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Effects of cannabinoid drugs on the reinforcing properties of food in gestationally undernourished rats

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ABSTRACT

Involvement of the endocannabinoids in hyperphagia has been demonstrated, however, behavioral characterization of its role in food reinforcement is limited. The present study investigated whether 2-arachidonoyl glycerol, an endocannabinoid ligand, and rimonabant, a CB1 antagonist, change the reinforcing properties of food in gestationally undernourished rats (a putative model of obesity) vs controls. Albino dams were food deprived by 0 to 45% of their free-feeding weights up to day 18 of their gestational period. Their offspring were allowed to free-feed until postnatal day 75. Then, behavior of the offspring was placed under progressive ratio schedules of sucrose reinforcement. After baseline data were established, intraperitoneal injections of 2-AG (0.03–3.75 mg/kg), and rimonabant (SR141716, 0.3–3.0 mg/kg) were administered and compared across group. Results show gestationally undernourished (GU) rats as adults weighed less than controls at the time of testing and female offspring allowed to free-feed for over 35 weeks exhibited lower body weights than controls. Under baseline, GU rats had lower breakpoints than controls. 2-AG and rimonabant significantly increased and decreased, respectively, breakpoint and responses made per session, suggesting involvement of the cannabinoid system in food reinforcement. When comparing peak doses of 2-AG on breakpoint, gestationally undernourished rats exhibited lower peak doses than controls. These data suggest that under the gestation deprivation method employed, GU rats were thinner and had lower food reinforcer efficacy than controls, and may have heightened sensitivity to 2-AG.

1. Introduction

The endocannabinoid system is involved in feeding and hyperphagia (Cota et al., 2003; Di Marzo et al., 2001; Kirkham et al., 2002; Williams and Kirkham, 2002a,b). Two endogenous cannabinoids — anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) — have been shown to activate the CB_1 receptor and are linked to increases in food intake in rats (Jamshidi and Taylor, 2001; Kirkham et al., 2002; Williams and Kirkham, 1999). Additionally, following food deprivation, levels of AEA and 2-AG in the limbic forebrain and the hypothalamus are higher than in free-feeding conditions (Kirkham et al., 2002), which suggest involvement of these endocannabinoids in the initiation of feeding behavior. Furthermore, drugs that mimic AEA and 2-AG, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), increase food intake while cannabinoid antagonists are also effective in reducing food intake (Jamshidi and Taylor, 2001; Williams and Kirkham, 2002b; Williams et al., 1998).

The aforementioned studies suggest that cannabinoids may enhance the rewarding properties of food. However, to characterize a behavioral mechanism involved in cannabinoid-induced food intake, such as food reinforcer efficacy, it is not sufficient to show only an increase in feeding. Other contextual variables must also be considered, for example, the effort related to obtaining food. The progressive ratio schedule of reinforcement is a well-established procedure that characterizes motivation, or the relation between behavioral effort and the value of a particular reinforcer, e.g., food or drugs of abuse (Hodos, 1961; see also Markou et al., 1993; Stafford et al., 1998 for reviews). Under this schedule, the initial response requirement for a reinforcer is low, then the ratio requirement increases systematically within a single session. The "breakpoint" or ratio at which the animal no longer responds, is the referent for the value of the reinforcer, with higher breakpoints reflecting a more highly valued reinforcer. The progressive ratio schedule has been used extensively in determining the reinforcing efficacy for drugs of abuse (see Spealman and Goldberg, 1978; Stafford et al., 1998 for reviews). Its application to the conditions under which food functions as a reinforcer has been applied also to obesity. Obese Zucker rats, for example, exhibit higher breakpoints for grain-based reinforcement (Glass et al., 1999) and sucrose reinforcement (Rasmussen and Huskinson, 2008) compared to lean rats under progressive ratio schedules of reinforcement.

Because the value of a reinforcer is determined by the effort put forth to produce it, examining reinforcers in a low-price economy, where food is freely available without a large response cost provides

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an incomplete description of behavior. To provide a more complete characterization, one must examine food-contingent behavior across a range of costs, in which an increase in response requirement for food is observed.

Solinas and Goldberg (2005) investigated how the cannabinoid system affects food reinforcement using the progressive ratio schedule. After establishing behavior under this schedule, acute doses of the cannabinoid agonist, Δ^9-THC, and the cannabinoid antagonist, rimonabant (SR141716), were administered. The authors found that Δ^9-THC increased breakpoints for food reinforcement and rimonabant reduced breakpoints. Additionally, they established that the observed effects were selective to these drugs and not a result of increased motor function. These effects have been replicated in other studies using different species, strains, and operants (Higgs et al., 2005; Rasmussen and Huskinson, 2008; Ward and Dykstra, 2005).

While the economic environment is an important determinant of reinforcer sensitivity, some individual difference variables may also play a role. These differences can result from genetic variations. Genetically altered rodents, such as the obese Zucker *fa*/*fa* rat strain, have a deficient leptin receptor and exhibit sensitivity to food reinforcement in studies using free operant procedures (Greenwood et al., 1974; Rasmussen and Huskinson, 2008; Vasselli et al., 1980).

