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ORIGINAL INVESTIGATION

Acute behavioural effects of bupropion and naltrexone, alone and in combination, in non-deprived male rats presented with palatable mash

F. L. Wright · R. J. Rodgers

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Abstract
Rationale In appetite research, drugs frequently progress to clinical trials on the basis of outcome (reduced food intake/body weight gain) with insufficient attention to process (behavioural analysis). Although bupropion and naltrexone (alone and in combination) reduce food consumption in rodents and humans, their effects on behaviour *during* feeding tests have not been thoroughly investigated.
Objectives This study aimed to assess the behavioural specificity of anorectic responses to bupropion, naltrexone and their combination.
Methods Video analysis was employed to characterise the behavioural effects of acute systemic treatment with bupropion (10.0–40.0 mg/kg), naltrexone (0.1–3.0 mg/kg) and combined bupropion (20 mg/kg) plus naltrexone (0.1–1.0 mg/kg) in non-deprived male rats exposed for 1 h to palatable mash. Particular attention was paid to the behavioural satiety sequence (BSS).
Results In experiment 1, the anorectic response to 40 mg/kg bupropion was associated with significant psychomotor stimulation and a complete disruption of the BSS. In experiment 2, the anorectic response to 3 mg/kg naltrexone was associated with an accelerated but otherwise normal BSS. In experiment 3, the co-administration of 20 mg/kg bupropion and naltrexone (0.1 and 1.0 mg/kg) not only produced an additive anorectic profile (including a reduced rate of eating), but the addition of the opioid receptor antagonist also concurrently attenuated the psychomotor stimulant response to the atypical antidepressant.
Conclusions Low-dose co-treatment with naltrexone and bupropion produces a stronger suppression of appetite than that seen with either agent alone and has the additional advantage of reducing some of the unwanted effects of bupropion.

Keywords Bupropion · Naltrexone · Co-treatment · Behavioural satiety sequence · Food intake · Anorexia · Psychomotor stimulation · Rats

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Introduction

The history of anti-obesity drug development is less than impressive, with most initially approved agents sooner or later withdrawn due to unacceptable adverse effects (Halford et al. 2010; Heal et al. 2012; Kennett and Clifton 2010; Rodgers et al. 2012; Vickers et al. 2011). Examples include amphetamine, phentermine and fenfluramine (dependence and/or cardiovascular risk) and, more recently, rimonabant and sibutramine (psychiatric and cardiovascular risks, respectively). However, advances in our understanding of the neurobiology of appetite/energy homeostasis have led to the identification of novel targets and treatment strategies (Halford et al. 2010; Harrold et al. 2012; Kennett and Clifton 2010). While some initiatives focus on monotherapies (e.g. lorcaserin/Belviq®, targeting the serotonin 5-HT_{2C} receptor), polytherapy is now attracting substantial scientific and clinical interest (Rodgers et al. 2012). Advantages of targeting multiple mechanisms include the use of lower drug doses, possible additive or synergistic anorectic/weight loss effects, fewer/less serious adverse reactions and a reduced likelihood of counter-regulation (Greenway et al. 2009; Padwal 2009; Roth et al. 2010; Young 2012). Although various treatment options have been assessed, major interest is focused on Qsymia® (phentermine/topiramate), Empatic® (bupropion/zonisamide) and Contrave® (bupropion/naltrexone) (Heal et al. 2012; Rodgers et al. 2012).

Despite such developments, surprisingly little detail is available concerning the effects of the newer treatments on *behaviour* during tests of food intake. Indeed, past failures in drug development can in part be attributed to a lack of attention to the behavioural process/es whereby test compounds suppress appetite and/or weight gain (Halford et al. 2010; Kennett and Clifton 2010; Rodgers et al. 2010, 2012; Tucci et al. 2006; Vickers et al. 2011; Vickers and Clifton 2012). It is now widely appreciated that intake can be indirectly reduced as a function of nausea, pain, sedation or by the induction of competing behaviours such as hyperactivity or stereotypy (Blundell and McArthur 1981; Halford et al. 1998; Rodgers et al. 2010). In this context, although co-treatment with bupropion and naltrexone has been reported (in rodents and obese humans) to produce significantly greater reductions in intake and body weight than either drug given alone (Contrave NDA 2010; Greenway et al. 2009, 2010; Padwal 2009; Wadden et al. 2011), comparatively little is known about the effects of these agents, either alone or together, on *behaviour* within the feeding context.

Bupropion is structurally similar to psychostimulants such as amphetamine (Ascher et al. 1995) and acts both as a noradrenaline and dopamine reuptake inhibitor (Ferris et al. 1983) and a nicotinic receptor antagonist (Miller et al. 2002; Slemmer et al. 2000). Clinically, it is best known as an atypical antidepressant (Wellbutrin®) and smoking cessation aid (Zyban®) (Ascher et al. 1995; Horst and Preskorn 1998; Warner and Shoaib 2005; Zimmerman et al. 2005). Early indications that the compound may also have therapeutic value in weight management came from incidental observations during clinical trials for antidepressant efficacy (for a review, see Fava et al. 2005; Li et al. 2005). These reports in turn prompted several randomised, double-blind, placebo-controlled trials with obese patients which confirmed that bupropion can indeed produce mild weight loss equivalent to that seen with sibutramine and orlistat (Anderson et al. 2002; Gadde et al. 2001; Jain et al. 2002). Although bupropion also reduces food intake in rodents (Billes and Cowley 2007; Greenway et al. 2009; Stairs and Dworkin 2008; Zarrindast and Hosseini-Nia 1988), it shares many of the behavioural effects of psychostimulants. For example, it is self-administered, induces conditioned place preference and substitutes for drugs such as cocaine, amphetamine, methamphetamine, methylphenidate and diethylpropion (for a review, see Dwoskin et al. 2006). Importantly, it also produces profound psychomotor stimulation (Billes and Cowley 2007; Carrasco et al. 2004; Cooper et al. 1980; Gomez et al. 2008; Nielsen et al. 1986; Paterson et al. 2010; Redolat et al. 2005a, b; Santamaria and Arias 2010; Soroko et al. 1977; Zarrindast and Hosseini-Nia 1988; Zarrindast et al. 1996). Although it has been argued that the anorectic and psychomotor responses to bupropion are independent phenomena (Billes and Cowley 2007), it is noteworthy that both effects are typically seen at very similar dose levels.

Since the mid-1970s, research groups worldwide have confirmed Holtzman's (1974) report that the opioid receptor antagonist naloxone significantly suppresses deprivation-induced feeding in rodents. Such findings have been extended to other opioid receptor antagonists, other forms of feeding and other species and have been shown to be both stereospecific and largely centrally mediated (for reviews, see Cooper et al. 1988; Berridge 2009; Bodnar 2004). Further work has indicated that the endogenous opioid system is predominantly involved in the hedonics of feeding, a view supported by the identification of 'hot spots' for μ-opioid enhancement of taste hedonics not only in the nucleus accumbens but also the ventral striatum (for a review, see Berridge 2009; Berridge et al. 2010). To our knowledge, however, only two reports have examined in detail the effects of naloxone on behaviour within the feeding context. Kirkham and Blundell (1984) observed that naloxone (2.5–5.0 mg/kg) reduces food intake without significantly influencing feeding rate or locomotor activity. Nevertheless, arguments regarding behavioural selectivity were somewhat undermined by concomitant reductions in drinking, sniffing, grooming and rearing, as well as increases in resting. More recently, Tallett et al. (2008b) reported that naloxone (≥1.0 mg/kg) suppresses food consumption and feeding behaviour without influencing non-ingestive behaviours or the structural integrity of the behavioural satiety sequence (BSS). Rather, the principal drug effect was to accelerate the satiety sequence, a finding consistent with a reduction in orosensory reward that normally accompanies/follows the ingestion of palatable food (Berridge 2009; Bodnar 2004; Cooper 2004; Cota et al. 2006).

In contrast to these developments with naloxone, comparatively little is known about the behavioural selectivity of the anorectic response to naltrexone. In a runway situation, Kirkham and Blundell (1986a) found that this longer-acting opioid receptor antagonist significantly reduced the motivation to eat but only after initial experience of the test diet, while in a more standard context, Cooper and Turkish (1989) reported that naltrexone anorexia could not be explained by locomotor impairment. The need for a more comprehensive behavioural analysis of naltrexone anorexia is indicated both by known pharmacokinetic and pharmacodynamic differences between naloxone and naltrexone (Blumberg et al. 1967; Goldstein and Naidu 1989; Magnan et al. 1982; Martin et al. 1963; Raynor et al. 1994; Tepperman et al. 1983) and recent findings on appetite/weight loss effects of the compound when co-administered with bupropion (Contrave NDA 2010; Greenway et al. 2009, 2010; Padwal 2009; Wadden et al. 2011). The aim of the present study was to assess the effects of bupropion and naltrexone, alone and in combination, on

ingestive and non-ingestive behaviours in non-deprived male rats presented with palatable mash. The methodology employed incorporates the characterisation of treatment effects on the BSS (for a review, see Rodgers et al. 2010).

