced satiety, after the consumption of at least some food (e.g. Kirkham and Blundell 1984; Tallett et al. 2008a). Furthermore, while the BSS profiles of *m*CPP 0.1–1.0 mg/kg appear quite normal relative to vehicle control (including comparable eat-to-rest transitions), the profile observed at the highest dose (3 mg/kg) is quite unusual (Fig. 3). More specifically, there is no indication whatsoever of an acceleration (shift to the left) in the BSS as is typical of a wide range of behaviourally selective anorectic agents (for review, see Rodgers et al. 2010)—if anything, the shift is in the opposite direction. This pattern of effect seen with *m*CPP (3 mg/kg) is quite different to the accelerated BSS profiles reported at this dose level in rats (Kitchener and Dourish 1994) and mice (Hewitt et al. 2002; Lee et al. 2004). The reason for such a large discrepancy, especially between the rat studies, is unclear but may relate to differences such as genetic strain (Sprague–Dawley vs Lister hooded), behavioural scoring method (time sampling vs. continuous monitoring), test diet (chow vs mash) and/or nutritional status (food deprivation vs non-deprivation). These factors apart, it is clear that, in all three studies, *m*CPP (3 mg/kg) significantly altered behaviours other than feeding and that some of these changes (e.g. enhanced resting) were observed from the start of the test session.

Apart from the already discussed increase in grooming behaviour, the behavioural profile observed at 3.0 mg/kg *m*CPP in the current report is characterised by periodic bouts of feeding that continue throughout the test session—as distinct from an initial peak feeding response followed by a gradual decline. As nausea is a common side effect of 5-HT_{2C} receptor agonists (e.g. Cowen et al. 1995; O'Neill et al. 2012; Sargent et al. 1997; Walsh et al. 1994), it is interesting to note the similarity in profile between *m*CPP (3 mg/kg) and the established emetic, lithium chloride (90 mg/kg) (e.g. Ishii et al. 2004). At these dose levels, both compounds reduce intake by 40–50 %, reduce eating rate and suppress active behaviours such as locomotion and sniffing. However, whereas lithium increases the duration (but not frequency) of feeding while preserving the structural integrity of the BSS, *m*CPP reduces the frequency (but not duration) of feeding and disrupts the BSS. Furthermore, of the two agents, only *m*CPP induces periodic bouts of eating throughout the test session, a pattern reminiscent of that induced under present test conditions by quinine-adulterated diet (Ishii et al. 2003a). These drug 'signature' comparisons suggest that the anorectic effect of 3.0 mg/kg *m*CPP may be due, at least in part, to a combination of mild nausea and altered taste perception. As a similar, though statistically weaker, profile was observed at the intermediate dose of 1.0 mg/kg, the lowest dose of *m*CPP was selected for combination with NTX in experiment 2. The doses of NTX used (0.1 and 1.0 mg/kg) were drawn directly from very recent work in our laboratory (Wright and Rodgers 2013) to represent sub-anorectic and sub-maximal anorectic doses, respectively. As it turned out, the results of experiment 2 showed that NTX had somewhat more potent behavioural effects in the current study, with even the lower dose (0.1 mg/kg) inducing a modest though significant reduction in intake (~26 %; $p<0.05$) relative to vehicle control. The reason for this discrepancy is not immediately clear, although it is notable that basal intake in the NTX dose–response study reported by Wright and Rodgers (2013) was atypically low (14.91 g vs. 22.63 g in current experiment 2). Furthermore, the present study involved a double injection procedure which may have altered the stress background and drug response. While few other effects were found with the lower NTX dose, the higher dose per se induced a more robust and consistent anorectic action (~40 %; $p<0.001$) and significantly reduced the duration (but not frequency) of eating. Figure 5 confirms that, while the structure of feeding behaviour was fully preserved under both doses of NTX, there was a clear dose-dependent acceleration (shift to the left) in the BSS. This agrees well with our earlier NTX findings (Wright and Rodgers 2013) and with previous studies using naloxone (Tallett et al. 2008a).

