Cannabinol and cannabidiol exert opposing effects on rat feeding patterns.

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Abstract

Rationale

Increased food consumption following Δ^9tetrahydrocannabinol-induced cannabinoid type 1 receptor agonism is well documented. However, possible non-Δ^9tetrahydrocannabinol phytocannabinoid-induced feeding effects have yet to be fully investigated. Therefore, we have assessed the effects of the individual phytocannabinoids, cannabigerol, cannabidiol and cannabinol upon feeding behaviors.

Methods

Adult male rats were treated (p.o.) with cannabigerol, cannabidiol, cannabinol or cannabinol plus the CB_1R antagonist, SR141716A. Prior to treatment, rats were satiated and food intake recorded following drug administration. Data were analyzed for hourly intake and meal microstructure.

Results

Cannabinol induced a CB_1R-mediated increase in *appetitive* behaviors via significant reductions in the latency to feed, and increases in *consummatory* behaviors via increases in meal one size and duration. Cannabinol also significantly increased the intake during hour 1 and total chow consumed during the test. Conversely, cannabidiol significantly reduced total chow consumption over the test period. Cannabigerol administration induced no changes to feeding behavior.

Conclusion

This is the first time cannabinol has been shown to increase feeding. Therefore, cannabinol could, in the future, provide an alternative to currently used and psychotropic Δ^9tetrahydrocannabinol-based medicines since cannabinol is currently considered to be non-psychotropic. Furthermore, cannabidiol reduced food intake in line with some existing reports, supporting the need for further mechanistic and behavioral work examining possible anti-obesity effects of cannabidiol.

Keywords: cannabis, cannabigerol, cannabidiol, cannabinol, phytocannabinoids, feeding, appetite, behavio(u)r

1 *Abbreviations*

2-AG	2-arachidonoylglycerol
Δ^9THC	Δ^9-tetrahydrocannabinol
Δ^9THCV	Δ^9-tetrahydrocannabivarin
AEA	Anandamide
ANOVA	Analysis of variance
BDS	Botanical drug substance
C. sativa	*Cannabis sativa*
CB_1R	Cannabinoid type 1 receptor
CB_2R	Cannabinoid type 2 receptor
CBD	Cannabidiol
CBG	Cannabigerol
CBN	Cannabinol
CNS	Central nervous system
eCB	Endocannabinoid
i.p.	Intraperitoneal
pCB	Phytocannabinoid
p.o.	Per ora
s.c.	Subcutaneous

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Introduction

While *Cannabis sativa* (*C. sativa*) has been used on the Indian subcontinent and in China for thousands of years as a medicine, its use has been a source of controversy in Western medicine since its introduction in the 19th century due to widespread recreational use and abuse (O'Shaughnessey 1843; Wang et al. 2008). *C. sativa*'s pharmacological actions and psychotropic properties include sedation, analgesia, hypothermia, catalepsy and euphoria (Martin et al. 1981) alongside ravenous eating (Abel 1975).

Since the original identification of the cannabinoid type 1 and 2 receptors (CB_1 and CB_2R; Devane et al. 1988; Matsuda et al. 1990; Munro et al. 1993) and confirmation of an endogenous cannabinoid system following the discovery of the endogenous cannabinoids (eCBs; anandamide (AEA; Devane et al. 1992) and 2-arachidonyl glycerol (2-AG; Mechoulam et al. 1995; Sugiura et al. 1995)) research has largely focused on the effects of Δ^9tetrahydrocannbinol (Δ^9THC; Gaoni and Mechoulam 1964). Indeed, only limited research has considered the effects of the numerous other phytocannabinoids (pCBs) also present (Izzo et al. 2009). More recently, research has begun to examine the effects of these individual pCBs (for a review of cannabinoid pharmacology see Izzo et al. 2009). Currently, a range of possible cannabinoid-based therapies are being considered for a number of disorders (e.g. neurological and neurodegenerative, multiple sclerosis and anti-obesity (Glass 2001; Pryce et al. 2003; Van Gaal et al. 2005), for review see Amar 2006). Interestingly, this new research has made it apparent that these pCBs are likely to act at sites other than CB_1 and CB_2R due to their low binding affinities at these receptor sub-types (with the exceptions of Δ^9-tetrahydrocannabivarin (Δ^9THCV) and cannabinol (CBN); Petrosino et al. 2009). Importantly, the currently available literature gives no indication of non-Δ^9THC pCB psychoactivity (for reviews see Amar, 2006; Izzo et al., 2009).

Specifically in terms of feeding, and unlike the other pCBs, Δ^9THC has been relatively well studied. Indeed, some time ago it became apparent that CB_1R sites in the central nervous system (CNS; Herkenham et al. 1991) were responsible for Δ^9THC-mediated increases in feeding (Williams and Kirkham 2002a; Williams and Kirkham 2002b; Williams et al. 1998). Δ^9THC-induced CB_1R-mediated hyperphagia following a prefeed process is classically described by increases in consumption during the first hour of testing due to significant decreases in the latency to feed without concomitant increases in meal size and duration (Williams and Kirkham 2002b; Williams et al. 1998). Δ^9THC-induced hyperphagia has been shown to be CB_1R-mediated in

experiments which co-administered Δ^9THC alongside the CB_1R antagonist SR141716A (Rinaldi-Carmona *et al.*, 1994) even though in the same paradigm SR141716A alone was unable to alter feeding patterns (Williams and Kirkham 2002b). Similar alterations to feeding patterns have also been observed following exogenous AEA administration where AEA reduced the latency to feed but also increased meal size and duration (Hao et al. 2000; Jamshidi and Taylor 2001; Williams and Kirkham 2002a). As such, it has been suggested that CB_1R-mediated alterations to feeding patterns can be divided into *consummatory* (those which control intake quantity) and *appetitive* (those which control feeding pattern) behaviors (Farrimond et al. 2011a).

Recently in our lab, we have described alterations to feeding behaviors induced by a variety of non-Δ^9THC pCBs when administered as standardized cannabis extracts, i.e. botanical drug substances (BDS). Following administration of a Δ^9THC-rich standardized extract (high-Δ^9THC BDS; 67% Δ^9THC, 6.5% other pCBs), we observed a reduction in Δ^9THC-induced hyperphagia when our extract was compared to purified Δ^9THC alone (Farrimond et al. 2010a). Interestingly, in subsequent trials we demonstrated that a Δ^9THC-free extract analogue (non-Δ^9THC pCB content matched to the high-Δ^9THC BDS; Farrimond et al. 2011b) and a second standardized extract which contained little Δ^9THC (low-Δ^9THC BDS; 6.9% Δ^9THC, 14.2% other pCBs; Farrimond et al. 2010b) administered at Δ^9THC doses below those previously observed to alter feeding patterns could both increase feeding in male rats. Importantly, the Δ^9THC-free extract analogue altered feeding behaviors by reducing the latency to feed and increasing the quantity of food consumed during both the first hour of testing and the first meal in the same manner as the high-Δ^9THC BDS and purified Δ^9THC did, but without increases in first meal duration. However, the low-Δ^9THC BDS significantly increased *appetitive* behaviors, only inducing hyperphagia as a result of a highly significant decrease in the latency to feed but without concomitant increases in meal size and duration. These data have led us to suggest that non-Δ^9THC pCBs can not only modulate the feeding effects of Δ^9THC but also induce alterations to feeding behaviors by themselves. However, our previous data shed no light on the specific contributions made by the individual pCBs found in our BDS' to changes in feeding behaviors.