Another condition that may make behavior sensitive to the reinforcing properties of food is gestational undernutrition (GU). Epidemiological studies have demonstrated a link between reduced nutrition during gestation and later development of obesity. For example, people in the Netherlands experienced a seven-month famine imposed by the Nazi invasion during WWII. Women who were pregnant at the time received a fewer-than-1500-calorie food ration per day, and subsequently gave birth to infants who were of low birth weight. As the offspring aged, the incidence of obesity was higher by 19 years of age than matched controls who experienced no famine during gestation (Ravelli et al., 1976). Additionally, similar effects have been found in India where as a result of famine, offspring exposed to reduced gestational nutrition develop obesity during early adulthood (Yajnik, 2000).

While other epidemiological studies of obesity in humans reveal links between reduced nutrition during gestation and later obesity (e.g., Ravelli et al., 1976, 1999; Yajnik, 2000, 2004), some animal studies support causal relations between GU and obesity (e.g., Anguita et al., 1993; Desai et al., 2005; Jones et al., 1984, 1986; Jones and Friedman, 1982; Vickers et al., 2000). Much of the animal studies have shown that free-feeding adult mammals that were gestationally undernourished exhibit hyperphagia, larger body mass, and larger fat cells compared to adults rats from dams that free-feed during the gestational period (Beall et al., 2004; Jones and Friedman, 1982; Vickers et al., 2000). The observations in the animal studies, however, took place in environments in which the offspring, as adults, were given access to food that was readily available, i.e., the "cost" for food was low.

A behavioral analysis of the reinforcing properties of food comparing GU to control rats has not been examined. The present study aimed to expand research on the role of endocannabinoids in food procurement by investigating food reinforcer efficacy in an economy where the price for food increases within experimental session. It is possible that GU rats may exhibit differences in sensitivity to food reward, and to cannabinoid drugs because GU rats demonstrate an insensitivity to leptin, a neuropeptide that modulates hunger signals, such as neuropeptide Y (Desai et al., 2005; Vickers et al., 2000, 2001). Leptin resistance is important because it seems to influence endocannabinoid levels in the brain, thereby leading to hyperphagia (Di Marzo et al., 2001). No studies to date have been conducted that behaviorally evaluate cannabinoid sensitivity in GU rats. The current study represents a first attempt to begin elucidating this relation. The effects of acute administration of 2-AG and rimonabant on the value of food reinforcement in GU offspring were investigated also. It was hypothesized that the endocannabinoid ligand, 2-AG, would increase the reinforcing efficacy of food (as measured by breakpoint under a progressive ratio schedule of reinforcement) and rimonabant would reduce it. Moreover, we were interested in whether GU rats may show altered sensitivity to these drugs.

2. Methods

2.1. Subjects and breeding

2.1.1. Dams

For two weeks, 20 albino Sprague–Dawley adult dams (ISU Breeding Colony, Pocatello, ID) were allowed ad libitum lab chow. Baseline food intake (g/day) was determined by averaging daily food intake within a 24 h period for 10 consecutive days. Dams were randomly assigned to one of two groups: 1) free-feeding controls ($n=10$); or 2) food restriction ($n=10$). After baseline food intake was determined for each dam, one of the ten albino Sprague–Dawley sires was bred with one female from the control group and one female from the food-restricted group on alternating days. Thus, each male rat sired two dams and fathered a litter of pups from each group. Dams in the food-restriction group ($n=10$) were given a daily ration of 45% of their average daily free-feeding intake, i.e., each ration was individually determined. Food restriction of the dams began on gestational day 0 (GD0), when conception occurred (determined by the presence of a sperm plug located in the dam's vagina) and lasted until GD18. In addition, at GD18, the other 8 dams were given their predetermined ration plus an additional 10 g of food per day for the duration of gestation. (Note: A 50% food deprivation procedure was initially used across the entire gestational period, but after two dams died during parturition, the deprivation was changed to the one described above. Two 50% GU offspring were used in the present study because their data did not significantly differ from the offspring in the 45% GU litters.) At birth, all dams were allowed free access to lab chow and had free access to water at all times and litters were culled to six pups per litter. Of the ten control litters, one was excluded from analysis as a result of the development of blindness in offspring. From the remaining 19 dams, one was unable to conceive.

2.1.2. Offspring

In the present study, the offspring were the subjects of interest. Starting on postnatal day (PND) 2, litters were weighed. The average pup weight was determined by dividing the litter weight by the number of pups in the litter. Individual pup lengths (measurement from tip of nose to base of tail) were measured once a week until asymptote was reached (approximately 90 days of age). At 21 days of age the offspring were weaned and pair housed with a littermate of the same sex. Sixteen male offspring were selected for behavioral assessments. There were nine males from the control group (eight pups representing eight litters, 2 representing 1 litter) and 7 from the gestationally undernourished (GU) group (1 from each of 5 litters and 2 from two litters). Offspring had free access to water and were allowed to free-feed until 75 days of age. Weight was monitored daily. At 75 days of age offspring were individually housed in clear polyurethane cages. Weights were maintained at 320 g, a healthy weight that establishes food as a reinforcer. The colony room that held all rats (both dams and offspring) was humidity controlled and held at a constant temperature of 21 °C, on a 12-hour light/dark cycle beginning at 0800. The offspring remained in this colony throughout testing.

