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## Characterization of a shortened model of diet alternation in female rats: effects of the CB<sub>1</sub> receptor antagonist rimonabant on food intake and anxiety-like behavior

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### Abstract

The prevalence of eating disorders and obesity in Western societies is epidemic and increasing in severity. Preclinical research focused on the development of animal models which can mimic the maladaptive patterns of food intake observed in certain forms of eating disorders and obesity. This study was aimed at characterizing a recently established model of palatable diet alternation in female rats. For this purpose, female rats were fed either continuously with a regular chow diet (*Chow/Chow*) or intermittently with a regular chow diet for 2 days and a palatable, high-sucrose diet for 1 day (*Chow/Palatable*). Following diet cycling, rats were administered rimonabant (0, 0.3, 1, 3 mg/kg *i.p.*) during access to either palatable diet or chow diet and were assessed for food intake and body weight. Finally, rats were pretreated with rimonabant (0 – 3 mg/kg, *i.p.*) and tested in the elevated plus maze during withdrawal from the palatable diet. Female rats with alternating access to palatable food cycled their intake, overeating during access to the palatable diet, and under-eating upon returning to the regular chow diet. Rimonabant treatment resulted in increased chow hypophagia and anxiety-like behavior in *Chow/Palatable* rats. No effect of drug treatment was observed on the compulsive eating of palatable food in the diet cycled rats. The results of this study suggest that withdrawal from alternating access to the palatable diet makes individuals vulnerable to the anxiogenic effects of rimonabant and provide etiological factors potentially responsible for the emergence of severe psychiatric side-effects following rimonabant treatment in obese patients.

### Keywords

Eating disorders; diet cycling; palatable food; withdrawal; rimonabant; female; rat

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## Introduction

The prevalence of eating disorders and obesity in Western societies is epidemic and increasing in severity. One of the recognized causes of pathological overeating is the increased availability of palatable, calorie-dense foods, high in fats and refined sugars, which are more rewarding than low calorie foods. Individuals who repeatedly try to abstain from obesogenic foods by switching to safer low energy alternatives ultimately crave highly palatable foods, relapse and binge eat (Polivy and Herman, 1985). Vicious circles of abstinence and uncontrollable eating of palatable food have been proposed to share similarities with the intoxication/withdrawal cycles observed in drug addiction (Corwin and Grigson, 2009; Cottone *et al*, 2012).

In recent years, preclinical research has focused on the development of animal models that can mimic the maladaptive patterns of food intake observed in certain forms of disordered eating. A widely accepted approach to induce overeating is to provide intermittent access to highly palatable diets in free-fed experimental animals (Hagan and Moss, 1997; Avena *et al*, 2006; Cifani *et al*, 2009; Cottone *et al*, 2009b; Corwin *et al*, 2011; Parylak *et al*, 2012; Velazquez-Sanchez *et al*, 2014). This strategy allows the development of excessive intake of highly palatable food as well as undereating of safer alternatives. It is based on the relative palatability of the different diets provided to animals rather than on energy alternations induced by food restriction.

In an effort to better understand the etiological factors of maladaptive feeding behavior, we have recently developed a model of diet alternation in male Wistar rats which is characterized by short 3-day cycles of access to differentially rewarding diets (Dore *et al*, 2013b). In this procedure, subjects quickly escalated food intake and increased body weight gain when intermittently fed the palatable diet for 1 day. The palatable diet was cyclically withdrawn for 2 days and replaced with a regular chow diet, during which period male rats developed spontaneous and progressively increasing hypophagia and body weight loss (Dore *et al*, 2013b).

Whether female rats under a shortened palatable diet cycling procedure would show a maladaptive feeding behavior is currently unknown. The female sex is generally underrepresented in preclinical research, limiting the study of eating disorders, which have been extensively documented to occur to a greater extent in women compared to men (Oakley Browne *et al*, 2006; Hudson *et al*, 2007; Hay *et al*, 2008; Preti *et al*, 2009). Indeed, women show earlier onsets of eating disorders (Kessler *et al*, 2013) and worse outcomes compared to men (Stoving *et al*, 2011).

Therefore, the first aim of this study was to characterize a shortened model of diet alternation in female rats. For this purpose we monitored food intake, body weight and anxiety-like behavior in female rats which received cycles of 2 days of access to a regular chow diet (C phase) followed by 1 day of access to a highly palatable, sugary diet (P phase).

The endocannabinoid system represents a promising target for the development of novel pharmacological agents that reduce food intake (Di Marzo, 2008; Kirkham, 2009; Berner *et al*, 2011; DiPatrizio and Piomelli, 2012; Mendez-Diaz *et al*, 2012). Antagonism of the

type-1 cannabinoid (CB<sub>1</sub>) receptor has been demonstrated to be a successful pharmacological approach for overeating and obesity. However, the CB<sub>1</sub> receptor antagonist rimonabant, initially approved for the treatment of obesity, was withdrawn from the market shortly after due to serious psychiatric side effects, including the emergence of a negative emotional affect (Akbas *et al*, 2009; Moreira *et al*, 2009).

Therefore, the second aim of our study was to investigate whether the endocannabinoid system (eCB) plays a role in the consummatory and emotional outcomes of the shortened diet cycling procedure in female rats. For this purpose, we investigated the effects of systemic rimonabant administration on *i*) escalated excessive intake of palatable diet *ii*) regular chow hypophagia, and *iii*) anxiety-like behavior during withdrawal from palatable food.

## Methods

### Subjects

Female Wistar rats ( $N=20$ ), 41–47 days old at arrival weighing 126–150 g (Charles River, Wilmington, MA), were single housed in wire-topped, plastic cages (27 × 48 × 20 cm) in a 12-hour reverse light cycle (lights off at 11:00h), in an AAALAC-approved humidity- (60%) and temperature-controlled (22°C) vivarium. Rats had access to corn-based chow (Harlan Teklad LM-485 Diet 7012 [65% (kcal) carbohydrate, 13% fat, 21% protein, metabolizable energy 310 cal/100 g; Harlan, Indianapolis, IN]) and water *ad libitum* at all times, unless otherwise specified. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85-23, revised 1996) and the Principles of Laboratory Animal Care (<http://www.nap.edu/readingroom/books/labrats>), and were approved by the Institutional Animal Care and Use Committees (IACUC) of Boston University.

