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Physiology & Behavior 70 (2000) 397–405

PHYSIOLOGY & BEHAVIOR

Spontaneous meal patterns in female rats with and without access to running wheels

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Received 22 February 2000; received in revised form 14 April 2000; accepted 14 April 2000

Abstract

Rats display strong behavioral rhythms during the ovarian cycle. During estrus, food intake is minimal due to a decrease in meal size, and locomotor activity is maximal. To investigate how activity influences feeding patterns across the ovarian cycle, we used a computerized system to monitor spontaneous meal patterns in intact, cycling female rats with and without access to running wheels. We found that running wheel access decreased dark meal frequency, increased dark meal size, and increased 24-h water intake during each phase of the ovarian cycle. In contrast, body weight, 24-h food intake, and the ovarian rhythms of reduced food intake, meal size, and body weight during estrus were not affected by running wheel access. In particular, the reduction in food intake during estrus was due to a selective reduction in dark meal size, not dark meal frequency, and this occurred independent of wheel access. These data indicate that estrus-related changes in spontaneous meal patterns and locomotor activity are independently controlled and that the reduction in food intake during estrus involves a selective change in the neurobiological controls of meal size.

Keywords: Meal size; Food intake; Ovarian cycle; Locomotor activity; Circadian rhythms

1. Introduction

Many behaviors vary during the ovarian cycle. In most rat strains, the estrous phase is accompanied not only by the increase in sexual receptivity from which its name derives, but also by increased locomotor activity and aggressive behavior and by decreased food intake, water intake, and sodium intake [1–9]. Food intake has also been shown to decrease around the time of ovulation in other species including women [10,11].

Analyses of spontaneous patterns of feeding and activity have been central to the physiology of feeding behavior since Richter identified them as fundamental biological variables [12]. Available data indicate that the estrus-related decrease in food intake is due to decreased meal size [13–15]. In contrast, meal frequency either remains stable [15,16] or increases during estrus [13,17]. In none of these studies, however, were animals given opportunity to increase spontaneous activity (i.e., by housing them in larger cages or with access to running wheels) although such manipulations are known to increase the amount of activity occurring during estrus [6]. Therefore, the main goal of the present study was to extend previous work by determining, for the first time, the influence of running wheel access on spontaneous meal patterns in cycling female rats. Given the apparent plasticity of meal size and meal frequency, it is possible that rats might decrease meal frequency rather than meal size under the influence of the competing motivation to increase activity. Because meal size and meal frequency appear to be controlled by distinct physiological mechanisms [18–21], identifying how meal size and meal frequency change during the ovarian cycle is prerequisite for understanding the physiology of this phenomenon.

2. Experiment 1

This experiment characterized variations in spontaneous meal patterns during the ovarian cycle in rats that had free access to laboratory chow and running wheels.

2.1. Method

2.1.1. Subjects and housing

Eight female Long–Evans rats (Charles River Breeding Laboratory, Wilmington, MA), weighing between 300–330 g, were housed individually in Plexiglas cages (floor area 450–475 cm^2; height 40–50 cm) with stainless steel wire

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0031-9384/00/$ – see front matter
PII: S0031-9384(00)00278-X

mesh floors and perforated lids. A feeding niche (8 × 9 × 13 cm) protruded from each cage about 4 cm above the floor. A circular opening (4.5 cm in diameter) in the floor of each niche allowed access to a spill-resistant food bowl that was mounted on an electronic balance (EW 300, A&D, Tokyo, Japan; ±0.1 g) to facilitate the analysis of spontaneous meal patterns. Tap water was presented in drip-resistant bottles, and rats were given continuous access to ground rat chow (#5001, Ralston Purina, St. Louis, MO). The cages were connected to Wahmann rotating stainless steel running wheels (35 cm in diameter, no load) by a 5-cm Plexiglas tube. The room was maintained at 20 ± 2°C with a 12:12 LD cycle (lights off, 1300–0100 h). Six 34-W fluorescent lamps were lit during the light period and two red 40-W incandescent lamps provided dim illumination during the dark period. A white noise generator (Lafayette Instruments, Lafayette, IN) was run at moderate intensity to mask extraneous noise except from 0830–0930 h, when the procedures described below and daily maintenance were carried out.