Two female pups also were selected per litter ($n=18$ from control group, 14 from GU group) after weaning. Each was housed individually and allowed ad libitum access to lab chow and water for over 35 weeks. Weights were monitored daily for the first several months and then weekly. All animals in this study were treated in

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accordance with the Idaho State University Institutional Care and Use Committee guidelines.

2.2. Apparatus

Seven standard rat operant chambers (Coulbourn Instruments, Allentown, PA) enclosed in sound-attenuated boxes were used to conduct behavioral testing. The chambers were comprised of Plexiglas sidewalls, wire grid flooring, two levers positioned to the left and right sides of the magazine, and a 45 mg food-pellet dispenser. A speaker on the left wall in the top left corner of the operant chamber produced white noise during each session. On the opposite side of the left wall in the top right corner, a 2×2 inch fan operated. Sucrose pellets (45 mg) were used as reinforcers (Noyes Pellets, Research Diets Inc., New Brunswick, NJ). When a pellet was delivered, the houselight darkened and the magazine light was illuminated for 5 s and a pellet was dropped into the magazine. A response on the left lever did not result in reinforcement, but was recorded. An IBM-compatible computer with Graphic State™ software (Coulbourn Instruments, Inc., Allentown, PA) was used to control behavioral contingencies and record data in each chamber.

2.3. Operant sucrose reinforcement

2.3.1. Acquisition of lever press

When weights reached 320 g, (approximately 80 days of age) each rat offspring was trained to lever press under a 10-hour fixed ratio 1 (FR1) session. During this session, a response on the right lever produced a single 45 mg sucrose pellet. Lever press training was considered acquired when 100 responses were made per session. If a subject did not meet criterion within three sessions, hand shaping commenced. Once lever pressing was shaped, subjects were placed under a single FR1 schedule of reinforcement for 15 min. When the rat earned 70 reinforcers within this session, it was considered lever-press trained.

2.3.2. Operant sucrose-reinforced behavior under progressive ratio

On Sundays, Tuesdays, and Thursdays lever pressing was placed under a FR1 schedule until 25 reinforcers had been delivered (the response maintenance schedule). On Mondays, Wednesdays, and Fridays, a progressive ratio (PR) schedule was in effect. The PR schedule is one in which the number of lever presses for a reinforcer gradually increases within session in a systematic fashion. In the present experiment, the exponent $(5\times e^{(0.2\times \text{number of reinforcers})})-5)$ was programmed as the increment in response requirement (Roberts and Bennett, 1993). This exponent produced the following ratios: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, and 178. Each progressive ratio session lasted until a ratio had not been completed within a 20-minute period.

Baseline responding under the PR schedule was continued until stability on this schedule occurred. Stability in responding was defined as less than 10% deviation in the mean number of responses made per session within three consecutive sessions, and no trends were apparent. The number of responses made per session and the breakpoint, or the ratio at which the animal stopped responding during the session, was measured and recorded as baseline data. All sessions were conducted at the same time Sunday thru Friday and body weight was monitored daily prior to the beginning of a session.

2.3.3. Drug administrations

After baseline data were collected in the progressive ratio condition, drug administrations commenced. Prior to the beginning of a session, the subject was administered an intraperitoneal (IP) injection of the specified dose of a drug or vehicle (placebo) in a 1 ml/kg solution. All subjects received individual, acute injections of the cannabinoid ligand 2-AG (0.03–3.75 mg/kg). 2-AG was purchased from Sigma Aldrich (St. Louis, MO), and was dissolved in an acetonitrile solution by the company, and then mixed with saline in our laboratory. The cannabinoid antagonist rimonabant (0.3–3.0 mg/kg; [National Institute of Mental Health, Research Triangle Park, NC]) was dissolved in a 1:1:18 solution of ethanol, cremaphor and saline.

A single acute injection of one of the two drugs was administered prior to commencement of a PR session (2-AG, 30 min; rimonabant, 60 min). Following the injection, the rat was placed in its home cage for the specified absorption time, after which the rat was placed in the operant chamber and tested under the PR schedule. Subsequent administrations of the same drug (administered no fewer than two days apart) increased in dose by half-log units until the highest dose was reached or until behavior was reduced by 50% of baseline, at which point, dosing was terminated. A one-week washout period, in which no drug injections were administered, occurred between the completion of one drug profile and the beginning of the next. All animals received all doses of all drugs and the placebo, though order of drug (2-AG, rimonabant) was counterbalanced — half of the rats in each group received the rimonabant determination first; half received 2-AG first.