Consistent with results obtained in experiment 1 and numerous other reports in rodents (e.g. Hewitt et al. 2002; Kennett et al. 1987; Kennett and Curzon 1988a, b; Kitchener and Dourish 1994; Lee et al. 2004; Samanin et al. 1979; Simansky and Vaidya 1990; Ward et al. 2008), *m*CPP (0.1 mg/kg) did not when given alone induce any significant behavioural effects when compared with VV control. However, it is interesting to note that (as for a higher dose in experiment 1) it tended to marginally delay (rather than accelerate) the BSS (Fig. 5; top right panel). Furthermore, there was little evidence that the combination of *m*CPP with either dose of NTX resulted in a stronger effect on intake or behaviour than seen in response to NTX alone. Certainly, relative to vehicle control, the combination treatments produced greater effects than those seen in response to *m*CPP alone but this would be expected since the latter per se did not significantly differ from vehicle control. As can clearly be seen in Table 2, Figs. 4 and 5, the profile for the combination treatments can be largely (if not totally) understood in terms of the NTX component. Therefore, under present test conditions and at the dose levels currently used, our results would not support a positive anorectic interaction between the 5-HT_{2C} receptor agonist *m*CPP and the opioid receptor antagonist NTX. Although it might be argued that a higher dose of *m*CPP would have yielded a different result, it is important to emphasise the

significant main effects (experiment 2) of the currently used dose on food intake and the rate of eating. These (larger sample) effects would suggest that 0.1 mg/kg was close to the anorectic threshold for *m*CPP (see Kennett and Curzon 1988b; Ward et al. 2008) and thus entirely appropriate for use in combination with threshold and sub-maximal doses of NTX. It might also be argued (e.g. Somerville et al. 2007) that a negative result on intake does not negate a possible effect on motivation to work for food (as assessed, e.g. by breakpoint analysis). While we cannot rule out this possibility, it would not seem to be compatible with the current lack of combined treatment effect on food approach and eat latencies.

In conclusion, present results confirm the dose-dependent anorectic efficacy of the 5-HT_{2C} receptor agonist *m*CPP and the opioid receptor antagonist NTX. However, whereas the hypophagic response to NTX appeared primary in nature, the suppression of intake by *m*CPP seemed less behaviourally selective as it was accompanied by other behavioural changes including disruption of the BSS. Indeed, the behavioural signature of *m*CPP included elements similar to those seen individually in response to lithium chloride and quinine-adulterated diet, thus suggesting the involvement of nausea and/or altered taste perception. Finally, the combination of a sub-anorectic dose of *m*CPP with one of two NTX doses (threshold and sub-maximal) failed to provide any evidence of a positive anorectic interaction. While this negative outcome corresponds well with the lack of a positive anorectic interaction between naloxone and the dual 5-HT/noradrenaline reuptake inhibitor sibutramine (Tallett et al. 2010), it contrasts markedly with reports of additive anorectic interactions between naloxone and both fluoxetine (Hagan et al. 1997) and 5-HTP (Fernandez-Tome et al. 1988). However, both latter manipulations would have had the effect of rather selectively increasing overall levels of 5-HT thereby potentially influencing multiple receptor subtypes throughout the neuraxis. It is therefore pertinent to note some early work with naloxone and 5-HT receptor subtype antagonists suggesting involvement of 5-HT_3 receptors in modulating endogenous opioid effects on food intake (Beczkowska and Bodnar 1991). Future research should therefore (a) address the possibility that, as recently reported for CB1 receptor antagonist/ inverse agonists (e.g. Tallett et al. 2008b, 2009a) and bupropion (Wright and Rodgers 2013), NTX co-treatment may counter at least some of the unwanted effects of higher doses of *m*CPP; (b) assess the generality of current findings to more recently developed 5-HT_{2C} receptor agonists, such as lorcaserin, Ro 60-0175, CP-809101 and/or VER23779 and (c) broaden the scope of the current strategy to include other 5-HT receptor subtypes and complementary research designs such as isobolographic or dose-addition analyses (e.g. Rowland et al. 2001; Ward et al. 2008).

