Effects of 2-AG on the reinforcing properties of wheel activity in obese and lean Zucker rats

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The endocannabinoid system plays a role in obesity, primarily by its role in food reward. Activity, also involved in obesity, seems to be at least partially controlled by the endocannabinoid system, but the relevant behavioral and neurochemical mechanisms have not been well established. This study represents an attempt to begin elucidating these mechanisms by examining the effects of an endogenous cannabinoid ligand, 2-arachidonoylglycerol (2-AG), on the reinforcing properties of exercise reinforcement in lean and obese Zucker rats. Ten obese and 10 lean Zucker rats pressed a locked door under a progressive ratio schedule of reinforcement that, when unlocked, provided access to a running wheel for 2-min periods. After baseline breakpoints were established, doses of 2-AG (0.3–3 mg/kg) were administered before experimental sessions. Obese rats exhibited lower breakpoints for wheel activity, lower response rates, and fewer revolutions compared with lean rats. 2-AG decreased breakpoints, response rates, and revolutions for obese rats, and revolutions only for lean rats. These data suggest that 2-AG may reduce the reinforcing properties of activity, and that obese Zuckers may show a greater sensitivity to 2-AG. The data also suggest that endocannabinoids may play a role in the reinforcing properties of exercise. *Behavioural Pharmacology* 21:292–300 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The endocannabinoid (ECB) system has been implicated in obesity, mostly through its association with excessive feeding and enhancement of food reinforcement (e.g. DiMarzo *et al.*, 2001; Kirkham *et al.*, 2002; Friede *et al.*, 2005; Kirkham, 2005; Solinas and Goldberg, 2005; Keeney *et al.*, 2008; Rasmussen and Huskinson, 2008; Wakley and Rasmussen, 2009). Inactivity, also linked to obesity (e.g. Pelleymounter *et al.*, 1995; Ahima *et al.*, 1999, Coppari *et al.*, 2005; Must and Tybor, 2005), seems to be at least partially regulated by the ECB system. For example, a high density of CB1 receptors is present in the basal ganglia, which suggests that the ECB system is used in the control of voluntary movement (DiMarzo *et al.*, 2000). Moreover, CB1-deficient mice exhibit lower basal levels of locomotor activity compared with wild types (Li *et al.*, 2009).

It would be reasonable to assume that drugs that affect the ECB system would have predictable effects on activity; however, the literature is somewhat puzzling. One may consider research on locomotion in an open field as an example. CB1 agonists, for example, have mixed effects on locomotion. In some studies, endogenous CB1 ligands, such as anandamide and the exogenous CB1 agonists δ-9 THC and WIN 55,212-2, enhance locomotor activity in rodents (Wiley, 2003; Pandolfo *et al.*, 2007). In other studies, δ-9 THC decreases locomotion (Järbe *et al.*, 2002; Smirnov and Kiyatkin, 2008). The effects of CB1 antagonists, such as rimonabant, on locomotion also produce mixed results: in some cases, locomotor activity increases (Compton *et al.*, 1996), and in others, it decreases (Tallet *et al.*, 2007).

The role of the ECB system in activity, then, seems complicated, and the mixed results are likely a function of procedural variants, such as strain-related and age-related characteristics of the individuals, pharmacological properties of the various substances used, and differences in the manner that locomotion was measured and quantified. Further complicating the picture is the role of reward in locomotor activity. In the above-mentioned studies, there was no experimenter-manipulated reward in the open-field activity measures, so one could make an argument that motivation could be ruled out as a contributing variable. However, the behavior of moving may be itself reinforcing. Consider a study by Zangen *et al.* (2006), in which δ-9 THC was injected into the posterior ventral tegmental area and the nucleus accumbens shell, both of which are areas of the brain involved in reward. In addition to finding that THC enhanced self-administration of THC and development of conditioned place preference (both are well-established measures of reward), THC also dose-dependently increased locomotor activity. Besides further supporting the interactive systems involved in reward and locomotion, an additional interpretation of this finding is that THC injected in reward areas may make many phenomena, including movement itself more reinforcing.

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It is difficult, then, to discern specific behavioral mechanisms that cannabinoids may affect in locomotor studies because the measure is too general. An increase in locomotor activity, for example, can be interpreted as a heightened ability to move (motor), heightened motivation to move, or even reduced fear of open space, as ECBs in some studies have been shown to affect sensitivity to aversive stimuli (i.e. fear and anxiety, Haller *et al.*, 2002; Mikics *et al.*, 2006). To begin clarifying how cannabinoids affect specific behavioral mechanisms, attempts should be made to use measures that better isolate these mechanisms. One way to begin might be to determine whether ECBs affect the reinforcing properties of activity.