2.4. Data analysis

Birth weight, litter size, and weight at adulthood (day 76; prior to beginning of food deprivation for operant tasks) were analyzed using an independent samples *t*-test. Female growth curves were analyzed using curve estimation regression models (comparing best fit functions). Parameters of the best fit function were compared between groups. Dose-response curves for each drug using the mean number of responses per session and breakpoints for each group were created. These data were also analyzed as percent of vehicle (behavior under drug/behavior under vehicle) for each animal under each dose of the drug. Analyses of main effects of GU (between-subjects variable) and dose (within-subjects variable) and interactions were conducted using a two-way repeated measures ANOVA. For all instances in which sphericity was violated, the sphericity estimate was less than 0.75 and therefore the Greenhouse–Geisser correction was used. Analyses for which this correction was used include degrees of freedom that are not integers.

3. Results

3.1. Weight and litter size

Control and GU offspring were not significantly different in average birth weight or litter size ($ps>0.05$). Fig. 1 shows the mean weight of the control group (black bars) and GU group (white bars) at

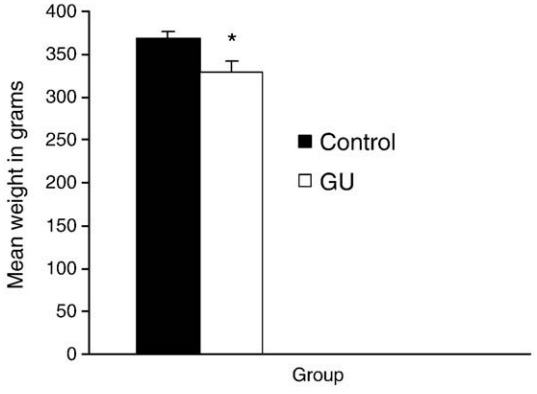

Fig. 1. Mean weight of subjects at adulthood (day 76). The black bar represents the control group ($n=9$); white bar represents GU group ($n=7$). Error bars represent one standard error of the mean. (*$p<0.05$ when compared to controls).

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approximately 76 days of age (after free-feeding and prior to beginning of operant training). At adulthood, the GU group (329.09 ± 12.50) weighed significantly less than the control group (368.72 ± 7.21) [$t(14) = 2.90$, $p < 0.05$].

Weights (g) from the female offspring that were allowed to free-feed for over 35 weeks are shown as a function of age in weeks in Fig. 2. During the first 8 weeks, the pups are difficult to distinguish in the figure, but the curves begin to diverge by week 10. By week 25, the control rats exhibited an asymptote in weight of approximately 295 g; the GU rats exhibited an asymptote around week 20 at about 255 g. Logarithmic functions were the best fit for both groups (control mean $r^2 = 0.84$; GU $r^2 = 0.78$). Weight increased significantly with age for the control group [$-119.65 + [0.89 * \ln 89.91$, $F(2036) = 8506.44$, $p < 0.01$) and for the GU group ($-103.11 + [0.80 * \ln 51.77]$, $F(1470) = 2680.81$, $p < 0.01$]. There was also a significant difference in growth between groups [$F(3507) = 210.37$, $p < 0.01$].

3.2. Baseline

The top of Fig. 3 shows the mean breakpoint + 1 SEM for baseline conditions under the food delivery condition (progressive ratio; left) and the no-food delivery condition (extinction; right); the bottom of Fig. 3 shows the mean responses per session + 1 SEM. Black bars represent the control group and white bars represent the GU group. Under the first baseline that took place before the initial drug administrations (Baseline 1), there was no significant difference in mean breakpoint [$p = 0.62$] between the control (19.78 ± 2.47) and GU (18.00 ± 2.41) groups. For Baseline 1 the mean responses per session for the control group was 86.89 ± 13.29 and GU group was 77.00 ± 12.19 and this difference was also not significant [$p = 0.60$]. In the re-establishment of baseline that took place between the first and second dose-response determinations (Baseline 2), and in the re-establishment after the second dose-response determination (Baseline 3) the mean breakpoint and responses made per session increased for both groups from initial baseline responding. A two-way repeated measures ANOVA revealed a significant main effect of baseline number (1 vs 2 vs 3) on breakpoint [$F(1.42, 18.46) = 14.49$, $p < 0.01$] and responses made per session [$F(1.25, 16.33) = 13.20$, $p < 0.01$]. Contrasts revealed significant differences between Baseline 1 vs 2 [$F(1, 13) = 13.13$, $p < 0.01$; $F(1, 13) = 11.68$, $p < 0.01$] and 1 vs 3 [$F(1, 13) = 42.74$, $p < 0.001$; $F(1, 13) = 37.97$, $p < 0.001$] but, not 2 vs 3 [p = ns] for breakpoint and responses, respectively. A main effect of group approached significance, but did not meet criteria of traditional significance levels [$p = 0.08$; $p = 0.09$ breakpoint and responses, respectively]. A marginal interaction between group and baseline number [$p = 0.07$] was found on breakpoint only.