### Development of a shortened, ad libitum palatable diet alternation in female rats

Intermittent, extended access to a palatable diet procedure in female rats was performed using a protocol previously described in male rats only (Dore *et al*, 2013b) and shortened from previous protocols (Cottone *et al*, 2008a, 2009a,b; Iemolo *et al*, 2012), in order to produce a faster experimental cycling procedure. After 1 week of acclimation, rats were divided into two groups of ten subjects, matched for food intake, body weight and feed efficiency from a 3–4 days baseline period. One group was then provided a chow diet ('Chow') *ad libitum* (Chow/Chow), whereas a second group was provided chow *ad libitum* for 2 days, followed by 1 day of *ad libitum* access to a highly palatable, chocolate-flavored, high-sucrose diet ('Palatable'; Chow/Palatable). The chow diet was the above described Harlan Teklad LM-485 Diet 7012. The palatable diet was a nutritionally complete, chocolate-flavored, high-sucrose (50% kcal), AIN-76A-based diet that is comparable in macronutrient proportions and energy density to the chow diet [chocolate-flavored Formula 5TUL: 66.7% (kcal) carbohydrate, 12.7% fat, 20.6% protein, metabolizable energy 344 cal/100 g; TestDiet, Richmond, IN; formulated as 45 mg precision food pellets to increase its preferredness]. For brevity, the first 2 days (chow only) and the following 1 day (chow or palatable according to experimental group) are referred in all experiments as phases C and P.

Diets were never concurrently available. Food intake and body weight were measured daily. Average food intake was calculated as the kcal intake in a certain phase divided by the number of days of that phase (2 for phase C, 1 for phase P). Average body weight change was calculated as the difference between the body weight at the end and at the beginning of a phase divided by the number of days of that phase (2 for phase C, 1 for phase P). Average and cumulative feed efficiency was calculated as mg body weight gained in a certain time interval (phase or cycle) divided by the kcal food intake in the same time interval. Cumulative food intake and cumulative body weight gain was calculated as the kcal food intake or body weight gained at the end of each cycle since the beginning of the study. To evaluate the escalation of palatable diet intake, food intake was measured 1 h after the diet switch at the beginning of each P phase. For the time course studies, food intake was measured at the beginning of either the C or the P phase (at the time of the diet switch), and then 1, 3, 6 and 24 h later. As previously published (Cottone *et al*, 2008a, 2009a,b) the 5TUL Chocolate Diet (sugary palatable diet) was uniformly preferred compared to the Harlan LM-485 chow diet.

### Effects of rimonabant on intake of regular chow and palatable diet

Rimonabant (0, 0.3, 1, 3 mg/kg, *i.p.*, –30 minutes) was administered at the onset of either the P or the C phase, in a within-subject Latin square design. Rats were then provided with a pre-weighed amount of food, and intake was recorded 1, 3, and 24 h later. Body weights were recorded immediately before and 24 h following drug administration.

### Effects of rimonabant on anxiety-like behavior

The elevated plus-maze apparatus (Cottone *et al*, 2007, 2008b; Dore *et al*, 2013a) was made of black Plexiglas and consisted of four arms (50 cm long 10 cm wide). Two arms had 40-cm-high dark walls (enclosed arms), and two arms had 0.5-cm-high ledges (open arms). The maze was elevated to a height of 50 cm. Open arms received 1.5–2.0 lux of illumination. Animals were habituated to the anteroom the day before testing. On the day of testing, rats were kept in the quiet, dark anteroom for at least 2 h before testing. White noise (70 dB) was present throughout habituation and testing. For testing anxiety-like behavior induced by withdrawal from spontaneous palatable food, rats were placed individually onto the center of the maze facing a closed arm and were then removed after a 5-min period. The primary measures were the percentage of total arm time directed towards the open arms [i.e.,  $100 \times \text{open arm} / (\text{open arm} + \text{closed arm})$ ], a validated index of anxiety-related behavior (Fernandes and File, 1996) and the number of closed arm entries, a specific index of locomotor activity (Cruz *et al*, 1994). During the 12th cycle, *Chow/Palatable* rats were tested in a between-subjects design 5h after the switch from the palatable diet to chow. The elevated plus-maze test was repeated after 26 cycles of diet alternation, 5h after the switch from the palatable diet to chow. Allowing 3 weeks later or more before re-testing prevents an increase in the time spent in the open arms (Adamec and Shallow, 2000; Adamec *et al*, 2005; Walf and Frye, 2007). During this second assessment, subjects were pretreated with rimonabant (0, 3 mg/kg, *i.p.*, –30 minutes). *Chow/Chow* control rats were tested concurrently in a between-subjects design. Chow diet was available *ad libitum* until the time of testing.

## Drug

Rimonabant [SR141716A or 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide × HCl] was synthesized as reported previously (Aggarwal *et al*, 2008); it was solubilized in an 18:1:1 mixture of saline : ethanol : cremophor and administered i.p. (0, 0.3, 1, 3 mg/kg, 1 ml/kg, 30 minutes pre-treatment).