Wheel running may be a useful animal model for activity, as it allows the researcher to examine an ecologically valid behavior (running) in a controlled setting. Voluntary wheel running is observed in a variety of rodent species, indicating some level of intrinsic reinforcement to the animal (Eikelboom and Mills, 1988; Sherwin, 1998). The putative litmus test, however, for whether wheel running can serve as a reinforcer is to make its access contingent upon another behavior, such as lever pressing. Kagan and Brekun (1953) first demonstrated that rats would press a lever to unlock a running wheel. In addition, lever pressing for wheel activity has been placed under schedule control, and found to exhibit properties of schedule patterning (Collier and Hirsch, 1971; Iversen, 1993; Belke, 1996, 2004) similar to that generated by other reinforcers such as food, water, and sucrose (e.g. Guttman, 1953; Ferster and Skinner, 1957; DeGrandpre *et al.*, 1993).

In one study, (Pierce *et al.*, 1986), wheel activity was assessed as a reinforcer using the progressive ratio (PR) schedule of reinforcement with rats. The PR schedule is a well-established procedure that determines the value of a reinforcer by increasing the response requirement for access to it within the session (see Refs Hodos, 1961; Markou *et al.*, 1993; Stafford *et al.*, 1998 for reviews). The point at which ratio strain occurs is referred to as the breakpoint and is used as a measure of the value of the reinforcer. In the study by Pierce *et al.* (1986), breakpoints for 60-s access to a running wheel were found to be higher in female rats than in male rats, and deprivation (maintaining rats at 80% of their free feed weight) increased breakpoints from 30 to 55 responses under no food deprivation to 50–70 responses under the 80% food deprivation condition. Other studies suggest that lower body weights enhance wheel running as a reinforcer (through higher response rates) when access to a wheel is under schedule control (Belke, 1996, 2004; Belke *et al.*, 2004).

This study had two objectives: first, the value of wheel running as a reinforcer using PR schedules of reinforcement was established and compared in lean and obese Zucker rats. The obese Zucker rat has a recessive genotype, in which the *fa* allele is inherited from each parent. The phenotype is expressed as obesity, and includes hyperphagia (excessive eating), large body mass, leptin insensitivity, raised cholesterol, high phosphatide levels, and high blood lipid levels (Chua *et al.*, 1996; Phillips *et al.*, 1996; Koyama *et al.*, 1998). Obese Zuckers also showed lower energy metabolism during resting states (Rolland *et al.*, 2002) and lower levels of activity (e.g. Shimuzu *et al.*, 1991). In addition, obese Zuckers have higher levels of 2-AG, an ECB, in the hypothalamus, compared with lean controls (DiMarzo *et al.*, 2001), and larger CB1 receptor densities in the limbic areas of the brain. In this, we hypothesized that obese rats would have lower breakpoints for exercise-based reinforcement than leans.

Second, we examined the effects of 2-AG on the reinforcing properties of wheel running in the lean and obese Zucker rat. Although we were uncertain how 2-AG would affect wheel reinforcement (given that the research is difficult to interpret), we hypothesized that obese Zucker rats would show greater sensitivity to 2-AG because they have higher than normal amounts of cannabinoid activity in the brain (DiMarzo *et al.*, 2001; Thanos *et al.*, 2008).

Methods

Subjects

Ten female obese *fa/fa* Zucker rats and 10 female lean (*Fa/fa or Fa/Fa*) Zucker rats (approximately 4–6 weeks old) were purchased from Harlen (Los Angeles, California, USA) at 3–4 weeks of age and allowed to free feed for 7 weeks. They were singly housed in clear, plexiglass home cages and maintained in a temperature-controlled and humidity-controlled room (approximately 22°C) under a 12-h light/dark cycle. Animals were given free access to water when not in experimental sessions. Two weeks before operant testing commenced, rats were given free access to food for 2 h per day at the same time. By the end of this 2 weeks, lean rats exhibited a mean body mass of 211.63 g (SEM = 3.22) and obese rats 437.96 g (SEM = 10.58), and this difference in weight was significant [$t(10.65) = -20.47$, $P < 0.01$]. One obese female Zucker rat developed skin lesions after unsuccessful treatment and was euthanized. This rat did not complete a drug profile for 2-AG, but did complete baseline data.