References

Adan RAH (2013) Mechanisms underlying current and future anti-obesity drugs. TINS 36:133–140

Bagdy G, Makara GB (1995) Paraventricular nucleus controls 5-HT_{2C} receptor-mediated corticosterone and prolactin but not oxytocin and penile erection responses. Eur J Pharmacol 275:301–305

Bagdy G, Kalogeras KT, Szemeredi K (1992) Effect of 5-HT_{1C} and 5-HT_2 receptor stimulation on excessive grooming, penile erection and plasma oxytocin concentrations. Eur J Pharmacol 229:9–14

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152

Basbaum AI, Fields HL (1984) Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 7:309–338

Beczkowska IW, Bodnar RJ (1991) Naloxone and serotonin receptor subtype antagonists: interactive effects upon deprivation-induced intake. Pharmacol Biochem Behav 38:605–610

Bello NT, Kemm MH, Ofeldt EM, Moran TH (2010) Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. Am J Physiol Regul Integr Comp Physiol 299:R945–R952

Benjamin D, Lal H, Meyerson LR (1990) The effects of 5-HT_{1B} characterizing agents in the mouse elevated plus-maze. Life Sci 47:195–203

Berridge KC (2009) 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiol Behav 97:537–550

Berridge KC, Ho C-Y, Richard JM, DiFeliceantonio AG (2010) The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res 1350:43–64

Bhavsar S, Watkins J, Young A (1998) Synergy between amylin and cholecystokinin for inhibition of food intake in mice. Physiol Behav 64:557–561

Bodnar RJ (2004) Endogenous opioids and feeding behavior: a 30-year historical perspective. Peptides 25:697–725

Bojanowska E, Nowak A (2007) Interactions between leptin and exendin-4, a glucagon-like peptide-1 agonist, in the regulation of food intake in the rat. J Physiol Pharmacol 58:349–360

Bojanowska E, Radziszewska E (2011) Combined stimulation of glucagon-like peptide-1 receptor agonist and inhibition of cannabinoid CB1 receptor act synergistically to reduce food intake and body weight in the rat. J Physiol Pharmacol 62:395–402

Boozer CN, Leibel RL, Love RJ, Cha MC, Aronne LJ (2001) Synergy of sibutramine and low dose leptin in treatment of diet-induced obesity in mice. Metabolism 50:889–893

Clifton PG, Barnfield AM, Curzon G (1993) Effects of food deprivation and mCPP treatment on the microstructure of ingestive behaviour in male and female rats. J Psychopharmaol 7:257–264

Clifton PG, Lee MD, Dourish CT (2000) Similarities in the action of Ro 60-0175, a 5-HT_{2C} receptor agonist, and *d*-amphetamine on feeding patterns in the rat. Psychopharmacology 152:256–267

Cooper SJ, Turkish S (1989) Effects of naltrexone on food preference and concurrent behavioural responses in food deprived rats. Pharmacol Biochem Behav 33:17–20

Cooper SJ, Jackson A, Kirkham TC, Turkish S (1988) Endorphins, opiates and food intake. In: Rodgers RJ, Cooper SJ (eds) Endorphins, opiates and behavioural processes. Wiley, Chichester, pp 143–186

Cowen PJ, Sargent PA, Williams C, Goodall EM, Orlikov AB (1995) Hypophagic, endocrine and subjective responses to m-chlorophenylpiperazine in healthy men and women. Human Psychopharmacol 10:385–391

Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J et al (2009) A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 5:749–757

Dourish CT (1995) Multiple serotonin receptors: opportunities for new treatments for obesity? Obesity Res 3(Suppl 4):449S–462S

Fernandez-Tome MP, Gonzalez Y, Del Rio J (1988) Interaction between opioid agonists or naloxone and 5-HTP on feeding behaviour in food-deprived rats. Pharmacol Biochem Behav 29:387–392

Gadde KM, Allison DB (2009) Combination therapy for obesity and metabolic disease. Expert Opin Pharmacother 10:921–925

Gadde KM, Yonish GM, Foust MS, Wagner HR (2007) Combination therapy of zonisamide and bupropion for weight reduction in obese women: a preliminary, randomized, open-label study. J Clin Psychiatry 68:1226–1229

Garvey WT, Ryan DH, Look M, Gadde KM, Allison DB, Peterson CA et al (2012) Two-year sustained weight loss and metabolic benefits with controlled release phentermine/topiramate in obese and overweight adults (SEQUEL): a randomised, placebo-controlled, phase 3 extension study. Am J Clin Nutr 95:297–308

Giuliano C, Robbins TW, Nathan PJ, Bullmore ET, Everitt BJ (2012) Inhibition of opioid transmission at the μ-opioid receptor prevents both food seeking and binge-like eating. Neuropsychopharmacology 37:2643–2652

Greenway FL, Whitehouse MJ, Guttadauria M, Anderson JW, Atkinson RL, Fujioka K et al (2009) Rational design of a combination medication for the treatment of obesity. Obesity 17:30–39

