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Effects of mild food deprivation on the estrous cycle of rats

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Abstract

It has long been known that severe food deprivation disrupts the estrous cycle. One of the main problems with behavioral tasks that use food for reinforcement is the requirement that the animal be food deprived. This manipulation could be problematic in studies using female animals, since it may interfere with the estrous cycle of the animals. The purpose of the present study was to investigate: (1) the effect of mild food deprivation on four different strains of rats, (2) factors in the food deprivation procedure that could affect the estrous cycle, and (3) the possible effect of enriched diets during food deprivation on the estrous cycle. A comparison of the estrous cycle in four different rat strains revealed differences in the reliability of the estrous cycle even before the onset of food deprivation. Fischer, Long–Evans, and Sprague–Dawley rats all showed reliable cycle patterns. This was not the case for Brown Norway rats. During food deprivation, the cycle of the Fischer rats was disrupted, whereas the Long–Evans and Sprague–Dawley animals continued to cycle. Both the rate of weight loss and the percent of ad libitum body weight were related to cessation of the estrous cycle. However, enriching an animal's diet with sugar or oil additives delayed the disruption of the estrous cycle. Additionally, animals resumed cycling when returned to ad libitum weight levels. The present findings suggest that when animals need to be food deprived, preference should be given to using Long–Evans or Sprague–Dawley rats. If Fischer rats must be used, they should not be deprived below 90–95% of their ad libitum body weight. Strategies for future food deprivation studies are discussed, as well as a comparison of the effects of mild and severe food deprivation. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Strain; Diet; Females; Anorexia

1. Introduction

There are many types of tasks used to examine behavioral ability, only some of which use food as a motivator. For instance, the Morris water maze [19] requires the animal to escape from an aversive situation. In contrast, the radial arm land maze requires the animal to forage for a food reward. Reports of fluctuations in hippocampal synaptic density [33] have led to investigations of possible estrous cycle-related changes in hippocampal processing [18,29]. However, there are inconsistencies in the literature. For instance, there are reports of impairment in performance on the water maze during proestrus (Refs. [9,12,32]; however, see Ref. [2]) and in fear conditioning [18]. Conversely, estrogen replacement has been linked to enhanced performance on appetitive hippocampal tasks (e.g., Refs. [7,14]; however, see Ref. [26]). These data may indicate that the spine changes in the hippocampus interact differently with performance on aversive and appetitive tasks.

One of the main problems with tasks that use food for the reinforcement is the requirement that the animal be food deprived. When testing females this manipulation could be problematic, since it may interfere with the estrous cycle of the animal [17].

It is well known that severe food deprivation (50% of normal food intake) disrupts the estrous cycle (see Refs. [6,21,27]). For example, severely food deprived rats have lower pituitary, ovarian, and uterine weights [20]. Restricted feeding for a prolonged period affects ovulation rate, cyclic behavior, and reproductive receptivity (see Refs. [4,10,20,28,31]). In addition, severe chronic food deprivation affects gonadotropin levels, such as FSH [20]. The present study focused on the effects of mild food restriction, since this is the standard level used in many appetitive tasks.

The present experiments investigated: (1) the effect of mild food deprivation on the estrous cycle of four different strains of rats, (2) possible variables in the deprivation procedure that

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affect the cycle, and (3) the possible effect of food supplements during food deprivation on the estrous cycle.

2. Experiment 1

The first experiment examined the degree to which the cessation of the estrous cycle is a general phenomenon observed in rats or specific to a given rat strain. The effects of mild food deprivation on the estrous cycle were compared in four different strains of rats. The study examined the percent of cycling animals both before and during food deprivation.

2.1. Materials and methods

2.1.1. Subjects

All animals were 6-month-old virgin females obtained from Harlan Sprague–Dawley (Indianapolis, IN) and were maintained in a room with a 12:12-h light–dark cycle (lights off at 1700 h). The four strains (10 per group) were as follows: Sprague–Dawley (275 g ± 6.39 S.E.M.), Long–Evans Hooded (290 g ± 6.44 S.E.M.), Fischer 344 (Fischer) (181 g ± 3.35 S.E.M.), and Brown Norway (178 g ± 5.31 S.E.M.).

The animals were singly housed in transparent plastic tubs 4 days before food deprivation began. Male rats were kept in the same animal room to promote cyclicity (see Ref. [30]).

