

Cannabinol and cannabidiol exert opposing effects on rat feeding patterns

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Accepted Version

Farrimond, J. A., Whalley, B. J. and Williams, C. M. ORCID: <https://orcid.org/0000-0003-4452-671X> (2012) Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. *Psychopharmacology*, 223 (1). pp. 117-129. ISSN 0033-3158 doi: <https://doi.org/10.1007/s00213-012-2697-x> Available at <https://centaur.reading.ac.uk/28010/>

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To link to this article DOI: <http://dx.doi.org/10.1007/s00213-012-2697-x>

Publisher: Springer Verlag

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1 **Cannabinol and cannabidiol exert opposing effects on rat feeding patterns.**

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24 This research was supported in part by the University of Reading Research Endowment Trust Fund (to JAF).

25

1 **Abstract**

2 *Rationale*

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

7 *Methods*

8 Adult male rats were treated (p.o.) with cannabigerol, cannabidiol, cannabinol or cannabinol plus the CB₁R
9 antagonist, SR141716A. Prior to treatment, rats were satiated and food intake recorded following drug
10 administration. Data were analyzed for hourly intake and meal microstructure.

11 *Results*

12 Cannabinol induced a CB₁R-mediated increase in *appetitive* behaviors via significant reductions in the latency
13 to feed, and increases in *consummatory* behaviors via increases in meal one size and duration. Cannabinol also
14 significantly increased the intake during hour 1 and total chow consumed during the test. Conversely,
15 cannabidiol significantly reduced total chow consumption over the test period. Cannabigerol administration
16 induced no changes to feeding behavior.

17 *Conclusion*

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

23 *Keywords: cannabis, cannabigerol, cannabidiol, cannabinol, phytocannabinoids, feeding, appetite, behavio(ur)*

1 *Abbreviations*

2-AG	2-arachidonoylglycerol
Δ^9 THC	Δ^9 -tetrahydrocannabinol
Δ^9 THCV	Δ^9 -tetrahydrocannabivarin
AEA	Anandamide
ANOVA	Analysis of variance
BDS	Botanical drug substance
<i>C. sativa</i>	<i>Cannabis sativa</i>
CB ₁ R	Cannabinoid type 1 receptor
CB ₂ R	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

2

1 **Introduction**

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

7 Since the original identification of the cannabinoid type 1 and 2 receptors (CB₁ and CB₂R; Devane et al. 1988;
8 Matsuda et al. 1990; Munro et al. 1993) and confirmation of an endogenous cannabinoid system following the
9 discovery of the endogenous cannabinoids (eCBs; anandamide (AEA; Devane et al. 1992) and 2-arachidonyl
10 glycerol (2-AG; Mechoulam et al. 1995; Sugiura et al. 1995)) research has largely focused on the effects of
11 Δ^9 tetrahydrocannabinol (Δ^9 THC; Gaoni and Mechoulam 1964). Indeed, only limited research has considered the
12 effects of the numerous other phytocannabinoids (pCBs) also present (Izzo et al. 2009). More recently, research
13 has begun to examine the effects of these individual pCBs (for a review of cannabinoid pharmacology see Izzo
14 et al. 2009). Currently, a range of possible cannabinoid-based therapies are being considered for a number of
15 disorders (e.g. neurological and neurodegenerative, multiple sclerosis and anti-obesity (Glass 2001; Pryce et al.
16 2003; Van Gaal et al. 2005), for review see Amar 2006). Interestingly, this new research has made it apparent
17 that these pCBs are likely to act at sites other than CB₁ and CB₂R due to their low binding affinities at these
18 receptor sub-types (with the exceptions of Δ^9 -tetrahydrocannabivarin (Δ^9 THCV) and cannabitol (CBN);
19 Petrosino et al. 2009). Importantly, the currently available literature gives no indication of non- Δ^9 THC pCB
20 psychoactivity (for reviews see Amar, 2006; Izzo et al., 2009).

21 Specifically in terms of feeding, and unlike the other pCBs, Δ^9 THC has been relatively well studied. Indeed,
22 some time ago it became apparent that CB₁R sites in the central nervous system (CNS; Herkenham et al. 1991)
23 were responsible for Δ^9 THC-mediated increases in feeding (Williams and Kirkham 2002a; Williams and
24 Kirkham 2002b; Williams et al. 1998). Δ^9 THC-induced CB₁R-mediated hyperphagia following a prefeed
25 process is classically described by increases in consumption during the first hour of testing due to significant
26 decreases in the latency to feed without concomitant increases in meal size and duration (Williams and Kirkham
27 2002b; Williams et al. 1998). Δ^9 THC-induced hyperphagia has been shown to be CB₁R-mediated in

1 experiments which co-administered Δ^9 THC alongside the CB₁R antagonist SR141716A (Rinaldi-Carmona *et*
2 *al.*, 1994) even though in the same paradigm SR141716A alone was unable to alter feeding patterns (Williams
3 and Kirkham 2002b). Similar alterations to feeding patterns have also been observed following exogenous AEA
4 administration where AEA reduced the latency to feed but also increased meal size and duration (Hao *et al.*
5 2000; Jamshidi and Taylor 2001; Williams and Kirkham 2002a). As such, it has been suggested that CB₁R-
6 mediated alterations to feeding patterns can be divided into *consummatory* (those which control intake quantity)
7 and *appetitive* (those which control feeding pattern) behaviors (Farrimond *et al.* 2011a).

