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Binge sucrose-induced neuroadaptations: A focus on the endocannabinoid system

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Running title: Binge sucrose regulation of the ECS

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Abbreviations:

2-AG : 2-arachidonoylglycerol; AEA : anandamide ; BED: Binge-eating disorder; CB1R : cannabinoid receptor 1 ; CB2R : cannabinoid receptor 2 ; CPP: conditioned place preference; dorsal striatum; ECS: endocannabinoid system; HPC: hippocampus; NAc: Nucleus Accumbens ; PFC: prefrontal cortex; RIM : rimonabant [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide].

Key words: Binge eating disorder, sucrose, cannabinoid, gene expression, reward, addiction, conditioned place preference

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Abstract

Binge eating, the defining feature of binge eating disorder (BED), is associated with a number of adverse health outcomes as well as a reduced quality of life. Animals, like humans, selectively binge on highly palatable food suggesting that the behaviour is driven by hedonic, rather than metabolic, signals. Given the links to both reward processing and food intake, this study examined the contribution of the endocannabinoid system (ECS) to binge-like eating in rats. Separate groups were given intermittent (12h) or continuous (24h) access to 10% sucrose and food over 28 days, with only the 12h access group displaying excessive sucrose intake within a discrete period of time (i.e., binge eating). Importantly, this group also exhibited alterations in ECS transcripts and endocannabinoid levels in brain reward regions, including an increase in cannabinoid receptor 1 (CB1R) mRNA in the nucleus accumbens as well as changes in endocannabinoid levels in the prefrontal cortex and hippocampus. We then tested whether different doses (1 and 3mg/kg) of a CB1R antagonist, Rimonabant, modify binge-like intake or the development of a conditioned place preference (CPP) to sucrose. CB1R blockade reduced binge-like intake of sucrose and blocked a sucrose CPP, but only in rats that had undergone 28 days of sucrose consumption. These findings indicate that sucrose bingeing alters the ECS in reward-related areas, modifications that exacerbate the effect of CB1R blockade on sucrose reward. Overall, our results broaden the understanding of neural alterations associated with bingeing eating and demonstrate an important role for CB1R mechanisms in reward processing. In addition, these findings have implications for understanding substance abuse, which is also characterized by excessive and maladaptive intake, pointing towards addictive-like properties of palatable food.

1. Introduction

Binge-eating disorder (BED), now listed as an independent disorder in DSM-5, is characterized by uncontrollable episodes of eating within a discrete period of time, not followed by compensatory behaviors such as purging or physical exercise (APA, 2013). BED is the most common of all eating disorders, affecting approximately 3% of the general population (Hudson *et al.*, 2007; Kessler *et al.*, 2013; Solmi *et al.*, 2016). Binge eating also occurs in other eating disorders (Hudson *et al.*, 2007) and is a likely contributor to the growing obesity epidemic (Stojek & MacKillop, 2017). Although binge eating is linked to serious medical complications, including increased risk for other psychiatric disorders, treatment options for BED are minimal (Hudson *et al.*, 2018). Advancement in this area depends on understanding the etiology and mechanisms that drive binge eating.

Regulation of food intake in both humans and animals is closely linked to activity in the endocannabinoid system (ECS) (Ceccarini *et al.*, 2016), the endogenous system activated by Δ -9-tetrahydrocannabinol in the *Cannabis Sativa* plant. For example, blockade of the cannabinoid receptor 1 (CB1R) reduces food intake (Arnone *et al.*, 1997; Simiand *et al.*, 1998; Colombo *et al.*, 2002), specifically, binge-like eating in rats (Parylak *et al.*, 2012; Scherma *et al.*, 2013; Dore *et al.*, 2014), and decreases scores on a binge eating scale in humans (Pataky *et al.*, 2013). Most telling, infusions of the endogenous cannabinoid, 2-arachidonoylglycerol (2-AG), directly into the nucleus accumbens (NAc), dose-dependently increases feeding in rats, an effect that is blocked by CB1R antagonism (Kirkham *et al.*, 2002). Results from these animal studies linking the ECS to excessive eating is not surprising given that cannabis is well known to promote eating in humans (Abel, 1975; Williams *et al.*, 1998; Koch *et al.*, 2015).

In addition to the direct effects of cannabinoid compounds on feeding, maladaptive eating patterns, themselves, may disrupt ECS function (Gaetani *et al.*, 2008). The specific details are difficult to disentangle in that a number of studies have yielded conflicting results. In humans, BED is associated with increased plasma levels of the endogenous cannabinoid anandamide (AEA), but not 2-AG, although the same pattern was observed in anorexia nervosa (Monteleone *et al.*, 2005). In animal models, bingeing on a high fat diet decreases AEA levels in the dorsal striatum (DS), amygdala, and hippocampus (HPC), but increases 2-AG levels in the HPC (Satta *et al.*, 2018). Food restriction (Dazzi *et al.*, 2014) and continuous access to a high-fat diet (Blanco-Gandía, Aracil-Fernández, *et al.*, 2017) decrease both gene and protein expression of CB1Rs in the prefrontal cortex (PFC), whereas excessive intake of fat/sweet pellets increases CB1R expression in the same region (Mancino *et al.*, 2015). In the NAc, CB1Rs are upregulated, but CB1R transcripts are decreased, following either binge or continuous intake of high fat foods (Blanco-Gandía, Aracil-Fernández, *et al.*, 2017; Blanco-Gandía, Cantacorps, *et al.*, 2017). These seemingly contradictory findings likely reflect the use of different feeding protocols and/or type of palatable food, particularly as the biological mechanisms underlying fat and sucrose bingeing are distinct (Wong *et al.*, 2009).