Methods

Subjects

Adult male Lister hooded rats, weighing 200–220 g (Charles River, Kent, UK), were pair-housed (46×26.5×26 cm) for 1 week following arrival in the laboratory and then transferred to individual cages (45×20×20 cm) containing environmental enrichment (a polycarbonate rat tunnel; Datesand Ltd., Manchester, UK). Single housing facilitated both initial familiarisation with the test diet and daily body weight tracking. Rats were maintained on a 12-h light cycle (lights on at 0700 hours) in an environment controlled for temperature (21±1 °C) and humidity (50±2 %). A normal light cycle was employed in view of the much clearer BSS seen when rats are tested during the light, rather than dark, phase of the cycle (Tallett et al. 2009b). Animals were handled regularly during routine husbandry and, as described in the succeeding sections, were thoroughly habituated to all experimental procedures prior to drug testing. Pelleted chow (Bantin & Kingman Universal Diet, Hull, UK; digestible energy value=14 kJ/g) and tap water were available ad libitum in the home cages, with the exception of the injection–test interval during which food was removed. Body weights were recorded daily (A.M.) throughout the experiment. Naïve groups of rats were used in each experiment. All procedures were conducted under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

Drugs

Bupropion hydrochloride (BUP) and naltrexone hydrochloride (NTX), both obtained from Sigma-Aldrich (Manchester, UK), were dissolved in physiological saline (0.9 %) which, alone, served as vehicle control. All doses are expressed as the salt. For experiments 1 and 2, doses were selected from previous research on food intake to span the full range from ineffective to sub-maximal (BUP: Billes and Cowley 2007, 2008; Greenway et al. 2009; Liu et al. 2002; Stairs and Dworkin 2008; Zarrindast and Hosseini-Nia 1988; NTX: Apfelbaum and Mandenhoff 1981; Cooper and Turkish 1989; Hadjimarkou et al. 2004; Jackson and Sewell 1985a, b; Kirkham and Blundell 1986a, b, 1987; Kirkham et al. 1987; Marks-Kaufman et al. 1985; Sanger and McCarthy 1982). Data obtained in these initial studies were then used to select appropriate doses for the interaction analysis in experiment 3. All solutions were freshly prepared on test days and administered intraperitoneally (IP) in a volume of 1 ml/kg either 30 min (BUP) or 15 min (NTX) prior to testing.

Apparatus

Food intake studies were conducted in a glass arena large enough (60×30×45 cm) to allow animals the freedom to engage in a variety of behaviours (e.g. Ishii et al. 2003; Tallett et al. 2008a, b, 2009a, b). The arena floor was covered with wood shavings, a water bottle was suspended from one of the endwalls and a pre-weighed glass food pot was secured to the centre of the floor with Velcro™ and an annular metal mounting. The test diet (mash) was prepared freshly each morning by hydrating a powdered form of the maintenance diet (Bantin & Kingman Universal Diet, Hull, UK; 1 g dry=3.125 g mash; digestible energy value=4.48 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. 2003), while its consistency minimises spillage and hoarding (e.g. Blundell et al. 1985; Halford et al. 1998). Two video cameras, one positioned above the arena and the other horizontal to the front wall, recorded test sessions for subsequent behavioural analysis. Camera signals were fed via an image merger to a nearby monitor and DVD recorder. This split-screen view of the test arena facilitated scoring accuracy.

Procedure

Food intake sessions (habituation and test) were conducted during the light phase of the light/dark cycle (0700–1900 hours) under normal laboratory illumination (265 lx). During each session, two control food pots (positioned adjacent to the test arena) were used to assess loss of food mass through evaporation alone: these measurements confirmed minimal evaporation loss (over the three experiments reported: average=0.18 %; range, 0.09–0.35 %).

Habituation phase

After 10 days acclimatisation to local laboratory conditions, rats were familiarised with mash in their home cages for 3 h on two consecutive days. The following week, they were individually exposed to a pseudo-experimental procedure daily for 5 days. For experiments 1 and 2, this involved the removal of home cage food and enrichment, IP injection of saline (1 ml/kg) and return to the home cage for 30 min. For experiment 3 (combination study), animals were given two saline injections spaced 15 min apart and were returned to their home cages after each injection. After a total of 30 min, animals were 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 not only familiarised animals with all aspects of the experimental procedure, it also facilitated the development of stable mash consumption prior to the main experimental phase of the study.

Experimental phase

In all experiments, drug testing commenced within 3 days of the final habituation trial and was conducted according to a within-subjects (crossover) design. A Latin square was used to determine treatment order, with a washout period of at least 3 days between successive treatments (Blumberg et al. 1967; Martin et al. 1963; Suckow et al. 1986). On test days, rats were individually transported to a preparation room where they received IP drug treatment and were immediately returned to their home cages (chow and enrichment removed). After the drug-appropriate injection–test interval, they were transferred to an adjacent laboratory, individually placed in the test arena with pre-weighed 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).

Three experiments were conducted: experiment 1 assessed the dose–response profile of BUP (0, 10.0, 20.0 and 40.0 mg/kg; injection–test interval=30 min), experiment 2 assessed the dose–response profile of NTX (0, 0.1, 1.0 and 3.0 mg/kg; injection–test interval=15 min) and experiment 3 assessed the effects of co-treatment with BUP (30 min pre-test) and NTX (15 min pre-test) using sub-maximal doses of each agent as identified in the first two studies.

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. 2003; Tallett et al. 2008a, b, 2009a, b), 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 front paws); *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). It should be noted that levels of drinking and scratching were extremely low in these studies and that, as such, data for these variables are not reported.

In addition to examining treatment effects on global (i.e. 1 h total) behavioural scores, each 60-min test period was divided into 12×5-min timebins thereby permitting the analysis of treatment effects over time. Within these timecourse analyses, specific attention was paid to the BSS, i.e. the temporal relationship between eating, grooming and resting.

Test day body weight and post-treatment body weight gain

Body weights were recorded at the same time daily from day 1 of individual housing until 3 days post-dosing. This procedure was used not only to confirm the equivalence of test day body weights across the different treatment conditions but also to detect any effects of acute drug treatment on subsequent weight gain. In addition to analysing treatment effects on the 3-day absolute weight gain, finer-grain analysis was permitted by expressing body weights for each post-treatment day as a percentage of test day body weight (where test day=100 %).

Statistical analysis

For experiments 1 and 2, data for food intake (habituation and test), test day body weight, 1-h behaviour totals and 3-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 body weight gain over 3 days 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 3, habituation data were analysed by one-way repeated-measures ANOVA, whereas data for test intake, 1-h behaviour totals and 3-day absolute weight gain were analysed by two-way repeated-measures ANOVA (BUP×NTX). Effects on behavioural change over time, as well as on percentage body weight gain over 3 days post-dosing, were analysed by three-way repeated-measures ANOVA (BUP×NTX×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

Habituation

Mean body weight for the sample ($N=10$) was 209.4±1.9g on arrival and 397.0±9.5 g by the end of the study. Mash consumption differed significantly over the course of habituation ($F(4,36)=18.86$, $p<0.001$), with intake on the first two trials predictably lower ($p \leq 0.01$) than on the remaining trials (trial 1, 9.86±0.80 g; trial 2, 12.42±0.59 g; trial 3, 17.15±0.77 g; trial 4, 16.90±1.97 g; trial 5, 19.86±1.17 g). Although intake on the first trial differed significantly from trials 3, 4 and 5 ($p \leq 0.01$) and intake on trial 2 from trials 3 and 5 ($p \leq 0.01$), the development of a stable intake pattern was confirmed by the lack of difference across trials 3–5.

Bupropion dose–response

Test day body weight and food intake Test day body weights were comparable across the various treatment conditions (vehicle, 366.3±10.9 g; BUP10, 369.2±7.4 g; BUP20, 370.4±8.4 g; BUP40, 370.4±9.0 g ($F(3,27)=0.17$, NS)). Treatment effects on food intake are summarised in Table 1. Bupropion significantly influenced 1 h mash intake ($F(3,27)=7.03$, $p=0.001$), with Bonferroni comparisons confirming a significant suppression (21 % reduction relative to vehicle control, $p<0.03$) at the highest dose tested (40 mg/kg).

Total behavioural scores Data for feeding-related parameters (latency to locate food source, latency to eat, average duration of eating bouts and average rate of eating) are summarised in Table 1, while treatment effects on the total frequency and duration of ingestive and non-ingestive behaviours are shown in Fig. 1.

ANOVA revealed significant treatment effects on the average duration of eating bouts ($F(3,27)=7.19$, $p<0.001$); the frequency and duration of eating ($F(3,27) \geq 11.59$, $p \leq 0.001$), resting ($F(3,27) \geq 4.58$, $p \leq 0.01$), locomotion ($F(3,27) \geq 13.71$, $p \leq 0.002$) and sniffing ($F(3,27) \geq 11.72$, $p \leq 0.001$); and both the duration of grooming ($F(3,27)=10.46$, $p<0.001$) and the frequency of rearing ($F(3,27)=18.15$, $p<0.001$). Treatment did not significantly influence the frequency of grooming or the duration of rearing ($F(3,27) \leq 2.41$, $p>0.05$) nor did it affect the latency of locate the food source, the latency to commence eating or the average rate of eating ($F(3,27) \leq 1.40$, $p>0.05$). As shown in Table 1 and Fig. 1, Bonferroni comparisons confirmed that behaviour was significantly affected mainly at the highest dose of BUP (40 mg/kg), which reduced time spent eating ($p<0.05$), the average duration of eating bouts ($p<0.01$) and grooming duration ($p=0.052$), while significantly increasing the frequency of eating and rearing ($p \leq 0.01$) and both the frequency and duration of locomotion and sniffing ($p \leq 0.03$). The only exception to exclusive high-dose efficacy was an increase in locomotion frequency observed at 20 mg/kg ($p=0.021$).