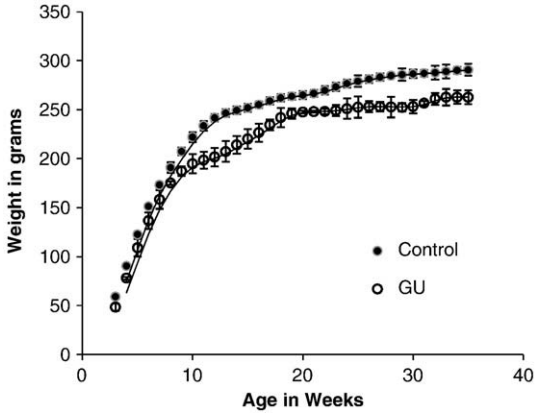

Fig. 2. Mean weight (smoothed) as a function of age in weeks for control (black circles) and GU (open circles) female rats that were allowed free access to food from birth until over 35 weeks of age. Error bars represent one standard error of the mean.

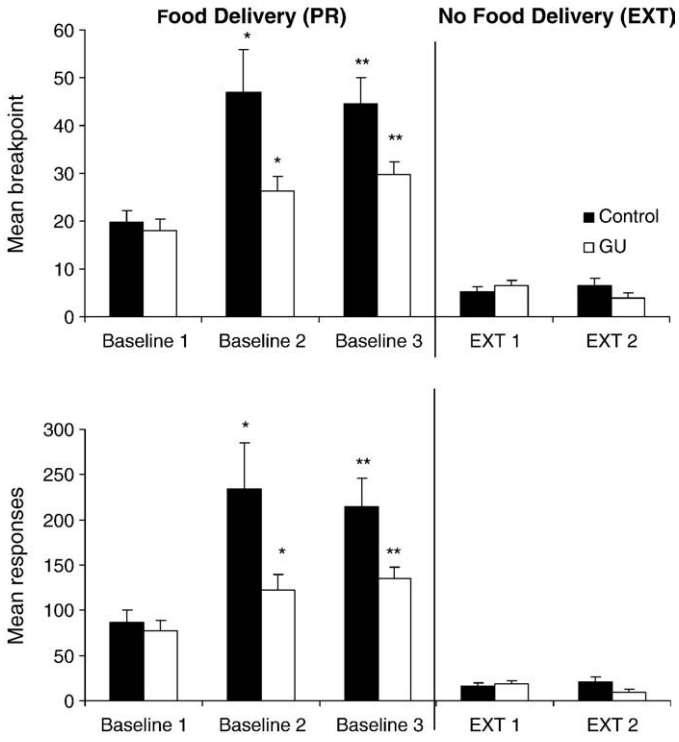

Fig. 3. Mean breakpoint (top) and responses per session (bottom) in baseline progressive ratio (left) and extinction (EXT; right) phases in GU and control groups. The black bars represent control group ($n = 9$), while the white bars represent the GU group ($n = 7$). Error bars represent one standard error of the mean. (*$p < 0.01$; **$p < 0.001$ when compared to Baseline 1).

3.3. Progressive ratio vs extinction

The right half of Fig. 3 (breakpoint = top; responses = bottom) shows behavior under extinction for both groups, which took place after each drug determination. The three data from the baseline condition were averaged into one datum for each rat, and the two data from extinction (no significant differences between them) were averaged into one datum for each rat, and these means were compared (these means are not shown). Extinction reduced breakpoint and responses significantly compared to the PR condition [$F(1, 14) = 77.50$, $p < 0.001$; $F(1, 14) = 64.04$, $p < 0.001$]. The mean breakpoint under PR conditions for the control group was 36.87 ± 4.87 and 24.68 ± 2.11 for the GU group. Under extinction, the mean breakpoint was 5.88 ± 1.04 for control and 5.21 ± 0.83 for GU rats; this was a marginally significant difference [$p = 0.06$]. The mean responses made per session under PR conditions for the control group was 177.78 ± 27.51 and 111.19 ± 10.77 for the GU group. Under extinction, the mean responses made per session were 18.55 ± 4.22 for control and 14.35 ± 2.62 for GU rats. There was no significant difference between group, extinction number and no extinction by group interaction [$ps > 0.05$] for either of these variables.

3.4. Drug effects

3.4.1. 2-AG

Fig. 4 shows dose-response curves for breakpoint (top) and responses per session (bottom) as a function of 2-AG dose. Baseline data (B) represent those that were collected immediately before the 2-AG dose-response determination for all rats. 2-AG dose-dependently affected breakpoint in a bi-phasic manner. A two-way repeated measures ANOVA (dose as within-subjects variable, group as between-subjects variable) revealed a main effect of 2-AG [$F(6, 84) = 2.30$, $p < 0.05$]. Specifically, contrasts revealed that breakpoints were

