

1975

Ovarian influences on the meal patterns of the female rat.

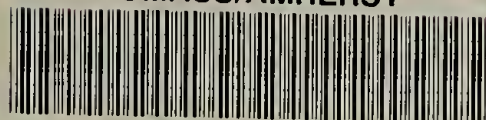
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OVARIAN INFLUENCES ON THE MEAL PATTERNS
OF THE FEMALE RAT

A Thesis Presented

By

Jeffrey D. Blaustein

Submitted to the Graduate School of the
University of Massachusetts in partial
fulfillment of the requirements for the degree of

MASTER OF SCIENCE

September 1975

Psychology

OVARIAN INFLUENCES ON THE MEAL PATTERNS
OF THE FEMALE RAT

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By

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ACKNOWLEDGEMENTS

I would, first of all, like to express my gratitude to my advisor, George Wade, whose enthusiasm sparked my initial interest in this research. He has made the past year of graduate school, most of all, a pleasant experience. The other members of my committee, Richard Gold and Edward Hirsch, deserve thanks for generating a number of once unanswered questions and for thoughtful criticisms both of the experiments and of the manuscript.

I also thank Edward Roy and R. Thomas Gentry, not only for comments on the manuscripts but for hours of discussion during the course of the experiments. I am grateful to John deCastro for his gift of the computer program. Thanks are also due to Elizabeth Silver and Cynthia Steinberg for technical assistance.

Lastly, I would like to thank my wife, Marilyn, whose help, encouragement and distractions facilitated rather than hindered the completion of this thesis.

ABSTRACT

A series of experiments examined the effects of estrous cycles, ovariectomy, and estradiol replacement on free-feeding meal patterns of female rats maintained on a liquid diet. The proestrous decrease in food intake was reflected in a decrease in meal size and a less than fully compensatory increase in meal frequency. The hyperphagia induced by ovariectomy was reflected in an increase in meal size and a decrease in meal frequency. When food intake returned to preoperative levels, meal size remained elevated while frequency decreased further. Estradiol benzoate (2 μ g/day) decreased meal size and temporarily decreased total daily food intake in long-term ovariectomized rats. The subsequent return to food intake levels of controls with continued estradiol treatment was due primarily to an increase in meal frequency. These results indicate that the transient changes in food intake caused by estradiol withdrawal and replacement are accompanied by permanent changes in meal size followed by compensatory changes in the number of meals consumed per day. They suggest that the decrease in meal size at proestrus is due to a direct effect of estradiol on the mechanisms that terminate short-term food intake and are not secondary to changes in the level of daily food intake.

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INTRODUCTION

The role of ovarian hormones in the body weight regulation of female rats has been extensively studied and comprehensive reviews are available on the subject (Wade, 1972, 1975b). During proestrus, just after the peak in estradiol secretion, food intake decreases relative to di-estrous levels (Brobeck, Wheatland & Strominger, 1947; Tarttelin & Gorski, 1971). This decrease in energy intake, along with a correlated increase in running wheel activity (Finger, 1969; Wang, 1973) and a decrease in thermoregulatory nest-building (Kinder, 1927; Blaustein, Note 1), two sources of energy expenditure to the environment, results in a net body weight decrease during proestrus (Brobeck et al., 1947).

Withdrawal of gonadal steroids by ovariectomy results in an increase in food intake and a concomitant increase in body weight (Tarttelin & Gorski, 1973). Estradiol-17 β injected systemically (Mook, Kenney, Roberts, Nussbaum & Rodier, 1972; Tarttelin & Gorski, 1973) or implanted in the area of the ventromedial hypothalamus (Beatty, O'Briant & Vilberg, 1974; Jankowiak & Stern, 1974; Wade & Zucker, 1970) reverses these effects, resulting in a decrease in food intake and decreased body weight. Systemic progesterone administered in the ovariectomized (OVX) rat is effective only when paired with estradiol injections, in which case it can block estradiol's suppressive effects on eating and body

weight (Wade, 1975a; Zucker, 1969).

The ovariectomy-induced hyperphagia is transient; food intake returns to control levels when the body weight levels off at an increased value (Gentry & Wade, in press; Mook et al., 1972). It is possible that the hyperphagia is not a primary effect of the steroid withdrawal. The primary effect may be to alter the level at which body weight is maintained. Similarly, the suppression in food intake resulting from estradiol replacement is transient. Total daily food intake returns to normal when the animal stabilizes at a lower body weight (Mook et al., 1972; Wade, 1975a). This indicates that the food intake-suppressing effect of estradiol may be secondary to its effects on body weight.

Few studies have examined the eating behavior of female rats over periods shorter than 24 hours. ter Haar (1972) measured food intake at two-hour intervals during the estrous cycle. He found that during estrus, metestrus and diestrus, there are two food intake peaks in the two-hour totals during the dark phase of the illumination cycle. There is an initial peak just as the lights are turned off and a secondary peak 6-10 hours later (Drewett, 1973; ter Haar, 1972). The proestrous decrease in food intake is due mainly to the absence of the first peak.

In an analysis of the meal patterns of female rats over the estrous cycle, it was found that the decrease in food intake during the night of proestrus was accomplished by a

decrease in the size of individual meals, which was partially compensated by a concomitant decrease in intermeal intervals (Drewett, 1974). The latter decrease is probably indicative of an increase in meal frequency, assuming rate of eating within a meal is somewhat constant, but these data are not reported. Conversely, withdrawal of ovarian hormones has been shown to increase meal size and to cause a less than fully compensatory decrease in meal frequency (Kenney & Mook, 1974).

Although analyses of microregulatory controls of meal parameters in the male rat (e.g., LeMagnen, 1971; Levitsky, 1970, 1974) and following central nervous system intervention (e.g. Balagura and Devenport, 1969; Becker and Kissileff, 1974; Teitelbaum & Campbell, 1958; de Castro, Note 2) have been extensive, there have been no other accounts of meal patterns as a function of gonadal hormone state than those already discussed. This approach has proven useful in the characterization of changes in feeding behavior following both ventromedial hypothalamic lesions (Becker and Kissileff, 1974) and lateral hypothalamic lesions (Kissileff, 1970; Snowdon and Wampler, 1974; de Castro, Note 2). The following series of experiments were undertaken in an attempt to describe the changes in meal-taking behavior of the female rat as a function of gonadal hormone state.