Griebel G, MIsslin R, Pawlowski M, Vogel E (1991) m-Chlorophenylpiperazine enhances neophobic and anxious behavior in mice. NeuroReport 2:627–629

Hagan MM, Holguin FD, Cabello CE, Hanscom DR, Moss DE (1997) Combined naloxone and fluoxetine on deprivation-induced binge-eating of palatable foods in rats. Pharmacol Biochem Behav 58: 1103–1107

Halford JCG, Wanninayake SCD, Blundell JE (1998) Behavioural satiety sequence (BSS) for the diagnosis of drug action on food intake. Pharmacol Biochem Behav 61:159–168

Halford JCG, Harrold JA, Boyland EJ, Lawton CL, Blundell JE (2007) Serotonergic drugs: effects on appetite expression and use for the treatment of obesity. Drugs 67:27–55

Halford JCG, Boyland EJ, Blundell JE et al (2010) Pharmacological management of appetite expression in obesity. Nature Revs Endocrinol 6:255–269

Harrold JA, Dovey TM, Blundell JE, Halford JCG (2012) CNS regulation of appetite. Neuropharmacology 63:3–17

Heal DJ, Gosden J, Smith SL (2012) What is the prognosis for new centrally-acting anti-obesity drugs? Neuropharmacology 63:132–146

Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL et al (2002) Activation of central melanocortin pathways by fenfluramine. Science 297:609–611

Heisler LK, Cowley MA, Kishi T, Tecott LH, Fan W, Low MJ et al (2003) Central serotonin and melanocortin pathways regulating energy homeostasis. Ann NY Acad Sci 994:169–174

Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z et al (2006) Serotonin reciprocally regulates melanocortin neurons to modulate food intake. Neuron 51:239–249

Hewitt KN, Lee MD, Dourish CT, Clifton PG (2002) Serotonin 2C receptor agonists and the behavioural satiety sequence in mice. Pharmacol Biochem Behav 71:691–700

Higgins GA, Silenicks LB, Robmann A, Rizos Z, Noble K, Soko AD et al (2012) The 5-HT_{2C} receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. Neuropsychopharmacology 37:1177–1191

Higgins GA, Silenicks LB, Lau W, de Lannoy IAM, Lee DKH, Izakova J et al (2013) Evaluation of chemically diverse 5-HT_{2C} receptor agonists on behaviours motivated by food and nicotine and on side-effect profiles. Psychopharmacology 226:475–490

Hinton V, Rosofsky M, Granger J, Geary N (1986) Combined injection potentiates the satiety effects of pancreatic glucagon, cholecystokinin and bombesin. Brain Res Bull 17:615–619

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ et al (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46: 157–203

Ishii Y, Blundell JE, Halford JCG, Rodgers RJ (2003a) Palatability, food intake and the behavioural satiety sequence in male rats. Physiol Behav 80:37–47

Ishii Y, Blundell JE, Halford JCG, Rodgers RJ (2003b) Effects of systematic variation in presatiation and fasting on the behavioural satiety sequence in male rats. Physiol Behav 79:227–238

Ishii Y, Blundell JE, Halford JCG, Upton N, Porter R, Johns A et al (2004) Differential effects of the selective orexin-1 receptor antagonist SB-334867 and lithium chloride on the behavioural satiety sequence in rats. Physiol Behav 81:129–140

Kennett GA, Clifton PG (2010) New approaches to the pharmacological treatment of obesity: can they break through the efficacy barrier? Pharmacol Biochem Behav 97:63–83

Kennett GA, Curzon G (1988a) Evidence that mCPP may have behavioural effects mediated by central 5-HT_{1C} receptors. Br J Pharmacol 94:137–147

Kennett GA, Curzon G (1988b) Evidence that hypophagia induced by *m*CPP and TFMPP requires 5-HT_{1C} and 5-HT_{1B} receptors: hypophagia induced by RU 24969 only requires 5-HT_{1B} receptors. Psychopharmacology 96:93–100

Kennett GA, Dourish CT, Curzon G (1987) 5-HT_{1B} agonists induce anorexia at a postsynaptic site. Eur J Pharmacol 141:429–435

Kennett GA, Whitton P, Shah K, Curzon G (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. Eur J Pharmacol 164: 445–454

Kennett G, Lightowler S, Trail B, Bright F, Bromidge S (2000) Effects or Ro 60-0175, a 5-HT_{2C} receptor agonist, in three animals models of anxiety. Eur J Pharmcol 387:197–204