The primary goal of this study was to investigate, in more detail, the relationship between ECS function and binge eating. We focused on sweet foods, using a validated model of sucrose bingeing in rats (Avena *et al.*, 2005; Avena, 2007; Avena, Bocarsly, *et al.*, 2008; Avena, Rada, *et al.*, 2008), in order to isolate the effects of this commodity from other palatable foods. A link between binge eating and the ECS likely occurs through brain reward circuits (Maldonado *et al.*, 2006) in that binge eating is driven by hedonic, rather than metabolic, signals. Thus, our first set of experiments examined whether binge eating alters gene expression of CB1R and cannabinoid

type 2 receptor (CB2R), as well as endocannabinoid levels (2-AG and AEA), in brain reward sites including the NAc, DS, PFC, and HPC. We also measured enzyme transcripts for endocannabinoid synthesis (NAPE-PLD/DAGL α) and degradation (FAAH/ MAGL) in all four regions. Based on our observation that sucrose bingeing alters CB1R gene expression, we extended these studies by testing whether blockade of CB1Rs alters sucrose bingeing or the rewarding effect of sucrose in the conditioned place preference (CPP) paradigm.

2. Materials and methods

2.1 Subjects

Two hundred and twenty-three male Wistar rats (Charles River Laboratories, Montréal QC and Janvier Laboratories, France), weighing 175 g at arrival, were habituated for two weeks to housing conditions in a temperature and humidity-controlled environment on a reverse 12 h light/dark cycle (lights OFF at 4:00 AM). Rats were group housed in standard polycarbonate cages with *ad libitum* access to food and water until 1 day before behavioral experiments. All animals were then single housed to provide accurate measures of individual food and solution consumption. All procedures and animal care were performed according to the European Union laws for animal studies and were in accordance with the guidelines for the ethical use of animals, outlined by the Canadian Council on Animal Care. Experiments were approved by the institutional ethics committee CREMEAS (Comité d'Éthique pour l'Expérimentation Animale de Strasbourg, France) (APAFIS#2019070816359145) and the Queen's University Animal Care Committee. A timeline displaying the sequence of experimental procedures for each set of experiments is shown in **Figure 1**.

2.2 Apparatus

Body weight and food consumption (g) were recorded with a standard scale. Liquid solutions were presented to rats in 100ml graduated glass drinking bottles fitted with rubber stoppers containing ball-tipped sipper tubes (Ancare Inc., Montreal, QUE) or in bottles (Techniplast, Milan, Italy) that were weighed to collect consumption measures. Sucrose was mixed in tap water, measured as g/L. Solution concentration was 10% sucrose. The CB₁ receptor antagonist, rimonabant [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] (RIM) (Cayman Chemicals, Burlington, ON) was dissolved in one drop vehicle of 0.3% polysorbate Tween-80 (Sigma-Aldrich Chemicals, St. Louis, MO) dissolved in 0.9% saline. Fresh solutions of 1mg/ml and 3mg/ml were prepared each day.

The CPP apparatus was made of plexiglass and consisted of two large compartments (46 x 46 x 30 cm) connected by a tunnel (19 x 38 x 30 cm). The two large compartments differed in wall colour (black and white stripes or solid white) and floor texture (striated or bumpy). The tunnel walls were clear plexiglass and the floor was made of sheet metal, spray painted with a matt white finish. Guillotine doors, that could be raised or lowered, separated the tunnel from each compartment. Cameras were mounted directly above each set of boxes so that the rats' movements throughout the entire compartment could be monitored and recorded. Movement was tracked using the video tracking software EthoVisonXT (Noldus Information Technology b.v. Wageningen, The Netherlands), which detects and extracts the size and position of the subjects from the digital image captured by the camera.

2.3 *Procedures*

2.3.1 *Effects of sucrose bingeing on neuroadaptations in the ECS*

2.3.1.2 Sucrose consumption

Eighty-three rats were assigned to one of three groups that determined daily access to sweet solution and food over 28 days (**Figure 1, experiment 1**).

For the primary experimental group, 12h sucrose (n=28), chow was removed from the cages 12h prior to the first access day. Then, beginning 4h after the onset of the active cycle, rats were provided with 12h access to standard chow and sucrose solution. The first control group (12h food, n=28) underwent the same protocol with no sucrose, providing a control for food restriction. The second control group (24h sucrose; n=27) had continuous access to standard chow and sucrose solution, which was introduced on day 1 (4h after the onset of the active cycle), providing a control for sucrose exposure.

All animals were weighed daily, prior to the presentation of solution and/or food. For intermittent access groups (12h sucrose, 12h food), solution intake (ml) was measured 1h after presentation and on removal (12h). For animals in the continuous access group (24h sucrose), solution intake was measured 1, 12, and 24h after presentation. Food intake (g) was measured at the end of the access period (12h or 24h).

2.3.1.2 Brain dissection

The day following the final sucrose access session, rats were given an overdose of pentobarbital (182 mg/kg, i.p), as required by regulations of the local animal ethics committee, followed by decapitation to perform brain extraction. This time point was selected to minimize acute effects of the last sucrose exposure and to avoid potential circadian effects. Coronal brain slices, 1mm thick, were cut using a brain matrix chilled on ice (Harvard apparatus, Holliston, MA, USA). Structures of interest were collected according to the rat brain stereotaxic atlas (Paxinos & Watson, 2007) by dissection (dorsal HPC, -2 to 4mm from bregma, 2 slices) or using punches (PFC, 3mm puncher, 4.68 to 2.5mm from bregma, 2 slices; NAc, 1.9mm puncher, 3 to

0.6mm from bregma, bilateral, 2 slices; DS, 3mm puncher, 2 to -0.96mm from bregma, bilateral, 3 slices). Samples were immediately frozen on dry ice and kept at -80°C. All extractions were performed in less than 20 min to avoid increased levels of AEA (Schmid *et al.*, 1995).