To date, there has only been limited research of the effects of the non-Δ^9THC pCBs on feeding behaviors. In 1976, Sofia and Knobloch reported that CBN (50.0 mg/kg; intraperitoneal injection (i.p.)) reduced food intake in rats, an effect that has yet to be recapitulated (Sofia and Knobloch 1976). However, one might expect that CBN could elicit hyperphagia because, like Δ^9THC, it exhibits CB_1R agonist properties (Felder et al. 1995). In

contrast, cannabidiol (CBD) exerts a superfluity of intracellular effects *in vitro* (e.g. modulation of Ca^{2+} homeostasis; Ryan et al. 2009 and AEA reuptake and FAAH inhibition; De Filippis 2008; Izzo et al. 2009) and has been employed in a small number of feeding studies. Wiley *et al.*, (2005) reported that CBD (3, 10, 30 and 100 mg/kg; i.p.) did not affect food intake in mice, a result confirmed by Scopinho et al. (2011) who demonstrated that CBD (1, 10 or 20 mg/kg; i.p.) did not affect feeding in rats. Similar data, in mice, were also recently described by Riedel (2009, 10.0 mg/kg; i.p.). Conversely however, Sofia and Knobloch (1976) reported a CBD-induced (50 mg/kg; i.p.) reduction in feeding in rats. Very recently, these data have been supported by the observation that CBD (2.5 and 5 mg/kg; i.p.) can reduce body weight gain in relatively young (260 ± 20 g at the start of testing) rats over a period of two weeks, a finding which suggests either reduced food consumption or increased activity over the test period (Ignatowska-Jankowska et al. 2010). As such, data describing the effects of CBD on feeding remains inconclusive and the mechanisms by which it could increase or decrease intake and/or body weight remain to be elucidated.

To our knowledge the possible effects of cannabigerol (CBG) on feeding have yet to be examined although such investigation is warranted since CBG shows partial agonism at CB_1R and/or CB_2R sites (Pertwee 2008), possible antagonism at CB_1R sites (Cascio et al. 2010), phospholipase A2 activation (Evans et al. 1987) and/or AEA reuptake inhibition (Ligresti et al. 2006). Therefore, it is conceivable that CBG administration could induce either hyper- or hypo-phagic effects.

Considering the poor side-effect profile of current Δ^9THC-based anti-anorectic agents (e.g. hallucinations; BNF 2006), and given the drive to produce new anti-obesity agents which do not cause unwanted side effects (*viz.* SR141716A; EMA 2009 or MK-0364; Clark 2009) it is clear that further research examining the possible feeding effects of pCB could prove therapeutically useful. Furthermore, considering the myriad of protocols thus far used to test possible feeding effects of pCBs, direct comparisons of these data are limited. To address this, we have administered CBD, CBG and CBN individually using the same prefeed protocol that we have successfully used to highlight hyperphagic actions of Δ^9THC. Furthermore, in order to assess possible CB_1R-mediation of any observed CBN effects we have also performed a CBN and SR141716A co-administration trial. We present an analysis of hourly intakes and critical meal parameters following drug administration.

General Methods

Animals

Thirty adult, male Lister-hooded rats (P > 40, 200 – 250 g at the start of testing, Harlan UK Ltd, England) were maintained in a temperature controlled environment (21 - 22 ^{o}C) under a 12:12 hour light:dark cycle (red light on at 10:30 hrs). Given the distinct pharmacological profiles of CBD, CBG and CBN (reviewed in Farrimond et al, 2011a), direct comparisons between the drugs on feeding behaviour would yield little pertinent data, thus rats were split into three groups of ten animals; with each group acting as its own control and receiving a different test substance (see table 1). Normal laboratory chow (PCD Mod C, Special Diet Services, Witham, England) was available *ad libitum* but on test days was removed for a three hour period and replaced with a prefeed mash for a two hour period (see '*Prefeed Procedure*') which was followed by one hour of food deprivation immediately after drug administration. Fresh tap water was available *ad libitum*. All procedures were performed in compliance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986.

Test Environment

All tests were performed during the dark light phase under low intensity red light (~4 lx). Testing took place in standard plastic cages, each fitted with a modified food hopper connected, via a strain gauge weighing device, to a computer running data acquisition and analysis software (The Feeding and Drinking Monitor v 2.16, TSE Systems GmbH, Bad Homburg, Germany) which permitted continuous monitoring of food intake. In addition, each cage was fitted with a CCTV camera positioned above each cage to allow an unimpeded view of rat behavior (distance from cage to camera approximately 10 cm). Food intake data were analyzed to provide information on hourly food intakes as well as critical meal parameters such as latency to onset of meals, individual meal size and duration. For the purposes of this study, a meal was defined as any feeding episode causing a change in food weight of $\geq$ 0.1 g, lasting at least 1 minute and separated by at least 15 minutes from any subsequent episode. These criteria have been previously used to facilitate the visualization and interpretation of drug effects on feeding behavior and to distinguish prolonged eating episodes from more transient, exploratory contacts with food (Williams and Kirkham 2002a). Consecutive feeding events separated by intervals of < 15 minutes were considered to be part of a single meal. Due to these criteria, in some

instances, animals have or have not chosen to consume meals during different test hours. Therefore, some ANOVA results have different degrees of freedom and F values than might be expected.

Drugs

Fresh solutions of CBG, CBD and CBN (GW Pharmaceuticals, Salisbury, England) were prepared 15 minutes before administration on each test day. All pCBs were dissolved in a sesame seed oil vehicle (Sainsbury's Supermarkets Ltd, London, England) and the doses specified in Table 1 were administered. The presented pCB doses were based on multiples of 1x (low), 10x (medium) and 100x (high) times the concentrations present in a low-Δ^9THC cannabis extract that we have previously shown to induce increases in appetitive behaviors (Farrimond et al. 2010b). Phytocannabinoids were delivered orally via a syringe placed into the rat's cheek pouch (*per ora*; p.o.).

In a second experiment, because of the likelihood of CB_1R involvement in any observed CBN effects, we co-administered 26.0 mg/kg CBN with SR141716A (1.0 mg/kg) and compared these data to that collected previously following CBN alone administration. SR141716A was administered in a 1:1:18 vehicle made as 1 part ethanol (Fisher Scientific UK Ltd, Loughborough, UK), 1 part cremophor (Sigma-Aldrich, St Louis, USA) and 18 parts 0.9% sodium chloride (Fisher Scientific UK Ltd, Loughborough, UK) saline via subcutaneous injection (s.c.).