Despite significant treatment effects on rest frequency and duration and the apparent elimination of resting from the behavioural profile at the highest dose of bupropion (Fig. 1), post hoc tests revealed only a significant difference between drug doses (10 vs 40 mg/kg) and not between drug and vehicle control. However, as resting is normally absent during the first half of the test session (Fig. 4), data were re-analysed focusing purely on the frequency and duration of resting during the second half of the session (timebins 7–12 inclusive). While significant treatment effects were confirmed (frequency: $F(3,27)=5.18$, $p<0.01$; duration: $F(3,27)=4.58$, $p<0.01$), the higher variability in these datasets once again precluded detection of a significant drug effect vs vehicle control.

Behavioural timecourses and behavioural satiety sequence Timebin analyses confirmed the normal temporal pattern of behaviour during the 1-h test, with a gradual reduction in most active behaviours and increase in resting as the session progressed (e.g. Ishii et al. 2003; Tallett et al. 2008a, b, 2009a, b). Thus, with the exception of groom frequency ($F(11,99)=1.27$, $p>0.05$), significant main effects of time were found for the frequency ($F(11,99) \geq 7.18$, $p \leq 0.001$) and duration ($F(11,99) \geq 2.70$, $p \leq 0.005$) of all behavioural measures. Significant treatment×time interactions were found for the duration of eating ($F(33,297)=3.26$, $p<0.001$), the frequency and duration of resting ($F(33,297) \geq 1.62$, $p \leq 0.02$) and the frequency of locomotion ($F(33,297)=1.49$, $p=0.05$), while the interaction term for locomotion duration closely approached significance ($F(33,297)=1.45$, $p=0.06$) (see Fig. 2). A series of one-way ANOVAs within each timebin indicated that 40 mg/kg BUP significantly reduced time spent

Table 1 The effects of bupropion hydrochloride (10–40 mg/kg, IP) on mash intake and feeding-related parameters in male rats tested for 1 h with palatable mash

Measure	Vehicle	BUP10	BUP20	BUP40
Mash intake (g)	17.89±1.08	19.05±0.92	18.85±0.81	14.21±1.67*
Latency to locate food (s)	7.60±1.20	12.39±3.15	9.02±2.02	8.62±2.97
Latency to eat (s)	34.13±4.68	25.27±6.29	18.83±4.32	25.36±7.18
Eat bout (s)	15.43±2.45	17.81±5.04	15.79±4.24	6.57±1.59**
Eat rate (g/min)	1.31±0.05	1.41±0.06	1.30±0.06	1.49±0.11

Data are presented as the mean values ± SEM. See text for full details

s seconds, *g* grams

*$p<0.05$, **$p<0.01$ vs vehicle

eating at several timepoints during the first half of the test session ($F(3,27)\geq4.05$, $p\leq0.02$) and increased both the frequency and duration of locomotion throughout the entire test (timebins 1–12; $F(3,27)\geq4.03$, $p\leq0.02$). Similar follow-up analyses of the treatment×time interactions for resting failed to reveal a significant effect of BUP on rest frequency or duration at any individual timepoint. This outcome is consistent with that of the analyses for total resting scores (see the previous section).

Treatment effects on the BSS are shown in Fig. 3. Consistent with previous work in our laboratory (Ishii et al. 2003; Tallett et al. 2008a, b, 2009a, b), the profile for the vehicle control condition shows a typical peak feeding response during the first half of the test. Over time, resting gradually replaces eating as the predominant behaviour, with a clear eat-to-rest transition occurring around timebin 8 (approximately 40 min). A very similar pattern of behaviour is evident in animals treated with 10 or 20 mg/kg BUP. However, at 40 mg/kg, behaviour is completely disrupted with the typical BSS replaced by high levels of locomotor activity, low levels of grooming and a virtual absence of resting behaviour.

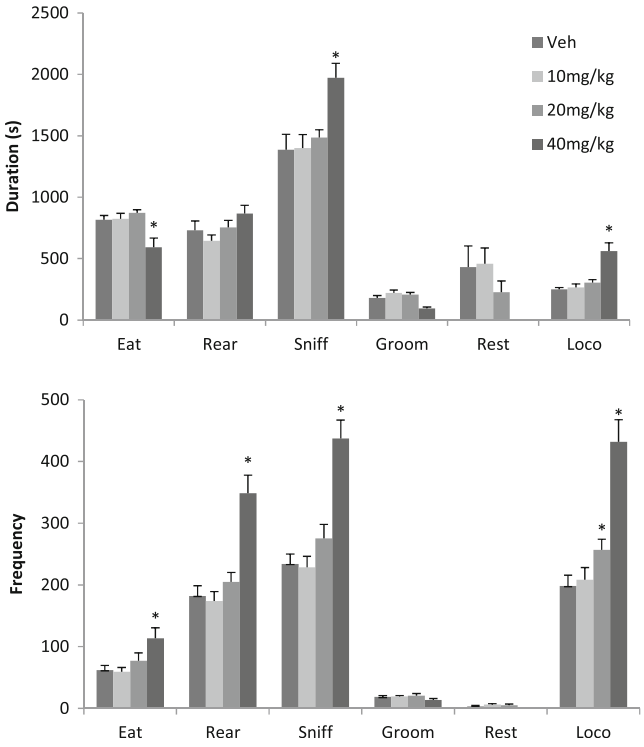

Fig. 1 Effects of acute bupropion HCl (10.0–40.0 mg/kg, IP) on behaviours displayed by male rats during a 1-h test with palatable mash. *Upper panel* total duration scores. *Lower panel* total frequency scores. Data are expressed as the mean values ± SEM. *s* seconds. *$p\leq0.05$ vs vehicle control. See text for details

Post-treatment body weight gain Data were not shown. ANOVA failed to reveal a significant effect of treatment on 3-day absolute weight gain ($F(3,27)=0.2$, $p>0.05$). To account for minor differences in test day body weight, datasets were converted to daily percent body weight changes from test day. While this finer-grain analysis confirmed a general increase in body weight over days (main effect of day: $F(2,18)=84.16$, $p<0.001$), it too failed to reveal an effect of treatment ($F(3,27)=0.44$, $p>0.05$) or an interaction ($F(6,54)=1.02$, $p>0.05$).

Experiment 2

Habituation

Although apparently quite healthy, one rat failed to consume any significant quantity of mash during habituation week and was, therefore, excluded from the study. For the remaining nine animals, mean body weight on arrival was 204.8±1.8 g on arrival and 418.1±8.2 g by the end of the study. Mash consumption differed significantly over the course of habituation ($F(4,32)=19.23$, $p<0.001$), with intake on trial 1 (10.28±0.77 g) significantly lower ($p\leq0.005$) than on trials 2–5 (trial 2, 15.35±1.23 g; trial 3, 17.56±0.90 g; trial 4, 17.82±0.86 g; trial 5, 16.52±0.93 g). The development of a stable intake pattern was confirmed by the lack of significant difference across trials 2–5.

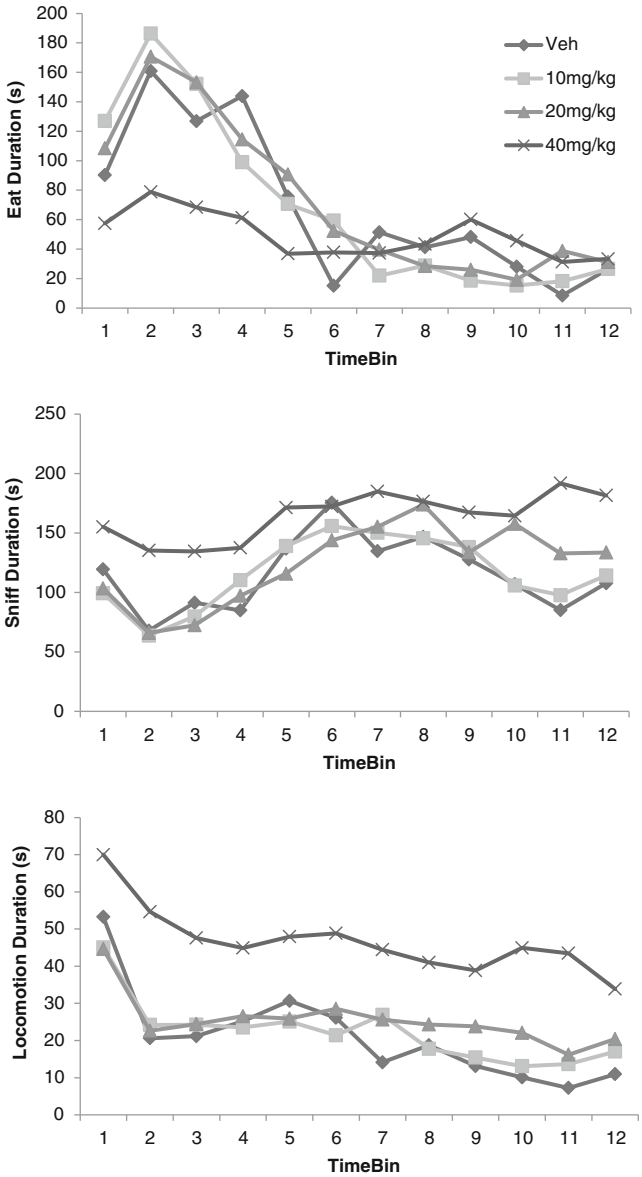

Fig. 2 Effects of acute bupropion HCl (10.0–40.0 mg/kg, IP) on the timecourses of eating, locomotion and sniffing in male rats during a 1-h test with palatable mash. Data are expressed as the mean duration of each behaviour in 12×5-min timebins. The highest dose of bupropion suppressed the peak feeding response in the early part of the test and stimulated locomotor activity and sniffing throughout the 1-h period. *s* seconds. See text for details

Naltrexone dose–response

Test day body weight and food intake Test day body weights did not vary significantly across treatment conditions (vehicle, 370.7±8.6 g; NTX0.1, 377.0±8.5 g; NTX1.0, 382.6±8.6 g; NTX3.0, 378.4±7.5 g; ($F(3,24)=0.77$, $p>0.05$)). Treatment effects on food intake are summarised in Table 2. NTX dose-dependently suppressed 1 h mash intake ($F(3,24)=18.13$, $p<0.001$), an effect statistically significant at the highest dose tested (3.0 mg/kg; 56v% reduction relative to vehicle control, $p=0.001$).