2.3.1.3 Quantitative real-time PCR

Total RNA was extracted using RiboZol (VWR, Fontenay-sous-bois, France) according to the manufacturer's instructions. RNA quality and quantity were measured with a NanoVue™ (GE healthcare) spectrophotometer (GE healthcare). Reverse transcription was performed on 750 ng of total RNA with iScript (iScript™ cDNA Synthesis Kit, Biorad, France). Real-time PCR was performed in triplicate using a CFX96 Touch™ apparatus (Biorad, France) and Sso Advanced™ Universal SYBR Green supermix (Biorad, France). Thermal cycling parameters were 30 sec at 95°C followed by 40 amplification cycles of 5 sec at 95°C and 45 sec at 60°C. Primer sequences for all tested genes are provided in **Table 1**. Expression levels were normalized to *Rplp0* housekeeping gene levels, classically used for brain or food related studies (Silberberg *et al.*, 2009; Li *et al.*, 2014) and compared between controls and treated samples using the $2^{-\Delta\Delta C_t}$ method (Livak, 2001).

2.3.1.4 Mass spectrometry

PFC, HPC, NAc and DS tissues (n=7-8/group) were sonicated in 200 µl of H₂O with a Vibra Cell apparatus (2 times 5 s, 90W; Sonics, Newtown, U.S.A.) and the homogenate was centrifuged (20,000g, 30 min, 4°C) to recover the supernatant. Protein concentration was determined (Protein Assay, Bio-Rad, Marnes-la-Coquette, France) and 150 µl of the supernatant was mixed with 50 µl of acetonitrile (ACN) 100% containing 400.26 pmol of D8-2-AG (sc-480539; Santa Cruz, Heidelberg, Germany) and 100.15 pmol of D4-AEA (Tocris/Biotechnique, Lille, France). Samples were centrifuged (20,000g for 30 min, 4°C) and the supernatants

collected and evaporated to dryness. Samples were re-suspended in 20 μ l of ACN 30% / H₂O 69.9% / formic acid 0.1% (v/v/v). Analyses were performed on a Dionex Ultimate 3000 HPLC system coupled with a triple quadrupole Endura mass spectrometer (Thermo Electron, San Jose, USA). The system was controlled by Xcalibur v. 2.0 software. Samples (3 μ l) were loaded onto a microbore C18 ODS column (1x100 mm, 3 μ m UniJet microbore ODS, ref MF8949, BioAnalytical Systems Inc., West Lafayette, U.S.A.) heated at 40°C. The presence of 2-AG, AEA, D8-2-AG and D5-AEA was studied using the multiple reaction monitoring mode (MRM). Elution was performed at a flow rate of 50 μ l/min by applying a linear gradient of mobile phases A/B. Mobile phase A corresponded to ACN 1% / H₂O 98.9% / formic acid 0.1% (v/v/v), whereas mobile phase B was ACN 99.9% / formic acid 0.1% (v/v) (see details in **Supplemental Table 1**).

Electrospray ionization was achieved in the positive mode (spray voltage set at 3,500 V). Nitrogen was used as the nebulizer gas. Desolvation (nitrogen) sheath gas was set to 10 Arb and Aux gas was set to 5 Arb. The Ion transfer tube was heated at 287°C. Q1 and Q2 resolutions were set at 0.7 FWHM, whereas collision gas (CID, argon) was set to 2 mTorr. Identification of the compounds was based on precursor ion, selective fragment ions and retention times obtained for 2-AG, AEA, D8-2-AG and D5-AEA. Selection of the monitored transitions and optimization of collision energy and RF Lens parameters were manually determined (see **Supplemental Table 2**). Qualification and quantification were performed with the MRM mode and quantification was obtained using Quan Browser software (Thermo Scientific). All amounts of endocannabinoids measured in samples fit within the standard curve limits, with typical analytical ranges from 1 fmol – 100 pmol to 150 fmol – 100 pmol. Precision (CV% between repeated injections of the same sample) values were <1% for same-day measurements and <5%

for inter-day measurements. The amount of 2-AG (nmol) and AEA (pmol) were normalized according to protein levels (mg).

2.3.2 *Effects of CB1 antagonism on sucrose bingeing*

Sixty rats were randomly assigned to 12h or 24h sucrose groups, which determined access to food and sucrose solution over 28 days using the protocol described previously (2.3.1.2). Within each access group, rats were randomly assigned to a drug dose (0, 1, or 3 mg/kg RIM) ($n=10$ each), and were then injected intraperitoneally (i.p.) with drug 30 min before the start of each sucrose access sessions on days 1-5 and 24-28 (**Figure 1, experiment 2**). No injections were administered on days 6-23 inclusive. Doses and timing of pre-treatment with RIM were based on previous studies showing behavioural effects of the drug, including an impact on food intake (Blasio, et al., 2014) and binge eating (Scherma, et al., 2013), but also on social play (Achterberg, et al., 2016), cocaine responses (Chaperon, et al., 1998; De Vries, et al., 2001; Yu, et al., 2011), and morphine reward (De Carvalho, et al., 2014).

2.3.3 *Effects of CB1 antagonism on sucrose reward*

Twenty-four rats, divided randomly into three drug doses ($n=8$ each) were used to examine the effect of RIM on sucrose reward in the CPP paradigm (**Figure 1, experiment 3**). Rats were food restricted for 48h prior to and throughout the CPP protocol. On day 1 (habituation), rats were placed in the tunnel and had free access to all three compartments for 30 min. Over the next 10 days, rats were confined to one test compartment or the other for 30 min where they had access to a water bottle containing either tap water or 20% sucrose solution. The assignment of sucrose-paired compartment and order of conditioning sessions (water- versus sucrose-paired) were counterbalanced within groups. Rats were injected with RIM (0, 1, or 3 mg/kg i.p.) 30 min prior to conditioning in the sucrose-paired compartment and with vehicle

solution (1 ml/kg i.p.) 30 min prior to conditioning in the water-paired compartment. The amount of fluid consumed during each conditioning session was measured. On test day, rats were placed in the tunnel and allowed free access to the entire apparatus. Water bottles were removed from both compartments and the time spent in each compartment was recorded across the 30-min session.