2.1.2. Ovarian cycles

Vaginal mucosal samples were obtained daily about 4 h prior to dark onset by inserting a cotton swab moistened with warm 0.15 M saline about 1.5 cm into the vagina. The sample was transferred to a microscope slide, fixed with alcohol (Surgipath Cytology Spray, Richmond, IL), stained with hematoxylin and eosin (HHS-32 and HT40-2-32, Sigma Diagnostics, St. Louis, MO), and examined microscopically (Olympus Provis AX, Tokyo, Japan; 10–40× magnification). Phases of the ovarian cycle were identified using standard criteria [7]. The first day of the cycle was characterized by a progression from leukocytes interspersed with small clusters of nonnucleated cornified cells (i.e., metestrus, lasting ~10 h) to leukocytes interspersed with nucleated epithelial cells (i.e., the onset of diestrus). This day was labeled diestrus 1. The second day (diestrus 2) was characterized by leukocytes interspersed with nucleated epithelial cells. Day 3, proestrus, was characterized by large clumps of round, nucleated epithelial cells, the absence of leukocytes, and occasional small clusters of cornified cells. Day 4, estrus, was characterized by large clumps of nonnucleated squamous cornified cells. Cycle phase labels were assigned to the 24-h period ending at the time of sampling. Thus, proestrus included the peak in estradiol secretion, and estrus included the subsequent dark period when female rats ovulate, increase sexual receptivity, and increase locomotor activity.

2.1.3. Meal patterns

Outputs from the balances were fed via an interface (Plus 8, Stargate Technologies, Solon, OH) into a computer (Dell 325D, Austin, TX) located in another room. A custom-designed program (VZM, Software Entwicklung Krügel, Munich, Germany) recorded the weight of each balance at 30-s intervals. Additional software (Café Mahlzeit, V1.03, T. A. Houpt) was used to convert individual feeding bouts into discrete meals. As used previously [15,20,21], a meal was defined as any feeding bout of at least 0.2 g that was separated from other feeding bouts by at least 15 min. This criterion resolved 24-h food intake into 8–12 spontaneous meals that accounted for 97–98% of food intake. Dark and light food intake, meal size, and meal frequency were examined across the ovarian cycle. To investigate the pattern of meal size through the dark, average meal size was calculated during each 3-h quartile of the dark period.

2.1.4. Procedure

Each day at 0830 h, the VZM program was halted. Phase of the ovarian cycle, body weight (±1 g), activity (number of revolutions in the running wheel), food intake (±0.1 g), and any food spillage (±0.1 g) were recorded for the preceding 23-h period. Water bottles were weighed (±0.1 g) and values were converted to volumes (±0.1 mL). Food bowls were refilled daily; water bottles and cage bedding were changed three times weekly. The VZM program was restarted at 0930 h, and rats were left undisturbed until the following day at 0830 h. After an 8–12-day adaptation period, testing began on diestrus 1 and was continued for three consecutive ovarian cycles. During this experiment, rats always had access to running wheels.

2.1.5. Data analysis

Two-factor (cycle number by cycle phase) repeated-measures analysis of variance procedures (ANOVAs) were used to analyze changes in 24-h food intake, water intake, body weight, and running wheel activity. One-factor (cycle phase) repeated-measures ANOVAs were used to analyze light food intake, dark food intake, and spontaneous meal patterns during one ovarian cycle that was randomly selected for each rat. A two-factor (dark quartile by cycle phase) repeated-measures ANOVA was used to analyze changes in meal size during the 3-h quartiles of the dark period. When significant effects were detected, differences between individual means were tested with Tukey's honestly significant-difference test. Differences were considered significant when $p < 0.05$. The standard error of the difference (SED) is presented as a measure of residual experiment-wide variability. Data were analyzed with the BMDP (SOLO V6.0; SPSS, Chicago, IL) and SAS (SAS, Cary, NC) statistical packages.

2.2. Results

2.2.1. Ovarian cycling, activity, body weight, and 24-h food and water intake

Daily samples of vaginal cytology appeared normal, and all rats displayed regular 4-day ovarian cycles. Large behavioral changes were detected between phases of the ovarian cycle (Fig. 1). Running wheel activity increased, whereas body weight, 24-h food intake, and 24-h water intake decreased during estrous compared to nonestrous phases, $F(3, 21) = 53.29$, $p < 0.0001$, SED = 1261 revolutions; $F(3, 15) = 16.03$, $p < 0.001$, SED = 2.0 g; $F(3, 21) = 21.68$, $p < 0.0001$, SED = 1.1 g, and $F(3, 15) = 9.83$, $p < 0.001$, SED = 3.0 mL, respectively. Each of these phasic changes occurred consistently during the three ovarian cycles with no main or interaction effect of cycle number.