2.1.2. Procedures

Females received daily vaginal lavages approximately 4–5 h before lights went off. The lavages were examined under a light microscope to estimate the relative preponderance of cornified epithelial cells, nucleated epithelial cells, and leukocytes [25]. Animals that displayed vaginal smears that contained predominately leukocytes ($\geq 60\%$) were classified as diestrus I or II. Smears that contained primarily nucleated epithelial cells ($\geq 60\%$) and no leukocytes ($\leq 10\%$) were classified as proestrus. Smears that contained primarily cornified cells ($\geq 90\%$) were classified as estrus. Smears that contained primarily cornified cells ($\geq 60\%$) with a significant amount of leukocytes ($\geq 20\%$) were classified as metestrus. This method has been reliably used in the past to assess stage of the estrous cycle (see Refs. [13,16,18]).

Previous experiments have evaluated lifetime changes in cyclicity by examining animals over periods of 2–3 weeks. This provided estrous cycle data over the course of four or five cycles, allowing for an estimation of the approximate *month* in which animals showed a transition in cyclicity (e.g., Refs. [11,13,16]). The aim of the current study was to examine for the onset of changes in cyclicity as a direct response to modifications in food intake. Consequently, the time frame of interest was considerably shorter, with an attempt to determine the approximate *day* in which the animal showed a transition in its cyclicity. Clearly, it is extremely difficult to estimate the specific day the animal shows a transition in cyclicity. Thus, it was imperative that the person coding these data was blind to the food deprivation status and the strain of the animals. Because of individual differences between animals (e.g., some exhibit a 4-day and others a 5-day cycle), the assessment was done for each animal individually in relation to its estrous cycle history. When an animal showed persistent days of only estrus or diestrus or skipped stages of the cycle, the first clear divergence from the normal cycle pattern was defined as the day the animal became irregular. Intermediate states that could not be readily defined were coded as missing data (for an example, see Table 1).

2.1.3. Food deprivation regimes

All food was removed from the animal's cage on day 0. The next day (day 1) was defined as the start of the food deprivation, which continued for 12 days. Each day the animal was weighed. Based upon the change in the animal's weight from the previous day and from its ad libitum weight, an individually calculated amount of laboratory rodent chow (Prolab RMH3000) was given. The status of the estrous cycle and body weight of each animal were monitored daily, with the goal of reaching 85% of ad libitum body weight.

2.1.4. Data analysis

In order to compare the estrous cycle disruption functions of the four strains a curve-fitting procedure was used. The analysis was similar to that used in drug dose–response studies when calculating the ED_{50} values (the dose that produces half the maximal effect) [5]. In the present experiment, the independent variable was the number of food deprivation days rather than dose level. Determination of the 50% cycle disruption effect (50% disruption day) was performed using the curve-fitting procedure in Graph Pad Prism v3.00. The calculation of these values allowed for a nonlinear comparison of the effect of food deprivation on the cycle status among the four different strains.

2.2. Results

Cycle status was verified for 13 days (two to three cycles) before food restriction. This allowed for the determination of cyclicity in the four different strains of rats. The cycling data from the last 5 days before food deprivation were analyzed. Out of the 5 days, the number of days each animal was cycling (maximum = 5 days) was calculated and

Table 1
Example of a rat's estrous cycle data and classification

Day	1	2	3	4	5	6	7	8	9	10	11	12—18	19	20	21	22	23
Stage	E	D	D	P	D	E	P	E	D	D	D	D—D	D	D	D	P	E
Classification	✓	✓	✓	✓	I	I	I	I	I	I	I	S—S	S	?	✓	✓	✓

Animals were classified as cycling (✓), irregular (I), or stuck (S) in diestrus or estrus. Estrous cycle stages that could not be readily defined were coded as missing data. D = diestrus, P = proestrus, E = estrus, ? = missing data.

averaged. Almost all the Sprague–Dawley (4.1 days ± 0.55 S.E.M.), Long–Evans (4.8 ± 0.20), and Fischer rats (4.8 ± 0.13 S.E.M.) were cycling on all 5 days. Conversely, Brown Norway rats showed disrupted cycle patterns (2.4 days ± 0.69). An ANOVA revealed a significant difference among the strains [$F(3,39) = 6.20$, $P < .01$]. Post-hoc analysis revealed a significant difference between the Brown Norway rats and the other three strains (Dunnett t tests, $P < .05$). Thus, the strains differed with respect to the estrous cycle even before the initiation of food deprivation.