## Statistical analysis

Average daily food intake, average daily body weight change and average daily feed efficiency were analyzed using three-way mixed analyses of variance (ANOVAs), with Diet Schedule (the schedule of food access, either continuous access to chow (*Chow/Chow*) or alternating access to chow and palatable diet (*Chow/Palatable*)) as a between-subjects factor, Phase (each period of 2 days (Phase C, chow only) or 1 day (chow or palatable according to experimental group)) and Cycle (a 3-day period consisting of a C and a P Phase) as within-subject factors. Intake in the first hour of palatable food consumption, cumulative food intake, cumulative body weight change and cumulative feed efficiency were analyzed using two-way ANOVAs with Diet Schedule as a between-subjects factor and Cycle as a within-subjects factor. To analyze the time course of ingestion following rimonabant treatment, three-way ANOVAs on incremental food intake were performed, with Diet Schedule as a between-subjects factor and Time (time points at which food intake was recorded) and Treatment (doses of the drug), as within-subjects factors. The effects of rimonabant on the percent of time spent in the open arms and the closed arms entries were analyzed using two-way ANOVAs, with Diet Schedule and Treatment as between-subjects factors. Pairwise effects were interpreted using Fisher's least significant difference tests. The software/graphic packages used were Systat 12.0, SigmaPlot 11.0 (Systat Software Inc., Chicago, IL), InStat 3.0 (GraphPad, San Diego, CA) and Statistica 7.0 (Statsoft, Inc., Tulsa, OK).

## Results

### Effects of diet alternation on food intake, body weight and feed efficiency

Alternating access to palatable food altered the daily food intake in a diet-specific manner [Cycle × Diet Phase × Diet Schedule:  $F(7,126)=8.66$ ,  $p<0.001$ ; Figure 1A]. Following the first access to the palatable food, the *Chow/Palatable* group started to overeat [ $t(18)=4.62$ ,  $p<0.001$ ], whereas the first withdrawal from palatable food induced significant hypophagia compared to control intake [ $t(18)=3.66$ ,  $p<0.02$ ]. Similarly to what was observed in male rats (Dore *et al*, 2013b), female rats with alternating access to food cycled their intake, overeating during access to palatable diet, and undereating upon returning to the regular chow diet. Both the overeating and the hypophagia progressively increased in magnitude as a function of the number of cycles. The magnitude of overeating in the *Chow/Palatable* group during the P phases [+213.03 kcal, 38.92% relative to the *Chow/Chow* group during the 1-day P phase, across 8 cycles] was not significantly different from the hypophagia of the regular chow during the C Phases [-200.94 kcal, -18.86% relative *Chow/Chow* group during the 2-day C phase across eight cycles], resulting in no significant difference in cumulative energy intake between the two groups [Diet Schedule  $F(1,18)=0.34$ , NS, Cycle × Diet Schedule:  $F(7,126)=0.04$ , NS; Figure 1B]. In addition, the *Chow/Palatable* group

cycled their body weight and feed efficiency [average body weight gain Cycle  $\times$  Diet Phase  $\times$  Diet Schedule  $F(7,126)=6.52$ ,  $p<0.001$ ; average feed efficiency Cycle  $\times$  Diet Phase  $\times$  Diet Schedule  $F(7,126)=6.20$   $p<0.001$ ; Figures 1C and 1E]. Beginning on the 3rd access to palatable food and until the 8th cycle, *Chow/Palatable* rats gained significantly more body weight compared to control *Chow/Chow* rats. In addition, *Chow/Palatable* rats started losing absolute body weight at the beginning of the 5th cycle. Finally, analysis of cumulative body weight [Diet Schedule  $F(1,18)=1.81$ , NS; Cycle  $\times$  Diet Schedule: [ $F(7,126)=1.64$ , NS; Figure 1D] and cumulative efficiency [Diet Schedule  $F(1,18)=0.64$ , NS; Cycle  $\times$  Diet Schedule: [ $F(7,126)=0.79$ , NS; Figure 1F] did not show any significant difference between groups throughout the eight cycles.

### Effects of diet alternation on escalation of palatable food consumption

Figure 2 shows the effects of diet alternation on food intake during the first hour of access to the palatable diet. The *Chow/Palatable* group progressively and dramatically escalated the intake of the palatable diet during the first hour of access, as a function of the number of cycles [Cycle  $\times$  Diet Schedule:  $F(7,126)=6.88$ ,  $p<0.001$ ]. The intake of diet-cycled rats became significantly higher than the intake of chow in control rats, starting from the first palatable diet access; by the 7th access, *Chow/Palatable* group were able to consume approximately 5.0-fold the intake of *Chow/Chow* rats [M  $\pm$  SEM;  $22.7 \pm 2.5$  kcal. versus  $4.9 \pm 1.1$  kcal respectively;  $t(18)=6.65$ ,  $p<0.001$ ]. This escalation was experience-dependent, as suggested by the strong fit with the sigmoidal associative learning curve (M  $\pm$  SEM: *min*:  $6.7 \pm 1.7$ , *max*:  $22.4 \pm 2.6$ , *ET*<sub>50</sub>:  $2.9 \pm 0.6$ , *Hill slope*:  $2.5 \pm 1.3$ ,  $r^2=0.93$ ,  $p<0.001$ ).

### Effects of rimonabant treatment on food intake and body weight

Administration of rimonabant reduced food intake in both *Chow/Chow* and *Chow/Palatable* rats regardless of the Diet Schedule [Dose:  $F(3,54)=21.24$ ,  $p<0.001$ ; Dose  $\times$  Diet Schedule:  $F(3,54)=0.33$ , NS; Figure 3]. However, the analysis of the time course of the anorectic effect of rimonabant revealed that drug treatment reduced food intake with different potency in the *Chow/Palatable* and *Chow/Chow* groups. Indeed, rimonabant dose-dependently reduced food intake and body weight in *Chow/Chow* rats with a minimum effective dose of 1 mg/kg across the 24 hour measurement window. However, drug treatment resulted in increased anorexia of chow during withdrawal from palatable food in *Chow/Palatable* rats, reducing food intake at the lowest dose injected (0.3 mg/kg) during the first hour of treatment, and therefore more potently compared to *Chow/Chow* control rats. During the first hour the effect size, calculated as % of reduction compared to vehicle condition following treatment with rimonabant 0.3, 1, 3 mg/kg, was 72.03%, 68.53% and 86.01% for the *Chow/Palatable* group and 31.82%, 57.05%, 69.55% for the *Chow/Chow* group. Conversely, when rimonabant was administered in *Chow/Palatable* rats during the P phase, only a nonsignificant trend towards a reduction was observed.