In a separate experiment, forty-eight rats, randomly divided into two access groups (12h and 24h sucrose), underwent sucrose consumption sessions using the protocol described in 2.3.1.2 (**Figure 1, experiment 4**). Rats were then randomly divided into three drug groups ($n=8$ each) and tested for a sucrose CPP as outlined above (0, 1, and 3 mg/kg RIM).

2.4 *Statistical analysis*

Solution and food intake (measured per body weight as ml/g and g/g) and weight were analyzed using a repeated-measures analysis of variance (ANOVA) in the mixed effect way (Afex package from R, Rstudio v1.3.1093). For the fixed effects part of the model, the explanatory variables were session as the repeated measure and sucrose access group and drug as between-subjects measures. The random effect part of the model accounts for the individual variation of the subjects across the sessions. In cases in which sphericity was violated (Machley's $p < 0.001$), the Greenhouse-Geisser correction was used. Post hoc analysis were conducted by multiple pairwise comparisons using the Sidak method for correction (lsmeans package from R).

Biochemical data were analyzed using ANOVA to assess group differences in ECS neuroadaptations, with separate analyses conducted in each brain region. Significant interactions were followed up with simple main-effects analyses and multiple pairwise comparisons using a Bonferroni correction (GraphPad Prism software).

For CPP data, consumption of solution (sucrose and water) during conditioning and time spent in each compartment during testing were analyzed using planned orthogonal comparisons, as described previously (SPSS v27) (Smail-Crevier *et al.*, 2018; Grenier *et al.*, 2019). A primary advantage of this technique is that it minimizes the number of comparisons to those of interest; because each comparison is independent and tests a unique hypothesis, it can be carried out regardless of the outcome of the overall ANOVA and no correction is made for multiple tests. As such a 5% risk of type I error is accepted for each comparison. Levene's F test was used to examine the assumption of homogeneity of variance and analyses were adjusted if significant. Significance level for all tests was set at $p \leq .05$.

3. Results

3.1 Effects of sucrose bingeing on neuroadaptations in the ECS

3.1.1 Sucrose consumption

Figure 2 shows data from the sucrose consumption sessions, with intake during the 1st hour of access being significantly higher in rats given intermittent, rather than continuous, access to sucrose, $F(1,32) = 55.11$, $p < .001$, general $\eta^2 = .39$ (group) (**Figure 2A**). The main effect of session, $F(9.84,314.85) = 4.41$, $p < .001$, general $\eta^2 = .08$, as well as the session x group interaction, $F(9.84,314.85) = 4.95$, $p < .001$, general $\eta^2 = .09$, were also statistically significant. With the exception of session 1, intake was higher in the 12h, compared to the 24h, sucrose group (*post-hoc* $ps < .0001$), verifying binge-like intake in rats given intermittent access to sucrose. In contrast, there was no significant difference in daily sucrose intake for rats in the 12h and 24h sucrose groups, $F(1,33) = 0.46$, $p = .501$, general $\eta^2 = .01$ (**Figure 2B**). Daily solution intake varied across sessions, $F(5.44,179.55) = 3.04$, $p < .01$, general $\eta^2 = .04$, but the effect did not interact with group, $F(5.44,179.55) = 1.70$, $p = .13$, general $\eta^2 = .02$.

Figure 2C shows that food intake of all groups declined across sessions, $F(11.92,453.05) = 132.49, p < .001$, general $\eta^2 = .71$, with higher overall intake in the food only group, $F(2,38) = 70.77, p < .001$, partial $\eta^2 = .53$. The significant session $\times$ group interaction, $F(23.84,453.05) = 6.97, p < .001$, partial $\eta^2 = .20$, reflected higher food intake in the 12h food group beginning on day 2 compared to both sucrose access groups, with no significant difference between the other two groups across sessions ($ps > .05$). Finally, body weight increased across sessions in all groups, $F(2.27,147.36) = 2176.71, p < .001$, general $\eta^2 = .77$. The significant main effect of group, $F(2,65) = 8.18, p < .001$, general $\eta^2 = .18$, and session $\times$ group interaction, $F(4.53,147.36) = 4.55, p = .001$, general $\eta^2 = .014$, were due to higher body weight in the 24h sucrose group beginning on day 2, compared to both 12h sucrose and 12h food groups ($ps < .05$), and no difference in body weight of 12h sucrose and 12h food groups across sessions ($ps > .05$) (**Figure 2D**).

3.1.2 Gene expression

Figure 3 shows the effects of sucrose bingeing on gene expression in the ECS throughout brain reward sites. *Cnr1* (CB1R coding gene) expression was increased in the NAc of rats in the 12h sucrose group, compared to either 12h food or 24h sucrose groups, $F(2,24) = 4.90, p < .05$ (**Figure 3A**). There were no differences in *Cnr1* expression in other regions (PFC: $F(2,20) = .84, p = .45$; DS: $F(2,21) = .041, p = .96$; HPC: $F(2,20) = 1.36, p = .28$) and no differences in *Cnr2* expression across any site (PFC: $F(2,20) = .77, p = .47$; NAc: $F(2,15) = .44, p = .65$; DS: $F(2,19) = 1.27, p = .30$; HPC: $F(2,25) = 1.26, p = .30$).