GENERAL METHOD

Adult female Sprague-Dawley rats were purchased from commercial suppliers (Camm Research Institute, Wayne, New Jersey or Charles River Breeding Laboratories, Wilmington, Massachusetts) and arrived in the laboratory weighing 200-250 g. All animals were housed individually in Wahmann LC-75 wire-bottom cages. Environmental illumination was provided from 0800 to 2000 hrs. daily, and room temperature was maintained at $22^{\circ}\text{C} \pm 1^{\circ}$. Inverted water bottles containing liquid diet were supported by C-clamps attached to the outside of the cage. The drinking tube was positioned 5 mm from the inside of the cage, and access was through a 1.1 cm x 1.1 cm opening in the stainless steel mesh. Clean bottles with fresh liquid diet were replaced and animals were weighed between 0800 and 1000 hrs. daily. Tap water was available ad libitum.

The diet was a more dilute version of that described by Becker and Kissileff (1974). Added to each can of Borden's Magnolia Brand sweetened condensed milk (300 ml) were 600 ml of tap water, .6 ml of Poly-vi-sol vitamin drops (Mead Johnson) and 2 ml of a solution containing 5.8 g/l copper sulfate, 25.8 g/l ferrous gluconate and 3.9 g/l manganese acetate. Formalin (.1%) was added to retard spoilage. Caloric density of the diet was 1.40 Kcal/ml.

Licks were detected by contact drinkometers attached to

the base of the drinking tubes. Each time the rat licked the sipper tube, a circuit was closed which was simultaneously counted by a Mod Comp I digital minicomputer and an Esterline Angus event recorder.¹ The computer was programmed to recognize a meal based on two parameters: a minimum number of licks, which set the lower limit for meal size, and a minimum intermeal interval. The minimum intermeal interval defines the duration that must elapse between two licks in order for them to be included in separate meals. Minimum meal size was defined as 25 licks, so that occasional contact with the sipper tube was not counted as a meal. Minimum intermeal interval was defined as 10 minutes. Kissileff (1970) has shown that in a free feeding situation changes in the value of the latter criterion between one minute and 40 minutes have little bearing on meal size distribution.

¹In order to determine the validity of using the number of licks recorded by the Mod Comp computer as an index of food intake, the following procedure was adopted. Four cycling female rats were placed in the meal pattern apparatus. Each day the volume of liquid diet consumed was measured. Corrected food intake was determined by subtracting the volume of spillage, collected under oil in a plastic reservoir located below the tip of the sipper tube. This procedure was followed for nine days. Pearson Product Moment Correlation Coefficients were then determined for the 32 daily food intakes and total daily licks (One rat's data for four days were omitted due to illness.). A highly significant correlation, $r = .871(30)$, $p < .001$, indicated that volume of food intake is, indeed, reflected in the total number of licks as recorded by the computer. A meal of 1,000 licks is equal to approximately 5.2 Kcal.

EXPERIMENT 1

Meal Patterns of Female Rats During the Four Stages of the Estrous Cycle

Drewett (1974) demonstrated that during the night of proestrus the rat's meal size decreased and meal frequency increased. That study was conducted using an operant procedure in which each bar-press was reinforced with a 45 mg Noyes pellet. Kissileff (1970) has shown that a bar-press procedure can result in an exceptionally high occurrence of small meals. This experiment attempted to replicate Drewett's work and extend his results to a free-feeding liquid diet situation.

PROCEDURE

Vaginal smears were taken by saline lavage on a population of Sprague-Dawley rats each morning when animals were weighed. Of these, 16 were selected that showed consistent four-day estrous cycles.² These animals were then given 10-14 days to adapt to the liquid diet as their sole source of nutrients. When food intake had stabilized, meal patterns were monitored for two estrous cycles.

²Estrus refers to the day on which a fully cornified vaginal smear was found and the subsequent dark phase of the illumination cycle. Proestrus refers to the light and dark phase prior to this. Sexual receptivity normally peaks during the dark phase of proestrus. Metestrus refers to the light and dark phase following estrus, and diestrus refers to the next full day.

Within-groups comparisons are evaluated by Wilcoxon matched-pairs, signed-ranks test (T) and are two-tailed. Differences were considered statistically significant if $p < .05$.

RESULTS

Total food intake and body weight. Food intake for the day of proestrus was significantly lower than the rest of the estrous cycle, $T(16) = 27$, $p < .05$. Body weight for the morning of estrus was significantly lower than for the rest of the cycle, $T(16) = 0$, $p < .01$ (Table 1).

Light-dark rhythms. In order to determine whether the light-dark feeding rhythm changed during the estrous cycle, the percentage of total food intake occurring in the dark phase of the illumination cycle was calculated for each stage of the estrous cycle. There were no significant changes in this parameter over the estrous cycle.

Meal patterns. Analyses are dissected into eating in the light and dark phases of the illumination cycle. In the dark, meal size at proestrus was significantly smaller than meal size for the rest of the cycle, $T(16) = 28.5$, $p < .05$. Although the meal size in the light decreased significantly from diestrus to proestrus, $T(16) = 29$, $p < .05$, it was not significantly lower than the rest of the cycle, $T(16) = 35$, $p > .05$. These data indicate that the decrease in food intake on the night of proestrus is achieved by a

decrease in the size of individual meals.

Table 1

Mean Meal Size, Meal Frequency, Daily Food Intake, and Body Weight (\pm Standard Errors of the Mean) of Female Rats During the Four Stages of the Estrous Cycle.

Stage of Cycle	DARK		LIGHT		DAILY	
	Meal Size (licks/meal)	Meal Frequency (meals/day)	Meal Size (licks/meal)	Meal Frequency (meals/day)	Food Intake (licks/day)	Body Weight ^a (grams)
Diestrus	962.8 (± 71.7)	9.1 ($\pm .9$)	976.0 (± 106.7)	3.2 ($\pm .4$)	10,735.2 (± 677.1)	304.0 (± 4.5)
Proestrus	728.9 (± 48.3)	10.4 (± 1.1)	795.5 (± 85.9)	3.1 ($\pm .5$)	9,554.5 (± 768.5)	291.0 (± 4.3)
Estrus	933.0 (± 60.8)	8.9 ($\pm .7$)	794.5 (± 76.1)	3.1 ($\pm .5$)	10,111.9 (± 667.0)	285.7 (± 4.5)
Metestrus	1,064.0 (± 71.8)	8.6 ($\pm .7$)	937.3 (± 83.4)	3.3 ($\pm .4$)	11,495.5 (± 647.6)	288.6 (± 4.5)

^aBody weight measured during the morning of each stage of the cycle. For example, body weight at estrus is a result of energy balance during proestrus.