Two-way ANOVA revealed that the anorectic effect of rimonabant lasted up to 24 h [Dose:  $F(3,54)=7.08$ ,  $p<0.001$ ; Figure 4A]; however, at this time point, drug treatment was selective for *Chow/Chow* control rats as it did not affect *Chow/Palatable* food intake. Similar results were obtained when body weight gain was analyzed [Dose:  $F(3,54)=3.28$ ,  $p<0.05$ ; Figure 4B].

## Effects of diet alternation and rimonabant treatment on anxiety-like behavior

*Chow/Palatable* rats were first tested for spontaneous anxiety-like behavior induced by palatable food withdrawal during the first day of the C phase of the 12th cycle. As shown in Figure 5A, diet-cycled rats did not show any spontaneous anxiety-like behavior when compared to control *Chow/Chow* rats [% open arm time,  $t(18)=0.07$ , NS; closed arm entries,  $t(18)=0.14$ , NS].

*Chow/Palatable* rats were also tested for rimonabant-induced anxiety-like behavior later in the cycling procedure, during the first day of the C phase of the 38<sup>th</sup> cycle. As shown in Fig. 5B, a two-way ANOVA revealed that rimonabant administration selectively reduced the percentage of time spent in the open arms, i.e. showing an anxiogenic-like effect, in *Chow/Palatable* rats, but not in *Chow/Chow* rats [Dose:  $F(1,15)=6.31$ ,  $p<0.05$ ]. No significant differences between groups were detected in the number of closed arm entries, an index of locomotor activity [Dose:  $F(1,15)=1.39$ , NS]

## Discussion

The results of the present study show that female rats, undergoing a protocol of shortened intermittent access to a palatable diet recently established in our laboratory (Dore *et al*, 2013b), developed feeding behavior adaptations characterized by cycles of overeating and spontaneous rejection of the otherwise acceptable chow diet. Our results are similar to those observed in significantly longer diet-cycling procedures (Cottone *et al*, 2009b). The present findings also show that blockade of CB<sub>1</sub> receptor resulted in increased anorexia of regular chow diet in palatable food withdrawn female rats, without affecting the intake of palatable food. In addition, cycled female rats withdrawn from the palatable diet did not show any sign of spontaneous anxiety-like behavior, but revealed a markedly increased vulnerability to the precipitating anxiogenic effects of CB<sub>1</sub> receptor blockade.

In this study, we show that female rats undergoing the shortened diet alternation procedure displayed a pattern of spontaneous cycling of food intake, body weight and feed efficiency which resembles the food adaptations observed during longer food alternation procedures (Cottone *et al*, 2009a,b; Iemolo *et al*, 2012). Similarly to what has been observed in male rats (Dore *et al*, 2013b), incremental food intake, body weight and feed efficiency of female rats dramatically cycled across the eight cycles of observation; however, cumulatively, these variables did not differ between groups. This represents a major advantage of the task, as potential energy-homeostasis confounding factors can then be excluded.

In this study, female rats dramatically escalated food intake during the first hour of access to the palatable diet throughout the eight cycles of the study. The escalated intake peaked during the 7th access to the palatable diet where *Chow/Palatable* rats ate ~5 fold the intake of *Chow/Chow* rats. Interestingly, the intake during the first hour of renewed access increased to a much greater extent (*Chow/Palatable* first hour intake as % increase compared to *Chow/Chow*: Cycle 1, ~+100%; Cycle 8, ~+378%), compared to the moderate increase in daily palatable food intake (*Chow/Palatable* daily intake as % increase compared to *Chow/Chow*: Cycle 1, ~+34%; Cycle 8, ~+53%), Therefore, the mechanism behind the escalation of palatable diet intake was mostly induced by a gradual shift of the intake

towards the beginning of the renewed access, rather than an overall increase in feeding behavior. “Escalation” is a phenomenon observed with extended access to many different drugs of abuse, such as cocaine and heroin (Ahmed *et al*, 2000; Park *et al*, 2013). It is believed that escalation towards uncontrolled taking is a key factor and a hallmark of drug addiction (Koob and Kreek, 2007).

The spontaneous and progressive under-eating of the otherwise acceptable chow diet has been demonstrated to be independent from energy-homeostasis mechanisms (Cottone *et al*, 2008a) and it has been proposed to result either from a devaluation process due to the recent experience with the more rewarding alternative (Flaherty and Rowan, 1986; Iemolo *et al*, 2013) or from the aversive state induced by a palatable food withdrawal, similarly to what is observed with drugs of abuse (Parylak *et al*, 2011).

A major difference between the findings of the present study and the results obtained using the same procedure in male rats (Dore *et al*, 2013b) is related to the intake of palatable food during the first access. While diet-cycled female rats significantly overate during the first hour of access to the palatable diet compared to chow control rats, male rats ate similarly to chow fed rats (Dore *et al*, 2013b). A possible explanation for the observed difference could be related to sex differences in neophobia (Cooke *et al*, 2007), an adaptive response that protects individuals from the possible harmful post-ingestive consequences of unfamiliar tastants (Corey, 1978; Birch, 1999).

In the present paper, we show that female rats withdrawn from the highly palatable diet did not differ from controls in the percent of open arm time in the elevated plus-maze test. This finding contrasts with the observation that female rats, which underwent an extended diet-cycling procedure, show spontaneous anxiety-like behavior when withdrawn from the highly palatable diet (Cottone *et al*, 2009b), and suggests that a shorter diet-cycling procedure is not sufficient to produce a spontaneous negative emotional state. However, when palatable food withdrawn female rats were systemically treated with rimonabant, a dramatic anxiogenic-like effect was observed, selectively in diet-cycled rats. Furthermore, rimonabant, administered during withdrawal from palatable food, also resulted in increased anorexia during the first hour of treatment (Blasio *et al*, 2013).