Further analysis showed *Faah* expression was decreased in the NAc, only in the 24h sucrose group, $F(2,21) = 4.03, p < .05$ (**Figure 3B**), whereas *Dagla* expression in this region was decreased in both sucrose groups, $F(2,21) = 10.22, p < .001$ (**Figure 3C**). There were no

significant differences in enzyme expression in the PFC (*Faah*: $F(2,20) = .09, p = .91$; *Dagla*: $F(2,20) = .58, p = .57$), DS (*Faah*: $F(2,18) = .57, p = .58$; *Dagla*: $F(2,18) = .13, p = .88$), or HPC (*Faah*: $F(2,20) = .87, p = .43$; *Dagla*: $F(2,20) = 1.67, p = .21$). Pearson's correlation analysis revealed no significant relationship between sucrose intake during the first hour of access and regulated genes for any group (data not shown).

3.1.3 Endocannabinoid levels

In assessing endocannabinoid levels following sucrose bingeing, we observed an increase of AEA in the PFC of the 12h sucrose group, $F(2,21) = 4.05, p < .05$, with no other differences in this measure (NAc: $F(2,21) = .04, p = .96$; DS: $F(2,21) = .16, p = .85$; HPC: $F(2,18) = .13, p = .87$) (**Figure 4A**). In contrast, 2-AG was decreased in the HPC of the 12h sucrose group, $F(2,18) = 3.75, p < .05$, with no group differences in other brain regions (PFC: $F(2,21) = .49, p = .62$; NAc: $F(2,21) = .20, p = .82$; DS: $F(2,21) = .21, p = .81$) (**Figure 4B**). Pearson's correlation analysis revealed no significant relationship between sucrose intake during the first hour of access and regulated eCB levels for any group (data not shown). We did not perform correlation analyses between gene expression and eCB levels as these measures were obtained on two distinct cohorts.

3.2 Effects of CBI antagonism on sucrose consumption

Figure 5A (left panel) shows that sucrose intake during the first hour of access increased across days, with the 12h sucrose groups consuming significantly more solution than the 24h sucrose groups. These effects were verified, statistically, by a significant session x group interaction, $F(12.74,688.12) = 10.22, p < .001$, general $\eta^2 = .11$, as well as significant main effects of session, $F(12.74,688.12) = 20.70, p < .001$, general $\eta^2 = .19$, and group, $F(1,54) =$

268.33, $p < .001$, general $\eta^2 = .65$. The main effect of drug, $F(2,54) = 3.40$, $p < .05$, general $\eta^2 = .04$, reflected decreased intake in sucrose groups following RIM, compared to vehicle, administration ($ps < .01$). The session x group x drug interaction was statistically significant, $F(25.49,688.12) = 1.88$, $p < .01$, general $\eta^2 = .042$. *Post-hoc* test revealed that intake of the 12h sucrose vehicle group was significantly higher than the 24h sucrose vehicle group ($p < .0001$); in the 12h sucrose groups, intake was significantly lower following RIM 3, compared to RIM 1, treatment ($p = 0.0002$) and there was no significant difference between intake of the 24h sucrose RIM 1 and 24h sucrose RIM 3 groups. The effects of RIM on binge intake were examined, in more detail, by comparing total intake during the first hour of days 1-5 and days 24-28 in the 12h sucrose group (**Figure 5A, right panel**). This analysis revealed no significant differences across the first 5 sessions, $F(2,96.8) = 1.23$, $p = .29$, but a significant effect of drug during the final 5 sessions, $F(295.2) = 8.56$, $p < .001$. Both doses of RIM significantly decreased sucrose intake during the first hour of these 5 sessions, compared to vehicle injections (post-hoc $ps < .01$).

Daily sucrose intake also increased across sessions, $F(8.32,449.33) = 81.80$, $p < .001$, general $\eta^2 = .42$ (**Figure 5B, top panel**) although there were no group or drug differences on this measure and no interaction between the two (main effects and interaction, $ps > .05$). The session x group x drug interaction was significant ($F(16.64,449.33) = 1.88$, $p = .02$, general $\eta^2 = .03$), as well as the session x group interaction ($F(8.32,449.33) = 2.94$, $p < .01$, general $\eta^2 = .03$), suggesting that a change in sucrose intake across days was dependent on both access condition and drug dose. Post-hoc analyses revealed lower intake in the 12h, compared to the 24h, sucrose group following vehicle administration ($p < .0001$), but no significant differences between these groups following RIM administration. In addition, both doses of RIM increased intake, compared to vehicle, in the 12h sucrose group ($ps < .0001$), with no significant differences

between intake of the two RIM-treated groups. The higher (RIM 3), but not the lower (RIM 1) drug dose decreased intake in the 24h sucrose group, compared to vehicle -treated rats ($p < .0001$), and intake of the 24h sucrose group administered RIM 3 was significantly lower than intake of the 24h sucrose group receiving RIM 1 ($p = .0006$).

Figure 5B (middle panel) shows that food intake declined across sessions in all groups, $F(13.21, 713.24) = 38.50$, $p < .001$, general $\eta^2 = .22$, with significant interactions between session and group, $F(13.21, 713.24) = 2.14$, $p = .01$, general $\eta^2 = .02$; and session and drug dose $F(26.42, 713.24) = 1.56$, $p = .04$, general $\eta^2 = .02$. The main effect of group, $F(1, 54) = 10.61$, $p = .002$, general $\eta^2 = .11$, was due to higher food intake in the 12h sucrose groups ($p < .0001$). *Post-hoc* tests revealed that the highest dose of RIM decreased food intake, compared to vehicle injections, in the 12h sucrose groups ($ps < .0001$). In contrast, RIM 1 increased food intake in the 24h sucrose groups ($p = 0.02$).

As shown in **Figure 5B (bottom panel)**, body weight increased across sessions in all groups, $F(3.36, 186.85) = 559.92$, $p < .001$, general $\eta^2 = .7$, and the effect interacted with drug, $F(6.92, 186.85) = 3.20$, $p = .003$, general $\eta^2 = .03$, indicating that the impact of drug on body weight varied across sessions. *Post-hoc* tests revealed lower body weights in the 12h sucrose group administered RIM 3, compared to RIM 1, on session average ($p < .0001$). In the 24h sucrose groups, vehicle-treated rats were heavier than rats treated with the higher and lower RIM doses ($ps < .0001$). None of the other main effects or interactions were statistically significant.