Only animals displaying a reliable cycle could be examined further for the effects of food deprivation. Consequently, 7/10 Sprague–Dawley, 9/10 Long–Evans, 10/10 Fischer, and 4/10 Brown Norway rats were food deprived.

Fig. 1a shows the percent of ad libitum body weight during the food deprivation period. The Brown Norway group was affected to a greater degree by the food restriction and dropped in weight faster than the other three groups. A repeated measures ANOVA showed a main effect for baseline weight loss over days [$F(11,286) = 639.57$, $P < .001$], an effect for strain [$F(3,26) = 5.78$ $P < .01$], and day by strain interaction [$F(33,286) = 2.64$ $P < .001$]. Further post-hoc tests (Dunnett t) showed a significant effect for days 2–9 between the Brown Norway rats and the other three strains ($P < .05$). Thus, all groups except the Brown Norway rats showed a similar rate of weight loss.

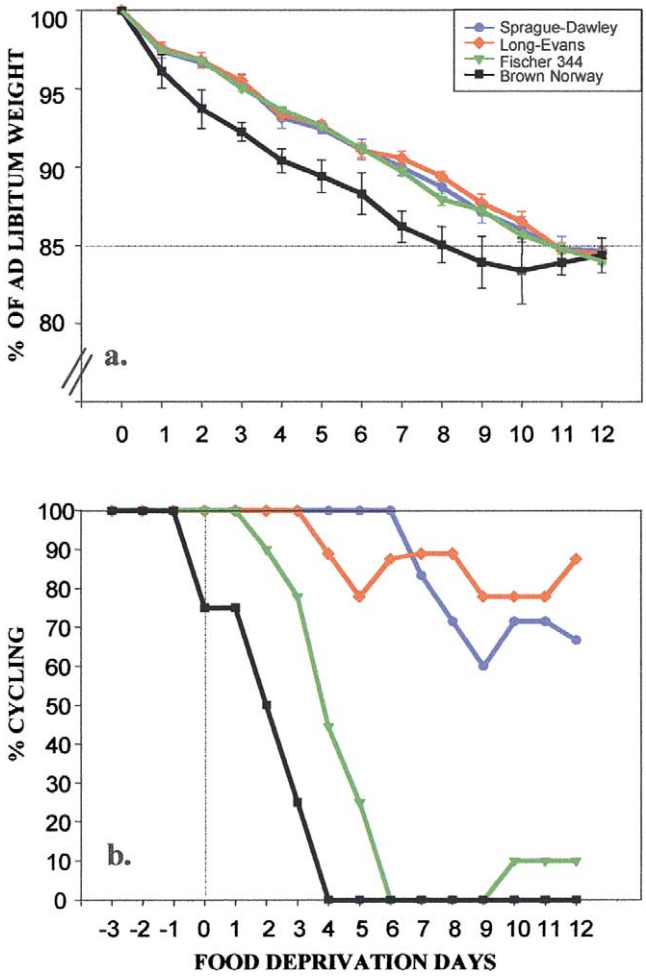

Fig. 1. (a) The percent of ad libitum body weight for the four different strains of animals during food deprivation. Three of the four groups showed similar weight loss throughout the food deprivation period. Brown Norway rats approached the 85% baseline target weight (dashed line) faster than the other strains of rats. (b) The percent of animals cycling throughout food deprivation. Both Sprague–Dawley and Long–Evans rats continued to cycle, while most Brown Norway and Fischer rats ceased cycling early during the food deprivation regime. The dashed line represents when the food was removed from the animals (day 0).

Fig. 1b shows the percent of cycling animals during the 12-day deprivation period. Both Long–Evans and Sprague–Dawley rats remained cycling throughout the food deprivation period. In contrast, by day 4, there were no Brown Norway rats cycling and, by day 6, there were no Fischer rats cycling. A comparison of the rate of cycle disruption using the curve-fitting analysis showed that the Fischer (day = 3.20, 95% Confidence Internal (CI) = 2.66–4.26) and Brown Norway rats (day = 2.96, 95% CI = 2.83–3.10) were similar to each other and outside the 95% confidence interval of the Sprague–Dawley (day = 13.55, 95% CI = 11.27–19.33) and Long–Evans rats (day = 25.57, 95% CI = 18.97–41.51).

Thus, there are strain differences in the susceptibility of the estrous cycle to food deprivation.