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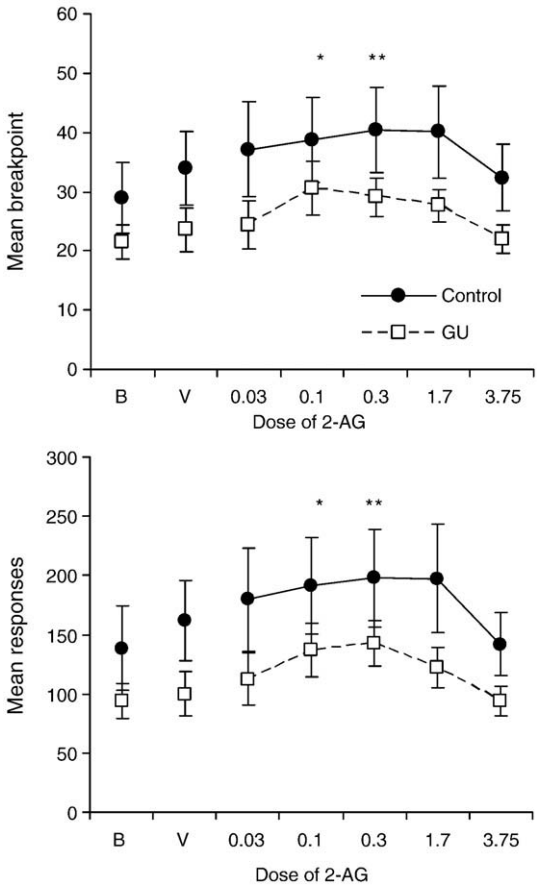

Fig. 4. Dose-response curves for 2-AG (mg/kg). Mean breakpoint (top) and responses per session (bottom) are represented on the *y*-axis. Black circles represent control group ($n=9$); white squares represent GU ($n=7$). Error bars represent one standard error of the mean. (*$p<0.05$; **$p<0.01$ when compared to Baseline).

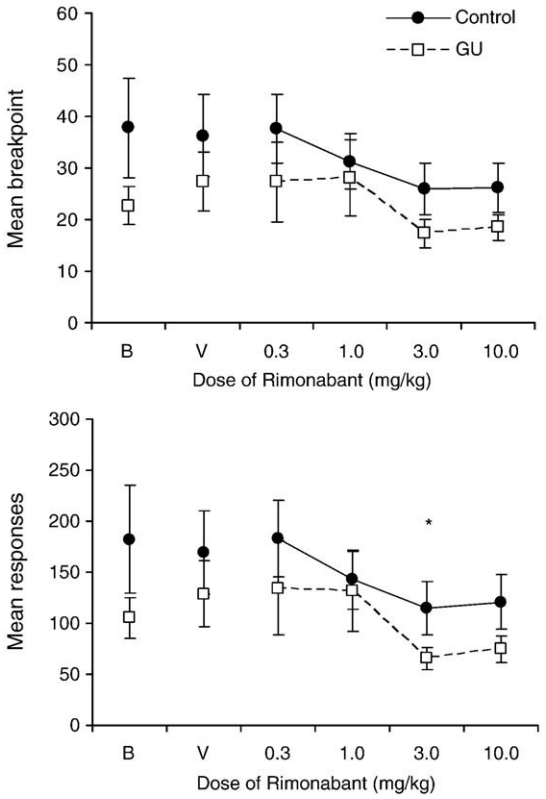

Fig. 5. Dose-response curves for rimonabant. Mean breakpoint (top) and responses per session (bottom) are represented on the *y*-axis. Black circles represent the control group ($n=9$); white squares represent the GU group ($n=7$). Error bars represent one standard error of the mean. (*$p<0.05$ when compared to Baseline).

increased from baseline at the 0.1 mg/kg and 0.3 mg/kg doses of 2-AG [$F(1, 14)=6.37$, $p<0.05$; $F(1, 14)=7.87$, $p=0.01$, respectively] by 30–50% for each group. There was no main effect of group [$p=$ ns] or an interaction [$p=$ ns]. 2-AG also dose-dependently increased responses per session [$F(3.68, 51.63)=2.74$, $p=0.05$], but there was no main effect of group or an interaction [$ps=$ ns]. Responses made per session were significantly increased from baseline by 25–30% at the 0.1 mg/kg and 0.3 mg/kg doses of 2-AG [$F(1, 14)=5.22$, $p<0.05$; $F(1, 14)=7.33$, $p=0.01$, respectively].

3.4.2. Rimonabant

Fig. 5 shows dose-response curves for breakpoint (top) and responses per session (bottom) for rimonabant. Rimonabant dose-dependently decreased breakpoint [$F(2.32, 32.52)=3.25$, $p=0.05$] and responses per session [$F(2.16, 30.37)=3.81$, $p=0.05$]. There was no significant main effect of group on breakpoints or responses, nor an interaction between group and dose.

3.5. Peak dose effects

Peak doses for 2-AG (dose that caused the greatest increase in breakpoint) and rimonabant (dose that caused the greatest decrease in breakpoint) were determined for each rat individually and each peak dose was administered to each rat after extinction ensued. The mean peak dose of 2-AG for control rats and GU rats was 1.48 mg/kg ± 0.48 and 0.42 mg/kg ± 0.21, respectively. Fig. 6 shows the mean breakpoint under the PR schedule (top panel) under the peak dose of 2-AG divided by vehicle, then multiplied by 100, reflecting a percent of vehicle. Data that fell above or below the horizontal line (100% of vehicle) reflect an increase or decrease, respectively in breakpoint under the peak dose. The peak dose of 2-AG raised breakpoint to 150% of vehicle on average for both control and GU rats. These increases in breakpoint were significantly higher than vehicle administrations [$F(1, 14)=16.07$, $p<0.01$]. No significant differences, in terms of the degree to which 2-AG increased breakpoints, were found between groups, nor was there a significant interaction between group and 2-AG peak dose [$ps>0.30$]. However, there was a marginal difference between groups in the average peak doses administered [$p=0.07$]. The mean peak dose for the control group was three and a half times higher than the mean peak dose for the GU group.