Apparatus

One of the five Coulbourn activity wheels (22.9 cm in diameter, 9 cm wide) was attached to one of the five standard two-lever operant chambers. Access to the wheel from the chamber was possible through a swinging door, which would lock under conditions specified in the procedures. Pressing this door when locked served as the operant for wheel access. Each wheel and chamber was enclosed in a sound-attenuating box. White noise was used during all operant sessions to mask any external stimuli. A Windows-based computer with Graphic State software (Coulbourn Instruments, Whitehall, Pennsylvania, USA) was used to collect data and control experimental contingencies.

Procedure

Wheel reinforcement training

Each rat was placed individually inside the operant chamber at the start of the session. Rats were required to press the locked door under a fixed ratio 5 (FR 5) schedule of wheel reinforcement. Here, five door presses unlocked the swinging door and the rat could enter the running wheel and run for a period of 2 min. After the 2 min elapsed, the wheel locked, and the door remained unlocked. If the rat had not returned to the chamber within 1 min the experimenter removed the rat from the wheel and placed the rat back inside the chamber. Once the rat was inside the chamber, the swinging door locked and the rat could begin the next ratio of door presses. Sessions lasted for 60 min. Sessions under the FR 5 schedule continued until stability was ensured. Stability was defined as three consecutive sessions in which the number of reinforcers earned did not deviate by more than 10% and there were no visible trends. Rats were allowed to free feed for 2 h after all operant sessions.

Progressive ratio schedule

After the rats were trained to press the door, door pressing was placed under a PR schedule of wheel reinforcement in which the response requirement for 2-min access to the wheel started at five door presses and increased in a systematic fashion within the session with each earned reinforcer. The response requirement progression for the PR schedule was 5, 15, 30, 50, 90, and 150, which was a progression used for saccharin and phencyclidine reinforcement in an earlier study (Carroll *et al.*, 1991) and allowed for sessions that were relatively short (45–60 min) to capitalize on peak drug effects. Each session ended when ratio strain occurred (defined as not completing a ratio within 20 min). Sessions were conducted at the same time (± 15 min) on Sundays through Fridays. Half of the rats completed baseline PR sessions on Mondays, Wednesdays, and Fridays; the other half completed on Sundays, Tuesdays, and Thursdays. FR 1 sessions, in which a single door press resulted in access to the running wheel for 2 min, were in effect on days between PR sessions. The implementation of an FR 1 schedule between PR sessions maintains the door-press response, as ratio strain under PR schedules may lead to extinction of the door press; this procedure has been used by others when assessing the reinforcing properties of food using PR schedules (Solinas and Goldberg, 2005; Wakley and Rasmussen, 2009). FR 1 sessions ended after five wheel reinforcers were earned. Once rats showed stability across three sessions under baseline PR sessions (breakpoints were ± 1 PR step without trends), drug administrations began. All procedures were approved by the Idaho State University Animal Welfare and Use Committee.

2-Arachidonoylglycerol

The endogenous CB1 ligand, 2-arachidonoylglycerol (2-AG), as acetonitrile solution (0.3–3.0 mg/kg), was purchased from Sigma-Aldrich (St Louis, Missouri, USA) and dissolved in a saline vehicle (1 ml/kg volume).

After baseline data were collected, vehicle sessions commenced in which acute doses of saline were administered intraperitoneally 30 min before a PR session. Once breakpoints stabilized under vehicle conditions, dose-response determinations for 2-AG commenced. A single acute drug injection was administered intraperitoneally 30 min before a session began, beginning with the smallest dose of the drug. Doses increased in half-log units in subsequent sessions. One dose was administered per rat per day, with no fewer than 2 days between injections.