3. Experiment 2

The findings from Experiment 1 suggest that the estrous cycles of both Sprague–Dawley and Long–Evans rats were minimally disrupted under mild food deprivation conditions, whereas Fischer and Brown Norway rats were severely disrupted. Fischer rats are commonly used in a wide range of behavioral studies. This strain showed reliable cycles before but not after food deprivation. Thus, the purpose of Experiment 2 was to explore possible strategies to maintain cyclicity in Fischer rats and the important parameters of food deprivation that affect the estrous cycle.

We examined three variables in the food deprivation procedure that could potentially affect the estrous cycle: (1) the number of grams lost, (2) the percent of weight lost from baseline (ad libitum body weight), and (3) the rate of weight loss. The data from a second group of Fischer 344 rats were examined to investigate the effects of food deprivation on the estrous cycle.

3.1. Materials and methods

3.1.1. Subjects

Twenty-two female Fischer 344 retired breeders (8 months old; 223 g ± 3.22 S.E.M.) were used. Housing and handling were similar to Experiment 1. These animals had previously been tested in a fear conditioning experiment before food deprivation. This included several adaptation procedures that involved placing the animal in a novel

location for up to 4 min and receiving a brief mild shock (0.4 mA).

3.1.2. Procedures

The assessment of the estrous cycle was the same as in Experiment 1. All animals were maintained at ad libitum weight for several weeks before food deprivation began and cycle status was verified for a minimum of two estrous cycles (i.e., 8–10 days) before food restriction.

3.1.3. Food deprivation regimes

Food deprivation procedures were similar to Experiment 1.

3.1.4. Data analysis

Three variables were calculated from the data: (1) number of grams lost — the absolute reduction in weight from baseline on a given day; (2) percent loss from baseline — the relative loss in weight (grams lost divided by the baseline weight); and (3) rate of weight loss — calculated by dividing the percent weight loss from baseline by the number of food restriction days.

3.2. Results

Fig. 2 shows the percent of cycling animals during the food deprivation period, as well as the percent of ad libitum body weight. There was a gradual reduction in the proportion of cycling animals. It took approximately 2 weeks for all animals to cease cycling. The data were examined to investigate the relation of when individual animals stopped cycling to the three variables: number of grams lost, percent weight loss from baseline, and rate of weight loss. All three variables were correlated with the cessation of the cycle (number of grams, $r=.82$, $P<.01$; percent weight loss from baseline, $r=.87$, $P<.01$; rate of weight loss, $r=-.87$, $P<.01$). A stepwise regression revealed that the two best predictors of cycle cessation were percent loss from baseline and rate of weight loss. The number of grams lost did not provide additional information to this model.

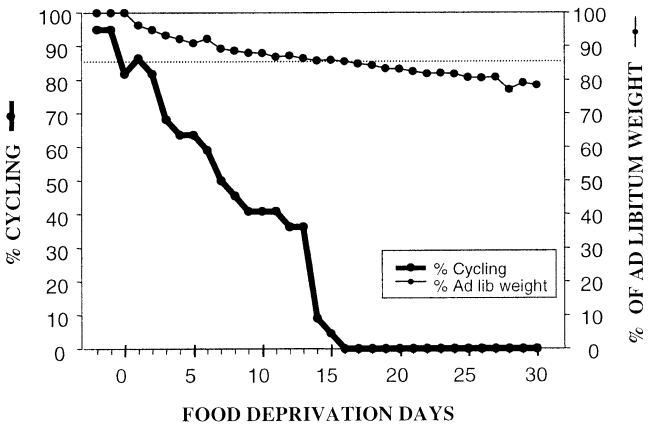

Fig. 2. The percent of Fischer rats cycling during food deprivation, and their percent of ad libitum body weight. The dashed line represents 85% baseline weight.

4. Experiment 3

Both the rate of body loss and the percent of ad libitum body weight were related to the cessation of the estrous cycle. Food enriched with glucose solution or vegetable shortening prevents many of the estrous cycle changes associated with food restriction in hamsters [24]. It is possible then that enriched food sources could reverse or delay the effects of mild food deprivation also in rats.

The third experiment used another group of Fischer rats to evaluate the potential protective effects of enriched diets during food deprivation. In this experiment, the normal diet was replaced with a diet higher in fat or carbohydrate content. In addition, the experiment allowed for the examination of whether returning animals to ad libitum body weight would affect their estrous cycle.

4.1. Materials and methods

4.1.1. Subjects

Twenty-one female Fischer retired breeders (approximately 6–7 months of age) were used. Housing and handling were similar to Experiment 1.