As shown in Fig. 6, the peak doses of rimonabant (controls = 5.88 mg/kg ± 1.31; GU = 3.84 mg/kg ± 1.65) significantly reduced breakpoints (compared to vehicle) by 40% for the controls and by about 50% for the GU. Breakpoints under the vehicle condition were significantly different from those of the peak dose of rimonabant [$F(1, 14)=13.21$, $p<0.01$]. There were no group differences in the amount of reduction [$p=0.29$] and no differences in the actual peak dose used [$p=0.34$].

4. Discussion

In the present study, lever pressing came under the control of progressive ratio schedules for gestationally undernourished rats and controls. Moreover, under extinction, lever pressing dropped substantially for both groups. These findings in the control group replicate what others have reported in standard laboratory strains (Shram et al., 2008; Solinas and Goldberg, 2005), and also extend what is known about gestationally undernourished rats, in which there are few (if any) studies using free operant procedures.

The present study also found that non-injected baseline performance under PR schedules changed across the experiment. There was an increase in breakpoints (and responses) from the initial baseline to

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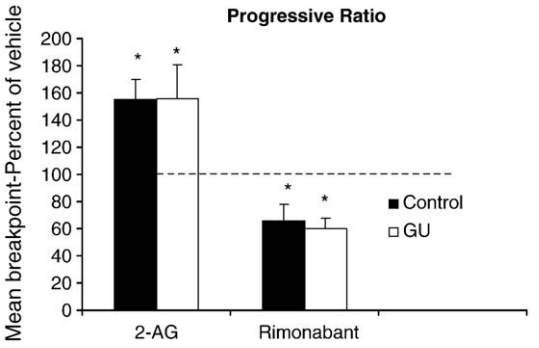

Fig. 6. Top panel shows mean percent of vehicle breakpoint as a function of peak dose of 2-AG and rimonabant in the progressive ratio condition. Data are represented as percent of vehicle condition (drug/vehicle); horizontal dashed line represents to change from vehicle. Black bars = control group ($n = 9$); white bars = GU group ($n = 7$). Error bars represent one standard error of the mean. (*$p<0.01$, **$p<0.001$ when compared to respective vehicle).

the first re-establishment of breakpoints after the first drug determination (Baseline 2), and after the second drug determination, breakpoint remained stable (Baseline 3). This increase in breakpoint was not linked to a particular drug as the drug order was counterbalanced. As such, this finding may suggest that food reinforcer efficacy increased with time. The control group demonstrated a time-related increase that was much larger than the increase observed in the GU group when baseline conditions were reinstated (59.5% increase in controls compared to 33.5% in GU).

Although it is unclear as to why baseline responding changed over time, at least two explanations are plausible. The subjects may have demonstrated a practice effect, therefore becoming more efficient on the progressive ratio schedule across the experiment. It is necessary to point out, however, that stable responding was achieved for each datum represented in Fig. 1, so a practice effect may be called into question as an explanation for this increase in baseline between drug determinations. A second interpretation could be an effect of aging such that as the animals aged, reinforcer efficacy increased. Recall that weights were held constant at 320 g when the operant portion of study began (76 days of age, or roughly 11 weeks). If growth continued after that age (which may have been likely, based on growth curves of the female rats), then it is possible that more food would be required to maintain that growth, which may manifest in higher food reinforcer efficacy. Further investigation is needed to determine which of these accurately explains the observed change in baseline responding over time.

2-AG increased breakpoints and responses for both groups. Importantly, no dose of 2-AG reduced breakpoints or responses compared to control conditions. Each rat's individual sensitivity to 2-AG varied, as reflected by the large amount of variability within each group at each dose of 2-AG. Part of this variance likely came from the spread in the data that was influenced by time. Consider, for example, that the baseline data alone contain breakpoints from rats that received 2-AG first (at 3 mo of age) and from rats that received it last (at least 4 mo), and that there were differences in these data. Because aging may have contributed to the large amount of variability in the data, we thought it important to examine each rat's peak effect to 2-AG, comparing it to his own vehicle condition (Fig. 6), which would eliminate time-related confounds. The peak dose of 2-AG increased breakpoints by 150% of vehicle in the control and GU groups. This is the first demonstration of the effects of an endogenous cannabinoid on operant behavior, specifically of behavior under PR schedules. Several studies to date have reported the effects of endogenous cannabinoids such as anandamide and 2-AG on food intake where food is freely available and have found that following administration of either drug, hyperphagia is induced (Jamshidi and Taylor, 2001; Kirkham et al., 2002; Williams and Kirkham, 1999, 2002a). The present study complements these data by showing that this intake is likely due to food having higher reinforcer efficacy. Additionally, the average 2-AG peak dose for the control group was about three and a half times higher than the average peak dose for the GU, a finding that was close to traditional significance levels. These results may suggest that some small level of sensitivity to 2-AG exists in GU rats, which may be related to possible differences in the endocannabinoid circuitry in GU rats. Research examining CB_1 receptor density in GU rats could determine this.