It is important to highlight that the primary goal of this study was to investigate the negative emotional state during abstinence from a highly palatable diet following rimonabant treatment rather than possible physical signs of withdrawal (i.e. wet-dog shakes, forepaw flutter, ptosis, etc.) (Gellert and Holtzman, 1978). The reason behind this choice is that extensive literature has highlighted the contribution to the addiction process of anxiety, irritability and dysphoria, when access to the drug is prevented (Koob and Le Moal, 2001; Koob and Volkow, 2010). Indeed, it has been argued that the development of such a negative affective state contributes to compulsivity through negative reinforcement mechanisms (Koob *et al*, 2001; Koob *et al*, 2010). Future studies will be needed to clarify whether rimonabant can also precipitate physical signs of withdrawal when access to palatable food is prevented.



Contrarily to what was observed with the regular chow diet, when the effects of rimonabant were evaluated on the overeating of the palatable diet, only a nonsignificant trend towards a reduction was observed. This result is consistent with previous observations showing that intermittent access to palatable diet decreases the effectiveness of anorectic effects of CB<sub>1</sub> receptor blockade (Parylak *et al*, 2012; Blasio *et al*, 2013). Indeed, treatment with the CB<sub>1</sub> receptor antagonist, SR147778, was less effective in reducing binge eating of a sweet fat diet in female rats (Parylak *et al*, 2012). Moreover, rimonabant was ineffective in reducing overeating of a palatable diet in female rats that underwent a longer palatable food cycling procedure (Blasio *et al*, 2013). On the other hand, we have previously shown that when using the same intermittent access procedure as used here in male rats, excessive palatable food intake is blocked with an increased potency compared to chow. Therefore, a possible explanation for the divergent effects observed could be due to the different sex tested, since female rats undergoing intermittent access to palatable food develop overeating, which is generally less sensitive to the anorectic effects of CB<sub>1</sub> receptor blockade.

In summary, we have shown the consummatory and emotional effects of a shortened diet alternation procedure in female rats. The procedure described here in female rats has the advantage of being considerably faster than previously established protocols of palatable diet alternation (Hagan *et al*, 1997; Cifani *et al*, 2009; Cottone *et al*, 2009b; Rossetti *et al*, 2013). In addition, our results show that withdrawal from alternating access to the palatable diet makes individuals vulnerable to the anxiogenic effects of rimonabant. More generally our findings support the hypothesis that the emergence of severe psychiatric side-effects following rimonabant treatment in obese patients (Akbas *et al*, 2009; Moreira *et al*, 2009) could result from a worsening negative emotional state in a subpopulation of individuals abstaining from palatable foods in the attempt to lose weight.