Kirkham TC, Blundell JE (1984) Dual action of naloxone on feeding revealed by behavioural analysis: separate effects on initiation and termination of eating. Appetite 5:45–52

Kirkham TC, Blundell JE (1986) Effect of naloxone and naltrexone on the development of satiation measured in the runway: comparisons with d-amphetamine and d-fenfluramine. Pharmacol Biochem Behav 25:123–128

Kirkham TC, Blundell JE (1987) Effects of naloxone and naltrexone on meal patterns of freely-feeding rats. Pharmacol Biochem Behav 26: 515–520

Kirkham TC, Williams CM (2001) Synergistic effects of opioid and cannabinoid antagonists on food intake. Psychopharmacology 153:267–270

Kitchener SJ, Dourish CT (1994) An examination of the behavioural specificity of hypophagia induced by 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 receptor agonists using the post-prandial satiety sequence in rats. Psychopharmacology 113:369–377

Lee MD, Somerville EM, Kennett GA, Dourish CT, Clifton PG (2004) Reduced hypophagic effects of *d*-fenfluramine and the 5-HT_{2C} receptor agonist *m*CPP in 5-HT_{1B} receptor knockout mice. Psychopharmacology 176:39–49

Nathan PJ, Bullmore ET (2009) From taste hedonics to motivational drive: central μ-opioid receptors and binge-eating behaviour. Int J Neuropsychopharmacol 12:995–1008

O'Neill PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J et al (2012) Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. Obesity 20:1426–1436

Padwal R (2009) Contrave, a bupropion and naltrexone combination therapy for the potential treatment of obesity. Curr Opin Invest Drugs 10:1117–1125

Paulik M, Hamilton B, Hommel J, Holt L, Herring C, Stroup A et al (2011) Combined long-acting PYY and GLP-1 agonism synergistically normalizes weight and glucose in obese and diabetic mice. Diabetes 60: A61, 224-OR

Pietras TA, Rowland NE (2002) Effect of opioid and cannabinoid receptor antagonism on orphanin FQ-induced hyperphagia in rats. Eur J Pharmacol 442:237–239

Ravussin E, Smith SR, Mitchell JA, Ahringarpure R, Shan K, Maier H et al (2009) Enhanced weight loss with pramlintide/metreleptin: an integrated neurohormonal approach to obesity pharmacotherapy. Obesity (Silver Spring) 17:1736–1743

Reidelberger RD, Haver AC, Apenteng BA, Anders KL, Steenson SM (2011) Effects of exendin-4 alone and with peptide YY (3–36) on food intake and body weight in diet-induced obese rats. Obes Silver Spring 19:121–127

Rodgers RJ, Cole JC, Cobain MR, Daly P, Doran PJ, Eells JR et al (1992) Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and *m*CPP. Behav Pharmacol 3:621–634

Rodgers RJ, Halford JCG, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JRS et al (2001) SB–334867, a selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats. Eur J Neurosci 13:1444–1452

Rodgers RJ, Holch P, Tallett AJ (2010) Behavioural satiety sequence (BSS): separating wheat from chaff in the behavioural pharmacology of appetite. Pharmacol Biochem Behav 97:3–14

Rodgers RJ, Tschöep MH, Wilding JPH (2012) Anti-obesity drugs: past, present and future. Dis Model Mech 5:621–626

Roth JD, Coffey T, Jodka CM, Maier H, Athanacio JR, Mack CM et al (2007) Combination therapy with amylin and peptide YY3-36 in obese rodents: anorexigenic synergy and weight loss additivity. Endocrinology 148:6054–6061

Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE et al (2008a) Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci U S A 105:7257–7262

Roth JD, Trevaskis JL, Wilson J, Lei C, Athanacio J, Mack C et al (2008b) Antiobesity effects of the beta-cell hormone amylin in combination with phentermine or sibutramine in diet-induced obese rats. Int J Obesity 32:1201–1210

Roth JD, Trevaskis JL, Turek VF, Parkes DG (2010) 'Weighing in' on synergy: preclinical research on neurohumoral anti-obesity combinations. Brain Res 1350:86–94

Rowland NE, Mukherjee M, Roberston K (2001) Effects of the cannabinoid receptor antagonist SR141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. Psychopharmacology 159:111–116