4.1.2. Procedures

The assessment of the estrous cycle was the same as in Experiment 1. Cycle status was verified for a minimum of two estrous cycles before food restriction. The animals were then given special food diets at the start of food deprivation. Unlike the animals in Experiment 2, these animals did not undergo any other behavioral procedures.

4.1.3. Food deprivation regime

The animals' weight was reduced to 85% of ad libitum body weight and monitored daily. The animals received one of three types of food: standard lab chow ($N=7$) (Prolab, RMH3000), standard lab chow mixed with 20% pure granulated sugar ($N=7$), or standard lab chow mixed with 20% corn oil ($N=7$). The amount of food given to each animal was measured daily. This ranged from 3 to 7 g depending on the animal. The goal was for the animal to lose only approximately 3 g of body weight per day.

After a minimum of 17 days of food deprivation, a subgroup ($N=15$, six from control, four from sugar, five from oil) of animals were given ad libitum access to the standard laboratory diet. The estrous cycle of these animals was monitored for 5 more days.

4.1.4. Data analysis

The nonlinear curve-fitting analyses were similar to the methods used in Experiment 1.

4.2. Results

Prior to food deprivation the three groups displayed similar body weights (control 224 g ± 4.40 S.E.M.; sugar 224 g ± 7.48 S.E.M.; oil 220 g ± 4.06 S.E.M.) The three groups demonstrated similar rates of weight loss (Fig. 3a). A repeated measures ANOVA showed a main effect for baseline weight loss over days [$F(16,288) = 218.34$, $P < .001$], no group differences [$F(2,18) = 0.38$, $P > .10$], and no day by group interaction [$F(32,288) = 0.61$, $P > .10$]. As found previously, the control animals stopped cycling as they approached 85% baseline loss, which occurred around day 11 (see Fig. 3b). The estrous cycle in the oil and sugar-treated groups were less affected by the loss of body weight. On day 11, both the sugar and oil groups still had approximately 50% of the animals cycling (see Fig. 3b). However, almost all animals in these groups had stopped cycling by day 17. Thus, enriching the food delayed but did not prevent the disruption of the cycle.

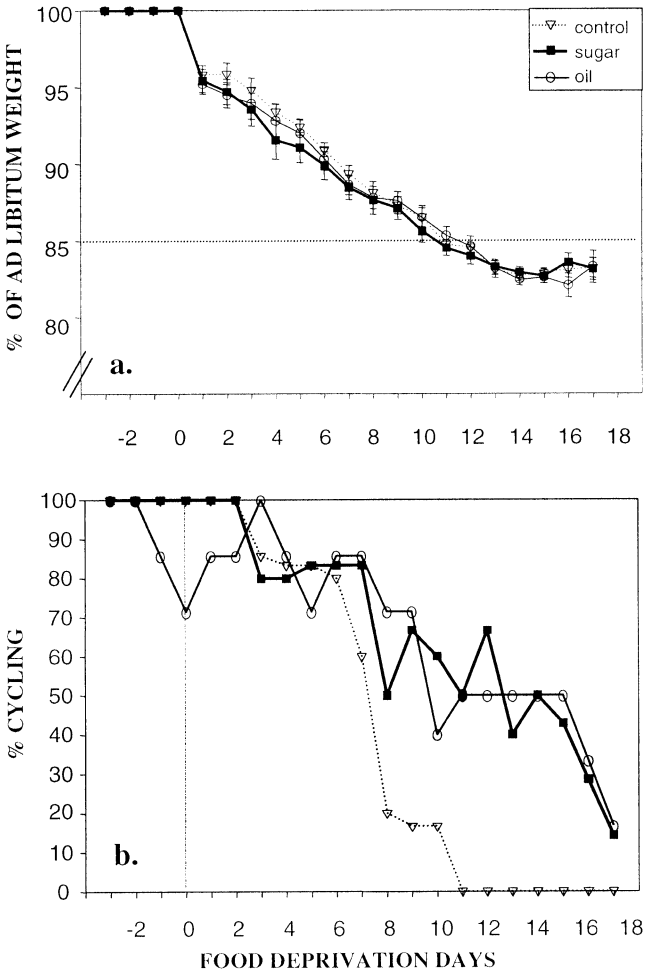

Fig. 3. (a) Percent of ad libitum body weight during food deprivation for control, sugar, and oil-treated animals. The three groups had similar weight loss throughout the food deprivation period. The dashed line represents 85% baseline weight. (b) The percent of animals cycling during the food deprivation regime, for control, sugar and oil groups, respectively. The dashed line represents when the food was removed from the animals (day 0).