3.3 Effects of CBI antagonism on sucrose reward

Figure 6A shows that all rats consumed significantly more sucrose than water across the CPP conditioning sessions (vehicle, $t(42) = 8.44$, RIM 1, $t(42) = 7.71$, RIM 3, $t(42) = 4.90$, all $ps < .01$). The significant main effect drug, $F(2, 42) = 3.57$, $p < .05$, partial $\eta^2 = .15$, indicated that

RIM significantly reduced overall consumption during conditioning sessions, with RIM 3 reducing total sucrose intake, compared to the vehicle group (*post-hoc*, $p < .05$). In contrast, RIM had no effect on the development of a sucrose CPP, $F(2,42) = .93$, $p = .40$, partial $\eta^2 = .15$, in that all three groups showed a preference for the sucrose-paired compartment (vehicle, $t(42) = 3.66$, $p < .01$; RIM 1, $t(42) = 2.13$, $p < .05$; RIM 3, $t(42) = 2.81$, $p < .01$) (**Figure 6B**).

We also tested the effects of RIM on a sucrose CPP in rats given either intermittent or continuous access to sucrose over 28 days. As with the previous two cohorts (see 3.1.1 and 3.2.), the 12h sucrose group developed binge-like intake (i.e., increased intake during the first hour of access) that was absent in the 24h group (data not shown). During subsequent CPP conditioning sessions, both groups consumed more sucrose than water across the 5 days (12h sucrose: vehicle, $t(84) = 6.62$, RIM 1, $t(84) = 5.28$, RIM 3, $t(84) = 6.33$; 12h sucrose: vehicle, $t(84) = 7.22$, RIM 1, $t(84) = 5.48$, RIM 3, $t(84) = 5.01$, all $ps < .01$), with no group $F(1,84) = .19$, $p = .66$, partial $\eta^2 = .01$ or drug, $F(2,84) = 1.38$, $p = .26$, partial $\eta^2 = .03$, differences on this measure (**Figure 6C**). During CPP testing (**Figure 6D**), however, only two groups displayed a significant preference for the sucrose-paired compartment: 24h sucrose plus vehicle, $t(84) = 4.11$, $p < .01$, and 24h sucrose plus RIM 1, $t(84) = 1.99$, $p < .05$. None of the other rats showed a preference for either the sucrose- or water-paired side during testing, all $ps > .05$.

4. Discussion

Our study confirms an important role for the ECS, specifically CB1Rs, in sucrose bingeing. More specifically, binge-like intake of sucrose modified ECS signaling in brain reward regions and CB1R blockade reduced this behavior in rats. CB1R antagonism, on its own, had no effect on the development of a sucrose CPP but blocked this effect in animals that had already consumed excessive amounts of sucrose. We propose that excessive sucrose intake modifies

ECS signals related to reward processing, rendering animals vulnerable to CB1R antagonism in the CPP paradigm. Taken together, our findings further the understanding of mechanisms sustaining sucrose bingeing, including how these relate to reward processing in brain regions that are also implicated in drug addiction.

Our study provides the first evidence that CB1R antagonism reduces binge-like intake of a sucrose solution, measured in animal models as excessive intake during the first hour of access (Avena *et al.*, 2005; Smail-Crevier *et al.*, 2018; Maracle *et al.*, 2019). The effect was manifested, specifically, during the final sessions of intermittent sucrose access following administration of RIM. Our findings corroborates previous evidence that CB1R blockade in rats dose-dependently decreases bingeing of palatable foods that are high in sugar (Dore *et al.*, 2014), sugar and fat (Parylak *et al.*, 2012), or fat only (Scherma *et al.*, 2013). The reduction in binge-like eating following RIM administration may reflect a general decline in palatable food intake (Dore *et al.*, 2014), although we did not observe an effect of drug on daily intake of sucrose, or on hourly intake in rats that did not exhibit binge-like eating (i.e., 24h sucrose group). Admittedly, these measures may be less sensitive to disruption by CB1R antagonism in that binge-like eating is an exaggerated response that occurs within a short period of time and, given the half-life of the drug, effects would have dissipated by two or three hours into the sessions (Jarbe, et al., 2010). Thus, although some details require further clarification, it is becoming increasingly clear that CB1Rs play an important role in over consumption of sweet foods.

Our post-mortem assessment revealed an interesting pattern of ECS-related changes following excessive sucrose intake. This included an upregulation of CB1R gene expression in the NAc, increased AEA levels in the PFC, and decreased 2-AG levels in the HPC; importantly, these changes were only apparent in rats that exhibited binge-like intake of sucrose. In contrast to

our findings, binge intake of high fat or high fat-high sugar foods decreased CB1R gene expression in the NAc (Bello *et al.*, 2012; Blanco-Gandía, Cantacorps, *et al.*, 2017), providing further evidence that the biological consequences of high fat and high sugar diets are dissociable (Avena, 2010). Our study is the first to assess brain endocannabinoid levels in an animal model of sucrose bingeing, supporting clinical reports of alterations in blood AEA levels in BED patients (Monteleone *et al.*, 2005). We also observed sucrose-induced decreases in ECS enzymes (FAAH and MAGL) that were not restricted to bingeing rats, suggesting that excessive sucrose intake, itself, may impact ECS function. Not surprisingly, these changes were limited to the NAc, an area that is closely associated with consumption of palatable food (Kelley *et al.*, 2005).