Total behavioural scores Data for the two latency measures, eat bout duration and eating rate, are summarised in Table 2, while treatment effects on the total frequency and duration of ingestive and non-ingestive behaviours are shown in Fig. 4.

NTX did not significantly alter latencies to locate the food source or to commence eating ($F(3,24)\leq1.60$, $p>0.05$) nor did it significantly affect the duration of eating bouts or the rate of eating ($F(3,24)\leq2.49$, $p>0.05$). Nevertheless, it is interesting to note the trend towards a dose-dependent reduction in the rate of eating with NTX (Table 2). ANOVA did, however, reveal significant treatment effects on the frequency and duration of eating ($F(3,24)\geq9.99$, $p<0.001$), locomotion ($F(3,24)\geq3.16$, $p\leq0.05$) and rearing ($F(3,24)\geq5.04$, $p\leq0.008$, as well as the duration of resting ($F(3,24)=6.63$, $p=0.012$) and the frequency of sniffing ($F(3,24)=6.53$, $p=0.002$). Treatment did not significantly influence any other behavioural measure ($F(3,24)\leq2.46$, $p>0.05$).

As shown in Fig. 4, and consistent with the effects on intake, NTX was behaviourally most effective at the highest dose tested (3.0 mg/kg) with significant reductions in both the frequency and duration of eating ($p\leq0.01$), as well as the frequency of locomotion and sniffing ($p\leq0.05$). This dose of NTX was also associated with a significant increase in the duration of resting ($p<0.02$). Very few effects were observed at lower doses of the opioid receptor antagonist, exceptions being suppression ($p\leq0.03$) of eat frequency at 1.0 mg/kg and of sniff frequency at 0.1 mg/kg. Despite significant ANOVA main effects for the duration of locomotion, as well as the frequency and duration of rearing, post hoc tests failed to identify any significant drug–vehicle differences for these measures.

Temporal patterns and behavioural satiety sequence Timebin analyses confirmed the gradual reduction in most active behaviours and increase in resting over the 1-h test session (e.g. Ishii et al. 2003; Tallett et al. 2008a, b, 2009a, b). Thus, except for the frequency and duration of grooming and scratching ($F(11,88)\leq1.81$, $p>0.05$), significant main effects of time were found for the frequency ($F(11,88)\geq10.98$, $p\leq0.001$) and duration ($F(11,88)\geq7.41$, $p\leq0.001$) of all other behavioural measures. Of potentially greater importance, significant treatment×time interactions were found for the frequency and duration of eating ($F(33,264)\geq1.60$, $p\leq0.03$), rearing ($F(33,264)\geq1.91$, $p\leq0.01$) and sniffing ($F(33,264)\geq1.54$, $p\leq0.05$), as well as for the duration of resting ($F(33,264)=1.60$, $p=0.024$) and the frequency of locomotion ($F(33,264)=1.58$, $p<0.03$).

Significant drug×time interactions were further explored by a series of one-way ANOVAs within each timebin

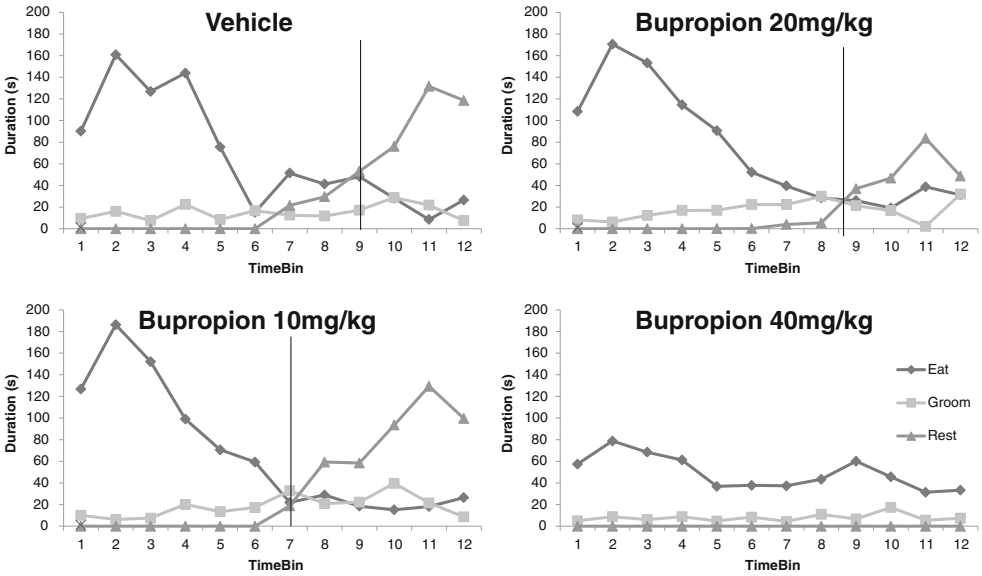

Fig. 3 Effects of acute bupropion HCl (10.0–40.0 mg/kg, IP) on the BSS in male rats tested for 1 h with palatable mash. Data are expressed as the mean duration scores in each of 12×5-min timebins comprising the 1-h test period. *s* seconds. The *vertical line* bisecting the *x*-axis is merely an aid to visualisation of the transition between eating and resting. The structural integrity of the BSS, unaffected by low (sub-anorectic) doses of bupropion (10–20 mg/kg), was totally disrupted at the highest dose tested. See text for details

(T1–T12) and, in view of their importance to interpretation, the outcomes of these analyses are reported in some detail. *Eating*. Significant treatment effects were found for eat frequency in T1–T3 ($F(3,24)\geq3.85$, $p\leq0.03$) and for eat duration in T1–T4 ($F(3,24)\geq4.50$, $p\leq0.02$). Somewhat unexpectedly, the lowest dose of NTX (0.1 mg/kg) significantly increased time spent eating in T1 ($p<0.05$) but reduced the frequency of eating in T4 ($p<0.01$), whereas the highest dose of NTX (3.0 mg/kg) reduced eat frequency in both T1 and T4 ($p\leq0.05$). *Resting*. NTX significantly affected rest duration in T8 and T9 ($F(3,24)\geq5.00$, $p\leq0.01$), with Bonferroni comparisons confirming significant enhancement of this measure by the highest dose of the compound ($p<0.02$). *Locomotion*. Significant effects of drug treatment were found for locomotion frequency in T1 and T8–T9 inclusive ($F(3,24)\geq3.40$, $p\leq0.04$), with significant suppression evident at the lowest dose in T1 ($p<0.03$) and at the highest dose in T8 and T9 ($p\leq0.03$). *Rearing*. Significant effects of NTX were found for rear frequency in T3–T9 inclusive ($F(3,24)\geq3.48$, $p\leq0.04$) and for rear duration in T1 and T5–T9 inclusive ($F(3,24)\geq3.24$, $p\leq0.04$). Post hoc tests showed that rear frequency was suppressed by NTX 1.0 mg/kg in T6 ($p<0.02$) and by the highest dose in T5, T8 and T9 ($p\leq0.05$), while rear duration was suppressed by the lowest dose in T1 ($p<0.01$) and by the highest dose in T5 ($p\leq0.005$). *Sniffing*. Significant treatment effects were found for sniff frequency in T1 and T3–T9 inclusive ($F\geq3.37$, $p\leq0.04$) and for sniff duration in T1, T9 and T12 ($F(3,24)\geq3.62$, $p\leq0.03$). Sniff frequency was significantly reduced by NTX 0.1 mg/kg in T1 ($p<0.001$), by NTX 1.0 mg/kg in T6 ($p<0.01$) and by NTX 3.0 mg/kg in T8 and T9 ($p\leq0.02$). In addition, sniff duration was suppressed by NTX 3.0 mg/kg but only in T9 ($p<0.04$).

Overall, these temporal analyses indicate that, while NTX suppressed eating in the early part of the session, resting was increased and other behaviours inhibited somewhat later in proceedings—a profile consistent with an increase in and earlier onset of postprandial resting. Figure 5 illustrates the timecourse patterns for time spent eating and resting, as well as the frequency of sniffing, locomotion and rearing.