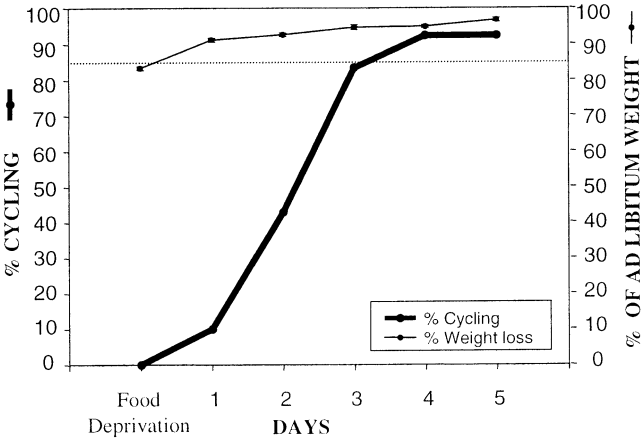

Fig. 4. The percent of animals cycling after the return to ad libitum food availability and the percent of ad libitum body weight. At 95% of ad libitum body weight, approximately 90% of the animals resumed cycling.

A comparison of the rate of cycle disruption using the curve-fitting analysis showed that the sugar (day = 11.67, 95% CI = 10.11–13.97) and oil (day = 11.85, 95% CI = 10.45–13.90) treated groups were similar to each other and outside the 95% confidence interval of the control group (day = 5.73, 95% CI = 4.87–7.33).

As can be seen in Fig. 4, after 4 days on full food, the animals were at approximately 95% of their ad libitum body weight. In addition, approximately 90% of the animals resumed their cycle.

4.3. Discussion

4.3.1. Comparison of estrous cycle in different strains of rats

A comparison was made among four commonly used rat strains: Fischer, Long–Evans, Sprague–Dawley, and Brown Norway rats. Specifically, Brown Norway rats had irregular cycle patterns even before the food manipulation and this cycle irregularity was increased with food deprivation. The other three strains had reliable cycles under full food conditions. However, cycle status of animals before food deprivation was not always predictive of cycle status during the food deprivation period. For example, the Fischer rats showed a reliable cycle before food deprivation, yet 75% of the rats stopped cycling by day 5 of deprivation. In contrast, most of the Long–Evans and Sprague–Dawley rats displayed a reliable cycle both before and during food deprivation. These findings may be related to the fact that both the Sprague–Dawley and Long–Evans rats weigh more (275 and 290 g, respectively) than the Fischer and Brown Norway rats (181 and 178 g, respectively). Presumably, the larger body mass could provide for greater body energy stores, making the animals less susceptible to food deprivation. However, given the fact that food deprivation schedules were adjusted to account for differences in initial body weight, it would seem unlikely that simple strain differences in body weight account for the results. A second possible source of these results is a difference between inbred and outbred strains. Both Fischer

and Brown Norway animals are inbred, whereas the Long–Evans and Sprague–Dawley rats, both of which are unaffected by food deprivation, are outbred.

4.3.2. Effects of mild and severe food deprivation on the estrous cycle

Investigating the effects of mild food restriction may allow for subtle effects, which are normally masked by severe food deprivation, to be expressed. In the present study, a mild food deprivation (body weight loss to 15%) over a long duration was used to examine the effects on cyclic behavior. The estrous cycle of the Sprague–Dawley rats was minimally disrupted under these conditions. Nakanishi et al. [20] showed that severe food deprivation (50% of normal intake) resulted in cessation of the estrous cycle in Sprague–Dawley rats. Similarly, Marin Bivens et al. [15] showed that 72-h fasting in Sprague–Dawley rats decreased lordosis behavior. Taken together, these findings suggest that while the estrous cycle of Sprague–Dawley rats is less susceptible to the effects of mild food deprivation, these rats are not immune to the effects of severe food deprivation.

Findings from Experiment 2 revealed that both the rate of body weight loss and the percent of ad libitum body weight were related to the cessation of the cycle in Fischer rats.