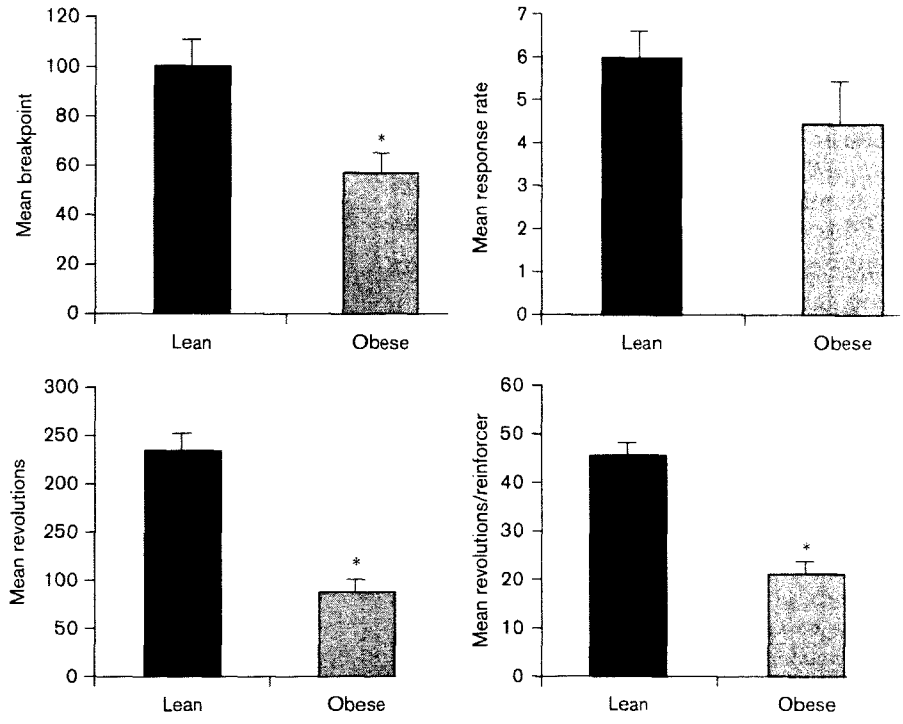
Extinction condition

Once all doses of 2-AG were completed under PR, an extinction condition took place, which was identical to a PR session, except that door pressing resulted in access to a locked wheel. The purpose of the extinction condition was to ensure that door pressing was maintained by wheel running, as opposed to escape from the chamber, for example. A single extinction session was attempted first, followed by a PR session (which allowed recapturing of baseline). After examining data from this single extinction session, it was determined that more sessions were necessary for extinction to take place for the majority of rats, so multiple extinction sessions were run until breakpoints decreased by at least 50% of baseline. Rats also completed a PR session (to recapture baseline) followed by extinction sessions again in which the rat's peak dose of 2-AG (dose that caused largest change from baseline) was administered 30 min before extinction sessions commenced. This was done to determine whether the effects of 2-AG were specific to behavior maintained by wheel running, as opposed to another mechanism, for example, motor function.

Analysis

Baseline and vehicle data from the last three stable PR sessions were averaged for each rat. Response rates were determined by subtracting wheel running time (2 min per reinforcer) from the total session duration and then dividing the number of door presses per session by the net number of minutes in the session. Mean breakpoint (last ratio completed before ratio strain ensued), door-press rates (door presses per minute), and wheel revolutions per session and per reinforcer were compared across group (obese vs. lean), dose of 2-AG, and between baseline and extinction conditions. As baseline data differed between groups (see Fig. 1), mean percent of baseline (calculated as each rat's drug dose datum/baseline datum) was compared across rat strain, drug, and dose, to standardize baseline group differences, such that drug sensitivity could be examined. 2-AG data were analyzed first using a two-way analysis of variance (ANOVA) with repeated measures to determine main effects of dose (within-subjects variable),

Fig. 1



Mean breakpoints (upper left), response rates (upper right), revolutions per session (lower left), and revolutions per reinforcer (lower right) for lean (dark bar) and obese (light bar) rats in baseline condition. Error bars represent 1 standard error of the mean. *$P<0.01$.

group (lean vs. obese, between-subjects variable), and group × dose interactions. As we saw strong evidence for greater sensitivity to 2-AG by the obese Zucker group (see Results), we reanalyzed each group separately, using a one-way ANOVA with repeated measures, and report specific dose reductions through contrasts. For extinction conditions, two-way repeated measures ANOVAs were conducted to determine main effects and interactions under (i) vehicle versus peak (3 mg/kg) dose of 2-AG, and group and (ii) PR versus extinction conditions, and group. Data from the last extinction session for each rat were used in the extinction analysis to show the reduction in behavior. The Greenhouse–Geiser or Huynh–Feldt (depending on estimate of sphericity) corrections were used when data violated sphericity.