8 Recently in our lab, we have described alterations to feeding behaviors induced by a variety of non- Δ^9 THC
9 pCBs when administered as standardized cannabis extracts, i.e. botanical drug substances (BDS). Following
10 administration of a Δ^9 THC-rich standardized extract (high- Δ^9 THC BDS; 67% Δ^9 THC, 6.5% other pCBs), we
11 observed a reduction in Δ^9 THC-induced hyperphagia when our extract was compared to purified Δ^9 THC alone
12 (Farrimond *et al.* 2010a). Interestingly, in subsequent trials we demonstrated that a Δ^9 THC-free extract analogue
13 (non- Δ^9 THC pCB content matched to the high- Δ^9 THC BDS; Farrimond *et al.* 2011b) and a second standardized
14 extract which contained little Δ^9 THC (low- Δ^9 THC BDS; 6.9% Δ^9 THC, 14.2% other pCBs; Farrimond *et al.*
15 2010b) administered at Δ^9 THC doses below those previously observed to alter feeding patterns could both
16 increase feeding in male rats. Importantly, the Δ^9 THC-free extract analogue altered feeding behaviors by
17 reducing the latency to feed and increasing the quantity of food consumed during both the first hour of testing
18 and the first meal in the same manner as the high- Δ^9 THC BDS and purified Δ^9 THC did, but without increases in
19 first meal duration. However, the low- Δ^9 THC BDS significantly increased *appetitive* behaviors, only inducing
20 hyperphagia as a result of a highly significant decrease in the latency to feed but without concomitant increases
21 in meal size and duration. These data have led us to suggest that non- Δ^9 THC pCBs can not only modulate the
22 feeding effects of Δ^9 THC but also induce alterations to feeding behaviors by themselves. However, our previous
23 data shed no light on the specific contributions made by the individual pCBs found in our BDS' to changes in
24 feeding behaviors.

25 To date, there has only been limited research of the effects of the non- Δ^9 THC pCBs on feeding behaviors. In
26 1976, Sofia and Knobloch reported that CBN (50.0 mg/kg; intraperitoneal injection (i.p.)) reduced food intake in
27 rats, an effect that has yet to be recapitulated (Sofia and Knobloch 1976). However, one might expect that CBN
28 could elicit hyperphagia because, like Δ^9 THC, it exhibits CB₁R agonist properties (Felder *et al.* 1995). In

1 contrast, cannabidiol (CBD) exerts a superfluity of intracellular effects *in vitro* (e.g. modulation of Ca²⁺
2 homeostasis; Ryan et al. 2009 and AEA reuptake and FAAH inhibition; De Filippis 2008; Izzo et al. 2009) and
3 has been employed in a small number of feeding studies. Wiley *et al.*, (2005) reported that CBD (3, 10, 30 and
4 100 mg/kg; i.p.) did not affect food intake in mice, a result confirmed by Scopinho et al. (2011) who
5 demonstrated that CBD (1, 10 or 20 mg/kg; i.p.) did not affect feeding in rats. Similar data, in mice, were also
6 recently described by Riedel (2009, 10.0 mg/kg; i.p.). Conversely however, Sofia and Knobloch (1976) reported
7 a CBD-induced (50 mg/kg; i.p.) reduction in feeding in rats. Very recently, these data have been supported by
8 the observation that CBD (2.5 and 5 mg/kg; i.p.) can reduce body weight gain in relatively young (260 ± 20 g at
9 the start of testing) rats over a period of two weeks, a finding which suggests either reduced food consumption
10 or increased activity over the test period (Ignatowska-Jankowska et al. 2010). As such, data describing the
11 effects of CBD on feeding remains inconclusive and the mechanisms by which it could increase or decrease
12 intake and/or body weight remain to be elucidated.

13 To our knowledge the possible effects of cannabigerol (CBG) on feeding have yet to be examined although such
14 investigation is warranted since CBG shows partial agonism at CB₁R and/or CB₂R sites (Pertwee 2008),
15 possible antagonism at CB₁R sites (Cascio et al. 2010), phospholipase A2 activation (Evans et al. 1987) and/or
16 AEA reuptake inhibition (Ligresti et al. 2006). Therefore, it is conceivable that CBG administration could
17 induce either hyper- or hypo-phagic effects.

18 Considering the poor side-effect profile of current Δ⁹THC-based anti-anorectic agents (e.g. hallucinations; BNF
19 2006), and given the drive to produce new anti-obesity agents which do not cause unwanted side effects (*viz.*
20 SR141716A; EMA 2009 or MK-0364; Clark 2009) it is clear that further research examining the possible
21 feeding effects of pCB could prove therapeutically useful. Furthermore, considering the myriad of protocols
22 thus far used to test possible feeding effects of pCBs, direct comparisons of these data are limited. To address
23 this, we have administered CBD, CBG and CBN individually using the same prefeed protocol that we have
24 successfully used to highlight hyperphagic actions of Δ⁹THC. Furthermore, in order to assess possible CB₁R-
25 mediation of any observed CBN effects we have also performed a CBN and SR141716A co-administration trial.
26 We present an analysis of hourly intakes and critical meal parameters following drug administration.

27

28

1 **General Methods**

2 *Animals*

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

13

14 *Test Environment*

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

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

3 *Drugs*

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

11 In a second experiment, because of the likelihood of CB₁R involvement in any observed CBN effects, we co-
12 administered 26.0 mg/kg CBN with SR141716A (1.0 mg/kg) and compared these data to that collected
13 previously following CBN alone administration. SR141716A was administered in a 1:1:18 vehicle made as 1
14 part ethanol (Fisher Scientific UK Ltd, Loughborough, UK), 1 part cremophor (Sigma-Aldrich, St Louis, USA)
15 and 18 parts 0.9% sodium chloride (Fisher Scientific UK Ltd, Loughborough, UK) saline via subcutaneous
16 injection (s.c.).