Both administration methods (p.o. and s.c.) were calculated to have an injection volume of 1.0 ml/kg. Each group of animals received their drug treatments according to a Latin Square design, counterbalanced for phytocannabinoid dose, with at least 48 hours between successive treatments. All drug groups used vehicle controls as part of the Latin square design. Drug administration began only after animals had been habituated to housing conditions, oral dosing, s.c. injections and all subsequent test procedures.

Throughout these tests, no non-specific behavioral effects of any drug at any dose were evident

Prefeed Procedure

In all experiments, rats were transferred from home cages to individual test cages immediately after dark onset (10:30 hrs) and presented with 30 g of a wet mash diet for 120 minutes as a prefeed. Any remaining wet mash and spillage was recovered after 120 minutes and weighed. Animals were fully habituated to the prefeed procedure before testing began and drug administration did not begin until prefeed intakes were stable as assessed by a non-significant one-way analysis of variance (ANOVA).

Procedure

Following removal of the prefeed at 12:30 hrs, all drugs were administered to the rats according to a Latin square design. Rats were then deprived of food until 13:30 hrs to allow for drug assimilation. At 13:30 hrs, 30 g of normal laboratory chow were placed into the food hoppers. Subsequent hourly food intake (calculated from: starting food mass – (remaining food mass + spillage)) was measured for four consecutive hours.

Statistical Analysis

Hourly food intake was analyzed by two-way ANOVA with four dose levels (vehicle, low, medium and high) and four time points (hours 1, 2, 3 and 4), where appropriate this analysis was followed by individual one-way ANOVA tests for each time point and bonferroni post-hoc tests. The data collected for each meal parameter following test substance administration were separately analyzed using one-way ANOVA with four dose levels (vehicle, low, medium and high), with bonferroni post-hoc tests performed where appropriate. Following SR141716A plus CBN co-administration, the same hourly intake and meal parameter data were analyzed by further one-way ANOVA with three drug levels (vehicle, CBN alone and CBN plus SR141716A), Bonferroni post-hoc tests were carried out when appropriate. All tests were performed using IBM SPSS Statistics 19 (International Business Machines Corp., Armonk, USA).

Results

Cannabinol alone and cannabinol co-administered with SR141716A

Before testing began, prefeed intakes were stabilized for both CBN-only administration ($F(12,122)=1.277$, $p=0.242$) and CBN-SR141716A co-administration ($F(4,49)=1.538$, $p=0.207$). During testing animals consumed 19.40 (± 0.57) and 19.50 (± 0.46) g of prefeed per day respectively. Furthermore, upon rearrangement of prefeed intakes by dose, no significant differences were apparent between any prefeed intakes for any individual dose of either CBN alone, CBN plus SR141716A or their respective vehicle-treatments ($F(5,57)=0.113$, $p=0.989$).

Hourly intake

Two-way analysis of variance failed to show significant effects of either dose ($F(3,60)=0.973$, $p=0.411$) or time ($F(3,20)=0.807$, $p=0.505$), however, there was a significant time by dose interaction ($F(9, 60)= 2.704$, $p=0.010$). Subsequent analysis of effects for each individual hour showed that CBN significantly increased chow consumption during the first hour (Figure 1 (panel A, white bars); $F(3,34)=7.663$, $p=0.001$) from a vehicle-treated intake of 0.86 ± 0.51 g to 2.87 ± 0.45 g at the 26.0 mg/kg dose. Post-hoc analysis revealed that intake following 26.0 mg/kg CBN was significantly greater than after vehicle treatment ($p=0.010$), no other doses induced significant hyperphagic effects. During the second hour of testing, a marginal effect was apparent (Figure 1 (panel A, light grey bars); $F(3,34)=2.391$, $p=0.088$) which is most likely due to the small increases in feeding seen following the 2.60 mg/kg dose compared to vehicle treatment; intakes increased from 0.86 ± 0.51 g following vehicle treatment to 1.44 ± 0.44 g at the 2.6 mg/kg dose. However, post-hoc tests show no significant differences between chow intakes following any CBN treatment when compared to vehicle treatments ($p \geq 0.938$ in all cases) during hour two. Significant increases were observed in all cumulative combinations of hourly intake (Figure 1 (panel B; light grey, grey and black bars), hours one and two; $F(3,34)=3.590$, $p=0.025$, hours one, two and three; $F(3,34)=4.635$, $p=0.009$ and all four hours; $F(3,34)=3.509$, $p=0.027$).

Co-administration of SR141716A with CBN blocked the previously observed CBN-mediated increases in hour one intake (Figure 2 (panel A, white bars); $p=0.696$). Furthermore, SR141716A co-administered with CBN also blocked the marginally significant increase in chow consumption observed during the second hour of testing

(Figure 2 (panel A, light grey bars); $F(2,27)=2.099$, $p=0.114$) and during each consecutive cumulative hourly arrangement of animals' intakes (Figure 2 (panel B; light grey, grey and black bars), hours one and two; $F(2,27)=1.351$, $p=0.227$, hours one, two and three; $F(2,27)=0.974$, $p=0.392$ and all four hours; $F(2,27)=2.112$, $p=0.142$). However, during the third hour of testing intakes recorded for the two vehicle-treated conditions (those animals which received both the sesame oil and 1:1:18 ethanol:cremophor:saline vehicles) varied such that SR141716A plus CBN co-administration vehicle-treated animal intakes were ~2 g higher than their CBN vehicle-treated counterparts (0.1 g). As such, during the third hour of CBN plus SR141716A treatment, a significant reduction in chow consumption compared to control was apparent ($F2,27)=3.940$, $p=0.033$) such that SR141716A plus CBN treated animals displayed significantly reduced intakes versus vehicle-treated animals ($p=0.038$).

Alterations to meal pattern

Following CBN administration, the observed increase in hour one food consumption (Figure 1, panel A, white bars) was due to a significant dose-dependent increase in the size of the first meal (Figure 1 (panel C); $F(3,34)=4.377$, $p=0.011$) and a reduction in the latency to the first meal (Figure 1, (panel D, light grey bars); $F(3,34)=5.217$, $p=0.005$) which shifted feeding into the first hour of the test. However, post-hoc Bonferroni tests revealed no significant differences in meal one size following any individual dose of CBN versus vehicle treatments, whilst the latency to meal one was significantly reduced from 96.7 ± 26.7 to 10.8 ± 4.6 min (at a dose of 26.0 mg/kg CBN; $p=0.038$). In conjunction with the dose-dependent increase in the size of the first meal, its duration was also significantly increased in a dose-dependent manner (Figure 1 (panel D, light grey bars); $F(3,34)=2.963$, $p=0.047$). Consistent with the previously reported abolition of the CBN effect upon hourly intake, SR141716A blocked the CBN induced increases in meal one size (Figure 2 (panel C): $p=0.374$), meal one duration (Figure 2 (panel D): $p=1.000$) and the latency to feed (Figure 2 (panel D): $p=1.000$), supporting a CB_1R-mediated mechanism for CBN. Analysis of second, third and inter-meal interval parameters is not included as less than four rats consumed second or third meals in any given dose group during this test.