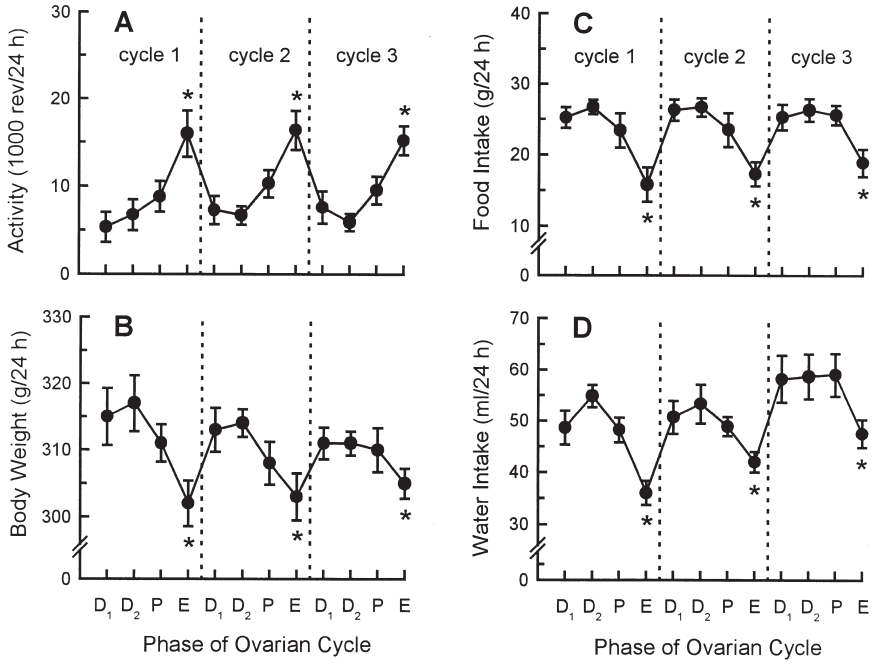

Fig. 1. Running wheel activity, body weight, 24-h food intake, and 24-h water intake vary with phase of the ovarian cycle. During estrus, running wheel activity increased (A), and body weight (B), food intake (C), and water intake (D), decreased. Data are means ± SE. *Significantly different than nonestrous phases (p s< 0.05). Abbreviations: in all figures, D_1 = diestrus 1; D_2 = diestrus 2; P = proestrus; and E = estrus.

2.2.2. Meal patterns

Dark food intake, which accounted for about 85% of the 24-h food intake, decreased during estrous compared to non-estrous phases, $F(3, 18) = 6.92$, $p < 0.01$, SED = 1.8 g (Table 1). Light food intake did not vary significantly with phase of the ovarian cycle (Table 1).

The decrease in dark food intake during estrus resulted from a decrease in average dark meal size, $F(3, 18) = 13.06$, $p < 0.001$, SED = 0.2 g, with no change in average dark meal frequency (Fig. 2). Dark meal size varied by quartile, $F(3, 18) = 10.16$, $p < 0.001$, as well as by cycle phase, $F(3, 18) = 12.02$, $p< 0.0001$, SED = 0.6 (Fig. 3). That is, meal size was larger late in the dark than early in the dark during all cycle phases, and meal size decreased during estrous compared to non-estrous phases during all quartiles. No interaction between quartile and cycle phase was detected.

The rats' light meal patterns did not vary significantly across the ovarian cycle (data not shown).

2.3. Discussion

This experiment confirms previous reports that locomotor activity increases [3,6,15,20], food and water intake decrease [5,8,9,15,20], meal size decreases [13–15,17,20], or body weight decreases [22,23] during estrus. We extend these reports by providing the first detailed description of spontaneous feeding patterns across the ovarian cycle in rats with access to running wheels. Our principal result was that food intake decreased during estrus due to a decrease in spontaneous dark meal size, with no change in dark meal frequency. Thus, when rats are given access to running wheels, estrus selectively affects the physiological controls of meal size, not those of meal frequency.

These results do not support the hypothesis that increased motivation to locomote during estrus might decrease meal frequency and thereby induce a compensatory increase in meal size. Despite a three-fold increase in running wheel activity during estrus, the rats initiated the same number of meals as during the other phases of the ovarian cycle when activity levels were lower. Because sexual receptivity is also increased during estrus, it would be interesting to see whether similar results would be obtained if rats had the opportunity to engage in reproductive behavior.

Another novel finding is that female rats with wheels increased meal size through the 12-h dark period during each phase of the ovarian cycle. A similar result was reported previously in adult female rats in which the phase of the

Table 1
Food intake during the light and dark periods during each phase of the ovarian cycle in rats with access to running wheels

	Light food intake (g/12 h)	Dark food intake (g/12 h)
Diestrus 1	3.7 ± 0.4	21.6 ± 2.1
Diestrus 2	3.3 ± 0.3	21.7 ± 1.3
Proestrus	3.7 ± 1.2	19.4 ± 1.2
Estrus	2.5 ± 0.6	14.4 ± 2.2*

Values are means ± SEM ($n = 7$ per group).

*Significantly less than diestrus 1, diestrus 2, and proestrus (ps $<$ 0.05). No significant differences in light food intake were detected.