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

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

23

24 *Prefeed Procedure*

1 In all experiments, rats were transferred from home cages to individual test cages immediately after dark onset
2 (10:30 hrs) and presented with 30 g of a wet mash diet for 120 minutes as a prefeed. Any remaining wet mash
3 and spillage was recovered after 120 minutes and weighed. Animals were fully habituated to the prefeed
4 procedure before testing began and drug administration did not begin until prefeed intakes were stable as
5 assessed by a non-significant one-way analysis of variance (ANOVA).

6

7 *Procedure*

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

12

13 *Statistical Analysis*

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

23

24

1 **Results**

2 *Cannabinol alone and cannabinol co-administered with SR141716A*

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

8

9 *Hourly intake*

10 Two-way analysis of variance failed to show significant effects of either dose ($F(3,60)=0.973$, $p=0.411$) or time
11 ($F(3,20)=0.807$, $p=0.505$), however, there was a significant time by dose interaction ($F(9, 60)= 2.704$, $p=0.010$).
12 Subsequent analysis of effects for each individual hour showed that CBN significantly increased chow
13 consumption during the first hour (Figure 1 (panel A, white bars); $F(3,34)=7.663$, $p=0.001$) from a vehicle-
14 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
15 following 26.0 mg/kg CBN was significantly greater than after vehicle treatment ($p=0.010$), no other doses
16 induced significant hyperphagic effects. During the second hour of testing, a marginal effect was apparent
17 (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
18 feeding seen following the 2.60 mg/kg dose compared to vehicle treatment; intakes increased from 0.86 ± 0.51 g
19 following vehicle treatment to 1.44 ± 0.44 g at the 2.6 mg/kg dose. However, post-hoc tests show no significant
20 differences between chow intakes following any CBN treatment when compared to vehicle treatments ($p \geq$
21 0.938 in all cases) during hour two. Significant increases were observed in all cumulative combinations of
22 hourly intake (Figure 1 (panel B; light grey, grey and black bars), hours one and two; $F(3,34)=3.590$, $p=0.025$,
23 hours one, two and three; $F(3,34)=4.635$, $p=0.009$ and all four hours; $F(3,34)=3.509$, $p=0.027$).

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

1 (Figure 2 (panel A, light grey bars); $F(2,27)=2.099$, $p=0.114$) and during each consecutive cumulative hourly
2 arrangement of animals' intakes (Figure 2 (panel B; light grey, grey and black bars), hours one and two;
3 $F(2,27)=1.351$, $p=0.227$, hours one, two and three; $F(2,27)=0.974$, $p=0.392$ and all four hours; $F(2,27)=2.112$,
4 $p=0.142$). However, during the third hour of testing intakes recorded for the two vehicle-treated conditions
5 (those animals which received both the sesame oil and 1:1:18 ethanol:cremophor:saline vehicles) varied such
6 that SR141716A plus CBN co-administration vehicle-treated animal intakes were ~2 g higher than their CBN
7 vehicle-treated counterparts (0.1 g). As such, during the third hour of CBN plus SR141716A treatment, a
8 significant reduction in chow consumption compared to control was apparent ($F(2,27)=3.940$, $p=0.033$) such that
9 SR141716A plus CBN treated animals displayed significantly reduced intakes versus vehicle-treated animals
10 ($p=0.038$).

11

12 *Alterations to meal pattern*

13 Following CBN administration, the observed increase in hour one food consumption (Figure 1, panel A, white
14 bars) was due to a significant dose-dependent increase in the size of the first meal (Figure 1 (panel C);
15 $F(3,34)=4.377$, $p=0.011$) and a reduction in the latency to the first meal (Figure 1, (panel D, light grey bars);
16 $F(3,34)=5.217$, $p=0.005$) which shifted feeding into the first hour of the test. However, post-hoc Bonferroni tests
17 revealed no significant differences in meal one size following any individual dose of CBN versus vehicle
18 treatments, whilst the latency to meal one was significantly reduced from 96.7 ± 26.7 to 10.8 ± 4.6 min (at a
19 dose of 26.0 mg/kg CBN; $p=0.038$). In conjunction with the dose-dependent increase in the size of the first
20 meal, its duration was also significantly increased in a dose-dependent manner (Figure 1 (panel D, light grey
21 bars); $F(3,34)=2.963$, $p=0.047$). Consistent with the previously reported abolition of the CBN effect upon hourly
22 intake, SR141716A blocked the CBN induced increases in meal one size (Figure 2 (panel C): $p=0.374$), meal
23 one duration (Figure 2 (panel D): $p=1.000$) and the latency to feed (Figure 2 (panel D): $p=1.000$), supporting a
24 CB_1R -mediated mechanism for CBN. Analysis of second, third and inter-meal interval parameters is not
25 included as less than four rats consumed second or third meals in any given dose group during this test.

26

27

1 *Cannabidiol administration*

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

4

5 *Hourly intake*

6 Here, 2-way ANOVA failed to show a significant effect of CBD treatment ($F(3,108)=1.380$, $p=0.253$) or any
7 dose by time interaction ($F(9,108)=1.412$, $p=0.192$). However, a significant effect of time was seen
8 ($F(3,36)=7.338$, $p=0.001$) indicating that chow intake did alter over the course of the experiment. However, 1-
9 way ANOVA for each individual hour showed that chow consumption following CBD administration did not
10 vary significantly from those observed for vehicle treatments during any individual hour (Figure 3, panel A:
11 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
12 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
13 hours one and two (0.77 ± 0.19 g) and one, two and three (2.31 ± 0.26 g) also showed no significant variation
14 from vehicle treatments induced by CBD administration (Figure 3, panel B, light grey and grey bars;
15 $F(3,39)=1.837$, $p=0.158$ and $F(3,39)=1.033$, $p=0.390$ respectively). However importantly, CBD induced
16 significant dose-dependent reductions in total food intake over the total four hour test period (Figure 3 (panel B;
17 black bars); $F(3,39)=3.343$, $p=0.030$). Vehicle treated animals consumed 4.06 ± 0.44 g of chow which was
18 reduced following administration of the highest CBD dose (4.40 mg/kg) to 2.59 ± 0.36 g during four hours.