4.3.3. Effect of enriched diets on maintaining the estrous cycle in food deprived animals

In the third experiment, animals were given enriched diets in place of their normal diet and the estrous cycle was monitored over this period. Animals receiving sugar or oil supplements were less disrupted than control animals. Although by design, all three groups showed similar rates of weight loss, the oil and sugar groups continued to show regular cycles for 6 more days. Thus, enriching the food with oil or sugar delayed disruption of the cycle, but did not prevent anestrus.

Similar results have been shown in the past. Leptin treatment has been shown to prevent fasting-induced anestrus in hamsters [22,23]. Leptin influences fuel metabolism by increasing both fatty acid oxidation and glucose oxidation [23]. While this method of treatment influences the estrous cycle, it is invasive and stressful for the animals.

Schneider and Wade [24] showed that when hamsters were deprived of their normal chow but provided with either glucose solution or vegetable shortening, these animals had a decreased incidence of anestrus. The present results extend their findings to rats.

4.3.4. Effect of return to free access to food on the estrous cycle

The return of ad libitum access to food for the Fischer animals restored the estrous cycle in most animals within 3–4 days. Nakanishi et al. [20] also showed a return to normal cycling (after 3–5 days) in Sprague–Dawley rats when given back full food after 30- or 60-day period of severe food restriction (50% of normal intake). Bronson [3] severely food deprived prepubertal Sprague–Dawley rats (45% of normal body weight) for approximately 30 days. When animals returned to unlimited access to food, they ovulated after only 2–4 days of ad libitum feeding. The present experiment extended on these studies to include Fischer rats. Thus, animals can quickly resume their normal cycling patterns if they are returned to ad libitum body weight.

4.3.5. Effects of mild food deprivation on the estrous cycle: possible role of prior experience and age

These studies were not specifically designed to examine the effects of prior experience or age on the estrous cycle. However, the fact that Fischer animals used in the three experiments varied in their behavioral history allows us to infer possible influences of experience.

4.3.5.1. Prior behavioral training. In the second experiment, all animals that were examined had previous training on behavioral tasks. In the first and third experiment, the animals had no prior training aside from the daily handling routine. A comparison of the results showed similar effects of food deprivation on the estrous cycle despite training history (compare Fig. 1 Fischer rats, with Fig. 2 Fischer rats, and with Fig. 3b control group).

4.3.5.2. Prior sexual experience. Although not the explicit goal of these studies, data were obtained from both retired breeders and virgin animals. The second and third experiment used retired breeders, while the first experiment examined virgin rats. Notably, the retired breeders (Experiments 2 and 3: 223 g ± 3.22 S.E.M., 224 g ± 4.40 S.E.M.) weighed more than the virgin animals (Experiment 1: 181 g ± 3.35 S.E.M.). However, in both cases, when animals were food deprived below 90% of ad libitum body weight the estrous cycle was severely disrupted. Consequently, these data indicate that differences in body weight or prior sexual experience do not appear to interact overtly with the disruptive effects of food deprivation on the estrous cycle.

4.3.5.3. Age of the animals. It has been shown in the past that the disruption of the estrous cycle begins around 8–12 months [8,13,16]. A comparison of the Fischer rats in all three experiments showed a similar effect of food deprivation on the estrous cycle. Specifically, both the 6- and 8-month-old animals showed a severe disruption of their cycle when they were food deprived below 90% of ad libitum body weight. Thus, there does not appear to be an overt effect of this range of ages (6–8 months) on the disruptive effects of food deprivation.

4.3.6. Implications for tasks requiring food reinforcement

Many behavioral tasks require mild food deprivation. Our results indicate that when food deprivation is required preference should be given to using Long–Evans, Sprague–Dawley, or other outbred rats, rather than Fischer or Brown Norway animals. In situations where Fischer rats must be

used, the current findings provide a number of strategies to minimize the disruptive effects of food deprivation on the estrous cycle. The weight of the animals should be monitored carefully and, optimally, the Fischer rats or other inbred strains should be maintained at no less than 90–95% of their ad libitum body weight. Additionally, enriching the normal diet with either a sucrose or fat additive is a noninvasive and relatively simple manipulation that should further ensure the maintenance of the cycle.

While there has been much research on the effects of severe food restriction on the estrous cycle, the current study examined the effects of relatively mild food deprivation. Investigating the effects of mild food restriction allowed for subtle effects such as strain differences or the effects of relatively minor changes to the diet to be expressed. Furthermore, while severe food restriction is a good animal model for conditions like anorexia [1], mild food deprivation studies can provide data on less extreme situations.

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