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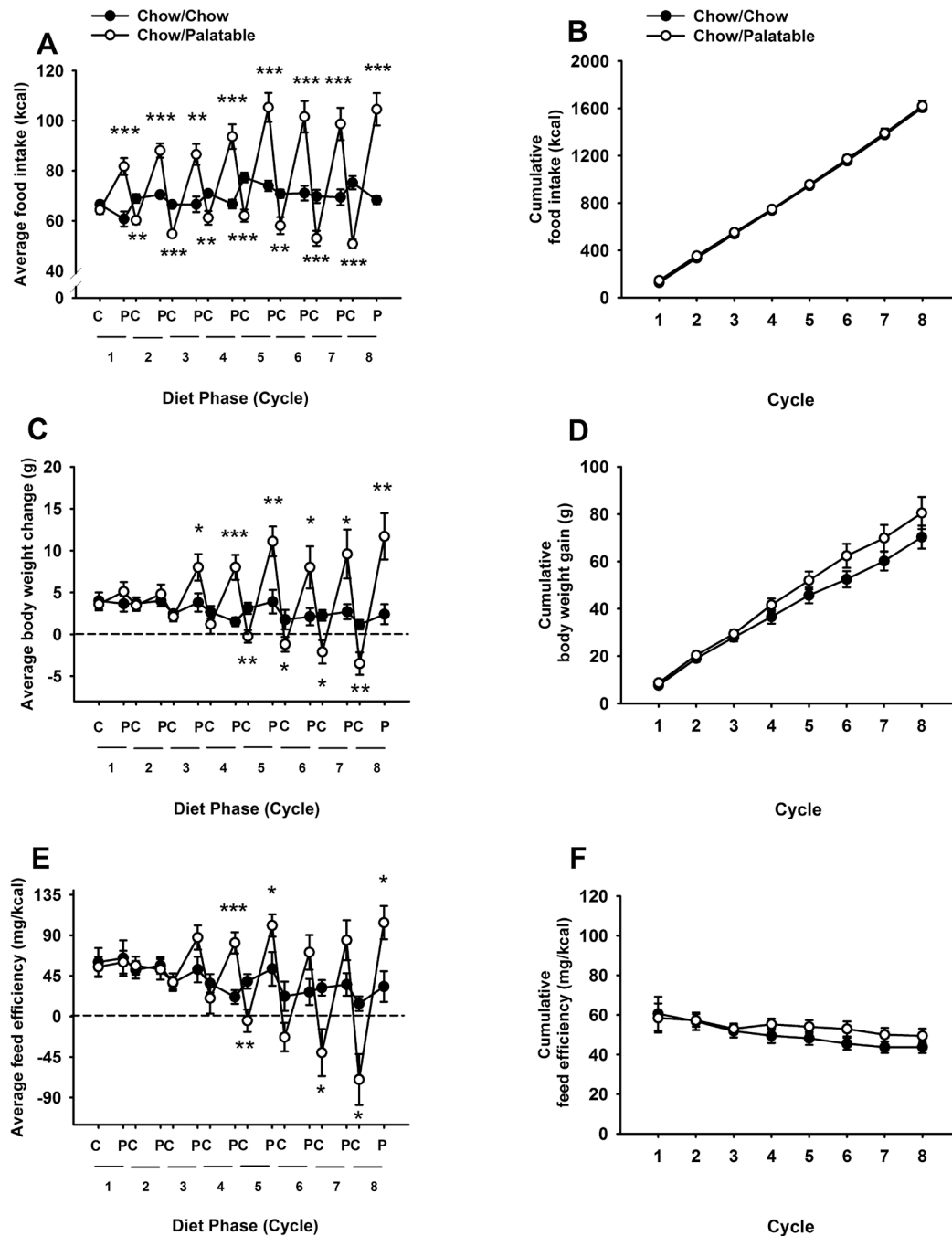
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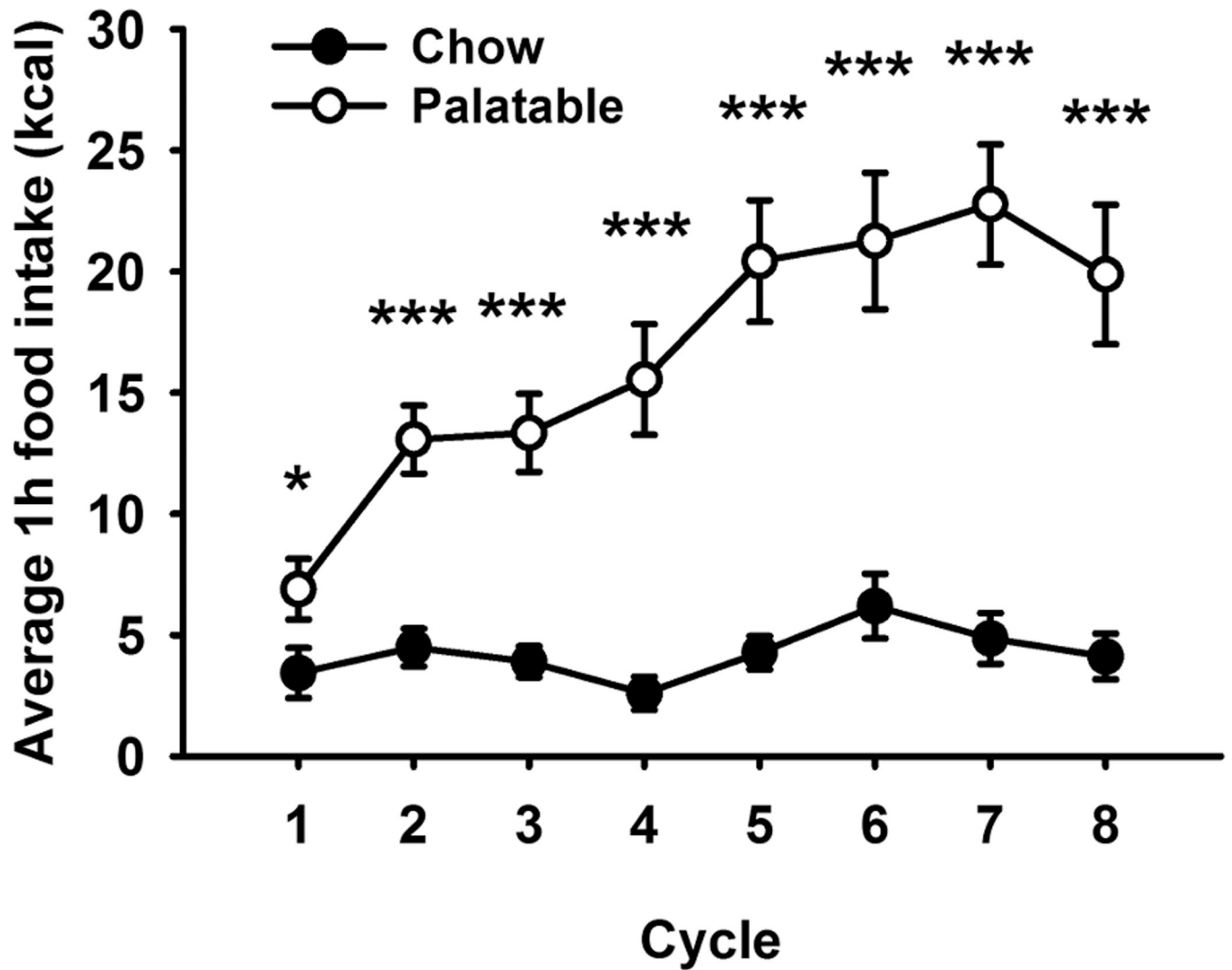
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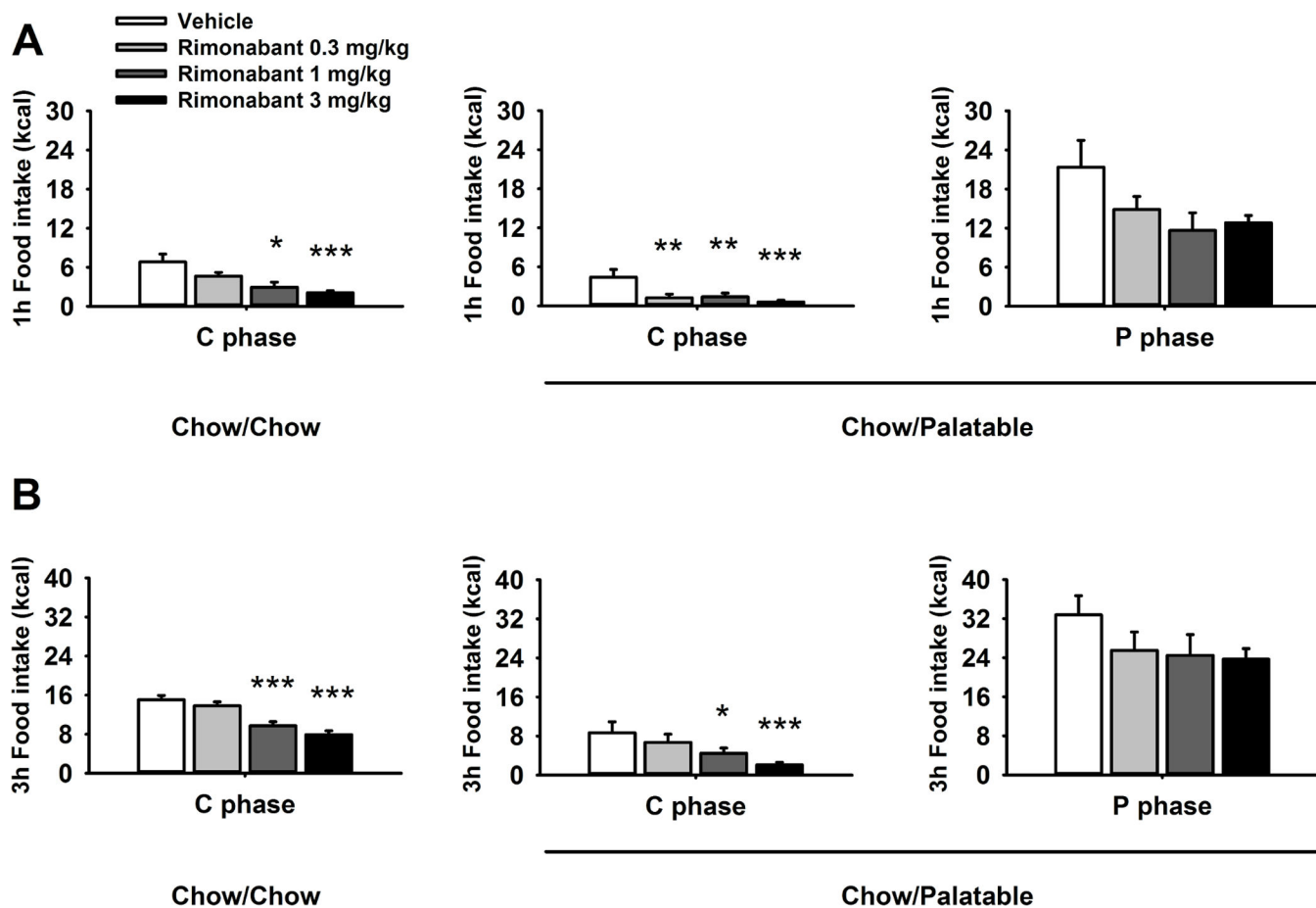
**Figure 1.**