EXPERIMENT 2

Meal Patterns of Female Rats After Ovariectomy

Since the decrease in meal size occurs concurrently with the decrease in daily food intake, it is possible that the same hormonal changes are responsible for both. This possibility is supported by the work of Kenney and Mook (1974) who demonstrated that meal size increased and meal frequency decreased following ovariectomy. In that study, meal patterns were monitored for a 10-day period following three days of recovery from surgery. However, the hyperphagia induced by ovariectomy is transient, lasting only until body weight levels off at its increased value. It would be of interest to know how the ovariectomized (OVX) rat adjusts its meal pattern to reduce its caloric intake to control levels. Food intake could return to normal by a total reversion to the preoperative meal pattern or by a decrease in either meal size or meal frequency alone. The second experiment investigated the long-term effects of ovariectomy on female rats' meal patterns.

PROCEDURE

Eight cycling rats from Experiment 1 were subjects in this experiment. Meal patterns for the two estrous cycles reported in Experiment 1 served as a preoperative baseline. Ovariectomies (n=5) were performed under sodium pentobarbital

anesthesia (40 mg/kg) through a single midventral incision. Sham ovariectomies (SHAMS; n=3) were identical to ovariectomies except that the uterine horns were not ligated nor were the ovaries excised.

Following a one-day recovery period, the animals were returned to the meal pattern apparatus, where meal patterns were monitored for 53 days. Since it was not possible to match the two groups on all variables of the meal pattern and food intake, data were converted to percentages of the eight-day baseline. Between-group comparisons were evaluated by Mann-Whitney U tests, and for within-groups comparisons, Wilcoxon matched-pairs, signed-ranks tests were used unless specified otherwise. All tests were two-tailed unless otherwise indicated.

RESULTS

Total food intake and body weight change. Following a postoperative decrease in both OVX and SHAM rats that has been reported previously (Gentry & Wade, in press), food intake in the OVX group showed the typical transient increase which lasted approximately until body weight leveled off (Figure 1). In order to best discuss changes in the meal pattern from the dynamic stage of body weight gain to the static phase, comparisons will be made between Days 10-21 postoperatively (dynamic stage = rapid weight gain) and Days 30-53 postoperatively (static stage; Figure 1).

During the dynamic stage, rate of body weight gain for the OVX group (1.48%/day) was significantly greater than the SHAM group (.47%/day), $U(5,3) = 0$, $p < .05$. During the static stage, these two values had decreased to .36%/day and .32%/day, respectively, $U(5,3) = 14$, $p > .05$ (Figure 1). During the period of rapid weight gain, food intake averaged 135% of baseline levels for the OVX group, $T(5) = p < .05$, one-tailed test, whereas food intake during the static period averaged 120%, not significantly above baseline levels, $T(5) = 3$, $p > .05$, one-tailed test.

Light-dark rhythms. The percentage of total food intake occurring in the dark phase of the illumination cycle did not reveal any significant light-dark alterations after ovariectomy either when OVX were compared to their own baseline or when compared to SHAMS.

Meal patterns. During the dark phase of the illumination cycle, the average meal size of the OVX group increased during Days 10-21 to 161% of baseline compared to SHAMS who decreased to 77%, $U(5,3) = 0$, $p < .05$ (Figure 2). This increase in meal size persisted during the static phase with the OVX group at 173% and the SHAMS at 88%, $U(5,3) = 0$, $p < .05$. Meal frequency in the dark phase decreased to 82% for the OVX group during Days 10-21 compared to 126% for SHAMS, $U(5,3) = 0$, $p < .05$. During the static phase, meal frequency decreased further to 73% for the OVX group, $t(4) = 3.829$, $p < .02$, compared to 126% for SHAMS,

$\underline{U}(5,3) = 0, p < .05$, (Figure 2).

In the light portion of the light-dark cycle, the OVX group's meal size increased to 189% during the dynamic phase and remained elevated during the static phase at 177% (Figure 3). Comparisons between these and the respective 64% and 91% for the SHAM group are statistically significant, $\underline{U}(5,3) = 0, p < .05$; $\underline{U}(5,3) = 0, p < .05$. Meal frequency tended to be much more variable in the light phase of the illumination cycle. The corresponding changes for the OVX group were 86% for Days 10-21 and 72% for Days 30-53. For SHAMS, these values were 169% and 144%. Neither the between-groups nor within-groups comparisons were statistically significant (Figure 3).

The data indicate that the increased food intake following OVX is accomplished by a permanent increase in meal size. There is a corresponding decrease in meal frequency which is initially less than compensatory, resulting in a net increase in food intake. Food intake returns to control levels by an additional decrease in meal frequency. The major finding of this experiment is that meal size increases during both phases of the light-dark cycle and remains greatly elevated even after rats return to control levels of food intake.