19

20 *Alterations to meal pattern*

21 Whilst CBD administration significantly reduced the total amount of food consumed in all meals combined, it
22 had no effect on all other meal parameters. Specifically, no significant effects of CBD administration were
23 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
24 (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;
25 $F(3,37)=0.523$, $p=0.670$ respectively), the cumulative intakes or durations of meals one and two combined (3.04
26 ± 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

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

4

1 *CBG administration*

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

4

5 *Hourly intake*

6 Two-way ANOVA failed to show any significant effect of dose ($F(3,72)=0.872$, $p=0.460$), time ($F(3,24)=2.135$,
7 $p=0.122$) or time by dose interaction ($F(3,72)=0.990$, $p=0.456$). CBG administration induced no significant
8 changes from vehicle-treated animal intakes during any hour of the test (Figure 4 panel A: hour 1 (white bars);
9 $F(3,33)=0.739$, $p=0.537$, hour 2 (light grey bars); $F(3,33)=2.105$, $p=0.121$, hour 3 (grey bars); $F(3,33)=1.278$,
10 $p=0.300$ and hour 4 (black bars); $F(3,33)=1.473$, $p=0.242$) or in any cumulative hourly arrangement of chow
11 intakes (Figure 4 panel B: hour 1 (white bars); $F(3,33)=0.739$, $p=0.537$, hours 1 and 2 (light grey bars);
12 $F(3,33)=0.810$, $p=0.498$, hours 1, 2 and 3 (grey bars); $F(3,33)=0.834$, $p=0.486$ and total intake (black bars);
13 $F(3,39)=1.563$, $p=0.215$).

14

15 *Alterations to meal pattern*

16 In conjunction with hourly intake quantities, CBG administration had no effect on meal patterns. Indeed, meal
17 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
18 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
19 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
20 parameters is not included as less than six rats consumed second or third meals in any given dose group during
21 this test.

22

1 **Discussion**

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

8 CBN induced a dose-dependent increase in chow consumption during the first hour, as illustrated by a
9 significant increase in intake versus vehicle-treated intakes at its highest dose. This significant increase in first
10 hour intake can be attributed to significant decreases in the latency to feed which altered the temporal
11 arrangement of feeding such that the first meal occurred in the first, rather than the second, hour of testing
12 (Figure 1). Furthermore, CBN increased the size and duration of the first meal but, importantly and unlike
13 Δ^9 THC (Farrimond et al. 2010a), also increased the total amount of food consumed during the test period.
14 Indeed, in the present case, total chow intake following CBN administration was significantly increased by
15 ~60% compared to vehicle-treatments during the test period, whereas previously, we observed a non-significant
16 change of ~2% following Δ^9 THC administration compared to vehicle-treatments over the same four hour period
17 (Farrimond et al. 2010a). CBN's effects upon appetitive aspects of feeding (i.e. decreased latency to feed
18 resulting in increased intake during the first hour of testing) and increases in the total amount of chow consumed
19 mirror the behavioral effects of administration of the eCB, AEA, which have been shown to be CB₁R-mediated
20 (Hao et al. 2000; Jamshidi and Taylor 2001; Koch and Matthews 2001; Williams and Kirkham 1999; 2002a).
21 Indeed, radioligand binding has demonstrated that CBN is a CB₁R agonist (Rhee et al. 1997) which justified our
22 co-administration of the CB₁R antagonist, SR141716A, with CBN. This co-administration duly blocked first
23 hour and first meal intake increases and the reduction in the latency to feed. These results conclusively
24 demonstrate that the changes to feeding patterns seen following CBN administration alone were CB₁R-
25 mediated. Indeed, we have demonstrated that SR141716A blocked CBN-induced changes to all meal parameters
26 and hour one intake and observed no significant effect of CBN alone administration in any subsequent hour.
27 Therefore, even though during the third hour of SR141716A CBN co-administration testing there were

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

3 It is interesting that CBN and Δ^9 THC administration induced different changes to feeding patterns, even though
4 both have been shown to affect feeding via a solely CB₁R-mediated mechanism (see Williams and Kirkham
5 2002b for CB₁R involvement in Δ^9 THC-mediated hyperphagia). In this test we have seen that the hourly effects
6 of CBN administration do not exhibit the reduction in second hour chow consumption which is characteristic of
7 Δ^9 THC-mediated modulation of feeding patterns. This lack of compensatory effect has led to increased total
8 chow consumption in this study. Indeed, Δ^9 THC has previously been found to significantly increase intakes
9 during the first hour of testing, but be followed by a significant reduction in feeding in the second hour; at the
10 highest administered Δ^9 THC dose (2.68 mg/kg; p.o.), intakes during hour two were ~17% of vehicle-treated
11 intakes (Farrimond et al. 2010a). The reason for this lack of compensatory mechanism may suggest that CBN
12 remained at higher concentrations in the brain for an increased period of time compared to Δ^9 THC (due to the
13 comparatively higher administered doses), or could be due to differences in the psychotropic properties of the
14 two pCBs. Due to CBN's lack of observed psychotropic side effects it is possible to administer CBN at higher
15 doses than Δ^9 THC without disruptions to feeding patterns caused by non-specific behaviors (i.e. motor
16 incoordination). These comparatively higher doses of CBN may have led to increased feeding behaviors with a
17 longer duration of action.