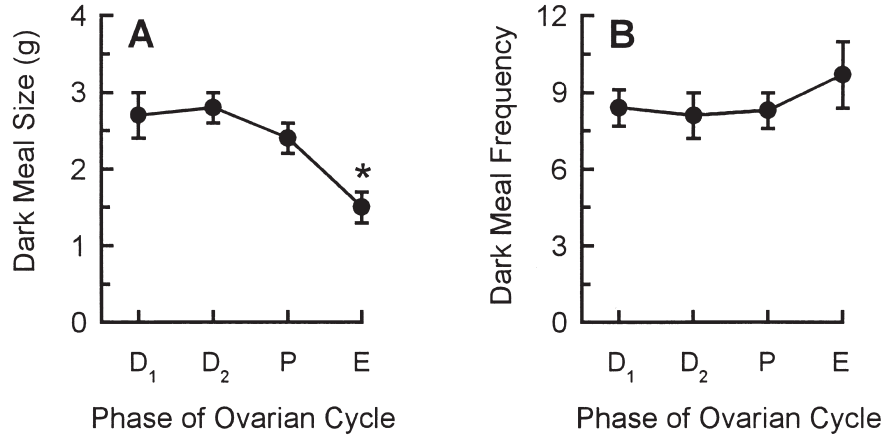

Fig. 2. Dark food intake decreased during estrus due to decreased meal size, not decreased meal frequency. (A) Dark meal size decreased during estrus. (B) Dark meal frequency did not vary significantly with cycle phase. Data are means ± SE. *Significantly different than non-estrus phases ($ps < 0.05$).

ovarian cycle was not monitored [16]. Our findings extend this report by demonstrating that this pattern of increasing meal size through the dark occurs similarly during each phase of the cycle.

We observed a decrease of about 20 g in 24-h food and water intake during estrus. This probably accounts for most or all of the 14-g decrease in body weight during estrus. The smaller change in body weight is likely due to decreased fecal and urinary output [8], but this was not monitored here.

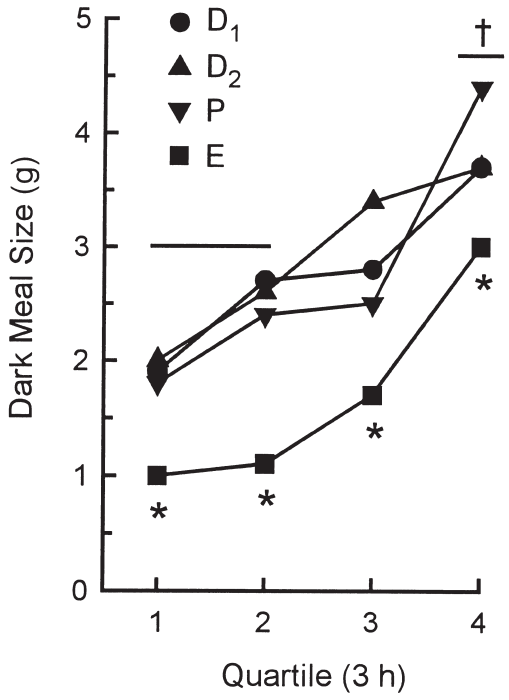

Fig. 3. Meal size increases through the dark period during each phase of the ovarian cycle. Data are mean meal size per 3-h dark quartile ± SE. *Significantly different than non-estrous phases ($ps < 0.05$). †Meal size during quartile 4 was significantly greater than meal size during quartiles 1 and 2 ($ps < 0.05$).

Although ovarian steroids potently affect body adiposity in ovariectomized rats [22,23], there is no evidence that any reduction in body adiposity contributes to the transient decrease in body weight during estrus in intact, cycling rats.

3. Experiment 2

The purpose of this experiment was to determine whether the spontaneous feeding patterns characterized in Experiment 1 depend on the availability of running wheels.

3.1. Method

3.1.1. Subjects and housing

Twelve new female Long–Evans rats, weighing 275–330 g, were assigned to two groups of six. One group had access to running wheels and one did not.

3.1.2. Procedure

Maintenance and daily collection of vaginal smears were identical to Experiment 1. After an 8–12-day adaptation period, the effects of differential access to running wheels on body weight, 24-h food and water intake, and spontaneous meal patterns were monitored for one ovarian cycle, beginning on diestrus 1.

3.1.3. Data analysis

A one-factor (cycle phase) repeated-measures ANOVA was used to analyze changes in running wheel activity across the ovarian cycle. Two-factor split-plot ANOVAs, with wheel availability as the between-subjects factor and cycle phase as the within-subjects factor, were used to analyze changes in 24-h food and water intake, meal patterns, and body weight. A three-factor split-plot ANOVA, with wheel availability as the between-subjects factor and cycle phase and dark quartile as the within-subjects factors, was used to analyze changes in meal size across the dark period. Post hoc tests of means were performed as described in Experiment 1.