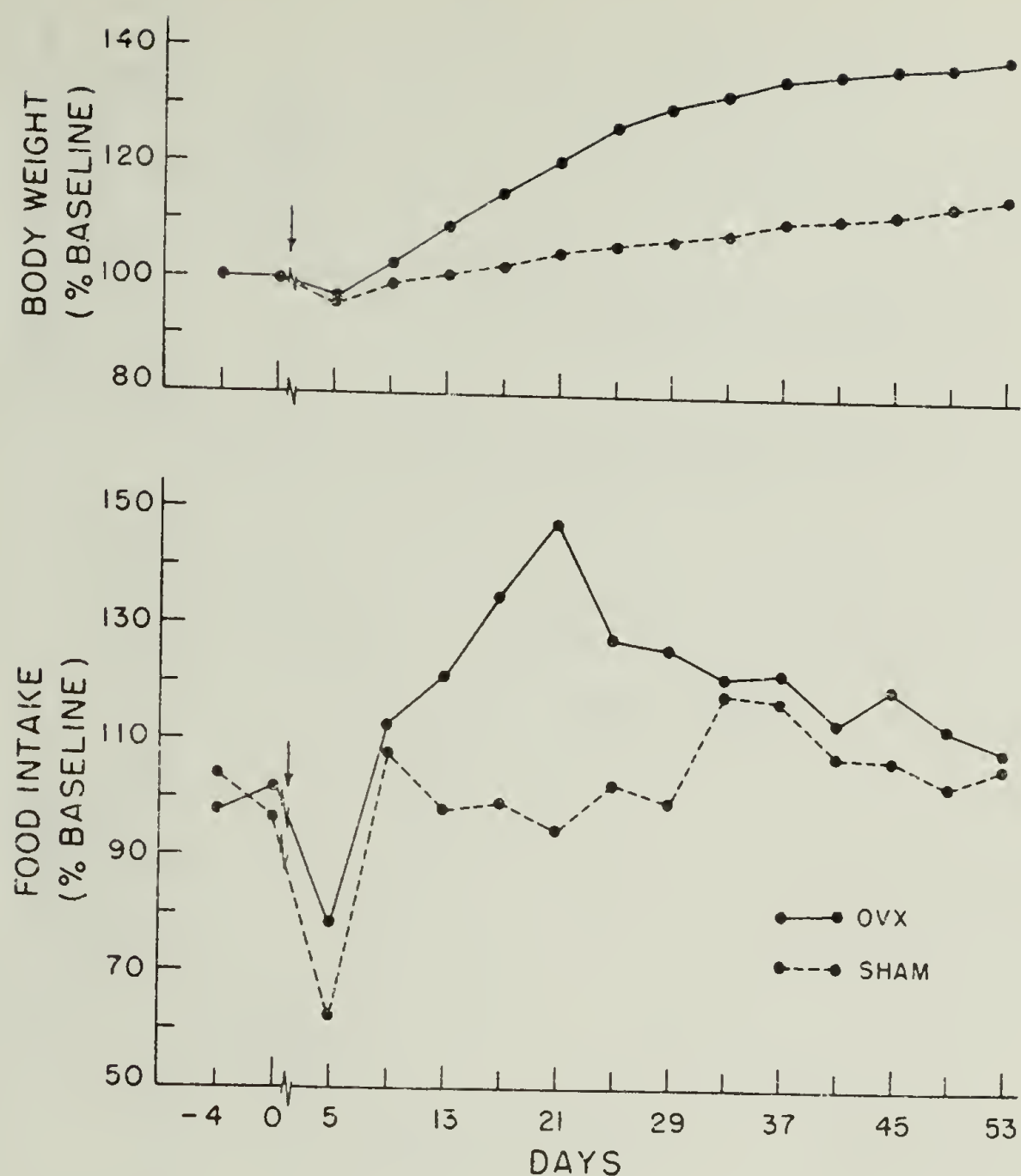


Figure 1. Body weight and daily food intake of ovariectomized (OVX) and sham-operated (SHAM) rats, expressed as percentage of 8-day baseline. Arrow indicates the day of surgery.

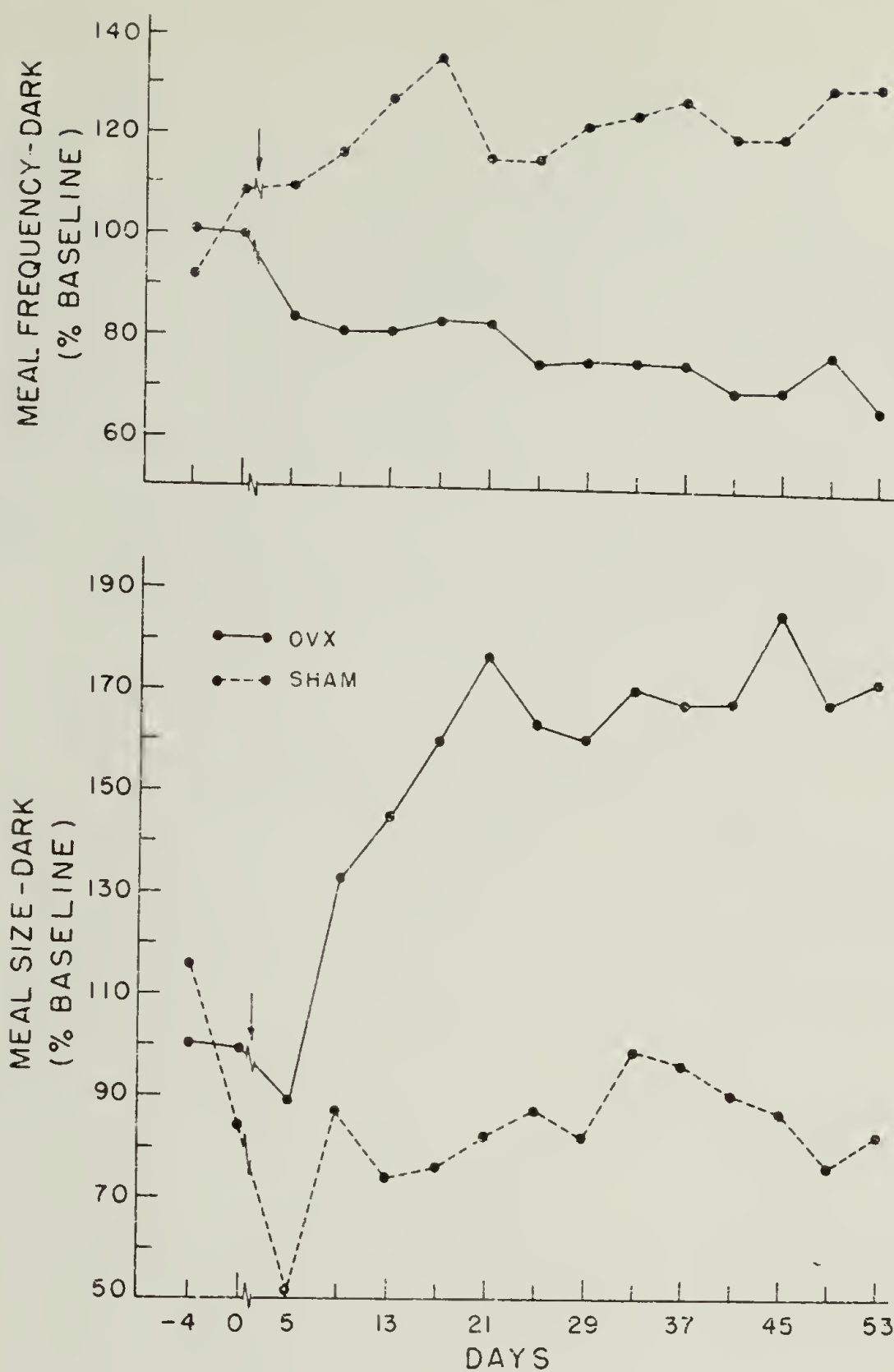


Figure 2. Meal frequency and meal size of ovariectomized (OVX) and sham-operated (SHAM) rats, for eating in the dark phase of the illumination cycle expressed as percentage of 8-day baseline. Arrow indicates day of surgery.