18 It is also intriguing that CBN's significant hyperphagic effects only manifested at a dose of 26 mg/kg versus
19 vehicle-treatments, and not at any lower dose. While CBN is an agonist at CB₁R, its disassociation constant (K_i)
20 is approximately five times greater than that of Δ^9 THC (CBN: 211.2 nM; Rhee et al. 1997 versus Δ^9 THC: 39.5
21 nM; Bayewitch et al. 1996). Therefore, CBN's lower affinity at CB₁R could explain the observed difference in
22 effective doses when compared with Δ^9 THC where a maximal effect in this paradigm is seen at 2.68 mg/kg;
23 Farrimond et al. 2010a). Furthermore, CBN's lower affinity for CB₁R could also explain why no evidence
24 supports psychotropic effects of CBN which are commonly associated with CB₁R activation. However, we must
25 accept that the gross visual analysis of behaviors we used here does not preclude the possibility of non-specific
26 behavioral side effects. Therefore, while we believe it is highly unlikely that the administered CBN had any
27 non-specific behavioral side effects for the previously mentioned reasons, we suggest that further experiments

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

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

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

20 It must also be noted that repeated Δ^9 THC administration has previously been linked to sensitization effects in
21 rat models. Both Cadoni *et al.*, (2001) and Runbino *et al.*, (2001) have observed that if rats are pretreated twice a
22 day for three or five days respectively with Δ^9 THC then, after a washout period, they react more strongly to
23 further Δ^9 THC administration compared to untreated controls, an effect that can be removed by administration
24 of SR141716A. As such sensitization has not yet been demonstrated following CBN, CBD or CBG
25 administration and given the lower affinity of CBN for CB₁R Δ^9 THC compared to Δ^9 THC, the distinct
26 pharmacologies of both CBD and CBG and since both Cadoni *et al.*, (2001) and Runbino *et al.*, (2001)
27 demonstrated sensitization following i.p. not p.o. administration in non-feeding behavioral tests it is unlikely
28 that non- Δ^9 THC pCB-induced behavioral sensitization is affecting the presented results.

1 Here we also present results which demonstrate that CBD administration can induce significant reductions in
2 chow consumption over a four hour period. Specifically, CBD administration induced only subtle, non-
3 significant reductions in animal intake during any individual hour of the test; however, together this led to a
4 significant reduction in total chow intake over the test period due to significant reductions in intake during all
5 meals. It is worthy of mention that these apparent late-onset of suppressive effects may reflect the relatively
6 slow pharmacokinetic profile of CBD. Indeed, Deiana et al (2011) recently showed that brain levels of CBD
7 continued to rise progressively for 4 hours following a 120 mg/kg oral dose. Despite these effects on hourly
8 intakes, CBD administration had no significant effect on any other critical meal parameter. Such behaviors have
9 been intimately linked to CB₁R activation, and since it is currently thought that CBD is unlikely to interact with
10 CB₁R (Hill *et al.*, 2011), these data may suggest that CBD can affect a feeding pathway which is unrelated to
11 CB₁R. Such data fit well with the reductions in chow consumption previously reported by Sofia and Knobloch
12 in 1976 who also demonstrated a CBD-mediated reduction in chow consumption. Very recently, Ignatowska-
13 Jankowska has shown that CBD (2.5 and 5.0 mg/kg; i.p.) can reduce body weight gain in young rats, suggesting
14 either reduced food intake or increased activity, and therefore indirectly supporting the reductions observed by
15 Sofia and Knobloch and the results presented here (Ignatowska-Jankowska et al. 2010). However, data which
16 describe the effects of CBD administration on feeding patterns are not yet conclusive. Recently, Wiley and
17 colleagues (Wiley et al. 2005) and Scopinho and colleagues (Scopinho et al. 2011) observed no effect of CBD
18 on feeding patterns in food restricted mice and normally fed and fasted rats respectively. Wiley used 3.0 – 100.0
19 mg/kg CBD, whilst Scopinho used 1.0 – 20.0 mg/kg CBD; both administered i.p.. Therefore, it seems likely that
20 the differences between the experimental protocols used by Wiley and Scopinho and those presented here could
21 feasibly explain the differences in reported effects. Indeed, due to the route of administration (p.o.) used in our
22 study, it is likely that peak cerebrospinal fluid concentrations were considerably lower than those achieved with
23 the i.p. route used by Wiley and Scopinho. Furthermore, since neither Wiley nor Scopinho used pre-fed animals
24 it is likely that differences in endocannabinergic tone between the models will have altered feeding behaviors,
25 and consequently, the animals' responses to CBD administration.

26 It seems apparent from the available literature that the functional effect of CBD to induce significant decreases
27 in chow intake could arise from the numerous intra- and extra-cellular mechanisms with which it is known to
28 interact, but are likely to be unrelated to traditional CB₁R-mediated feeding control. Such a theory is supported
29 by CBD administration's failure to affect any meal parameter or individual hourly intake in this test.

1 Unfortunately, the relatively wide spectrum of cellular and molecular mechanisms that have been proposed but
2 not definitively established *in vivo* make such suggestions highly speculative and further investigations that
3 probe the discrete mechanisms potentially involved are required to confirm the mechanisms underlying the
4 observed functional effects. Clearly, it would be most interesting to establish a non-CB₁R-mediated feeding
5 pathway that is modulated by a pCB, such as CBD, although the lack of pharmacological tools with which to
6 block CBD's putative AEA reuptake, FAAH inhibition and antagonism of Δ^9 THC at CB₁R separately renders
7 such an experiment challenging to undertake.