Cannabidiol administration

After habituation to test procedures prefeed intakes were stabilized ($F(6,69)=1.282$, $p=0.279$) and CBD administration commenced. During the test period animals consumed 16.57 ± 0.46 g of prefeed per test day.

Hourly intake

Here, 2-way ANOVA failed to show a significant effect of CBD treatment ($F(3,108)=1.380$, $p=0.253$) or any dose by time interaction ($F(9,108)=1.412$, $p=0.192$). However, a significant effect of time was seen ($F(3,36)=7.338$, $p=0.001$) indicating that chow intake did alter over the course of the experiment. However, 1-way ANOVA for each individual hour showed that chow consumption following CBD administration did not vary significantly from those observed for vehicle treatments during any individual hour (Figure 3, panel A: hour 1 (white bars); $F(3,37)=0.394$, $p=0.758$, hour 2 (light grey bars); $F(3,37)=2.088$, $p=0.120$, hour 3 (grey bars); $F(3,37)=0.868$, $p=0.467$ or hour 4 (black bars); $F(3,37)=0.481$, $p=0.698$). Cumulative food intakes in hours one and two (0.77 ± 0.19 g) and one, two and three (2.31 ± 0.26 g) also showed no significant variation from vehicle treatments induced by CBD administration (Figure 3, panel B, light grey and grey bars; $F(3,39)=1.837$, $p=0.158$ and $F(3,39)=1.033$, $p=0.390$ respectively). However importantly, CBD induced significant dose-dependent reductions in total food intake over the total four hour test period (Figure 3 (panel B; black bars); $F(3,39)=3.343$, $p=0.030$). Vehicle treated animals consumed 4.06 ± 0.44 g of chow which was reduced following administration of the highest CBD dose (4.40 mg/kg) to 2.59 ± 0.36 g during four hours.

Alterations to meal pattern

Whilst CBD administration significantly reduced the total amount of food consumed in all meals combined, it had no effect on all other meal parameters. Specifically, no significant effects of CBD administration were observed for the latency to meal one (121.8 ± 10.8 min, Figure 3 D; $F(3,37)=1.635$, $p=0.196$), the intake during (1.88 ± 0.21 g) or duration (7.8 ± 0.9 min) of meal one (Figure 3 C; $F(3,37)=0.570$, $p=0.638$ and Figure 3 D; $F(3,37)=0.523$, $p=0.670$ respectively), the cumulative intakes or durations of meals one and two combined (3.04 ± 0.25 g; $F(3,37)=0.957$, $p=0.424$ and 13.4 ± 1.5 min; $F(3,37)=1.250$, $p=0.307$ respectively) or total duration of

all consumed meals (17.1 ± 2.1 min; $F(3,37)=1.523$, $p=0.226$). Please note that quoted values are averages ± S.E.M. collapsed by dose. Analysis of second, third and inter-meal interval parameters is not included as less than four rats consumed second or third meals in any given dose group during this test.

CBG administration

Prefeed intakes were stabilized before testing began ($F(9,99)=1.395$, $p=0.202$). On each test day animals receiving CBG consumed 18.94 ± 0.44 g.

Hourly intake

Two-way ANOVA failed to show any significant effect of dose ($F(3,72)=0.872$, $p=0.460$), time ($F(3,24)=2.135$, $p=0.122$) or time by dose interaction ($F(3,72)=0.990$, $p=0.456$). CBG administration induced no significant changes from vehicle-treated animal intakes during any hour of the test (Figure 4 panel A: hour 1 (white bars); $F(3,33)=0.739$, $p=0.537$, hour 2 (light grey bars); $F(3,33)=2.105$, $p=0.121$, hour 3 (grey bars); $F(3,33)=1.278$, $p=0.300$ and hour 4 (black bars); $F(3,33)=1.473$, $p=0.242$) or in any cumulative hourly arrangement of chow intakes (Figure 4 panel B: hour 1 (white bars); $F(3,33)=0.739$, $p=0.537$, hours 1 and 2 (light grey bars); $F(3,33)=0.810$, $p=0.498$, hours 1, 2 and 3 (grey bars); $F(3,33)=0.834$, $p=0.486$ and total intake (black bars); $F(3,39)=1.563$, $p=0.215$).

Alterations to meal pattern

In conjunction with hourly intake quantities, CBG administration had no effect on meal patterns. Indeed, meal one intake remained constant at 2.20 ± 0.23 g (Figure 4, panel C: $F(3,29)=0.488$, $p=0.694$), the latency to the first meal at 110.9 ± 14.4 min (Figure 4, panel D: $F(3,29)=0.597$, $p=0.622$) and the duration of the first meal at 12.4 ± 1.9 min (Figure 4, panel D: $F(3,26)=0.123$, $p=0.945$). Analysis of second, third and inter-meal interval parameters is not included as less than six rats consumed second or third meals in any given dose group during this test.

Discussion

In this study, the effects of CBD, CBG and CBN on feeding patterns in adult male rats were investigated. Here, the results obtained demonstrate that CBN can stimulate feeding and alter meal patterns in rats whilst, CBD significantly reduces intake. CBG had no effect upon feeding patterns using the experimental paradigm employed here. It should be noted, however, that non-significant differences in intake under vehicle control conditions exist between our three experiments and as a consequence these differences may limit the extent of interpretation of the drug effects.

CBN induced a dose-dependent increase in chow consumption during the first hour, as illustrated by a significant increase in intake versus vehicle-treated intakes at its highest dose. This significant increase in first hour intake can be attributed to significant decreases in the latency to feed which altered the temporal arrangement of feeding such that the first meal occurred in the first, rather than the second, hour of testing (Figure 1). Furthermore, CBN increased the size and duration of the first meal but, importantly and unlike Δ^9THC (Farrimond et al. 2010a), also increased the total amount of food consumed during the test period. Indeed, in the present case, total chow intake following CBN administration was significantly increased by ~60% compared to vehicle-treatments during the test period, whereas previously, we observed a non-significant change of ~2% following Δ^9THC administration compared to vehicle-treatments over the same four hour period (Farrimond et al. 2010a). CBN's effects upon appetitive aspects of feeding (i.e. decreased latency to feed resulting in increased intake during the first hour of testing) and increases in the total amount of chow consumed mirror the behavioral effects of administration of the eCB, AEA, which have been shown to be CB_1R-mediated (Hao et al. 2000; Jamshidi and Taylor 2001; Koch and Matthews 2001; Williams and Kirkham 1999; 2002a). Indeed, radioligand binding has demonstrated that CBN is a CB_1R agonist (Rhee et al. 1997) which justified our co-administration of the CB_1R antagonist, SR141716A, with CBN. This co-administration duly blocked first hour and first meal intake increases and the reduction in the latency to feed. These results conclusively demonstrate that the changes to feeding patterns seen following CBN administration alone were CB_1R-mediated. Indeed, we have demonstrated that SR141716A blocked CBN-induced changes to all meal parameters and hour one intake and observed no significant effect of CBN alone administration in any subsequent hour. Therefore, even though during the third hour of SR141716A CBN co-administration testing there were

differences in third hour vehicle-treated intakes between the CBN alone and SR141716A CBN co-administration trials, such differences do not hinder the analysis of our data