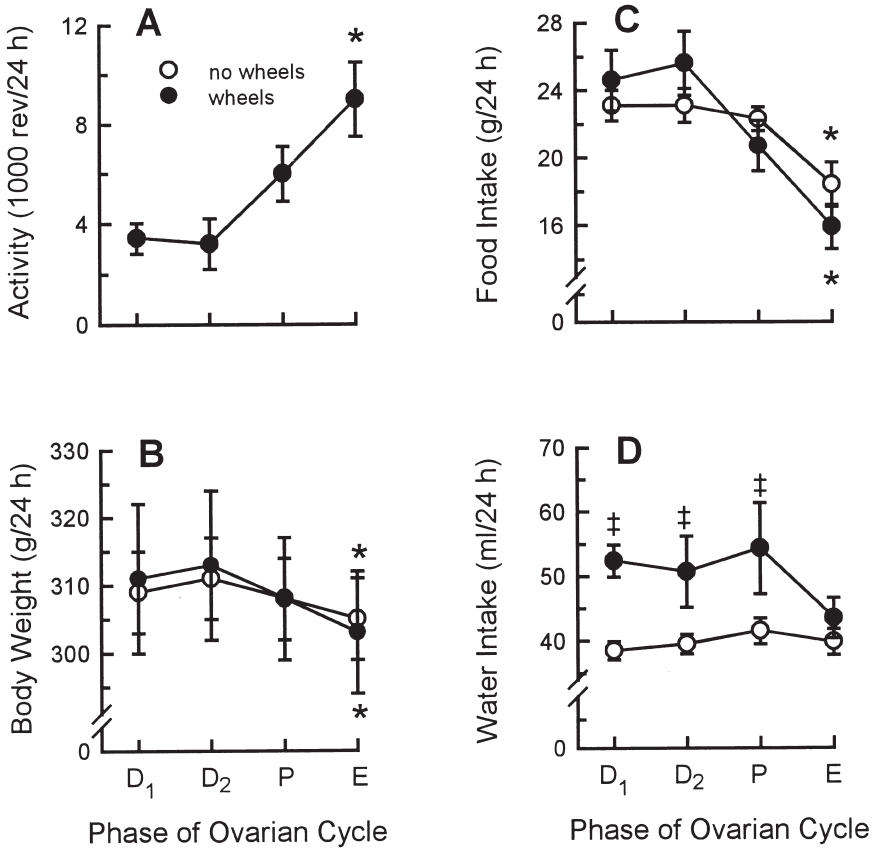

Fig. 4. Effects of running wheel access on body weight, 24-h food intake, and 24-h water intake across the ovarian cycle. Activity increased during estrus in rats with running wheels (A). Running wheel access did not significantly alter body weight (B) or food intake (C), but increased water intake during the non-estrous phases (D). Both groups reduced body weight and food intake during estrus. Data are means ± SE. *Significantly different than non-estrous phases within each group (ps < 0.05). ‡Significant group difference (ps < 0.05).

3.2. Results

3.2.1. Ovarian cycling, activity, body weight, and 24-h food and water intake

Daily samples of vaginal cytology appeared normal and rats displayed regular 4-day cycles ($n = 10$) or 5-day cycles ($n = 2$, one from each group). Both rats with 5-day cycles displayed diestrous vaginal smears for 3 days. Data from the third day of diestrus, which were similar to those on diestrus 1 and diestrus 2, were not included in the analysis. Activity increased during estrous compared to non-estrous phases in rats with wheels, $F(3, 15) = 11.32$, $p < 0.0005$, SED = 1144 revolutions (Fig. 4A). Access to running wheels did not modify the ovarian rhythm of body weight or 24-h food intake (Fig. 4B and C). Both groups reduced body weight and food intake during estrous compared to nonestrous phases, $F(3, 30) = 7.06$, $p < 0.001$, SED = 2.6 g, and $F(3, 30) = 14.05$, $p < 0.0001$, SED = 1.8 g, respectively, with no main or interaction effect of wheel availability. Rats with wheels increased 24-h water intake over rats without wheels during all cycle phases except estrus, $F(1, 8) = 7.71$, $p < 0.05$, SED = 4.2 mL, with no main or interaction effect of cycle phase (Fig. 4D).

Table 2
Food intake during the light and dark periods during each phase of the ovarian cycle in rats with and without access to running wheels

	Wheels	No wheels
Dark food intake (g)		
Diestrus 1	18.5 ± 1.3	17.7 ± 0.8
Diestrus 2	20.8 ± 1.8	18.3 ± 0.8
Proestrus	16.7 ± 1.6	17.8 ± 0.3
Estrus	11.1 ± 1.0*	15.0 ± 1.2†
Light food intake (g)		
Diestrus 1	6.2 ± 1.3	5.3 ± 0.5
Diestrus 2	4.9 ± 0.6	4.8 ± 0.9
Proestrus	4.1 ± 0.9	4.6 ± 0.6
Estrus	4.8 ± 1.2	3.4 ± 0.4

Values are means ± SE ($n = 6$ per group).
*Significantly less than diestrus 1, diestrus 2, and proestrus (ps < 0.05).
†Significantly less than diestrus 2 ($p < 0.05$). No significant differences in light food intake were detected.