Results

Baseline

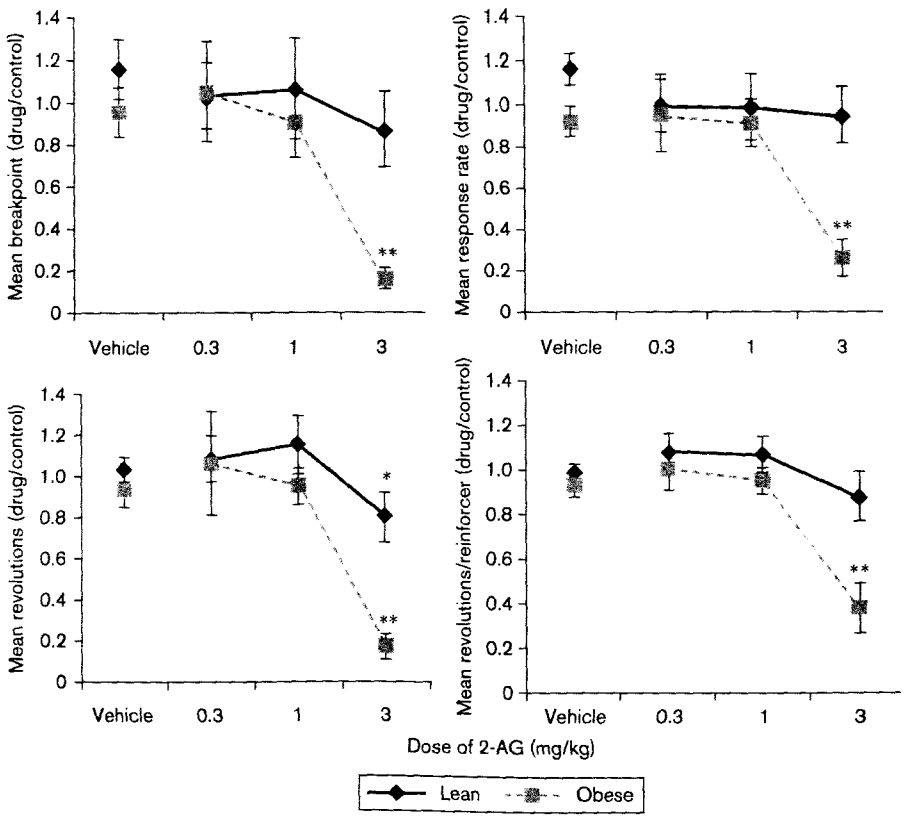
Figure 1 shows mean breakpoint (top left) under the PR schedule under baseline for lean (dark bar) and obese (light bar) rats. Independent samples t-tests revealed that breakpoints were significantly lower for obese rats, compared with lean controls [$t(18) = 3.28$, $P < 0.01$]. Obese rats also exhibited fewer revolutions (bottom left) and revolutions per reinforcer (bottom right) than leans [$t(18) = 6.96$, $P < 0.01$], [$t(18) = 8.00$, $P < 0.01$], respectively. Mean response rates (top right) did not significantly differ between lean and obese rats.

2-Arachidonoylglycerol

Figure 2 shows mean breakpoints (top left) as a percent of baseline as a function of 2-AG dose. There was a significant main effect of 2-AG on breakpoint [$F(3,51) = 7.19$, $P < 0.01$]. There was no significant effect of group; however, a dose × group interaction approached significance, [$F(3,51) = 2.62$, $P = 0.056$]. As the obese rats seemed more sensitive to 2-AG than the leans, one-way repeated measures ANOVAs were conducted on obese and lean rats separately and revealed that 2-AG dose-dependently decreased breakpoints for obese rats, [$F(4,32$, $P < 0.01$], but not lean rats, confirming the interaction from the two-way ANOVA analysis. Post-hoc contrasts conducted on each group revealed a significant decrease between the vehicle versus 3 mg/kg doses for the obese rats [$F(1,8) = 5.45$, $P < 0.05$].

Mean response rates are shown in the top right panel. There was a significant main effect of dose [$F(3,51) = 7.03$, $P < 0.01$], a marginal main effect of group, [$F(1,17) = 3.71$, $P = 0.07$], and a significant interaction [$F(3,51) = 4.01$, $P = 0 < 0.01$]. A one-way ANOVA with repeated measures conducted individually on each group indicated that 2-AG dose-dependently decreased response rates for

Fig. 2



Mean breakpoints (upper left), response rate (upper right), revolutions per session (lower left), and revolutions per reinforcer (lower right) as a function of 2-arachidonoylglycerol (2-AG) dose for lean (diamonds) and obese (squares) rats. Data are represented as percent of baseline (drug/baseline). Error bars represent 1 standard error of the mean. Asterisks refer to significant differences revealed by contrasts comparing each dose to its vehicle within group. *P<0.05, **P<0.01.

obese rats, [$F(3,24) = 10.02$, $P < 0.01$], but not lean rats. Post-hoc contrasts revealed a significant difference between the vehicle and 3 mg/kg dose for the obese rats [$t(1,8) = 31.60$, $P < 0.01$].