A role for the ECS in sucrose bingeing likely occurs through an interaction with brain reward systems. Indeed, both palatable food (Joseph & Hodges, 1990; Phillips *et al.*, 1993) and endocannabinoids (Solinas *et al.*, 2006) increase dopamine (DA) release in the NAc shell, with the latter acting via CB1Rs in this region (Sperlágh *et al.*, 2009). Moreover, NAc neurons encode signals related to food reward and this effect is attenuated by CB1R antagonism (Hernandez & Cheer, 2012; Thoeni *et al.*, 2020). CB1Rs appear to form heteromers with NAc opioid receptors (Rodriguez *et al.*, 2001; Rios *et al.*, 2006), which mediate the hedonic aspects of sweet foods (Peciña & Berridge, 2005). Intriguingly, excessive sucrose intake also increases binding of both mu opioid and DA D1 receptors in the NAc (Colantuoni *et al.*, 2001), pointing to an interaction between multiple transmitter systems in the control of palatable food intake. Finally, an ECS contribution to food reward may reflect a more general role for this system in reward processing in that both CB1Rs and endocannabinoids in the NAc play a role in reward linked to social interaction (Trezza & Vanderschuren, 2008; Trezza *et al.*, 2012; Wei *et al.*, 2015; Manduca *et al.*, 2016) and to a morphine CPP (Khaleghzadeh-Ahangar & Haghparast, 2015).

Modifications in ECS processes following extended sucrose access may explain our seemingly contradictory findings in the CPP paradigm. More specifically, in animals with no prior exposure to sucrose (i.e., rats that had not gone through the sucrose binge experiment), CB1R antagonism did not alter the development of a sucrose CPP. In contrast, the highest dose of RIM eliminated a CPP in rats that had consumed excessive amounts of sucrose during binge sessions (24h sucrose group). RIM had no effect in the 12h sucrose groups because these animals did not exhibit a CPP (i.e., there was nothing to block). The elimination of a sucrose CPP across all three drug conditions (vehicle, RIM 1, and RIM 3) in all 12h sucrose groups matches our earlier report (Smail-Crevier *et al.*, 2018), suggesting that the lack of a CPP in these animals is due to prior binge-like intake. We proposed, previously, that this behavior modifies brain reward systems, impacting the later expression of a CPP. Our current findings add to this discussion by indicating that excessive sucrose intake, itself, may also disrupt ECS function. That is, 28 days of sucrose consumption may have compromised ECS-linked reward processing, rendering animals susceptible to CB1R blockade in the CPP paradigm. This was manifested by the absence of a CPP in the 24h sucrose, 3 mg/kg RIM group.

An alternative interpretation is that the lack of CPP in RIM-treated animals reflects alterations in neural mechanisms of anxiety in that sucrose bingeing increases anxiety-like behavior in rats (Avena, Bocarsly, *et al.*, 2008), and the ECS plays an important role in regulating stress-related responses, such as anxiety (Morena *et al.*, 2016). In support of this idea, the 24h sucrose group exhibited ECS changes in the mPFC and HPC, brain regions that are particularly vulnerable to stress manipulations. Finally, the fact that CB1R antagonism reduced sucrose consumption during conditioning, but did not block a CPP during testing (i.e., in rats who had no prior experience with sucrose), is consistent with evidence showing a dissociation in

mechanisms that control these two processes (White & Carr, 1985; Papp *et al.*, 1991; Ågmo *et al.*, 1995; Smail-Crevier *et al.*, 2018).

To the extent that preclinical research is applicable to drug development, our findings suggest that cannabinoid compounds may be appropriate tools to treat BED. CB1Rs could be the most promising target in that CB1R expression is elevated in eating disorder patients (Frieling *et al.*, 2009). Moreover, CB1R antagonists reduce binge eating in both rats (Parylak *et al.*, 2012; Scherma *et al.*, 2013; Dore *et al.*, 2014) and humans (Pataky *et al.*, 2013), although at least one drug, SR141716 (Rimonabant), produces adverse events in BED patients (Pataky *et al.*, 2013). Selective targets of peripheral, rather than central, CB1Rs may mitigate these negative side effects. As an example, AM6545, a peripherally-restricted CB1R antagonist, diminishes intake of a high fat-high sugar diet in mice, with no alteration in consumption of standard chow (Argueta & DiPatrizio, 2017). In addition, cannabidiol, a CB1R antagonist with CB2R agonist activity, reduces sucrose intake in mice, an effect that is potentiated by CB1R blockade and inhibited by CB2R blockade (Bi *et al.*, 2020). Currently, there are only two drugs approved by the US food and drug administration to treat BED: lisdexamfetamine (Vyvanse), an inactive prodrug promoting the release of monoamines (McElroy *et al.*, 2015; Gasior *et al.*, 2017), and dasotraline, a DA and norepinephrine reuptake inhibitor (Grilo *et al.*, 2020). Lisdexamfetamine produces multiple adverse effects (Ward & Citrome, 2018) and dasotraline was recently withdrawn from drug development. Given the paucity of alternative therapies for BED (Hutson *et al.*, 2018), a strategic focus on ECS mechanisms may provide the best options for treating this disorder.

Finally, our study adds to the growing discussion concerning addictive-like properties of palatable food. On a general level, our model of sucrose bingeing shares a number of

commonalities with drug addiction (Bocarsly & Avena, 2013), including escalation of intake (Colantuoni *et al.*, 2001), signs of somatic withdrawal (Colantuoni *et al.*, 2002; Avena, Bocarsly, *et al.*, 2008), and neurochemical changes in the NAc, such as increased DA release (Avena *et al.*, 2005; Rada *et al.*, 2005) and reduced DA D2 receptor availability (Colantuoni *et al.*, 2001). Most telling, sucrose bingeing induces compulsive responding and electrophysiological changes in the bed nucleus of the stria terminalis (Maracle *et al.*, 2019) that match behavioral and biological consequences of cocaine self-administration in rats (Krawczyk, Georges, *et al.*, 2011; Krawczyk, Sharma, *et al.*, 2011). Further, food craving in humans is associated with enhanced activity in the HPC, NAc, insula, and orbitofrontal cortex (Pelchat *et al.*, 2004; Rolls & McCabe, 2007), the same regions that are activated in drug craving (Koob & Volkow, 2010; Everitt & Robbins, 2016). In addition, future experiments will investigate possible sex differences in the effects of binge intake on ECS processes. Given that baseline levels of endocannabinoids and reactivity towards cannabinoids are sex-dependent (for review see Fattore & Fratta, 2010; Craft *et al.*, 2013), we may expect distinct patterns of ECS changes in males and females following excessive intake of palatable food. These differences may contribute, at least partially, to the higher prevalence of eating disorders in females than males (Bulik *et al.*, 2015).