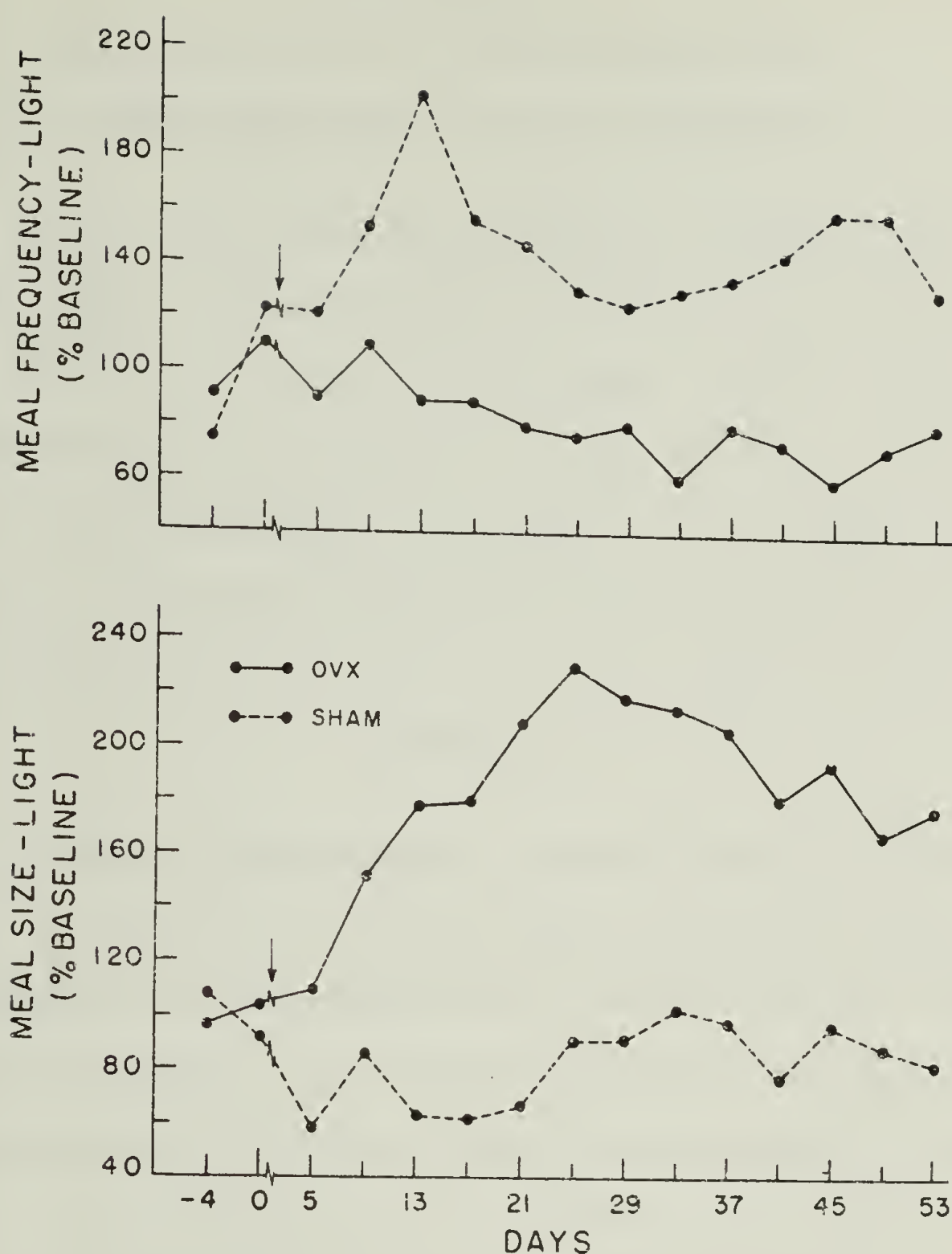


Figure 3. Meal frequency and meal size of ovariectomized (OVX) and sham-operated (SHAM) rats, for eating in the light phase of the illumination cycle, expressed as percentage of 8-day baseline. Arrow indicates the day of surgery.

EXPERIMENT 3

Meal Patterns of Ovariectomized Rats
During Estradiol Benzoate Treatment

In the rat, estradiol injections reverse the elevation in body weight brought about by ovariectomy (Mook et al., 1972; Tarttelin & Gorski, 1973; Wade, 1975a). This has led to speculation that estradiol is the critical gonadal steroid involved in body weight regulation in the female rat. Experiment 3 tests the hypothesis that estradiol injections will reverse the ovariectomy-induced changes in meal patterns.

PROCEDURE

Eighteen Sprague-Dawley female rats were ovariectomized as in Experiment 2. Approximately two months later, rats were adapted to the liquid diet for 10-14 days. After this adaptation period, .1cc sesame oil was injected subcutaneously for the four days of baseline recording of meal patterns. On the fifth day, 11 of the animals were switched to daily injections of 2 µg estradiol benzoate (EB)/.1cc sesame oil and seven rats were maintained on oil injections (OIL). All injections were administered between 0800-1000 hrs., and at the same time animals were weighed, bottles cleaned, and fresh liquid diet given.

RESULTS

Total food intake and body weight change. As in the

previous experiment, the results were divided into the peak of the dynamic stage, Days 3-8 following the first injection, and the static stage, Days 19-24 after the first injection. During the dynamic stage, the EB group lost weight at a rate of .52%/day and OILS gained at the rate of .24%/day, $U(11,7) = 0$, $p < .01$. By the static phase, the EB group was still losing weight albeit at a reduced rate (.07%/day) and the OIL group was still gaining (.23%/day). This difference is not statistically significant, $U(11,7) = 24$, $p > .05$. Food intake for the EB group during the dynamic stage averaged 65% of baseline, significantly below the 96% for the OIL group, $U(11,7) = 8$, $p < .01$ (Figure 4). This depression was transient; food intake rose to 84% for the EB group during Days 19-24 as opposed to 91% for the OIL group, $U(11,7) = 29$, $p > .05$.

Light-dark rhythms. The percentage of total food intake consumed in the dark phase of the illumination cycle increased in the EB group from 78% during pretreatment to 86% during Days 3-8, whereas the OIL group did not (79% and 77%). Neither the within-groups nor between-groups comparison, however, was statistically significant.