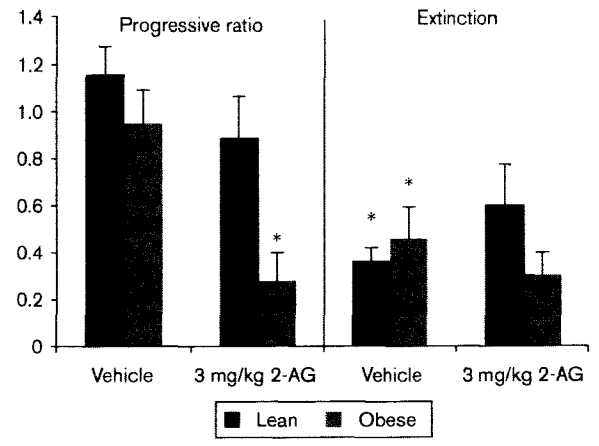
2-AG dose-dependently reduced the number of revolutions (bottom left) emitted by obese and lean rats [$F(2.02, 34.37$, note: d.f. reflect Greenhouse–Geiser adjustment) = 12.35, $P < 0.01$]. A two-way repeated measures ANOVA revealed that the main effect of group and the interaction term approached significance [$F(1,17) = 4.05$, $P = 0.06$], [$F(2.02,34.37) = 3.02$, $P = 0.06$], respectively. One-way ANOVAs conducted on each group separately revealed significant effects of 2-AG for lean [$F(3,27) = 3.26$, $P < 0.05$] and obese rats [$F(3,24) = 8.74$, $P < 0.01$]. Post-hoc contrasts yielded significant differences between the vehicle and the 3 mg/dose for lean [$t(1,9) = 5.56$, $P < 0.05$] and obese [$t(1.8) = 74.78$, $P < 0.01$], though the obese rats exhibited a stronger decrease than leans.

2-AG dose-dependently decreased the number of revolutions per reinforcer (bottom right) [$F(3,51) = 14.71$, $P < 0.01$]. There was a significant main effect of group [$F(1,17) = 5.94$, $P < 0.05$], and a significant interaction [$F(3,51) = 4.51$, $P < 0.01$]. One-way ANOVAs conducted individually on each group showed a significant effect of 2-AG on revolutions for obese rats only [$F(3,24) = 12.36$, $P < 0.01$]. Post-hoc contrasts revealed significant differences between the vehicle and 3 mg/kg dose for obese rats only [$t(1,8) = 21.32$, $P < 0.01$].

Extinction

Figure 3 summarizes means for breakpoints (percent of baseline) under PR (left) versus extinction (right) conditions. In the PR condition, the peak dose of 2-AG decreased mean breakpoints significantly from vehicle [$F(1,17) = 5.91$, $P < 0.05$]. There also was a significant main effect of group [$F(1,17) = 15.97$, $P < 0.01$], but no significant 2-AG × group interaction. The right half of Fig. 3 shows that extinction reduced mean breakpoints significantly compared with the PR condition [$F(1,17) = 32.62$, $P < 0.01$]. There was no significant main effect of group, and no significant interaction ($P = 0.08$). Extinction took between one and six sessions, and there were

Fig. 3



Mean breakpoint (proportion of baseline) as a function of 2-arachidonoylglycerol (2-AG) in progressive ratio (PR) and extinction conditions for lean (dark bars) and obese (light bars) rats. Error bars represent 1 standard error of the mean. *$P<0.05$ compared with baseline PR condition.

no group-related differences in the number of sessions to extinction. The main effects of 2-AG on behavior under extinction, group differences, and interaction were all nonsignificant.

Discussion

Baseline data

The PR schedule of wheel running maintained the behavior of door pressing for both lean and obese rats, until breakpoints were evident. During the 2-min reinforcer intervals, rats completed an average of 20 (obese) to 45 (lean) revolutions per reinforcer. Further, under extinction conditions, in which the wheel was locked, breakpoints for wheel access decreased by greater than 65%. We did not use a stability criterion for behavior under extinction, so it is uncertain whether behavior under extinction would have continued to decrease past 65%. Nonetheless, these results indicate that wheel running was a reinforcer for obese and lean Zucker rats, extending the findings of Pierce *et al.* (1986). Further, the results from this study extend the literature in at least two important ways. First, the data extend the literature on wheel running as a reinforcer in standard laboratory rodent strains (Skinner, 1932; Kagan and Berkun, 1953; Collier and Hirsch, 1971; Jennings and McCutcheon, 1974; Stewart *et al.*, 1985; Eikelboom and Mills, 1988; Belke, 1997; Sherwin, 1998) to the genetically obese Zucker rat strain. Second, the results also extend the literature on PR schedules by showing that the reinforcing efficacy of wheel running, like food, water, and drugs (Glass *et al.*, 1999; Solinas *et al.*, 2003; Solinas and Goldberg, 2005; Madden *et al.*, 2007; Rasmussen and Huskinson, 2008; Wakley and Rasmussen, 2009) can be characterized using PR schedules.