Samanin R, Mennini T, Ferraris A, Bendotti C, Borsini F, Garattini S (1979) m-Chlorophenylpiperazine: a central serotonin agonist causing powerful anorexia in rats. Naunyn-Schmiedeberg's Arch Pharmacol 308:159–163

Sargent PA, Sharpley AL, Williams C, Goodall EM, Cowen PJ (1997) 5-HT2C receptor activation decreases appetite and body weight in obese subjects. Psychopharmacology 133:309–312

Simansky KJ, Vaidya AH (1990) Behavioral mechanisms for the anorectic action of serotonin (5-HT) uptake inhibitor sertraline in rats: comparison with directly acting 5-HT agonists. Brain Res Bull 25:953–960

Somerville EM, Horwood JM, Lee MD, Kennett GA, Clifton PG (2007) 5-HT_{2C} receptor activation inhibits appetitive and consummatory components of feeding and increases brain *c-fos* immunoreactivity in mice. Eur J Neurosci 25:3115–3124

Tallett AJ, Blundell JE, Rodgers RJ (2008a) Behaviourally-selective hypophagic effects of naloxone in non-deprived male rats presented with palatable food. Behav Brain Res 187:417–427

Tallett AJ, Blundell JE, Rodgers RJ (2008b) Endogenous opioids and cannabinoids: system interactions in the regulation of appetite, grooming and scratching. Physiol Behav 94:422–431

Tallett AJ, Blundell JE, Rodgers RJ (2009a) Effects of acute low dose combined treatment with naloxone and AM 251 on food intake, feeding behaviour and weight gain in rats. Pharmacol Biochem Behav 91:358–366

Tallett AJ, Blundell JE, Rodgers RJ (2009b) Night and day: diurnal differences in the behavioural satiety sequence in male rats. Physiol Behav 97:125–130

Tallett AJ, Blundell JE, Rodgers RJ (2010) Sibutramine and naloxone: infra-additive interaction in the regulation of appetite? Behav Brain Res 207:174–181

Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL (2005) Peripheral exendin-4 and peptide $YY_{3–36}$ synergistically reduce food intake through different mechanisms in mice. Endocrinology 146:3748–3756

Vickers SP, Clifton PG (2012) Animal models to explore the effects of CNS drugs on food intake and energy expenditure. Neuropharmacology 63:124–131

Vickers SP, Jackson HC, Cheetham SC (2011) The utility of animal models to evaluate novel anti-obesity agents. Br J Pharmacol 164: 1248–1262

Walsh AES, Smith KA, Oldman AD, Williams C, Goodall EM, Cowen PJ (1994) m-Chlorophenylpiperazine decreases food intake in a test meal. Psychopharmacology 116:120–122

Ward SJ, Lefever TW, Jackson C, Tallarida RJ, Walker EA (2008) Effects of cannabinoid$_1$ receptor antagonist and serotonin$_{2C}$ receptor agonist alone and in combination on motivation for palatable food: a dose-addition analysis study in mice. J Pharmacol Exp Ther 325:567–576

Weintraub M, Sundaresan PR, Madan M, Schuster B, Balder A, Lasagna L et al (1992) Long-term weight control study. I (weeks 0–34). The enhancement of behavior modification, caloric restriction, and exercise by fenfluramine plus phentermine versus placebo. Clin Pharmacol Ther 51:586–594

Weiss SM (1995) Pharmacological and behavioural examination of the defensive reactions of laboratory mice to the calls of the Tawny owl. Ph.D. thesis, School of Psychology, University of Leeds (UK)

Weiss GF, Rogacki N, Fuel A, Buchen D, Suh JS, Wong DT et al (1991) Effect of hypothalamic and peripheral fluoxetine injection on natural patterns of macronutrient intake in the rat. Psychopharmacology (Berlin) 195:467–476

Westenberg HGM, den Boer JA (1994) The neuropharmacology of anxiety: a review on the role of serotonin. In: Sitsen JMA, den Boer J (eds) Handbook of anxiety and depression. A biological approach. Marcel Dekker, New York, pp 405–446

Wright FL, Rodgers RJ (2013) Acute behavioural effects of bupropion and naltrexone, alone and in combination, in non-deprived male rats presented with palatable mash. Psychopharmacology. doi:10.1007/s00213-013-3036-6

Young AA (2012) Brainstem sensing of meal-related signals in energy homeostasis. Neuropharmacology 63:31–45