Meal patterns. During the dark phase of the illumination cycle, the average meal size of the EB group decreased to 60% of pretreatment levels for the dynamic stage and remained depressed during the static stage at 67% (Figure 5). This contrasts with 97% and 105% respectively for the OIL

group, $\underline{U}(11,7) = 2$, $p < .01$, $\underline{U}(11,7) = 2$, $p < .01$. Although the EB group's meal frequency increased during the dynamic stage above OIL control levels (130% versus 97%), these differences only attained significance in the static stage (134% versus 84%), $\underline{U}(11,7) = 69.5$, $p < .01$ (Figure 5).

Similar changes were evident in the light phase of the illumination cycle. Meal size decreased to 48% during Days 3-8 for the EB group and remained depressed at 68% during the static stage (Figure 6). The comparable values for controls are 103% and 108%. Both of the values for the EB group are significantly below the level for OILS, $\underline{U}(10,7) = 7$, $p < .01$; $\underline{U}(11,7) = 13$, $p < .05$. Meal frequency for the EB group decreased to 76% for Days 3-8 and then rose to 169% for Days 19-24. For the OIL group, these values were 120% and 105%. Although the EB group did not differ from the OIL group, $\underline{U}(11,7) = 24.5$, $p > .05$, $\underline{U}(11,7) = 58.5$, $p > .05$, the increase in meal frequency during treatment for the EB group was significant, $\underline{T}(11) = 3$, $p < .01$ (Days 3-8 versus Days 19-24).

This experiment demonstrates that estradiol replacement results in an immediate and sustained depression in the size of individual meals. The depression differs from the change in total food intake, which is transient. Food intake returns to control levels primarily by a compensatory increase in meal frequency. Although meal size increases slightly during treatment, it remains depressed to a level well below the level of OIL-controls.

Postprandial correlations. The postprandial correlation refers to the correlation between meal size and subsequent intermeal interval. It has been postulated that this correlation is one of the fundamental mechanisms of food intake regulation in the rat (e.g., Le Magnen, 1969). In order to determine if hormonal state exerted any influence on this parameter, Pearson Product Moment Correlation Coefficients were calculated for each rat at all stages of the experiments. Correlations for the dark phase of the illumination cycle were calculated independently of the light because different regression equations characterize each phase (Le Magnen & Devos, 1970). Thus, statistically significant correlations are only found when each phase is considered independently (Le Magnen, Gaudelliere, Devos, Louis-Sylvestre & Tallon, 1973). R to z transformations were employed which allowed for calculation of weighted group means of the correlation coefficients (Edwards, 1950; McNemar, 1962). Statistical tests on these weighted means (McNemar, 1962) revealed statistically significant correlations for all groups at all stages of the experiments (Table 2). In addition, between-groups comparisons were made on the mean slopes of the linear regressions. The slope at proestrus did not differ significantly from the mean slope for the other stages of the estrous cycle. The only comparison that attained statistical significance was that between EB and OIL during the dynamic stage, $U(11,7) = 63$, $p < .05$.

(Table 2). This can be interpreted as evidence that for an equal meal size the dynamic-stage EB-treated rat delays the onset of its subsequent meal for a longer period of time than the OIL-treated controls. Although during the static stage, the slope is still elevated, it is not statistically different than OIL-controls.

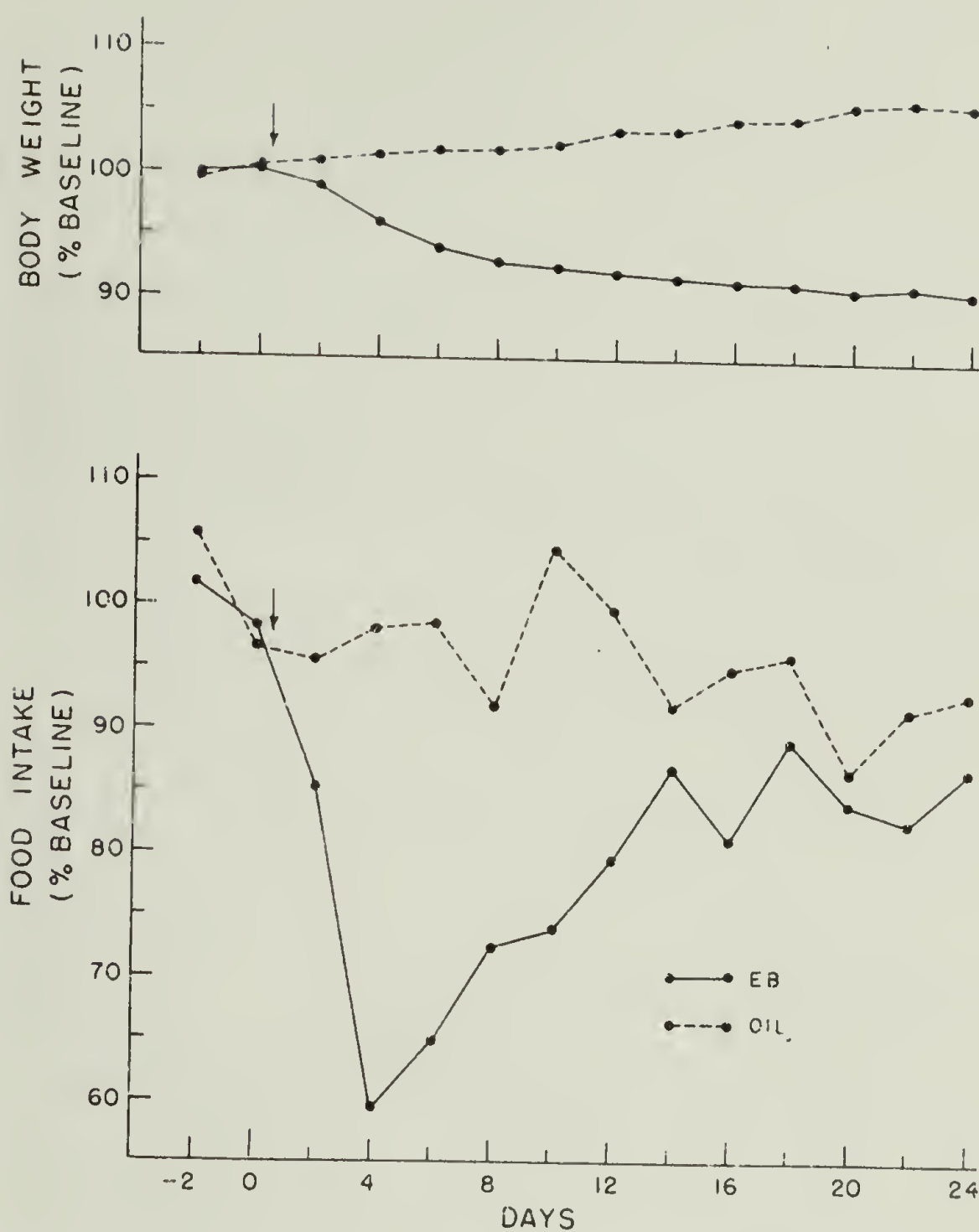


Figure 4. Body weight and food intake of ovariectomized rats injected daily with sesame oil (OIL) or 2 μ g estradiol benzoate (EB), expressed as percentage of 4-day baseline. Arrow indicates start of EB treatment.