Effects of intermittent, extended access to a palatable diet on (A) average daily food intake, (B) cumulative food intake, (C) average daily body weight change, (D) cumulative body weight change, (E) average daily feed efficiency, and (F) cumulative feed efficiency in female Wistar rats ( $N=20$ ). Values for phase C represent the average of 2 days access to chow food. Data represent mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , relative to the Chow/Chow group.

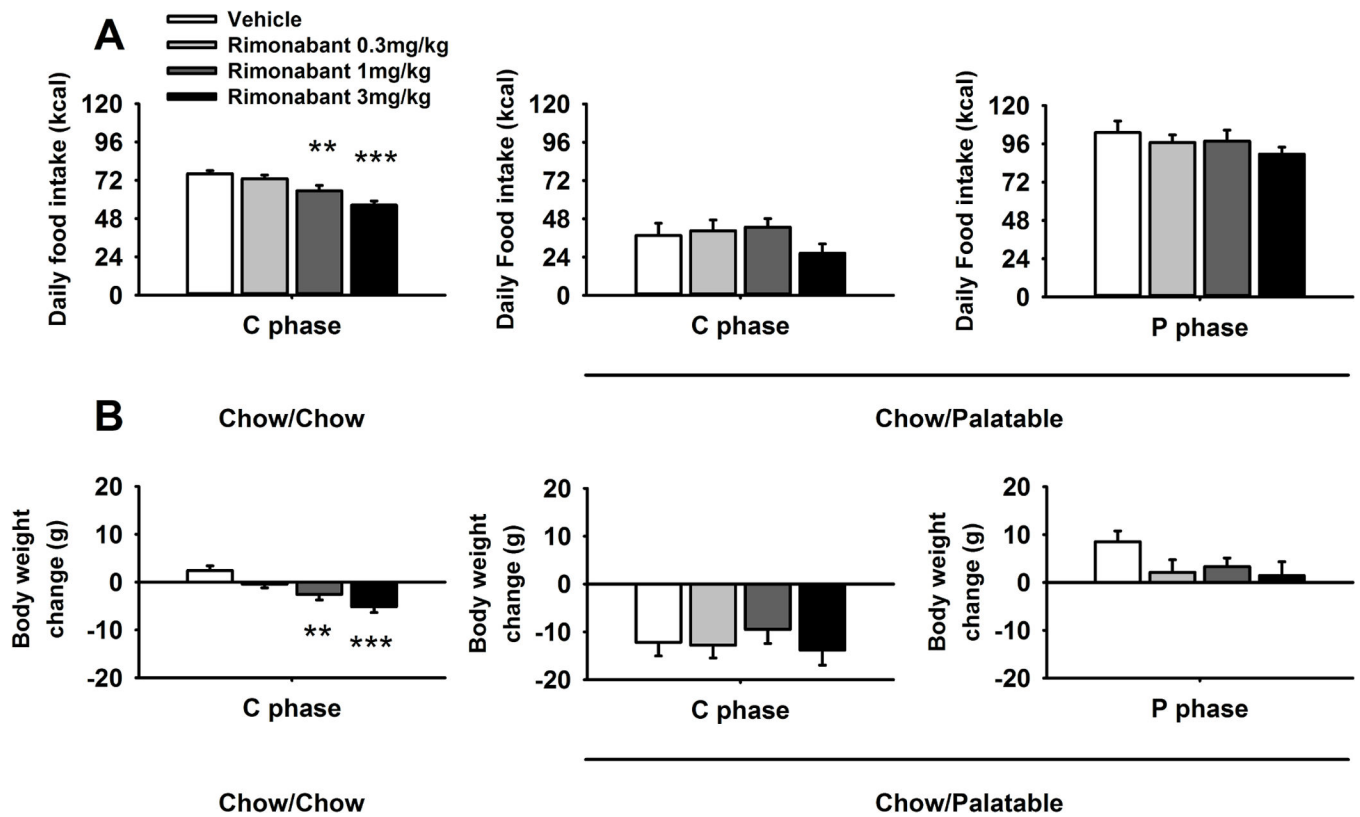


**Figure 2.**

Effects of intermittent, extended access to a palatable diet on food intake during the first hour of the P phase (when *Chow/Palatable* rats are fed the sugary, palatable diet) in female Wistar rats ( $N=20$ ). Data represent mean  $\pm$ SEM. \*  $p < 0.05$ , \*\*\* $p < 0.001$ , relative to the *Chow/Chow* group.