The ECS may provide a link between responses to palatable food and addictive behaviors, particularly as cannabinoid mechanisms are intimately linked to drug addiction (Silveira *et al.*, 2017). In support of this idea, CB1R antagonism reduces the proportion of animals that transition from controlled to compulsive feeding in a mouse model of addictive-like eating (Mancino *et al.*, 2015). At the same time, the concept of food addiction is not universally accepted (Ziauddeen *et al.*, 2012; Westwater *et al.*, 2016; Treasure *et al.*, 2018), with a growing awareness that research should focus on identifying biological underpinnings of specific

behavioral patterns that lead to excessive intake of food or drugs (Dileone *et al.*, 2012). Our findings inform this line of investigation by highlighting how endocannabinoid mechanisms in brain reward regions may contribute to binge eating.

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Author's contribution

Conceptualization: DN, MCO, KB designed the experiments and wrote the paper. Investigation: DN, VA, DF, KB performed the experiments; DN, RB, KB analyzed the data and designed the figures; GA and MCO conducted statistical analysis; YG, VA contributed to the design and analysis of the mass spectrometry experiment. Supervision and funding acquisition: MCO and KB supervised the study and obtained the funding. All authors approved the final version of the manuscript.

Conflict of interest. The authors declare no conflict of interest.

Data availability statement

All the data supporting the findings of this study can be provided upon request from the corresponding author.

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Figure Captions

Figure 1. Timeline of experimental procedures. Four separate experiments examined: ECS alterations following sucrose bingeing (Experiment 1), the effect of RIM on sucrose bingeing on days 1-5 and 24-28 of a 28-day sucrose access protocol (Experiment 2), the effect of RIM on a sucrose CPP (Experiment 3), and the effect of RIM on a sucrose CPP following sucrose bingeing (Experiment 4). CPP: conditioned place preference; ECS: endocannabinoid system; RIM: rimonabant.

Figure 2. Intermittent access to sucrose (12h sucrose) induces binge-like intake. (A) Solution intake (mL) per body weight (g) during the first hour of access across 28 days. (B) Daily solution intake (mL) per body weight (g) across sessions. (C) Daily food intake (g) per body weight (g) across sessions. (D) Body weight (g) across sessions. Data are presented as group means ($\pm$ SEM).

Figure 3. Intermittent access to sucrose modulates endocannabinoid gene expression in the NAc. Gene expression was examined in the PFC (n=7-8/group), NAc (5-10/group), DS (n=6-8/group), and HPC (n=7-10/group). (A) CB1R gene expression was significantly increased in the NAc of the 12h sucrose group compared to the other groups. CB2 gene expression remained unchanged. (B) FAAH gene expression was significantly reduced in the NAc of the 24h sucrose group. (C) DAGL α gene expression was significantly decreased in the NAc of both 12h and 24h sucrose groups. Data are presented as group means ($\pm$ SEM). * $p < .05$, ** $p < .01$, *** $p < .001$ compared to 12h food; # $p < .05$, compared to 24h sucrose group. CB1/2: cannabinoid receptor 1/2; DS: dorsal striatum. HPC: hippocampus; NAc: nucleus accumbens; PFC: prefrontal cortex.

Figure 4. Intermittent access to sucrose alters endocannabinoid levels. Endocannabinoid levels were examined in the PFC, NAc, DS, and HPC. (A) AEA levels were significantly

increased in the PFC of the 12h sucrose group. **(B)** 2-AG levels were significantly lower in the HPC of the 12h sucrose group. Data are presented as group means (+SEM). * $p < .01$: compare to 12h food. 2AG: 2-arachidonoylglycerol; AEA: anandamide; DS: dorsal striatum; HPC: hippocampus; NAc: nucleus accumbens; PFC: prefrontal cortex.

Figure 5. Rimonabant decreases binge-like intake of sucrose. **(A)** Solution intake (mL) per body weight (g) during the first hour of access across 28 days (left), and total intake on the drug injection days (days 1-5 and 24-28) for the 12h sucrose group (right). **(B)** Daily solution intake (mL) per body weight (g) (top), daily food intake (g) per body weight (g) (middle), body weight (g) (bottom) across sessions, for animals under a 12 h (right panels) or 24h sucrose access (left panels) protocol. Data are presented as group means ($\pm$ SEM). RIM 1: rimonabant 1 mg/kg; RIM 3: rimonabant 3 mg/kg; Veh: vehicle.

Figure 6. Rimonabant blocks a sucrose CPP following excessive sucrose consumption. **(A)** Effect of RIM on total solution intake across conditioning sessions (Experiment 3). **(B)** Time spent in each compartment during CPP testing (Experiment 3). **(C)** Effect of RIM on total solution intake across conditioning sessions, following sucrose consumption sessions (Experiment4). **(D)** Time spent in each compartment during CPP testing following sucrose consumption sessions (Experiment 4). Data are presented as group means (+SEM). * $p < .05$, ** $p < .01$. CPP: conditioned place preference; RIM 1: rimonabant 1 mg/kg; RIM 3: rimonabant 3 mg/kg; Veh: vehicle

Table 1. Primer sequences for reference (Rplp0) and endocannabinoid system genes.