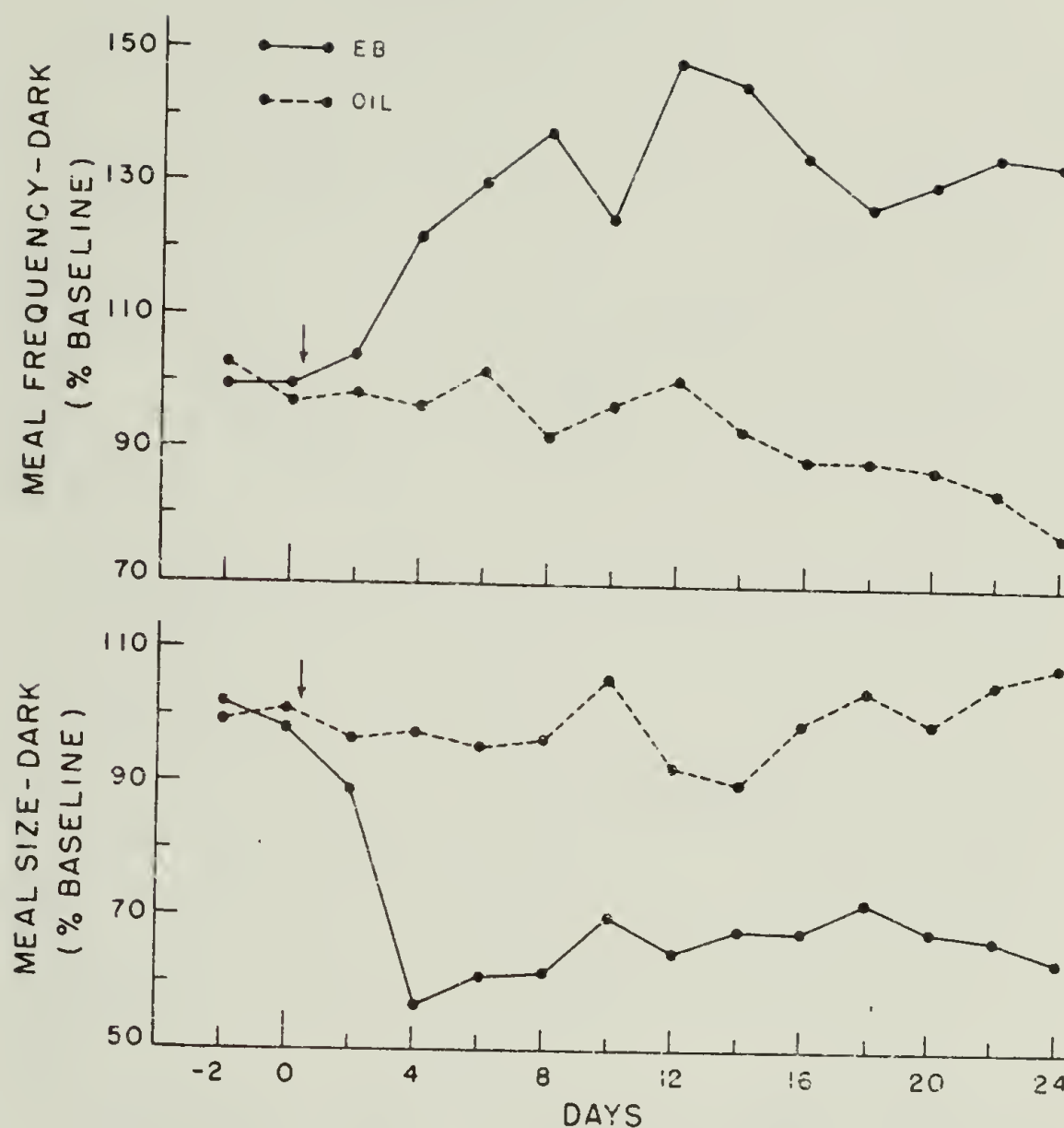


Figure 5. Meal frequency and meal size of ovariectomized rats injected daily with sesame oil (OIL) or 2 ug estradiol benzoate (EB), for eating in the dark phase of illumination cycle, expressed as percentage of 4-day baseline. Arrow indicates start of EB treatment.