Table 2 Effects of naltrexone hydrochloride (0.1–3.0 mg/kg IP) on mash intake and feeding-related parameters in male rats exposed for 1 h to palatable mash

Measure	Vehicle	NTX0.1	NTX1.0	NTX3.0
Mash intake (g)	14.91±0.90	14.19±0.92	11.68±1.05	6.57±1.25***
Latency to locate food (s)	5.70±2.25	5.29±1.84	4.93±1.95	6.65±2.84
Latency to eat (s)	27.67±6.57	57.84±12.48	37.50±11.43	41.28±12.08
Eat bout (s)	10.24±1.15	11.12±1.08	12.29±1.13	13.25±1.57
Eat rate (g/min)	1.42±0.10	1.40±0.08	1.25±0.08	1.10±0.12

Data are presented as the mean values ± SEM. See text for full details

s seconds, *g* grams

***$p<0.001$ vs vehicle

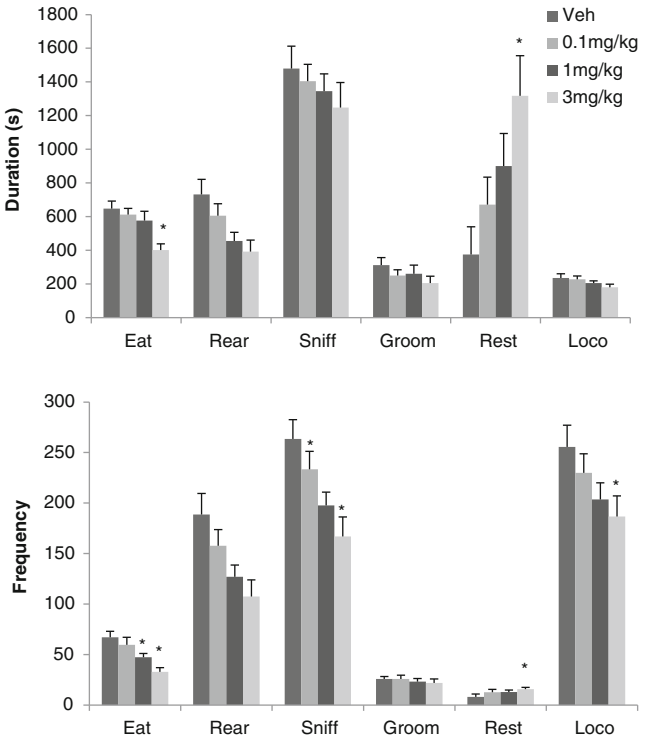

Fig. 4 Effects of acute naltrexone HCl (0.1–3.0 mg/kg, IP) on behaviours expressed by male rats during a 1-h test with palatable mash. *Upper panel* total duration scores. *Lower panel* total frequency scores. Data are expressed as the mean values ± SEM. *s* seconds. *$p \leq 0.05$ vs vehicle control. See text for details

The effects of NTX (0.1–3.0 mg/kg) on the BSS are summarised in Fig. 6. As seen in experiment 1, the vehicle profile shows a peak feeding response during the first 20 min of the test. Over time, resting gradually begins to replace eating as the predominant behaviour with the first clear eat–rest transition occurring approximately at T6. Although the structural integrity of the BSS is fully maintained under all doses of NTX, Fig. 8 shows a dose-dependent acceleration (shift to left) in the entire behavioural pattern, an effect most evident at the highest dose tested (3.0 mg/kg; T4). It is particularly important to note that increased resting seen in response to NTX occurred after (and not before) the ingestion of food.

Post-treatment body weight gain Data were not shown. ANOVA failed to reveal a significant effect of NTX on 3-day absolute weight gain (vehicle, 8.63±1.77 g; NTX0.1, 8.26±1.14 g; NTX1.0, 8.93±0.94 g; NTX3.0, 7.46±0.69 g; $F(3,24)=0.32$, $p>0.05$). Similarly, while the analysis of percent body weight changed over days post-treatment confirmed a general increase in body weight over time (main effect of day: $F(2,16)=127.49$, $p<0.001$), it too failed to support a significant main effect of treatment ($F(3,24)=1.49$, $p>0.05$) or a treatment×day interaction ($F(6,48)=0.94$, $p>0.05$).

Experiment 3

Habituation

Mean body weight on arrival was 205.1±2.2 and 444.3±9.5 g by the end of the study. Intake again differed significantly over the course of habituation ($F(4,36)=8.82$, $p<0.001$), with intake on trial 1 (13.83±0.85 g) significantly lower ($p \leq 0.05$) than on trials 2, 3 and 5 (trial 2, 17.58±0.94 g; trial 3, 19.00±1.27 g; trial 4, 19.29±1.98 g; trial 5, 20.36±1.29 g). However, the lack of significant difference across trials 2–5 confirmed the development of a stable intake pattern.

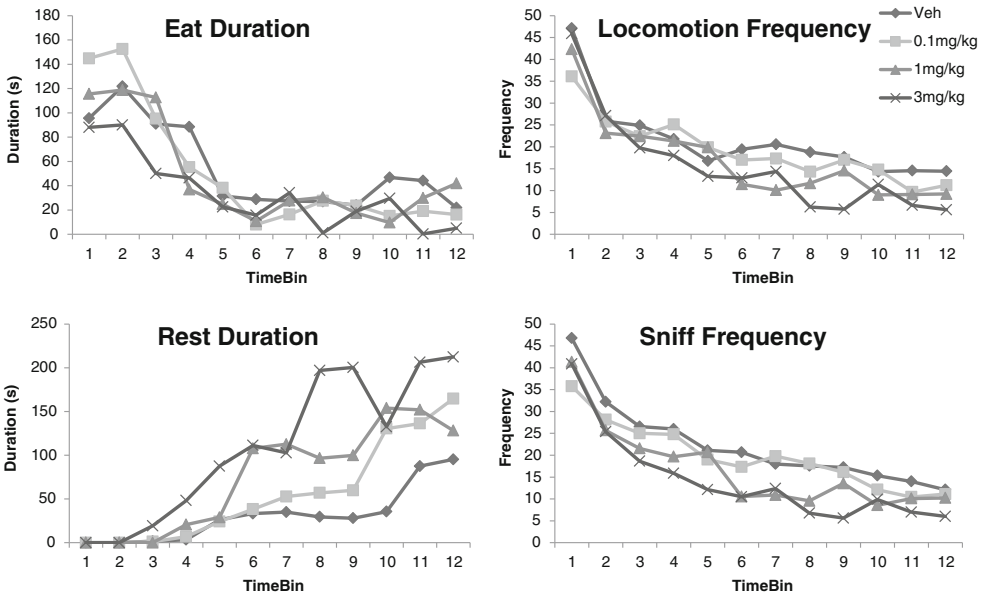

Fig. 5 Effects of acute naltrexone HCl (0.1–3.0 mg/kg, IP) on the duration of eating and resting as well as the frequency of locomotion and sniffing over the course of the 1-h test session. Data are expressed as the mean scores in each of 12×5-min timebins. While ingestive behaviour was mainly suppressed during the first third of the test session (0–20 min), resting was enhanced and activity/sniffing during the second half of the test session (25–50 min). See text for details

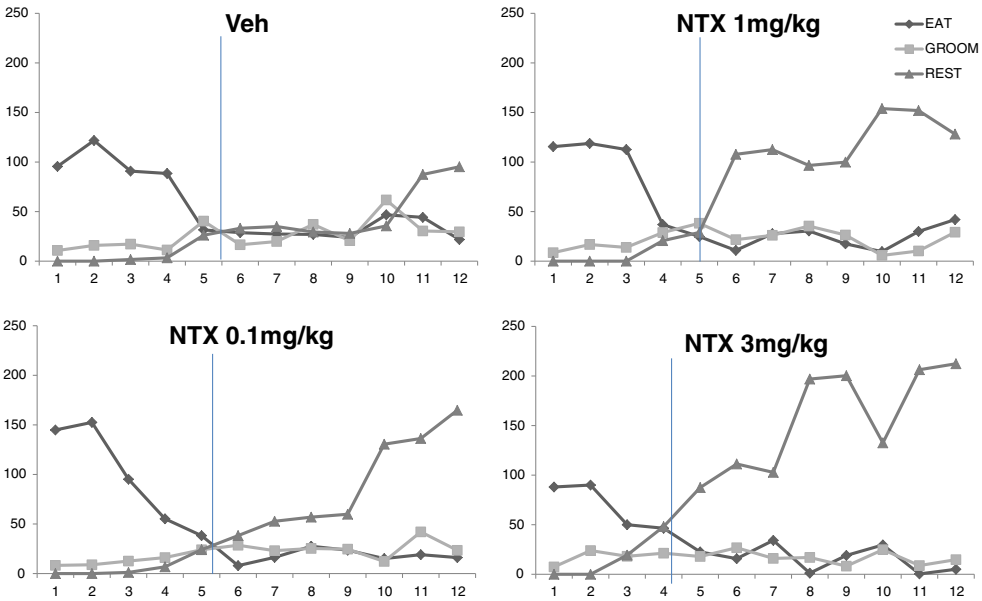

Fig. 6 Effects of acute naltrexone HCl (0.1–3.0 mg/kg, IP) on the BSS in male rats tested for 1 h with palatable mash. Data are expressed as the mean duration scores (*s* seconds) in each of 12×5-min timebins comprising the 1-h test period. The *vertical line* bisecting the *x*-axis is merely an aid to visualisation of the transition between eating and resting. Without affecting behavioural structure, naltrexone produced a dose-dependent acceleration in behavioural satiety, as evidenced by a shift to the left in the sequence. See text for details

Bupropion–naltrexone interaction

Treatment effects on test day body weight and food intake Test day body weights did not differ significantly across treatment conditions: V/V, 399.2±14.3 g; V/NTX0.1, 399.0±12.4 g; V/NTX1.0, 401.1±11.3 g; BUP20/V, 403.9±7.2 g: BUP20/NTX0.1, 408.0±10.8 g; BUP20/NTX1.0, 403.4±12.3 g (main effect of BUP: $F(1,9)=0.30$, $p>0.05$; main effect of NTX: $F(2,18)=0.08$, $p>0.05$; interaction: $F(2,18)=0.05$, $p>0.05$).