Obese Zucker rats exhibited baseline breakpoints for wheel access that were 40% lower than lean rats' breakpoints. Moreover, obese rats made 60% fewer revolutions per session than leans. These data suggest that wheel running is less reinforcing for obese rats compared with leans, and may expand other studies that show obese animals are less physically active (Mayer, 1953; Stern and Johnson, 1977; Shimuzu *et al.*, 1991). This study, however, also suggests a motivational mechanism as at least part of the explanation for lower levels of activity.

No group differences were observed in response rate, which suggests that door pressing occurred at a similar speed in both groups of animals. This finding was surprising, given that there were group differences in other data (e.g. breakpoint, revolutions). The measure of response rate indicates a global measure of response speed across the session (responses per minute), and does not reflect moment-to-moment changes in rate. It may, in the future, be interesting to examine response bouts within small (e.g. 10 s) bins to determine whether there were group-related differences across the session. For example, higher response rates may have occurred early in the session for obese rats and slowed as the session progressed. This level of analysis could unmask group differences that may be evident at a more local level.

At least one question arises from the data on revolutions per reinforcer. The observation that obese rats valued wheel reinforcement less than leans may have to do with their contact with the wheel during reinforcer intervals. During each reinforcer interval, the obese Zuckers made fewer (about half as many) revolutions per reinforcer than the lean rats. Research on behavioral momentum (Nevin *et al.*, 1983) is relevant here. Nevin *et al.* (1983) showed that every contact with a reinforcer increases its value, that is, it gains momentum in terms of its ability to strengthen responding and its resistance to extinction. Though the reinforcer interval was held constant in this study (2 min), the number of revolutions was not held constant during the 2 min. It is possible, if one considers that a revolution (rather than the time on wheel) is the unit of reinforcement, that obese rats did not experience the reinforcer to the same extent as the leans (indeed, half as much), and that could have contributed to the difference in breakpoint. Other research may raise questions about interval of time as an appropriate unit of wheel reinforcement. Belke (2006), for example, showed that longer duration access to wheel reinforcement is not preferred to shorter duration access, a finding that contradicts that high-magnitude reinforcers are more preferred than those of low magnitude. Together, these findings suggest that another property of running may better characterize wheel running as a reinforcer, and this may be revolutions. In future studies, researchers could hold the revolutions per reinforcer interval constant to determine whether the number of revolutions is the appropriate measure of wheel reinforcement.

One aim of this study was to determine whether obese rats differed from lean rats in their sensitivity to wheel reinforcement. Although the aim of the study was not to determine the specifics of why obese rats devalue exercise reward compared with leans, the strong differences in the behavior of obese and lean rats is reflected by the fact that there are weight differences, and not deprivation differences. Some studies have indeed shown that deprivation is related to behavior involved with producing wheel running reinforcement and wheel running. Prefeeding rats before wheel running reduces response rates for wheel running, depending on the prefeed load (Belke *et al.*, 2004). Moreover, the more body weights approach free-feeding levels, the lower response rates are in producing wheel running reinforcement (Belke, 1996). In this study, we did not prefeed our rats before the operant sessions. Moreover, they were equally food deprived, as we used a 2-h free-feed session that took place 21 h before the experimental session, which resulted in rats in both groups consuming about 2.3% of their body weights during the free-feed sessions (Rasmussen and Huskinson, 2008). Thus, the differences in behavior in this study seem to be due solely to body weights, rather than not deprivation. Moreover, the differences in behavior seem to be motivation based (as evidenced by breakpoint), and therefore a behavioral mechanism was identified.

2-Arachidonoylglycerol effects

2-AG significantly and dose-dependently decreased breakpoints, response rates, revolutions per session, and revolutions per reinforcer for obese rats; for lean rats, 2-AG reduced revolutions per session only at the 3 mg/kg dose. 2-AG did not increase behavior at any dose. Some studies have shown that 2-AG at a similar dose range enhances food intake (e.g. Kirkham *et al.*, 2002) and increases breakpoints under PR schedules for food reinforcement (e.g. Wakley and Rasmussen, 2009). This is the first study (to our knowledge), however, to show the effects of 2-AG on exercise reinforcement.