It is interesting that CBN and Δ^9THC administration induced different changes to feeding patterns, even though both have been shown to affect feeding via a solely CB_1R-mediated mechanism (see Williams and Kirkham 2002b for CB_1R involvement in Δ^9THC-mediated hyperphagia). In this test we have seen that the hourly effects of CBN administration do not exhibit the reduction in second hour chow consumption which is characteristic of Δ^9THC-mediated modulation of feeding patterns. This lack of compensatory effect has led to increased total chow consumption in this study. Indeed, Δ^9THC has previously been found to significantly increase intakes during the first hour of testing, but be followed by a significant reduction in feeding in the second hour; at the highest administered Δ^9THC dose (2.68 mg/kg; p.o.), intakes during hour two were ~17% of vehicle-treated intakes (Farrimond et al. 2010a). The reason for this lack of compensatory mechanism may suggest that CBN remained at higher concentrations in the brain for an increased period of time compared to Δ^9THC (due to the comparatively higher administered doses), or could be due to differences in the psychotropic properties of the two pCBs. Due to CBN's lack of observed psychotropic side effects it is possible to administer CBN at higher doses than Δ^9THC without disruptions to feeding patterns caused by non-specific behaviors (i.e. motor incoordination). These comparatively higher doses of CBN may have led to increased feeding behaviors with a longer duration of action.

It is also intriguing that CBN's significant hyperphagic effects only manifested at a dose of 26 mg/kg versus vehicle-treatments, and not at any lower dose. While CBN is an agonist at CB_1R, its disassociation constant (K_i) is approximately five times greater than that of Δ^9THC (CBN: 211.2 nM; Rhee et al. 1997 versus Δ^9THC: 39.5 nM; Bayewitch et al. 1996). Therefore, CBN's lower affinity at CB_1R could explain the observed difference in effective doses when compared with Δ^9THC where a maximal effect in this paradigm is seen at 2.68 mg/kg; Farrimond et al. 2010a). Furthermore, CBN's lower affinity for CB_1R could also explain why no evidence supports psychotropic effects of CBN which are commonly associated with CB_1R activation. However, we must accept that the gross visual analysis of behaviors we used here does not preclude the possibility of non-specific behavioral side effects. Therefore, while we believe it is be highly unlikely that the administered CBN had any non-specific behavioral side effects for the previously mentioned reasons, we suggest that further experiments

be performed using a battery of behavioral tests (e.g. balance bars) which would fully determine any psychoactive properties of CBN.

Previously, we administered CBN (0.26 mg/kg) with Δ^9THC (0.27 mg/kg) and various other pCBs and observed significant hyperphagia (Farrimond et al. 2010b). When CBN was administered alone at 0.26 mg/kg in this study, and purified Δ^9THC alone at 0.34 mg/kg previously (Farrimond et al. 2010a), we observed no significant alterations to feeding patterns. This comparison clearly suggests that Δ^9THC and CBN interact synergistically in some way to induce changes to feeding patterns at doses which have previously been shown to be ineffective when administered alone. Further studies are required to fully characterize the behavioral adjustments induced by CBN Δ^9THC co-administration and any mechanisms via which CBN and Δ^9THC may interact to alter feeding patterns. Co-administration of Δ^9THC and CBN could have the therapeutic advantage that similar increases in feeding could be induced by doses of Δ^9THC below those currently used which induce unwanted side effects.

The data presented here contradicts, to an extent, that previously published by Sofia and Knobloch (Sofia and Knobloch 1976) where a significant reduction in feeding at a CBN dose twice as high as the highest presented here (50.0 mg/kg; i.p.) was reported. However, not only were Sofia's experiments conducted over a considerably different time scale (daily food intake measurements over a six day period, rather than a four hour test) but the route of administration (i.p.) would have caused a more rapid increase in plasma/brain concentrations of CBN which would have reached a higher maximum concentration than via p.o. administration employed here.

It must also be noted that repeated Δ^9THC administration has previously been linked to sensitization effects in rat models. Both Cadoni *et al.,* (2001) and Runbino *et al.,* (2001) have observed that if rats are pretreated twice a day for three or five days respectively with Δ^9THC then, after a washout period, they react more strongly to further Δ^9THC administration compared to untreated controls, an effect that can be removed by administration of SR141716A. As such sensitization has not yet been demonstrated following CBN, CBD or CBG administration and given the lower affinity of CBN for CB_1R Δ^9THC compared to Δ^9THC, the distinct pharmacologies of both CBD and CBG and since both Cadoni *et al.,* (2001) and Runbino *et al.,* (2001) demonstrated sensitization following i.p. not p.o. administration in non-feeding behavioral tests it is unlikely that non-Δ^9THC pCB-induced behavioral sensitization is affecting the presented results.

Here we also present results which demonstrate that CBD administration can induce significant reductions in chow consumption over a four hour period. Specifically, CBD administration induced only subtle, non-significant reductions in animal intake during any individual hour of the test; however, together this led to a significant reduction in total chow intake over the test period due to significant reductions in intake during all meals. It is worthy of mention that these apparent late-onset of suppressive effects may reflect the relatively slow pharmacokinetic profile of CBD. Indeed, Deiana et al (2011) recently showed that brain levels of CBD continued to rise progressively for 4 hours following a 120 mg/kg oral dose. Despite these effects on hourly intakes, CBD administration had no significant effect on any other critical meal parameter. Such behaviors have been intimately linked to CB_1R activation, and since it is currently thought that CBD is unlikely to interact with CB_1R (Hill *et al.*, 2011), these data may suggest that CBD can affect a feeding pathway which is unrelated to CB_1R. Such data fit well with the reductions in chow consumption previously reported by Sofia and Knobloch in 1976 who also demonstrated a CBD-mediated reduction in chow consumption. Very recently, Ignatowska-Jankowska has shown that CBD (2.5 and 5.0 mg/kg; i.p.) can reduce body weight gain in young rats, suggesting either reduced food intake or increased activity, and therefore indirectly supporting the reductions observed by Sofia and Knobloch and the results presented here (Ignatowska-Jankowska et al. 2010). However, data which describe the effects of CBD administration on feeding patterns are not yet conclusive. Recently, Wiley and colleagues (Wiley et al. 2005) and Scopinho and colleagues (Scopinho et al. 2011) observed no effect of CBD on feeding patterns in food restricted mice and normally fed and fasted rats respectively. Wiley used 3.0 – 100.0 mg/kg CBD, whilst Scopinho used 1.0 – 20.0 mg/kg CBD; both administered i.p.. Therefore, it seems likely that the differences between the experimental protocols used by Wiley and Scopinho and those presented here could feasibly explain the differences in reported effects. Indeed, due to the route of administration (p.o.) used in our study, it is likely that peak cerebrospinal fluid concentrations were considerably lower than those achieved with the i.p. route used by Wiley and Scopinho. Furthermore, since neither Wiley nor Scopinho used prefed animals it is likely that differences in endocannabinergic tone between the models will have altered feeding behaviors, and consequently, the animals' responses to CBD administration.