Treatment effects on food intake are summarised in Table 3. ANOVA confirmed significant main effects for BUP ($F(1,9)=33.74$, $p<0.001$) and NTX ($F(2,18)=10.71$, $p=0.005$), but no significant interaction ($F(2,18)=0.64$, $p>0.05$). Post hoc comparisons revealed that mash intake was significantly suppressed both by BUP20 and NTX1.0 when given alone ($p\leq0.01$ vs V/V) and by BUP20 in combination with each dose of NTX ($p\leq0.001$ vs V/V). The apparently greater suppressant effect of co-administration is supported by significant differences between (a) the lower dose of NTX in the presence vs absence of (BUP20/NTX0.1 vs V/NTX0.1; $p<0.01$) and (b) BUP20 in the presence vs absence of the higher doses of NTX (BUP20/NTX1.0 vs BUP20/V; $p<0.05$). An additive anorectic effect of co-treatment is further supported by comparisons between the actual percentage reductions in intake (relative to V/V control) for the treatment combinations and those predicted by simply adding the effects of the individual treatments: V/NTX0.1 (14.3 %); V/NTX1.0 (26 %); BUP20/V (23.1 %); BUP20/NTX0.1 (37.7 % actual vs 37.4 % calculated); BUP20/NTX1.0 (40.8 % actual vs 49.1 % calculated).

Treatment effects on total behavioural scores Data for feeding-related measures are summarised in Table 3, while treatment effects on the total frequency and duration of ingestive and non-ingestive elements are shown in Fig. 7. Significant BUP×NTX interactions were found for the frequency of rearing and sniffing and for time spent grooming

Table 3 Effects of bupropion hydrochloride (0 or 20 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/ NTX0.1	Vehicle/ NTX1.0	BUP20/ Vehicle	BUP20/NTX0.1	BUP20/NTX1.0
Mash intake (g)	19.30±1.25	16.57±1.46	14.28±1.40**	14.84±1.42**	12.02±1.16***, +	11.20±1.39***, #
Latency to locate food (s)	7.14±1.74	7.90±1.79	7.03±2.19	11.95±3.11	5.93±1.93	7.87±3.69
Latency to eat (s)	20.65±3.87	28.33±4.66	30.18±7.35	32.10±9.41	22.57±4.06	18.90±5.00
Eat bout (s)	11.12±1.63	10.42±1.36	9.97±1.55	6.97±1.12	9.26±1.97	9.19±1.80
Eat rate (g/min)	1.70±0.07	1.66±0.14	1.63±0.13	1.66±0.13	1.36±0.15	1.15±0.11*

Data are presented as the mean values ± SEM. See text for full details

s seconds, *g* grams

*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs vehicle/vehicle; $^{+}p<0.01$ vs vehicle/NTX0.1; $^{\#}p<0.05$ vs BUP20/vehicle

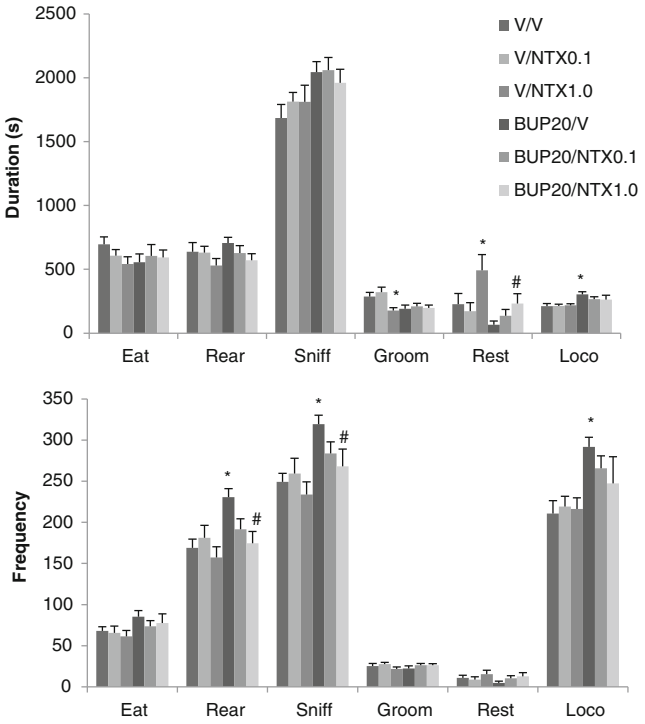

Fig. 7 Effects of bupropion HCl (20 mg/kg, IP) and naltrexone HCl (0.1 or 1.0 mg/kg, IP), alone and in combination, on behaviours expressed by male rats during a 1-h test with palatable mash. *Upper panel* total duration scores. *Lower panel* total frequency scores. *V* vehicle, *BUP* bupropion 20 mg/kg, *NTX0.1* 0.1 mg/kg naltrexone, *NTX1.0* 1.0 mg/kg naltrexone. Data are expressed as the mean values ± SEM. *s* seconds. *$p \leq 0.05$ vs vehicle control; #$p \leq 0.05$ vs BUP20/V. See text for details

($F(2,18) \geq 10.42$, $p \leq 0.01$), while significant main effects of BUP were found for the frequency of eating ($F(1,9) = 12.44$, $p < 0.01$), eat bout duration ($F(1,9) = 14.01$, $p = 0.005$), eating rate ($F(1,9) = 13.64$, $p = 0.005$), the duration of sniffing ($F(1,9) = 50.57$, $p < 0.001$) and both the frequency and duration of locomotion ($F(1,9) \geq 29.61$, $p \leq 0.001$). In addition, significant main effects of NTX were found for eating rate ($F(2,18) = 8.37$, $p = 0.003$), rear duration ($F(2,18) = 8.04$, $p < 0.005$) and both the frequency and duration of resting ($F(2,18) \geq 4.49$, $p \leq 0.05$). No other interactions or main effects were significant.

As summarised in Fig. 7, BUP increased the frequency of rearing and sniffing ($p \leq 0.01$, BUP20/V vs V/V), effects that were blocked by the intrinsically inactive higher dose of NTX ($p \leq 0.01$; BUP20/NTX1.0 vs BUP20/V). Relative to V/V control, BUP by itself also increased the frequency ($p < 0.01$) and duration ($p < 0.05$) of locomotion, effects that were non-significantly attenuated by co-administration of the higher dose of NTX. No significant pairwise contrasts were found in post hoc follow-ups to the reported main effects of BUP on eat frequency, eat bout duration or sniff duration or of NTX on rest frequency and rear duration. Although both BUP and NTX tended to individually reduce the rate of eating relative to V/V control, the largest effects were seen in animals receiving combined treatment (Table 3), with post hoc comparisons confirming a significant reduction in eat rate only for the combination of BUP and the higher dose of NTX (BUP20/NTX1.0 vs V/V, $p < 0.05$). It is also worth noting that the difference in eating rate between the latter condition and BUP given alone (BUP20/V) approached statistical significance ($p = 0.072$). Finally, relative to V/V control, the higher dose of NTX reduced the duration of grooming and increased the duration of resting ($p \leq 0.05$). Despite the observation that the latter effect of the opioid receptor antagonist was significantly attenuated by co-administration of BUP (BUP20/NTX1.0 vs V/NTX1.0; $p < 0.05$), it should be noted that BUP by itself (albeit non-significantly) reduced time spent resting and that a simple cancellation effect most probably occurred (Fig. 7).

Treatment effects on temporal patterns and behavioural satiety sequence With the solitary exception of groom frequency ($F(11,99) < 1.13$, $p > 0.05$), significant main effects of time were found for the frequency ($F(11,99) \geq 8.03$, $p \leq 0.001$) and duration ($F(11,99) \geq 4.08$, $p \leq 0.001$) of all behavioural measures. Very few parameters showed significant drug interactions involving time, the exceptions being three-way (BUP×NTX×time) interactions for the frequency of rearing and sniffing ($F(22,198) \geq 1.58$, $p \leq 0.05$) and a two-way (BUP×time) interaction for rest duration ($F(11,99) = 2.40$, $p = 0.01$). The NTX×time interaction for rest frequency also closely approached statistical significance ($F(22,198) = 1.57$, $p < 0.06$). Significant interactions were further explored by a series of two-way ANOVAs (and post hoc tests) within each timebin. These analyses showed that BUP alone significantly increased rear frequency in timebins 2, 3 and 9 (BUP20/V vs V/V; $p \leq 0.03$), effects that were significantly blocked by the co-administration of the higher dose of NTX (BUP20/NTX1.0 vs BUP20/V; $p \leq 0.05$). Similarly, sniff frequency was significantly increased by BUP alone (BUP20/V vs V/V) in timebins 1 and 2 ($p \leq 0.04$), with additional increases in timebins 3 and 8 closely approaching significance ($p \leq 0.07$). Given the pattern of results for rearing, it is interesting to note that the BUP20-induced increases in sniff frequency in timebins 1 and 3 were almost significantly attenuated by co-administration of the higher dose of NTX (BUP20/NTX1.0 vs BUP20/V, $p \leq 0.08$). Despite the significant main effects and/or interactions for resting parameters (indicating overall increases under NTX and decreases under BUP), high within-timebin variance largely precluded the detection of meaningful significant pairwise contrasts.