3.2.2. Meal patterns

Access to running wheels did not affect the proportion of food intake consumed during the dark period (77% in rats with wheels and 80% in rats without wheels) and did not modify the ovarian rhythm of decreased dark food intake during estrus, $F(3, 30) = 9.75$, $p < 0.0001$, SED = 1.8 g

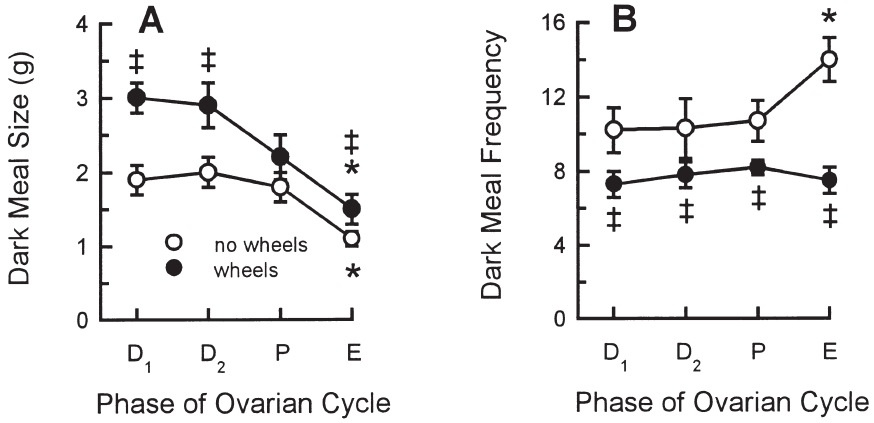

Fig. 5. Effects of running wheel access on mean dark meal size and dark meal frequency across the ovarian cycle. (A) Access to running wheels increased dark meal size during all cycle phases except proestrus. Both groups decreased dark meal size during estrus. (B) Access to running wheels decreased dark meal frequency during all cycle phases. Rats without wheels increased dark meal frequency during estrus, whereas rats with wheels did not modulate dark meal frequency across the ovarian cycle. Data are means ± SE. *Significantly different than non-estrous phases within each group ($ps < 0.05$). ‡Significant group difference ($ps < 0.05$).

(Table 2). Light food intake did not vary significantly with the phase of the ovarian cycle in either group (Table 2).

Access to running wheels increased average dark meal size and decreased average dark meal frequency (Fig. 5). Although meal size was larger in rats with wheels, $F(1, 10) = 11.93$, $p < 0.01$, SED = 0.3 g, both groups decreased meal size during estrus, $F(3, 30) = 13.40$, $p < 0.0001$ (Fig. 5A). These changes were nearly additive (the mean difference in meal size between non-estrous and estrous phases was 0.8 g in rats without wheels and 1.1 g in rats with wheels), and there was no interactive effect of wheel availability and cycle phase. Access to running wheels decreased meal frequency during all cycle phases and suppressed the increase in dark meal frequency during estrus that was apparent in rats without wheels, as indicated by a significant interaction, $F(3, 30) = 3.29$, $p < 0.05$, SED = 1.0 meal (Fig. 5B).

Meal size increased late in the dark period compared to early in the dark period in rats with wheels during all cycle