It seems apparent from the available literature that the functional effect of CBD to induce significant decreases in chow intake could arise from the numerous intra- and extra-cellular mechanisms with which it is known to interact, but are likely to be unrelated to traditional CB_1R-mediated feeding control. Such a theory is supported by CBD administration's failure to affect any meal parameter or individual hourly intake in this test.

Unfortunately, the relatively wide spectrum of cellular and molecular mechanisms that have been proposed but not definitively established *in vivo* make such suggestions highly speculative and further investigations that probe the discrete mechanisms potentially involved are required to confirm the mechanisms underlying the observed functional effects. Clearly, it would be most interesting to establish a non-CB_1R-mediated feeding pathway that is modulated by a pCB, such as CBD, although the lack of pharmacological tools with which to block CBD's putative AEA reuptake, FAAH inhibition and antagonism of Δ^9THC at CB_1R separately renders such an experiment challenging to undertake.

We believe this to be the first time that possible CBG effects on feeding have been examined, although no significant CBG-mediated effects were observed. It is unlikely that CBG administration can exert any effects via direct CB_1R binding since it has a very low affinity for CB_1R (disassociation constant: 440 nM; Gauson et al. 2007, c.f. Δ^9THC: 39.5 nM; Bayewitch et al. 1996). However, CBG is a known AEA reuptake inhibitor (Ligresti et al. 2006) such that CBG could induce increased brain concentrations of AEA which could conceivably produce similar effects to that seen following administration of exogenous AEA. However, in the presented experimental paradigm, we had reduced eCB tone using a prefeed. Therefore, even if CBG was inducing functionally effective AEA reuptake blockade, little endogenous AEA would be present in the CNS and so reuptake blockade would be unable to potentiate CBG-mediated behavioral effects. *Id est*, were CBG to be tested using a food restricted paradigm which elevated eCB production, then its putative effects on AEA reuptake inhibition may begin to induce significant effects on feeding patterns. As such, while the data we now present suggests that CBG administration has no effect on feeding patterns, different results may be found using a different experimental paradigm.

In summary, following the administration of CBN alone we observed significant increases in appetitive, consummatory and total intake behaviors. Thus we suggest that a balance exists between endocannabinergic tone and pCB-mediated CB_1R activation. This balance manifests as increasing feeding behaviors (appetitive, consummatory and total chow intake increase) with increasing CB_1R activation, and decreasing feeding behaviors with decreasing CB_1R activation. The data we have presented suggests that as CB_1R activation is reduced, feeding behaviors decay and the weakest behaviors are lost first (increased total chow consumption < increased meal one duration < increased meal one chow intake < increased hourly intake & reduced latency to the first meal). Such a theory is supported by currently available literature since only recently have significant

effects on Δ^9THC-mediated meal pattern changes in rats been observed, but AEA-induced increases in total chow intake, appetitive and consummatory behaviors have been demonstrated (see Farrimond et al. 2011a for review). Furthermore, we have also demonstrated significant, short-term CBD-mediated reductions in feeding which, we suggest, are due to reduced consummatory behaviors following CBD administration. However, given CBDs pharmacological profile, such effects are unlikely to be CB_1R-mediated. Finally, we have observed that the administration of CBG induces no significant alterations to feeding patterns in the presented paradigm. A direct comparison between these three drug treatments is necessarily limited by the large variability in response seen under vehicle conditions, and as such, the robustness of the effects we describe here should be confirmed by a fully randomized replication of our study.

Conclusion

Using a prefeed paradigm, CBN induced significant CB_1R-mediated hyperphagia in male rats via significant reductions in the latency to feed and significant increases in the food consumed during the first hour and meal, alongside significant increases in the total amount of food consumed when compared to vehicle-treatments. Conversely, CBD administration reduced total feeding over a four hour period. Neither Δ^9THCV nor CBG administration exerted effects on feeding behaviors in this paradigm.

As CBN has not so far been shown to have psychoactive properties it could be a useful anti-anorexic agent, since in this study CBN administration significantly increased intake over the total test period. Clearly, further experiments are required to fully characterize the effects of both chronic and acute CBN administration on food consumption and body weight. Moreover, the data we have presented here when compared to some of our previous data (Farrimond et al. 2010b) suggests that CBN and Δ^9THC, when co-administered, may synergistically induce powerful hyperphagic effects. Therefore, co-administration of CBN and Δ^9THC may also exhibit anti-anorexic properties.

Given CBD's well documented non-psychotropic nature and its high tolerability in humans, further characterization of its effects on feeding reduction and the mechanisms via which CBD induces such effects are also clearly warranted. Such tests may provide an interesting insight into the subtle feeding effects of CBD we have observed here and it would be particularly interesting to identify a non-eCB system-mediated mechanism of action of CBD in relation to feeding behaviors.

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Ethical compliance

All procedures were performed in compliance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986 and all other applicable laws and standards in the U.K.

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#	Phytocannabinoid	Doses (mg/kg)
1	Cannabidiol	0.00, 0.04, 0.44 & 4.40
2	Cannabigerol	0.00, 0.176, 1.76 & 17.60
3	Cannabinol	0.00, 0.26, 2.60 & 26.00 & 26.00 + 1.00 SR141716A

Table 1: Phytocannabinoid doses employed in this study. All phytocannabinoids were administered p.o. while SR141716A was administered s.c.. All drugs were administered at an injection volume of 1.0 ml/kg, one hour before testing began.