Figure 8 illustrates the BSS profiles for each of the six treatment conditions. Although the absolute level of resting in the second half of the session was not as great as seen in earlier experiments, the control BSS profile (V/V; top left panel) nevertheless shows the typical peak feeding response in the first 15–20 min of the test. Feeding gradually gives

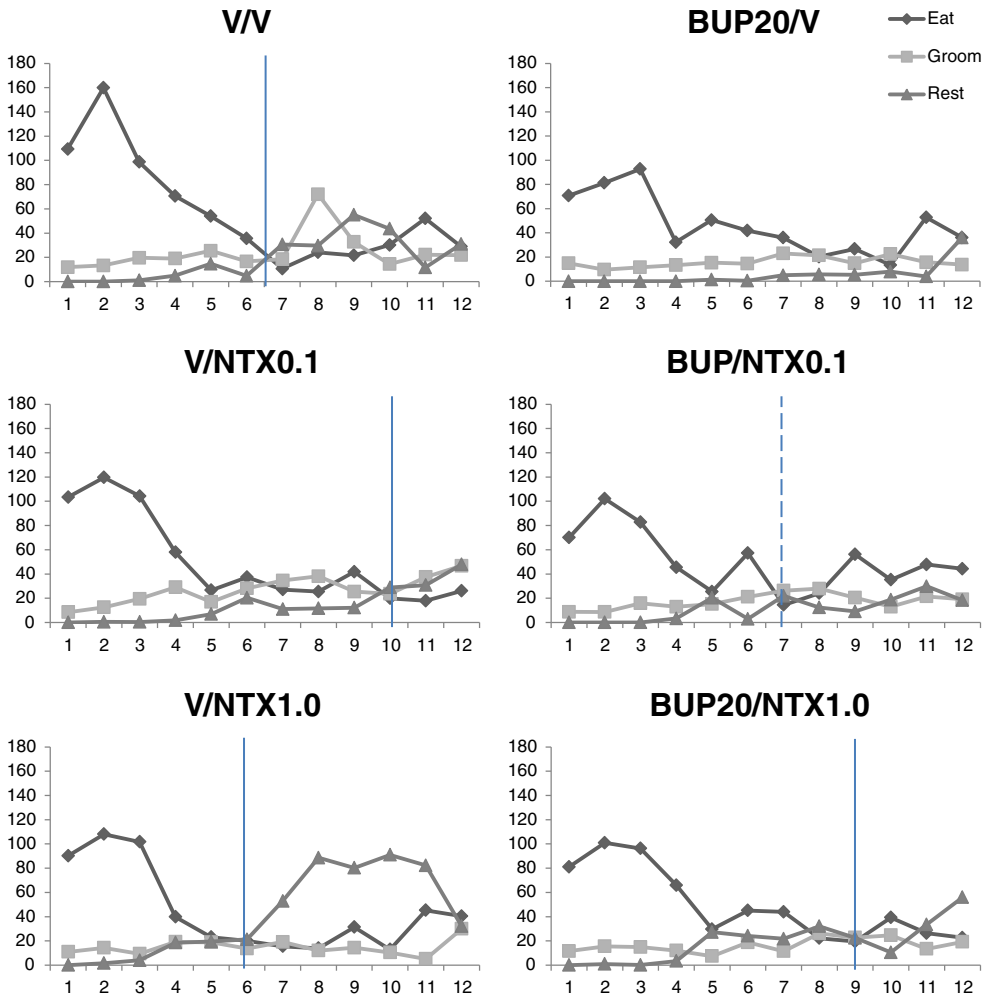

Fig. 8 Effects of bupropion HCl (20 mg/kg, IP) and naltrexone HCl (0.1 or 1.0 mg/kg, IP), alone and in combination, on the BSS in male rats tested for 1 h with palatable mash. Data are expressed as the mean duration scores (*s* seconds) in each of 12×5-min timebins comprising the 1-h test period. The *vertical line* (*dashed line* where unstable) bisecting the *x*-axis is merely an aid to visualisation of the transition between eating and resting. *V* vehicle, *BUP* bupropion 20 mg/kg, *NTX0.1* 0.1 mg/kg naltrexone, *NTX1.0* 1.0 mg/kg naltrexone. See text for details

way to grooming and resting as time progressed, with an eat-to-rest transition occurring around 35 min into the test. Although neither dose of NTX when given alone interfered with normal behavioural structure (centre and bottom left panels), V/NTX1.0 modestly accelerated the sequence (shift to the left) by suppressing the peak feeding response and producing an earlier transition to, as well as higher levels of, resting. Given alone, BUP20/V also suppressed the peak feeding response but virtually eliminated resting behaviour, a pattern consistent with behavioural disruption (top right panel). Interestingly, while still displaying a reduction in the peak feeding response, co-administration of either dose of NTX with BUP (centre and bottom right panels) appeared to reinstate a more normal behavioural structure with eat-to-rest transitions once again discernible around 35–40 min.

Post-treatment body weight gain Data were not shown. ANOVA failed to reveal any significant main effects or interactions for the 3-day absolute weight gain—animals generally gained between 9 and 10 g irrespective of treatment condition (main effect of BUP: $F(1,9)=0.96$, $p>0.05$; main effect of NTX: $F(2,18)=0.07$, $p>0.05$; interaction: $F(2,18)=0.24$, $p>0.05$). Although an analysis of percent body weight change over days post-treatment confirmed normal growth patterns (main effect of day: $F(2,18)=71.36$, $p<0.001$), it too failed to reveal any significant drug main effects or interactions.

Discussion

Early clinical trial reports that the atypical antidepressant and smoking cessation aid, BUP, also reduces appetite and weight gain have since been supported by several randomised, double-blind, placebo-controlled studies (for reviews, see Fava et al. 2005; Li et al. 2005; Padwal 2009). However, the relatively modest average weight loss (average of 2.8 kg over 24–52 weeks) under BUP falls short of the current regulatory criterion of a minimum 5 kg weight loss for marketing approval (Heal et al. 2009; Kennett and Clifton 2010). Somewhat similarly, although the opioid receptor antagonist NTX acutely suppresses appetite in humans, it has not by itself proved clinically useful in the management of obesity (Yeomans and Gray 2002). It is, therefore, of considerable

interest to note the proposal that that BUP's influence on weight gain may be self-limiting due to an opioid receptor-mediated negative feedback effect on POMC neurons in the arcuate nucleus (e.g. Greenway et al. 2009). This hypothesis has received support from animal and human studies wherein combined treatment with BUP and the opioid receptor antagonist NTX produced greater effects on food intake and weight gain than either compound alone (Greenway et al. 2009, 2010; Padwal 2009; Wadden et al. 2011). Despite such developments, however, little is known about the effects of BUP on behaviour within the feeding context. In view of this surprising gap in the literature, the current studies have employed BSS methodology (for a review, see Rodgers et al. 2010) to systematically and comprehensively profile the effects of BUP and NTX, alone and in combination, on ingestive and multiple non-ingestive behaviours in non-deprived male rats exposed to palatable mash.

Consistent with previous research (Billes and Cowley 2007; Greenway et al. 2009; Stairs and Dworkin 2008; Zarrindast and Hosseini-Nia 1988), experiment 1 confirmed that acute BUP (40 mg/kg) suppresses food intake in rodents. The observation that this modest anorectic response (20 % suppression relative to saline control) was not associated with a reduction in post-treatment weight gain is undoubtedly due to the acute nature of the treatment, the relatively low dosages involved and the short biological half-life of the compound (Suckow et al. 1986). Behavioural analysis showed that, although the acute appetite suppression was associated with a significant reduction in time spent feeding and the average duration of feeding bouts, BUP did not alter either the time taken to locate the food source or to commence eating nor did any dose alter weight gain over the 3-day period following treatment. However, at the same dose level (but not 10–20 mg/kg), the compound significantly suppressed grooming while markedly stimulating rearing, sniffing and locomotion. Interestingly, this behavioural activation extended to the frequency of eating even though actual time spent eating was significantly reduced. Very similar psychostimulant effects of BUP have previously been observed, although almost invariably in non-feeding contexts (Carrasco et al. 2004; Cooper et al. 1980; Gomez et al. 2008; Nielsen et al. 1986; Paterson et al. 2010; Redolat et al. 2005a, b; Santamaria and Arias 2010; Soroko et al. 1977; Zarrindast and Hosseini-Nia 1988; Zarrindast et al. 1996). One exception is a study by Billes and Cowley (2007) in which telemetry was used to record locomotor activity during tests of food intake. Although BUP produced both anorectic and locomotor stimulant effects, the authors argued for an independence of these two effects. Despite the largely parallel dose–response relationships for anorexia and locomotor stimulation, attention was drawn to the fact that (a) increasing doses of BUP that caused incremental increases in activity (i.e. 0–10 and 20–40 mg/kg) did not also significantly decrease food intake and (b) incremental reductions in food intake between 10 and 20 mg/kg were not correlated with a significant increase in activity level. However, the authors did not report any statistical correlations while their 'incremental dose comparison' argument assumes equivalent sensitivity of the measures of food intake and activity. The latter seems improbable given major differences in the units of measurement and base rates for intake and activity.