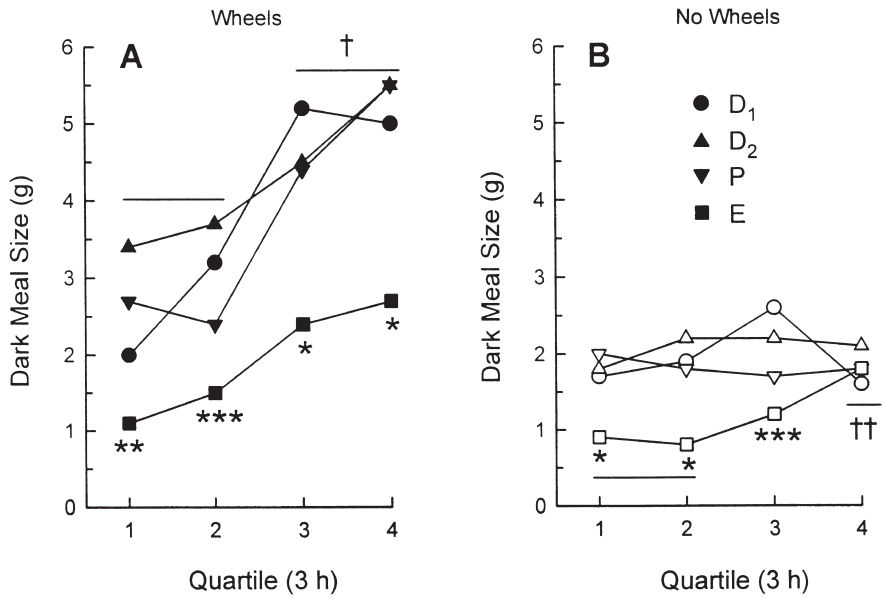

Fig. 6. Effects of running wheel access on average meal size per 3-h dark quartile across the ovarian cycle. (A) Rats with wheels increased meal size through the dark period during all cycle phases. (B) Rats without wheels did not modulate dark meal size during the non-estrous phases but increased meal size through the dark during estrus. Data are means ± SE. *Significantly different than non-estrous phases ($ps < 0.05$). **Significantly different than diestrus 2 and proestrus ($p < 0.05$). ***Significantly different than diestrus 1 and diestrus 2 ($ps < 0.05$). †In rats with wheels, meal size during quartiles 3 and 4 were significantly greater than meal size during quartiles 1 and 2 during all cycle phases ($ps < 0.05$). ††In rats without wheels, meal size during quartile 4 was significantly greater than meal size during quartiles 1 and 2 during estrus ($ps < 0.05$).

phases, $F(1, 10) = 16.06$, $p < 0.005$, SED = 0.6 g. Although a similar pattern was apparent in rats without wheels during estrus ($p < 0.05$), there was no main effect of meal size through the dark period, $F(1, 10) = 2.13$, NS, SED = 0.3 g (Fig. 6). Both groups reduced meal size during estrous compared to nonestrous phases [wheels: $F(3, 44) = 8.58$, $p < 0.001$; no wheels: $F(3, 44) = 5.77$, $p < 0.01$].

The rats' light meal patterns were not significantly affected by the availability of running wheels or cycle phase (data not shown).

3.3. Discussion

This experiment revealed that access to running wheels decreased dark meal frequency, increased dark-meal size, produced progressively larger meals through the dark, and increased 24-h water intake. Access to wheels, however, did not affect either 24-h food intake or body weight. During estrus, 24-h food intake, dark meal size, and body weight were reduced in both rats with and without wheels, whereas dark meal frequency was increased only in rats without wheels.

Rats both with and without wheels decreased 24-h food intake and dark meal size similarly during estrous compared to non-estrous phases, despite the fact that activity levels varied considerably between groups. Thus, locomotor activity does not appear to control these estrus-related changes in feeding. Rather, the stability of 24-h food intake across several ovarian cycles (Experimental 1) suggests a strong involvement of the hypothalamic–pituitary–ovarian (HPO) axis.

Running wheel access influenced the pattern of meal size through the dark. Rats with wheels increased average meal size through the dark during all cycle phases, whereas rats without wheels did so only during estrus. This is similar to the pattern of 2-h interval intakes reported by Ter Haar [9], who found that cycling rats without access to running wheels increased 2-h interval intakes relatively continuously through the dark during estrus, but displayed a bimodal pattern, with peaks just after dark onset and prior to light onset, during other phases. In another study in which the phase of the ovarian cycle was not monitored [16], no changes in average meal size through the dark were detected in rats without running wheels. In rats with access to running wheels, however, meal size did increase through the dark, similarly to what we observed here. These increases in meal size through the dark in rats with wheels or during estrus in rats without wheels may be related to activity levels. That is, perhaps rats increase meal size through the dark when activity levels are high, either during estrus or because running wheels are available. A more detailed analysis of the relationship between feeding bouts and running bouts should further illuminate the relationship between feeding and locomotor activity in female rats.

Running wheel access also influenced meal frequency during the dark phase. Rats with wheels decreased dark meal frequency and failed to show the increase in dark meal frequency during estrus that occurred in rats without wheels here and previously [13,14,17]. These findings indicate that increased activity decreased the frequency of meal initiation in female rats.

Running wheel access did not affect 24-h food intake or body weights, despite the presumed increase in energy expenditure in rats with wheels. A similar result was reported previously [2], and contrasts with the rapid loss of body weight in male rats given access to wheels [24]. The cause of this sex difference is not known. In a longer term study (3 weeks), running wheel access did increase female rats' food intake [2]. Thus, several weeks of running wheel access may be required to induce a compensatory increase in food intake in female rats. It is also possible that sex differences in activity-induced changes in metabolic rate or partitioning of nutrients between lean and adipose tissue contributes to the apparent difference in the feeding responses to increased physical activity in male and female rats.

Water intake was also differentially affected by running wheel access. Despite their similar 24-h food intakes, rats with wheels drank more water than rats without wheels during non-estrous phases. It is not clear whether this increase represents an increase in prandial drinking or drinking that is not associated with meals. The ovarian rhythm of reduced water intake during estrus was also influenced by running wheel access. Rats with wheels decreased water intake during estrus, whereas rats without wheels did not. This contrasts with previous studies using Sprague–Dawley rats without wheel access, in which there have been cyclic declines in water intake during estrus [4, 8]. Our lack of effect may be related to the more variable and smaller cyclic decrease in water intake than food intake [8], the small number of rats in our experiment, or a strain difference.