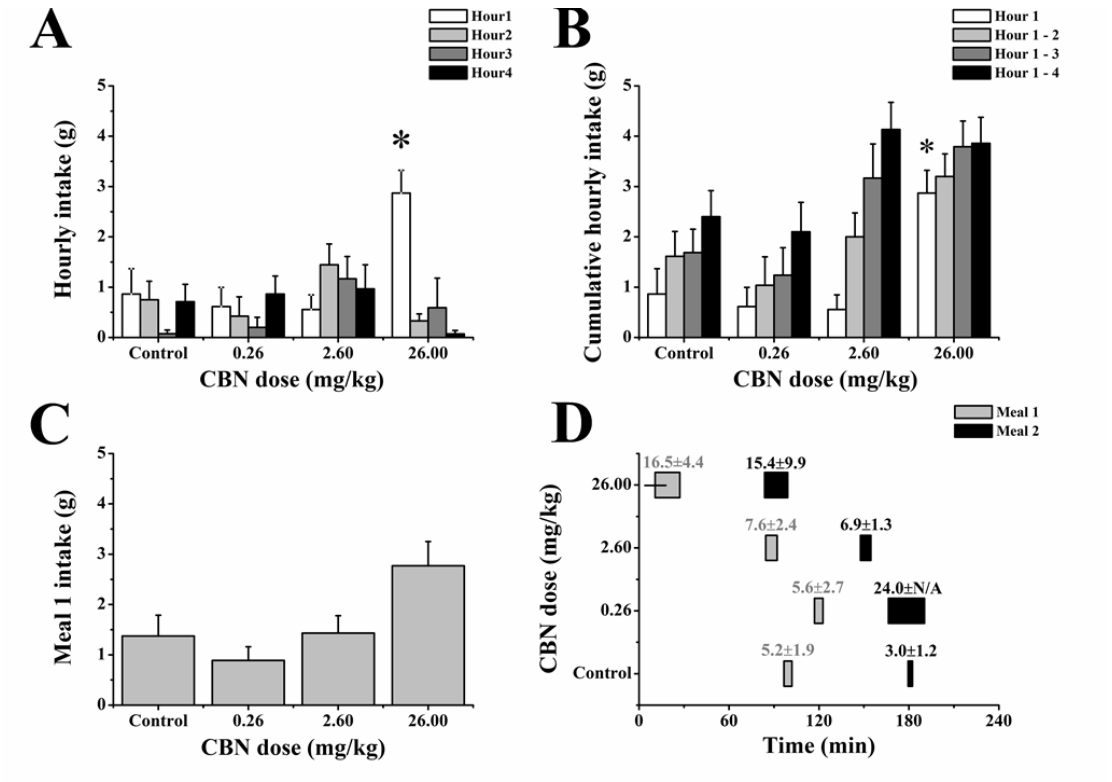

Figure 1: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and meal pattern (D) following administration of CBN (0, 0.26, 2.60 and 26.00 mg/kg; p.o.).

CBN administration significantly increased hour one intake (A: white bars) and chow consumption over all cumulative hourly arrangements (B). Furthermore, following CBN administration significant increases in chow consumption during the first meal (C) and highly significant decreases in the latency to feed (D) were observed. No statistical analyses have been performed on second meal data as animals consumed too few second meals. Meal 2 bars are included for reference only. In panel D meal duration is represented by the length of bar and is provided numerically above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ± SEM. * denotes $p \leq 0.05$ in Bonferroni post-hoc test following a significant one-way ANOVA result versus vehicle-treatment. – denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on meal 1 latency.

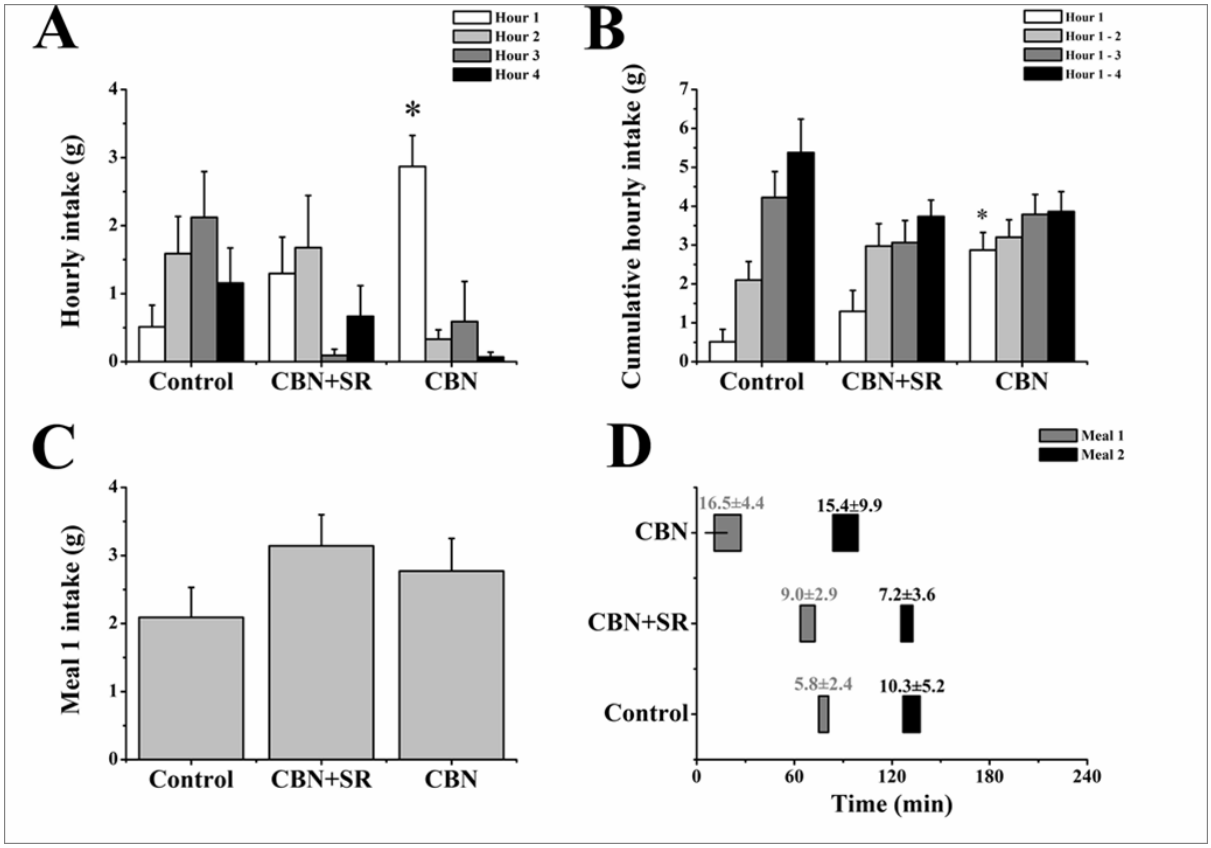

Figure 2: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and meal pattern (D) following administration of CBN and SR14171A (0 and 26.00 CBN mg/kg; p.o. and 1.0 mg/kg SR141716A; s.c.).

The response recorded previously (see figure 1) following the highest dose of CBN (26.00 mg/kg; p.o.) is compared to those following CBN (26.00 mg/kg; p.o.) and SR141716A (1.0 mg/kg; s.c.) co-administration. Co-administration of SR141716A with CBN blocked CBN-mediated increases in hour one intake (A: white bars), meal one size (C) and duration (D) and the latency to feed (D). No statistical analyses have been performed on second meal data as animals consumed too few second meals. Meal 2 bars are included for reference only. In panel D meal duration is represented by the length of bar and is provided numerically above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ± SEM. * denotes $p \leq 0.05$ in Bonferroni post-hoc test following a significant one-way ANOVA result versus vehicle-treatment. – denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on meal 1 latency.