Although locomotor stimulation does not always lead to a suppression of food intake (e.g. Cooper and van der Hoek 1993; van Rossum and Simons 1969), it would seem parsimonious to argue that the acute anorectic effect of BUP (40 mg/kg) in experiment 1 may have been secondary to the marked increase in non-ingestive behaviours. Thus, in tests of finite duration, significant increases in time spent on non-ingestive behaviour logically leave less time for eating (for a review, see Rodgers et al. 2010). Such an interpretation of BUP anorexia appears consistent with Figs. 2 and 3 which show that, at 40 mg/kg (but not lower doses), BUP completely disrupts the structural integrity of the BSS. Thus, not only was the peak feeding response suppressed, grooming inhibited and resting eliminated, the overall behavioural profile is characterised by a marked stimulation of locomotor activity and sniffing. This behavioural signature is reminiscent of that reported both for D-amphetamine (Blundell and McArthur 1981; Halford et al. 1998) and cocaine (Cooper and van der Hoek 1993). Nevertheless, it remains to be determined whether BUP would produce similar effects in a rodent (genetic or dietary) model of obesity and/or following sub-chronic dosing. The limited evidence available suggests comparable sensitivity to the acute anorectic effects of BUP in lean and DIO mice (e.g. Billes and Cowley 2007; Greenway et al. 2009). Interestingly, one report suggests that, despite increased activity and thermogenesis, sub-chronic BUP produces a compensatory increase in food intake thereby negating any significant weight loss (Billes and Cowley 2008).

Confirming numerous previous reports (e.g. Apfelbaum and Mandenhoff 1981; Cooper and Turkish 1989; Hadjimarkou et al. 2004; Jackson and Sewell 1985a, b; Kirkham and Blundell 1986a, b; Kirkham et al. 1987; Marks-Kaufman et al. 1985; Sanger and McCarthy 1982), the results of experiment 2 show that NTX dose-dependently reduced mash consumption. Our observation that the compound also reduced the frequency and duration of feeding behaviour similarly agrees well with the reports of Kirkham and Blundell (1987) and Cooper and Turkish (1989) and with our own earlier report on naloxone (Tallett et al. 2008b). Although the suppressant effects of NTX on food intake and feeding duration were statistically significant at the highest dose (3.0 mg/kg) only, it should be noted that the lower dose of 1.0 mg/kg actually reduced intake by 22 % and significantly decreased the frequency of eating episodes. Consistent with

previously reported effects for naloxone (Tallett et al. 2008b), NTX did not significantly alter the time taken to locate the food or to commence eating nor did it affect the average duration of eating bouts or eating rate. In addition to effects on ingestive elements, NTX significantly impacted most other behaviours displayed during testing. However, post hoc comparisons identified significant drug vs vehicle differences only for the duration of resting (increased) and for the frequency of locomotion and sniffing (decreased). Nevertheless, as these effects were observed at the same NTX dose (3.0 mg/kg) that inhibited food intake and eating behaviour, the behavioural selectivity of the anorectic response might be questioned. However, close examination of treatment effects over time clearly revealed that, whereas eating was chiefly suppressed during the first 15–20 min of the test, locomotion, rearing and sniffing were most affected later in the session—a pattern consistent with the significant increase in time spent resting towards the end of the test (Fig. 5; see also Cooper and Turkish 1989). The latter effect can also be seen in the BSS profiles (Fig. 6) where, without compromising behavioural structure, NTX treatment led to a modest acceleration (gradual shift to the left in the eat-to-rest transition) in the satiety sequence. Clearly, under present test conditions, acute NTX produces a more potent and behaviourally selective profile than does acute BUP (experiment 1). Furthermore, while the currently observed profile for NTX is quite similar to that previously obtained with naloxone (Tallett et al. 2008b), NTX was less potent in terms of the minimum effective anorectic dose (3.0 vs 1.0 mg/kg) but had a wider range of behavioural activity. As all other aspects of methodology were fairly constant between the two studies (general BSS protocol as used in our laboratory; Rodgers et al. 2010), the noted variations in behavioural signature most probably reflect pharmacokinetic and pharmacodynamic differences between the two opioid receptor antagonists (e.g. Blumberg et al. 1967; Goldstein and Naidu 1989; Magnan et al. 1982; Martin et al. 1963; Raynor et al. 1994; Tepperman et al. 1983).

The results of the first two experiments informed the design of experiment 3,which assessed the effects of combined treatment with low doses of BUP (20 mg/kg) and NTX (0.1 or 1.0 mg/kg). Somewhat unexpectedly, BUP20 by itself exerted significant anorectic activity in experiment 3 and produced evidence consistent with psychomotor stimulation (e.g. increased locomotion, rearing and sniffing). Given the lack of effect of this dose in experiment 1, this result can only reflect batch differences in sensitivity to bupropion. Indeed, the behavioural profile of BUP20 in the third study appeared intermediate between the patterns of effect induced by BUP20 and BUP40 in experiment 2 (see also Zarrindast and Hosseini-Nia 1988). The lower dose of NTX (0.1 mg/kg) was selected for the interaction study in view of its lack of anorectic activity in experiment 2, a sub-anorectic profile fully confirmed in experiment 3. The inclusion of the higher NTX dose (1.0 mg/kg) in experiment 3 was in recognition of its marginal anorectic profile in experiment 2, i.e. a modest, though non-significant, 22 % reduction in mash intake coupled with a significant reduction in eat frequency. A similar (though statistically significant) 26 % suppression of intake was evident in experiment 3. Despite the intrinsic anorectic activity of BUP20 and NTX1.0, the original aim of experiment 3 remained intact, namely, to study the behavioural effects of these two drugs when co-administered at sub-maximal dose levels. Indeed, it could be argued that the intrinsic activity of the higher NTX dose was fortuitous in permitting analysis of the effects of a sub-maximal dose of BUP when co-administered with either a sub-anorectic (0.1 mg/kg) or sub-maximal (1.0 mg/kg) dose of NTX. Our results showed that these combinations produced significantly greater reductions in mash intake (37–41 %) than those produced by either agent alone (14–26 %). However, unlike the theoretically predicted synergistic interaction for the BUP/NTX combination (Greenway et al. 2009), our findings are indicative of an additive interaction in that the reductions seen in response to combined treatment closely matched those simply calculated by the addition of reductions in response to the constituent agents. Interestingly, while Greenway et al. (2009) also observed an additive BUP/NTX interaction in lean mice, they reported a stronger (possibly synergistic) interaction in obese mice. The reason for this discrepancy is not immediately clear, but the greater effect of the combination in obese mice is certainly consistent with effects reported by the same authors for weight loss in obese humans.

In addition to the results on food intake per se, detailed analysis of behaviour during the feeding tests in experiment 3 yielded a number of other interesting findings. First, while BUP20 and NTX1.0 each tended to reduce time spent feeding, these individual drug effects were not statistically significant when compared with vehicle control, nor were the associated changes in feeding-related parameters (latencies, average bout length and eating rate). This finding indicates that food intake can be pharmacologically reduced in the absence of dramatic changes in any single measure of ingestive behaviour. Second, and consistent with the intake data per se, combined treatment led to an NTX dose-dependent reduction in the rate of eating (significant for the high-dose combination; BUP20/NTX1.0 vs V/V). Third, and most unexpectedly, the higher dose of NTX counteracted the stimulant effects of BUP20 on locomotion, rearing and sniffing, an effect statistically significant for the latter two measures. This pattern is reminiscent of the ability of another opioid receptor antagonist, naloxone, to counteract the compulsive scratching and grooming syndrome seen with cannabinoid CB1 receptor antagonist/inverse agonists (Tallett et al., 2007, 2008a, 2009a; Wright and Rodgers 2012) and may reflect known interactions between opioid and catecholamine substrates (e.g. Berridge et al. 2010;

Chien et al. 2012; Wiskerke et al. 2011). And, finally, BSS profiles confirmed the normality of behavioural structure under NTX treatment, the behaviourally disrupting effects of BUP20 and the apparent ability of NTX co-treatment to attenuate such disruption.

In summary, the present results show that acute treatment with BUP (20–40 mg/kg) significantly (though modestly) reduces food intake and time spent feeding in non-deprived male rats tested with palatable mash. However, these effects are accompanied by psychostimulant-like increases in sniffing, rearing and locomotor activity coupled with reductions in grooming and resting. Current results also confirm that the opioid receptor antagonist NTX alone suppresses food intake and feeding behaviour (significant mainly at 3 mg/kg). Although this action is associated with changes in other behaviours, including reductions in several active elements and an increase in resting, the latter occurred after (and not before) food consumption, while BSS profiles were modestly accelerated but otherwise normal. As suggested previously for naloxone anorexia (e.g. Tallett et al. 2008b), this pattern would be consistent with an inhibitory action of NTX on the reinforcing effects of palatable food. Finally, the combination of sub-maximal doses of the two agents was seen to result in an additive anorectic effect, with behavioural analysis additionally indicating an NTX-induced attenuation of the psychostimulant effects of BUP and a resultant normalisation of the BSS. This profile clearly suggests that acute BUP anorexia is not simply a consequence of psychomotor stimulation. Together, the present results would appear to support the therapeutic potential of this particular drug combination in the clinical management of obesity.

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