Behavioral measures were consistently higher for lean rats compared with obese rats, no matter what dose of 2-AG was examined. Owing to this difference, percent of baseline was calculated to normalize baseline differences and compare 2-AG effects across dose and group. Data represented as percent of baseline showed that the highest dose of 2-AG (3.0 mg/kg) reduced behavior to a greater degree from baseline for obese rats, compared with leans, across all measures (breakpoint, response rate, revolutions, and revolutions per reinforcer). This difference supports the hypothesis that obese rats exhibit a greater sensitivity to 2-AG than lean rats. This finding also supports other research showing that obese Zuckers are more sensitive to cannabinoid drugs, such as the antagonist rimonabant (Rasmussen and Huskinson, 2008). Sensitivity to 2-AG and rimonabant may be linked to obese Zuckers having higher basal levels of 2-AG in their brains (DiMarzo *et al.*, 2001) and higher densities of CB1 receptors in limbic areas of the brain (Thanos *et al.*, 2008).

Some recent findings with the drug rimonabant, a cannabinoid antagonist that blocks the CB1 receptor, seem worthy of mention in light of the effects described in this study. Keeney *et al.* (2008) showed that rimonabant dose-dependently increased free-wheel running in mice (though there was no response-contingent access to the wheel). The results from our study on revolutions mirror the effects reported in Keeney *et al.* (2008) by showing that substances that enhance ECB activity reduce wheel running, whereas [as Keeney *et al.* (2008) showed] substances that block ECB activity in the brain increase wheel running. It seems that ECBs are involved in wheel running, and may have predictable effects on this very specific behavior.

Obese and lean rats showed a reduction in revolutions at the 3 mg/kg dose of 2-AG, though only the obese rats showed this reduction when revolutions per reinforcer were analyzed. A decrease in revolutions and rate may suggest a reduction in motivation to run or a motor effect, or both. Two observations, however, lead us to interpret this as a motivational effect. First, there were no 2-AG-related effects on behavior under extinction. Extinction was placed in effect after PR sessions to ensure that wheel running maintained the door-pressing response and also to determine whether the wheel reinforcer was necessary to 2-AG's suppressive effects. Extinction significantly decreased breakpoint from baseline PR conditions for both obese and lean rats, suggesting that wheel running was necessary to maintain door pressing. Importantly, the peak dose of 2-AG on behavior under extinction had very little effect, suggesting that the 2-AG effects were specific to the availability and use of the wheel, and supports that 2-AG affects motivation specifically. It should be mentioned, however, that the lack of effect of 2-AG on behavior under extinction could also be because of the low rates of behavior under extinction (i.e. a floor effect or possibly a rate-dependent effect). Naloxone, an opioid antagonist, has been shown to reduce free-wheel running in situations in which basal-wheel-running rates were low (Sisti and Lewis, 2001), but not in situations in which free-wheel-running rates were high (e.g. Carey *et al.*, 1981). Therefore, rate dependence cannot be completely ruled out.

A second observation that supports the effects of 2-AG as motivational comes from another study. Wakley and Rasmussen (2009) reported on a dose range of 2-AG that was identical to the one used in this study; in that study, 2-AG increased breakpoints for food reinforcement, and no dose of 2-AG suppressed behavior. Owing to the absence of motor effects reported in that study, the suppression effect in this study can probably be attributed to motivation. Another study that compares food-based and exercise-based reinforcement, however, would need to be conducted to ascertain this.

Finally, it is important to point out that, because differences in baseline breakpoints were observed in lean versus obese rats, and differences in their sensitivity to 2-AG, the baseline data may explain the differential effects of 2-AG on the behavior of lean versus obese rats. In other words, it may be the case that lower breakpoints may combine with 2-AG in a manner that leads to greater sensitivity to the ligand; conversely, higher baseline breakpoints may lead to lessened sensitivity to 2-AG. To resolve this possibility, a study that equalizes behavior between the two groups could allow 2-AG-related sensitivity effects on lean and obese Zuckers to be ascertained.

In summary, this study was the first to measure effects of an endogenous cannabinoid on wheel running reinforcement using a contingent behavioral response (door pressing). 2-AG showed the strongest effects on wheel running reinforcement with obese rats, suggesting a strain-related sensitivity to 2-AG. Interestingly, the results of this study identified behavioral suppression effects of 2-AG, which have not been reported earlier. It may be the case that excessive ECB activity in the context of obesity may not only increase overeating by making food more reinforcing (Solinas and Goldberg, 2005; Rasmussen and Huskinson, 2008; Wakley and Rasmussen, 2009), but may also decrease activity by suppressing the reinforcing properties of exercise. Future studies may further examine this relationship.

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