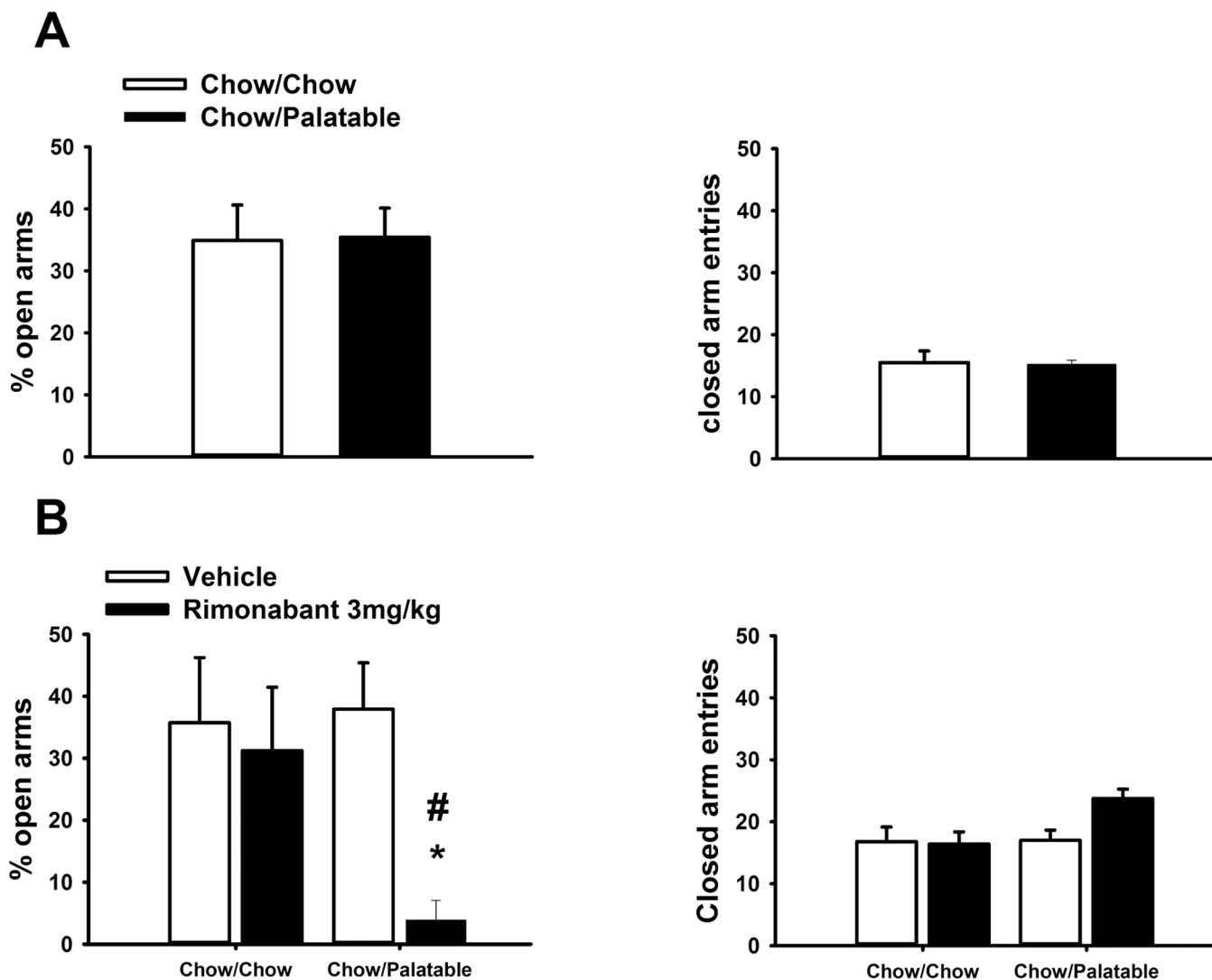


**Figure 3.** Effects of pre-treatment (–30 min) with rimonabant (0, 0.3, 1 and 3 mg/kg, *i.p.*) on (A) 1 h, and (B) 3 h food intake of *Chow/Chow* and *Chow/Palatable* female Wistar rats ( $N=20$ ). Drug treatment was performed in both C (first day of chow diet) and P phases. Data represent mean + SEM. Symbols denote significant difference from the group \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , relative to the Vehicle-treated group.



**Figure 4.** Effects of pre-treatment (–30 min) with rimonabant (0, 0.3, 1 and 3 mg/kg, *i.p.*) on 24 h (A) food intake and (B) body weight change of *Chow/Chow* and *Chow/Palatable* female Wistar rats ( $N=20$ ). Drug treatment was performed in both C (first day of chow diet) and P phases. Data represent mean  $\pm$  SEM.  $**p < 0.01$ ,  $***p < 0.001$  relative to the Vehicle-treated group.





**Figure 5.** Effects of pretreatment with rimonabant (30 min pretreatment, 0, 3 mg/kg, *i.p.*) on anxiety-like behavior in female Wistar rats ( $N=20$ ) withdrawn from chronic, intermittent access to palatable diet. (A) Spontaneous anxiety-like behavior in the first day of Phase C during the 12<sup>th</sup> cycle, measured using the elevated plus-maze test. (B) Rimonabant-induced anxiety-like behavior in the *Chow/Palatable* group, in the first day of Phase C during the 38<sup>th</sup> cycle, measured using the elevated plus-maze test. Data represent mean  $\pm$ SEM. \* $p < 0.05$  versus *Chow/Chow* vehicle-treated group; #  $p < 0.05$  versus *Chow/Palatable* vehicle-treated group.