Rimonabant dose-dependently decreased breakpoints and responses made per session, for both GU and controls. The peak dose reduced breakpoints by about 40% for controls and 45% for GU rats. This finding replicates other studies that show rimonabant decreases the reinforcing efficacy of food (Foltin and Haney, 2007; McLaughlin et al., 2003; McMahon et al., 2005; Rasmussen and Huskinson, 2008; Solinas and Goldberg, 2005) and extends to animals that are gestationally undernourished. It is important to note that the GU and control animals in this study appeared equally sensitive to this drug, as noted by the lack of a difference in the peak dose of rimonabant, and the behavioral response to it. One limitation of the present study is the omission of data demonstrating the antagonism of 2-AG's effects by rimonabant. Although this would have provided additional support to previous studies demonstrating reversal of hyperphagia induced by cannabinoid agonists (Jamshidi and Taylor, 2001; Williams et al., 1998; Williams and Kirkham, 2002b), we still believe that the present study does reveal important information about the effects of cannabinoid drugs when given alone on behavior under PR schedules.

Finally, rats exposed to GU demonstrated lower weights as adults compared to controls before the time of operant testing. Moreover, female GU offspring that were allowed to free-feed for over 35 weeks exhibited lower weights than control offspring. These data do not support previous claims of greater weight gain in GU offspring (e.g., Desai et al., 2005; Jones et al., 1984, 1986; Vickers et al., 2000). Moreover, GU rats exhibited lower baseline breakpoints and fewer responses per session under progressive ratio schedules of sucrose reinforcement as they aged, suggesting that food has a lower reinforcer efficacy compared to that of controls. While some of the data from the operant tasks did not reach the traditional 0.05 alpha level (particularly, when the rats were younger) they were noteworthy because they do not support obesity-related hypotheses relevant to GU. It would be expected that the GU animals would exhibit higher breakpoints and responses because food would be more reinforcing to them, based on literature that suggests food intake is higher in this group (e.g., Desai et al., 2005; Jones et al., 1984, 1986; Jones and Friedman, 1982). Indeed, just the opposite held: GU rats not only weighed less, they seemed to have lower food reinforcer efficacy than controls in the current study, and this was observed in baseline and drug-related conditions.

Not all studies have shown hyperphagic-like outcomes with GU offspring. A recent study published by Sébert et al. (2009) found that GU sheep raised in free-feeding, but confined environments (in which sedentary activity is likely, similar to laboratory studies with rats) demonstrated lower food intake at one year of age compared to control sheep. Moreover, GU offspring had lower levels of neuropeptide Y, complementing the lowered food intake. Interestingly, Sébert et al. (2009) did not find GU-related differences in weight in these offspring at birth or at 1 year of age.

Some reasons for the discrepancy in the findings from the present study vs others may have to do with parameters involving gestational deprivation. Most of our rats in this group were under an 18-day, 45% restriction during gestation. Though very few studies directly compare different deprivation levels, the general literature on GU suggests that differing levels and time periods of gestational food deprivations may differentially influence the level of hyperphagia and weight gain in offspring (Anguita et al., 1993; Desai et al., 1996; Jones et al., 1984,

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1986; Jones and Friedman, 1982). Generally, deprivation levels between 30% and 50% of baseline free-feeding are implemented during approximately two-thirds of the gestation cycle, which results in increased food intake and weight gain in the offspring as they age. They also exhibit larger fat cells, and later obesity. The deprivation level used in present study was consistent with other protocols used where a protocol of 45–50% reduction of normal daily food intake occurred for a majority of the rat's gestational cycle (18 days). However, because we used a longer deprivation period (an additional 4 days), this may explain the difference between our results and those from other studies.

Another explanation in the differences found in our study vs others on GU may lie with the environmental arrangement of food in the offspring as adults. Most of the studies on GU involve free-food intake in which the animal simply moves toward the food aperture in the home cage and eats large amounts of freely available grain-based food (e.g., Desai et al., 2005; Jones et al., 1984, 1986; Jones and Friedman, 1982; Vickers et al., 2000). The present study used a more effortful measure of food consumption — the progressive ratio schedule, in which the rat must move toward the lever, emit presses, the requirement of which increases with each reinforcer earned. Moreover, we used a 45 mg sucrose (not grain-based) food pellet for each response requirement that was met, so food palatability also differed. It may be that as a result of gestational food restriction, offspring may not be motivated to obtain sweetened food at higher response costs, but will readily consume food if freely available (and less palatable). A study that systematically manipulates effort to gain access to food between sessions and food palatability with gestationally undernourished offspring may be able to answer this question. More research is necessary to elucidate how GU effects manifest in operant behavior.

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