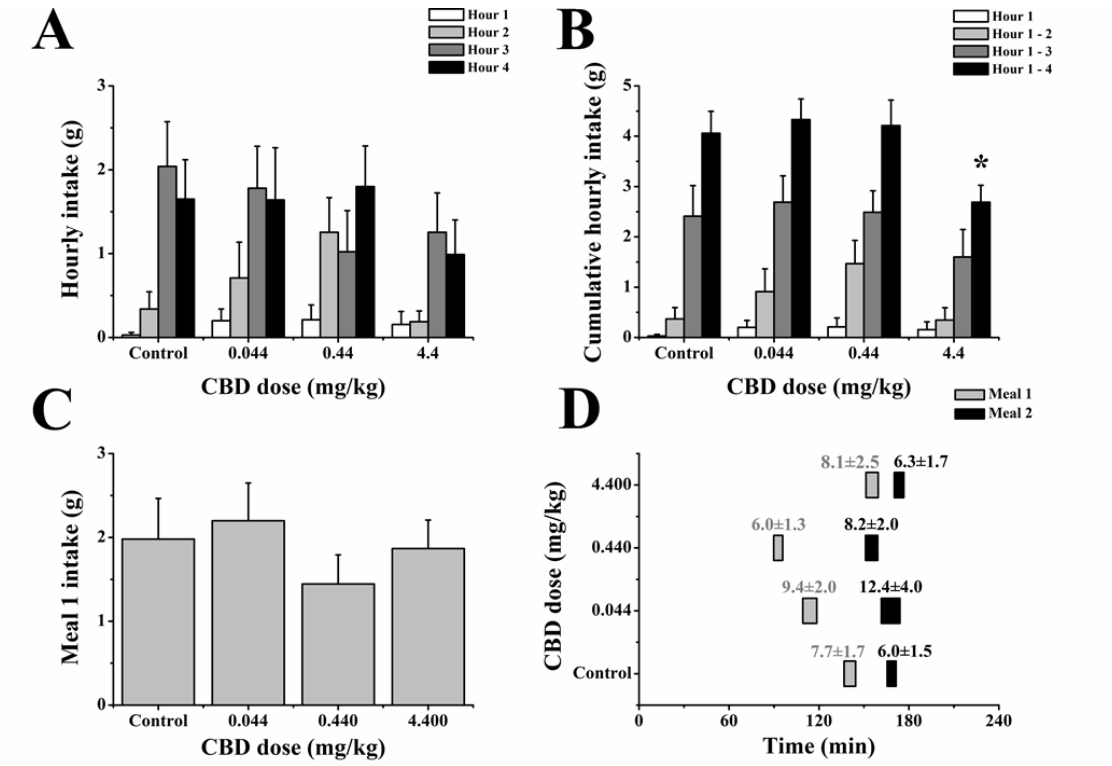

Figure 3: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and meal pattern (D) following administration of CBD (0.00, 0.044, 0.44 and 4.40 mg/kg; p.o.).

CBD administration significantly reduced chow intake over the period of the test (B). No statistical analyses have been performed on second meal data as animals consumed two few second meals. Meal 2 bars are included for reference only. In panel D meal duration is represented by the length of bar and is provided numerically above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ± SEM. * denotes $p \leq 0.05$ in Bonferroni post-hoc test following a significant one-way ANOVA result versus vehicle-treatment. – denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on meal 1 latency.

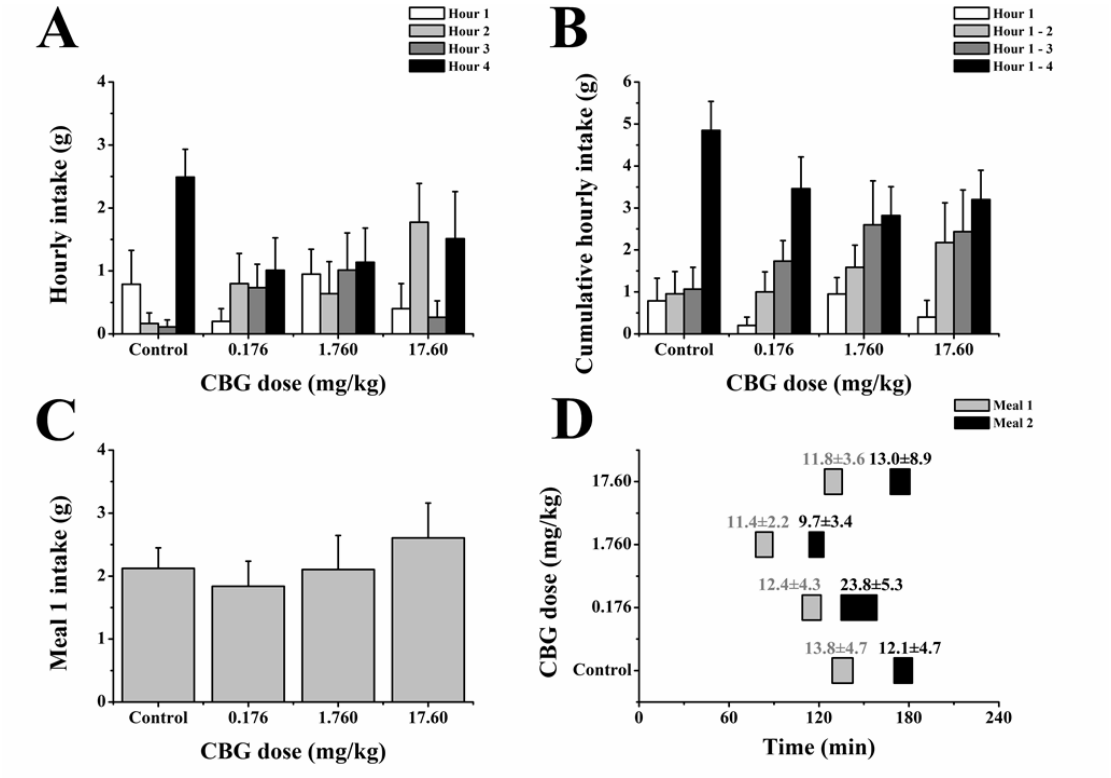

Figure 4: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and meal pattern (D) following administration of CBG (0.00, 0.176, 1.76 and 17.60 mg/kg; p.o.).

Administration of CBG induced no significant deviations from vehicle-treatments for any measure. No statistical analyses have been performed on second meal data as animals consumed too few second meals. Meal 2 bars are included for reference only. In panel D meal duration is represented by the length of bar and is provided numerically above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ± SEM. * denotes $p \leq 0.05$ in Bonferroni post-hoc test following a significant one-way ANOVA result versus vehicle-treatment. – denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on meal 1 latency.