8 We believe this to be the first time that possible CBG effects on feeding have been examined, although no
9 significant CBG-mediated effects were observed. It is unlikely that CBG administration can exert any effects via
10 direct CB₁R binding since it has a very low affinity for CB₁R (disassociation constant: 440 nM; Gauson et al.
11 2007, c.f. Δ^9 THC: 39.5 nM; Bayewitch et al. 1996). However, CBG is a known AEA reuptake inhibitor
12 (Ligresti et al. 2006) such that CBG could induce increased brain concentrations of AEA which could
13 conceivably produce similar effects to that seen following administration of exogenous AEA. However, in the
14 presented experimental paradigm, we had reduced eCB tone using a prefeed. Therefore, even if CBG was
15 inducing functionally effective AEA reuptake blockade, little endogenous AEA would be present in the CNS
16 and so reuptake blockade would be unable to potentiate CBG-mediated behavioral effects. *Id est*, were CBG to
17 be tested using a food restricted paradigm which elevated eCB production, then its putative effects on AEA
18 reuptake inhibition may begin to induce significant effects on feeding patterns. As such, while the data we now
19 present suggests that CBG administration has no effect on feeding patterns, different results may be found using
20 a different experimental paradigm.

21 In summary, following the administration of CBN alone we observed significant increases in appetitive,
22 consummatory and total intake behaviors. Thus we suggest that a balance exists between endocannabinergic
23 tone and pCB-mediated CB₁R activation. This balance manifests as increasing feeding behaviors (appetitive,
24 consummatory and total chow intake increase) with increasing CB₁R activation, and decreasing feeding
25 behaviors with decreasing CB₁R activation. The data we have presented suggests that as CB₁R activation is
26 reduced, feeding behaviors decay and the weakest behaviors are lost first (increased total chow consumption <
27 increased meal one duration < increased meal one chow intake < increased hourly intake & reduced latency to
28 the first meal). Such a theory is supported by currently available literature since only recently have significant

1 effects on Δ^9 THC-mediated meal pattern changes in rats been observed, but AEA-induced increases in total
2 chow intake, appetitive and consummatory behaviors have been demonstrated (see Farrimond et al. 2011a for
3 review). Furthermore, we have also demonstrated significant, short-term CBD-mediated reductions in feeding
4 which, we suggest, are due to reduced consummatory behaviors following CBD administration. However, given
5 CBDs pharmacological profile, such effects are unlikely to be CB₁R-mediated. Finally, we have observed that
6 the administration of CBG induces no significant alterations to feeding patterns in the presented paradigm. A
7 direct comparison between these three drug treatments is necessarily limited by the large variability in response
8 seen under vehicle conditions, and as such, the robustness of the effects we describe here should be confirmed
9 by a fully randomized replication of our study.

10

1 **Conclusion**

2 Using a prefeed paradigm, CBN induced significant CB₁R-mediated hyperphagia in male rats via significant
3 reductions in the latency to feed and significant increases in the food consumed during the first hour and meal,
4 alongside significant increases in the total amount of food consumed when compared to vehicle-treatments.
5 Conversely, CBD administration reduced total feeding over a four hour period. Neither Δ⁹THCV nor CBG
6 administration exerted effects on feeding behaviors in this paradigm.

7 As CBN has not so far been shown to have psychoactive properties it could be a useful anti-anorexic agent,
8 since in this study CBN administration significantly increased intake over the total test period. Clearly, further
9 experiments are required to fully characterize the effects of both chronic and acute CBN administration on food
10 consumption and body weight. Moreover, the data we have presented here when compared to some of our
11 previous data (Farrimond et al. 2010b) suggests that CBN and Δ⁹THC, when co-administered, may
12 synergistically induce powerful hyperphagic effects. Therefore, co-administration of CBN and Δ⁹THC may also
13 exhibit anti-anorexic properties.

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

19

20

1 **Acknowledgements**

2 This research was supported in part by the University of Reading Research Endowment Trust Fund (to JAF).

3 The authors thank Ms. Pam Rummings and her team for technical assistance and GW Pharmaceuticals for the
4 kind gift of purified phytocannabinoids.