4. General discussion

Decreased feeding and increased locomotor activity during estrus are among the most prominent behavioral rhythms displayed by cycling female rats [2,3,5,6]. Our goal here was to determine how varying the opportunity to express locomotor activity influences spontaneous feeding patterns through the ovarian cycle. The principal findings were that running wheel access decreased the frequency and increased the size of spontaneous meals, and that meal size and 24-h food intake decreased similarly during estrus whether running wheels were available or not.

Our data do not support the reasonable supposition that the decrease in food intake during estrus might arise from a decrease in meal frequency caused by competition from urges to locomotor or engage in sexual behavior with the urge to initiate meals. Rather, meal frequency was constant through the ovarian cycle in rats with wheels and actually increased during estrus in rats without wheels. Thus, the decreased food intake during estrus results from a selective change in the neurobiological controls of meal size rather

than being secondary to an increase in competing behaviors. This conclusion is consistent with other evidence that HPO function exerts independent controls on feeding, locomotion, and sexual behavior. For example, the threshold doses of peripheral estradiol to affect sexual responsivity and feeding in ovariectomized rats and the latencies of the effects are different [5,25], and estradiol's influences on feeding activity and sexual behaviors may originate in different brain sites [26,27].

There were two interactions between increased opportunity to express locomotor activity and spontaneous meal patterns during estrus. First, rats with access to running wheels increased meal size through the 3-h quartiles of dark feeding during each phase of the ovarian cycle, whereas rats without access to running wheels did so only during estrus. Second, rats with access to running wheels did not increase meal frequency during estrus, whereas rats without access to running wheels did. The relationship between these effects and the control of meal size during estrus is unknown.

Another goal of this study was to examine whether procedural differences may have influenced previous reports of spontaneous meal patterns of cycling female rats. Our demonstration that meal size decreases during estrus in rats whether or not they had access to running wheels demonstrates that previous reports of such estral decreases did not depend on the use of operant paradigms [14] or sweet, liquid diets [13], or access to running wheels. That food intake decreased during estrus in Long–Evans rats here as it did previously with Sprague–Dawley and Wistar rats also suggests that the lack of an estral decrease in food intake in Fischer 344 rats [17] may be a peculiarity of that strain, perhaps related to its small adult size.

The reduction in meal size during estrus appears to be dependent on the preovulatory rise in estradiol. Plasma estradiol concentration increases during diestrus and peaks late in proestrus [28]. Reduction in meal size and increases in sexual receptivity and locomotor activity are first detected during the following dark period (i.e., during estrus). The time lag between increased estradiol secretion and the reduction in meal size is presumed to reflect the time required for expression of target genes regulated by occupied estrogen receptors and for completion of the physiological cascades initiated by them. Meal pattern analysis in ovariectomized rats provides further support for a link between estrogen and meal size. The permanent increase in meal size induced by ovariectomy can be normalized by peripheral estrogen replacement alone [13]. Furthermore, a cyclic regimen of estradiol replacement is sufficient to restore the phasic reduction in meal size that is associated with estrus in intact, naturally cycling rats [29]. Together, these studies provide strong evidence that estradiol causes the reduction in meal size during estrus.

Little is known about how estradiol controls meal size. The most extensively studied mechanism involves cholecystokinin (CCK). CCK is released from the small intestine during a meal and binds to low-affinity CCK_A receptors on vagal afferents of the pylorus and proximal duodenum to initiate a satiety signal [30]. Estradiol may influence meal size by modulating the central processing of this signal [31–33, 38]. Recently, we have found that the satiating potency of endogenous CCK is enhanced during estrus in intact rats [15] and by estradiol treatment in ovariectomized rats [34]. Consistent with this, estradiol increases the expression of feeding- and CCK-induced c-*fos*–like immunoreactivity in the nucleus of the solitary tract, paraventricular nucleus of the hypothalamus, and central nucleus of the amygdala of ovariectomized rats [35,36]. Additional research is required to elucidate other possible mechanisms underlying estradiol's potent inhibitory effects on meal size.

In summary, our results indicate that the reduction in food intake during estrus is due to a reduction in dark meal size in rats with access to running wheels, that access to running wheels increases meal size and decreases meal frequency during all cycle phases, and that there is little interaction among these effects. These results suggest that the estrus-related reduction in food intake results from a selective change in the neurobiological controls of meal size that is independent of the controls of feeding exerted by locomotor activity. Because spontaneous meal size and meal frequency are under different controls [18,21,37], our results should facilitate analysis of the mechanisms underlying the reduction of food intake during estrus in female rats.

Acknowledgments

This research was supported by a Natural Sciences and Engineering Research Council of Canada Fellowship (L.A.E.) and by NIH Grants DK03198 (T.A.H.) and MH 51135 (N.G.).

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