5

6 **Ethical compliance**

7 All procedures were performed in compliance with the requirements of the United Kingdom Animals (Scientific

8 Procedures) Act 1986 and all other applicable laws and standards in the U.K.

9

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19

#	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

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

4

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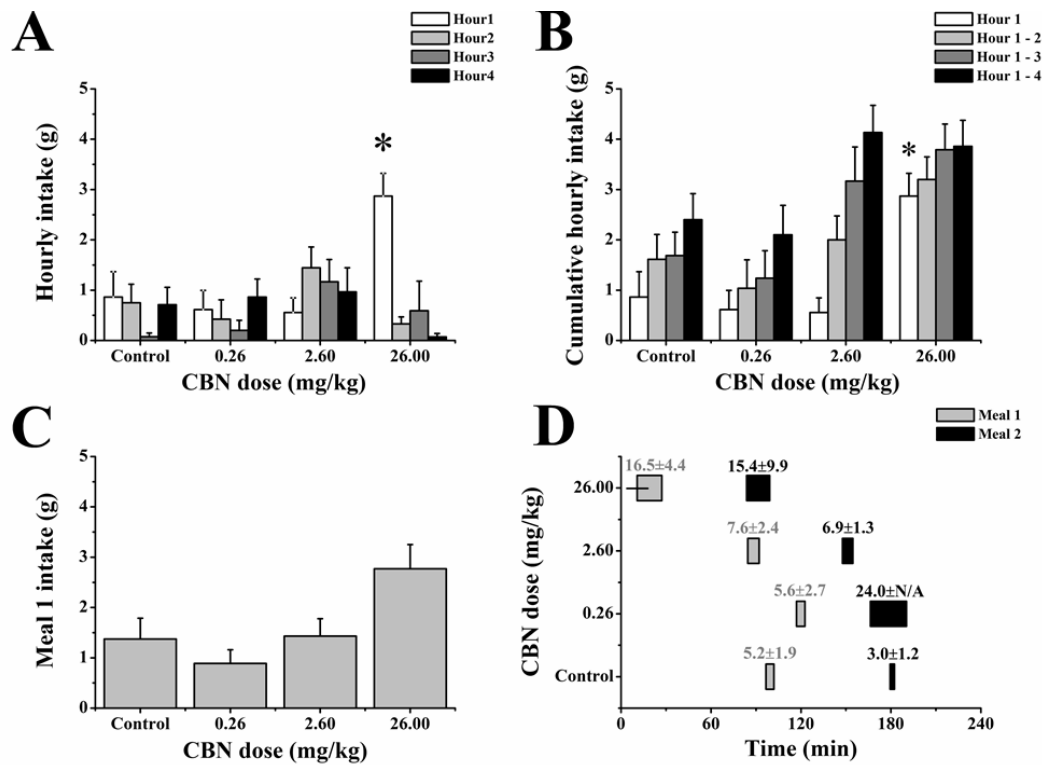
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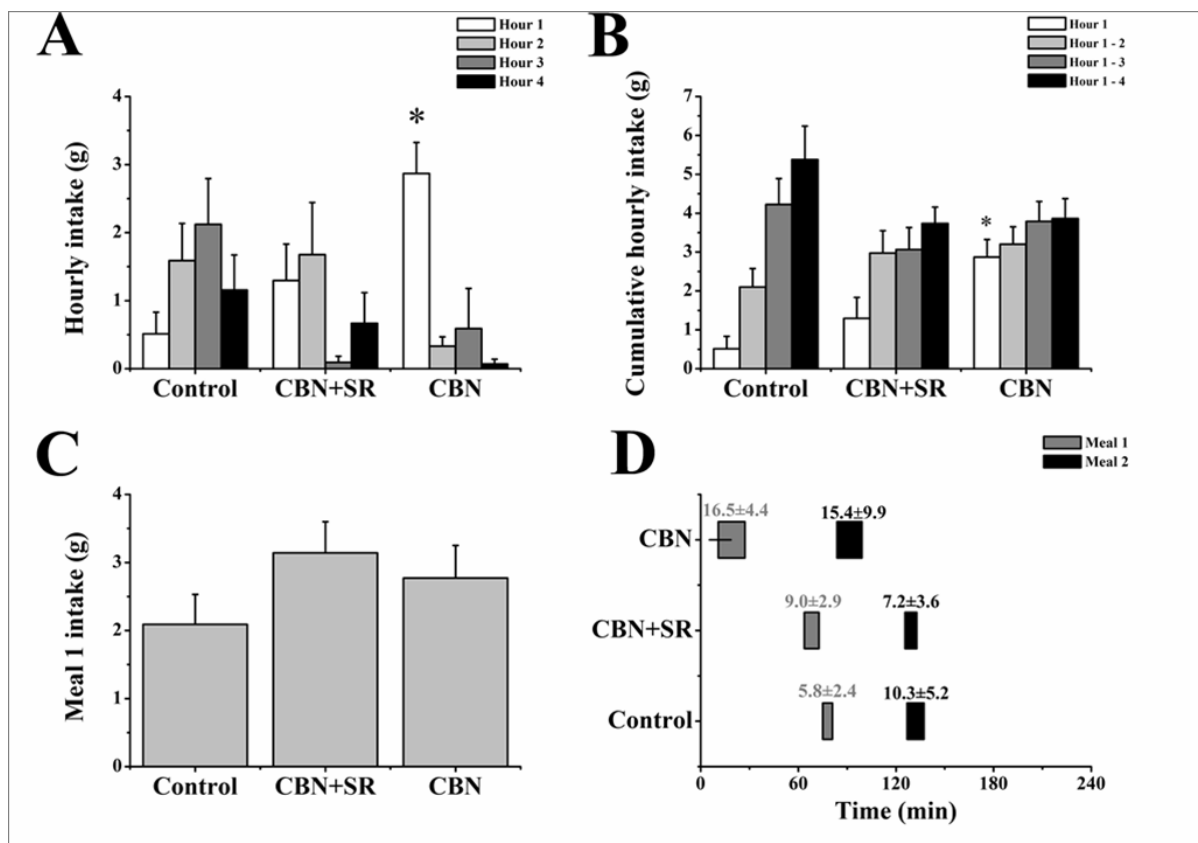


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2 **Figure 1: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and**
 3 **meal pattern (D) following administration of CBN (0, 0.26, 2.60 and 26.00 mg/kg; p.o.).**

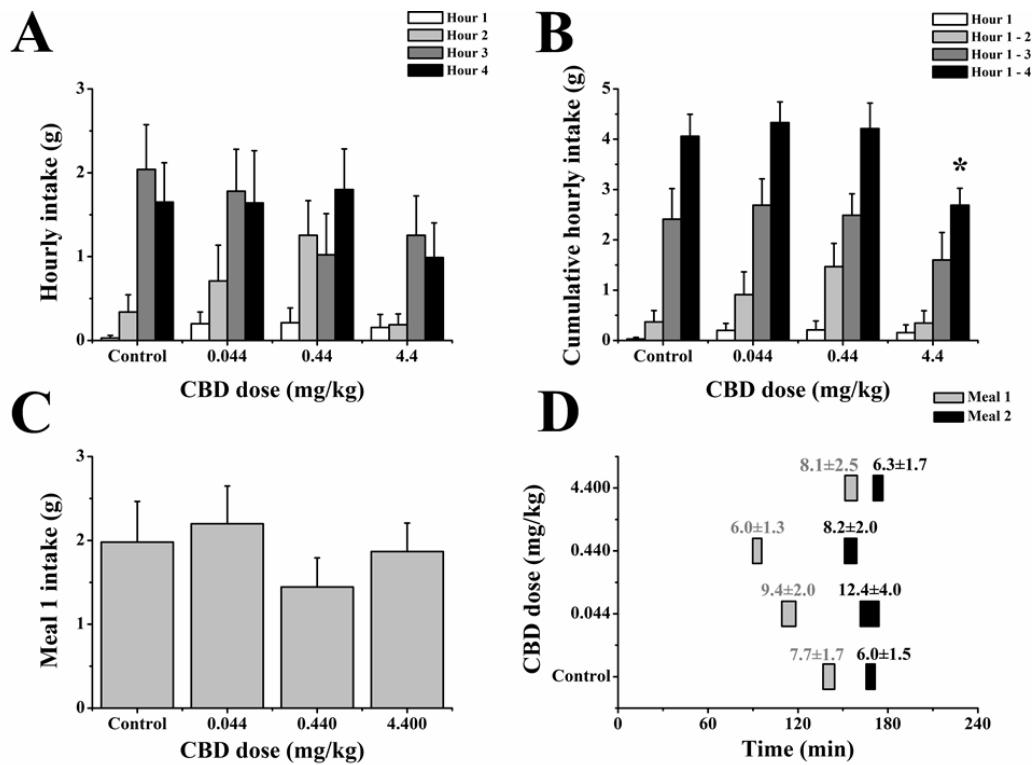
4 CBN administration significantly increased hour one intake (A: white bars) and chow consumption over all
 5 cumulative hourly arrangements (B). Furthermore, following CBN administration significant increases in chow
 6 consumption during the first meal (C) and highly significant decreases in the latency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

13



1
 2 **Figure 2: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and**
 3 **meal pattern (D) following administration of CBN and SR14171A (0 and 26.00 CBN mg/kg; p.o. and 1.0**
 4 **mg/kg SR141716A; s.c.).**

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

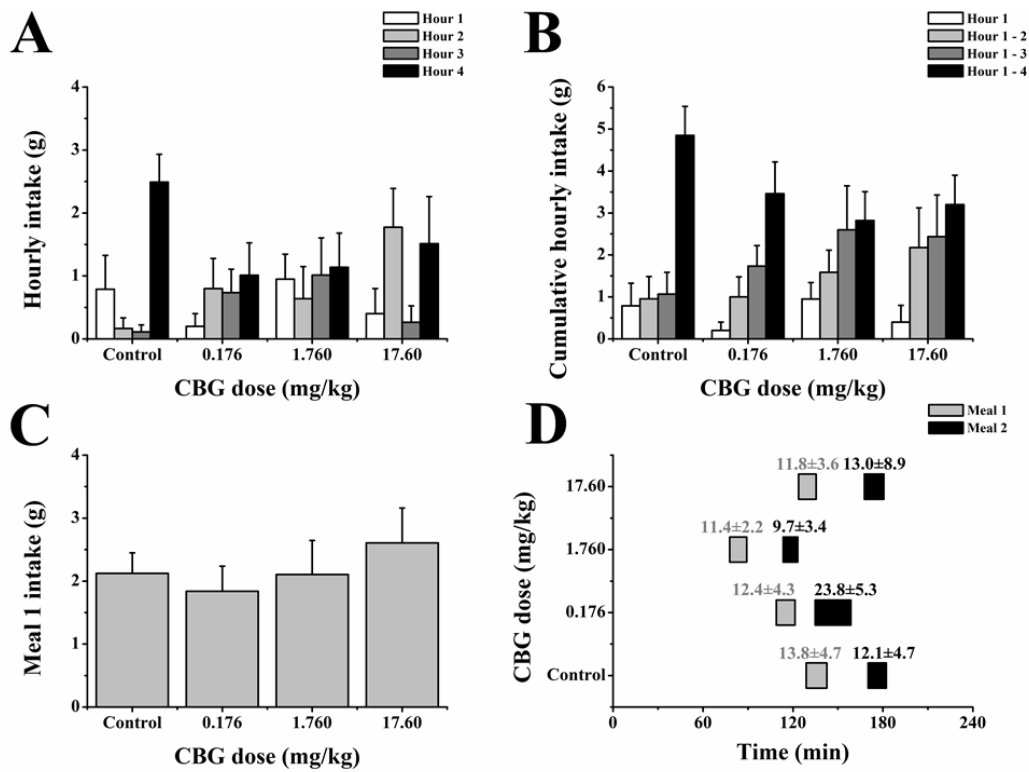


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2 **Figure 3: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and**
 3 **meal pattern (D) following administration of CBD (0.00, 0.044, 0.44 and 4.40 mg/kg; p.o.).**

4 CBD administration significantly reduced chow intake over the period of the test (B). No statistical analyses
 5 have been performed on second meal data as animals consumed two few second meals. Meal 2 bars are included
 6 for reference only. In panel D meal duration is represented by the length of bar and is provided numerically
 7 above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ± SEM. * denotes $p \leq$
 8 0.05 in Bonferroni post-hoc test following a significant one-way ANOVA result versus vehicle-treatment. –
 9 denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on meal 1 latency.

10



1
2 **Figure 4: Mean hourly chow (A), cumulative hourly chow (B) and meal one chow consumption (C) and**
3 **meal pattern (D) following administration of CBG (0.00, 0.176, 1.76 and 17.60 mg/kg; p.o.).**

4 Administration of CBG induced no significant deviations from vehicle-treatments for any measure. No
5 statistical analyses have been performed on second meal data as animals consumed too few second meals. Meal
6 2 bars are included for reference only. In panel D meal duration is represented by the length of bar and is
7 provided numerically above each bar (min ± SEM). Chow intake (A, B and C) is represented as mean intake ±
8 SEM. * denotes $p \leq 0.05$ in Bonferroni post-hoc test following a significant one-way ANOVA result versus
9 vehicle-treatment. – denotes a significant Bonferroni post-hoc test following a significant one-way ANOVA on
10 meal 1 latency.