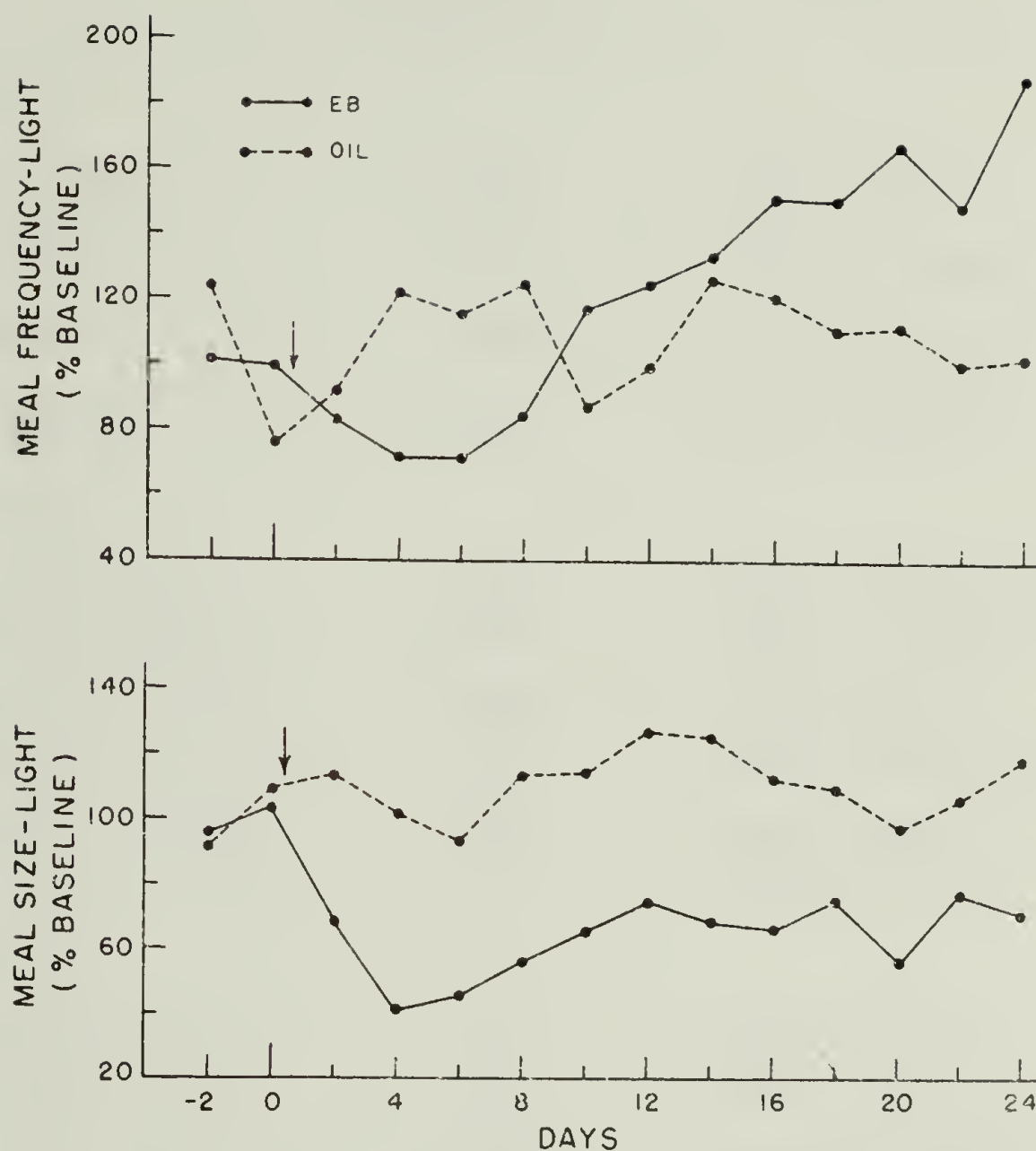


Figure 6. Meal frequency and meal size of ovariectomized rats injected daily with sesame oil (OIL) or 2 ug estradiol (EB), for eating in light phase of illumination cycle, expressed as percentage of 4-day baseline. Arrow indicates start of hormonal treatment.

Table 2

Postprandial Correlation Coefficients and Slopes of Regression Lines for Eating in the Dark Phase of the Illumination Cycle for Female Rats in Different Hormonal States

	Weighted Mean of r	Slope (\pm SEM)
Experiment 1		
Diestrus	.337 ¹	2.256 (\pm .443)
Proestrus	.271 ¹	2.563 (\pm .725)
Estrus	.418 ¹	3.527 (\pm .589)
Metestrus	.379 ¹	2.419 (\pm .435)
Experiment 2		
OVX-Baseline	.373 ¹	2.229 (\pm .705)
SHAM-Baseline	.490 ¹	4.404 (\pm .868)
OVX-Days 14-21	.397 ¹	1.598 (\pm .600)
SHAM-Days 14-21	.504 ¹	3.149 (\pm .880)
OVX-Days 46-53	.368 ¹	2.074 (\pm .784)
SHAM-Days 46-53	.422 ¹	2.747 (\pm .352)
Experiment 3		
EB-Baseline	.417 ¹	2.461 (\pm .456)
OIL-Baseline	.557 ¹	3.400 (\pm .646)
EB-Days 5-8	.633 ¹	5.309 (\pm .654) ²
OIL-Days 5-8	.496 ¹	3.387 (\pm .508)
EB-Days 21-24	.605 ¹	4.637 (\pm .875)
OIL-Days 21-24	.548 ¹	2.368 (\pm .919)

¹ p < .001

² Comparison of EB to OIL group, p < .05.

DISCUSSION

Experiment 1 corroborated the work of Drewett (1974) by demonstrating that the rat's decrease in food intake at proestrus is accomplished by a decrease in meal size and a less than fully compensatory increase in meal frequency. It extended Drewett's (1974) experiment by obtaining this pattern in a free-feeding situation. The replication of the same meal pattern with the present procedure indicates that Drewett's results were not due to his use of an operant procedure which has been shown to result in an exceptionally high occurrence of small meals (Kissileff, 1970). It also demonstrates that the same pattern is obtained whether Noyes pellets or condensed milk is used as the source of nutrition.

Experiment 2 demonstrated that following ovariectomy, there is a permanent modification in the meal pattern of the rat. Kenney and Mook (1974) had shown that during Days 4-13 postoperatively, meal size increases approximately 27%. This experiment demonstrated that the size of meals actually increased over 60% in the dark phase of the illumination cycle during Days 10-21 postoperatively (Figure 2). In addition, meal frequency in the dark decreased below the levels of sham-operated controls. The increase in meal size is also evident in the light phase of the illumination cycle. Furthermore, Experiment 2 demonstrated that this alteration in meal pattern is permanent. When food intake returns to

sham-operated control levels, meal size remains elevated. Food intake returns to normal by an additional decrease in the number of meals. The meal pattern does not revert to its preoperative pattern.

The results of Experiment 3 bore out the prediction that EB treatment would reverse the effects of ovariectomy. This experiment showed that estradiol treatment causes a decrease in meal size; food intake subsequently returned to oil-injected control levels primarily by an increase in meal frequency. When food intake returned to normal, meal size was still depressed well below that of controls.

Experiment 3 suggests that estradiol is the principal ovarian hormone affecting short-term food intake (meal size). Similar to its effects on body weight (Mook et al., 1972; Tarttelin & Gorski, 1973), estradiol permanently reverses the effects of ovariectomy on short term food intake. First, meal size is altered, and then meal frequency changes in a less than fully compensatory manner. When total food intake returns to normal and body weight stabilizes at its new level, meal frequency has adjusted in full compensation for the meal size alteration.

It has previously been demonstrated by correlative data (ter Haar, 1972), by ovariectomy (Tarttelin & Gorski, 1971) and by estradiol replacement (Mook et al., 1972; Wade, 1975a) that estradiol is the ovarian hormone that depresses food intake at proestrus in the intact female rat. Estradiol

secretion peaks around noon on the day of proestrus (Butcher, Collins, & Fugo, 1974). Food intake begins to decline shortly after this peak; the greatest depression is on the night of proestrus (Drewett, 1973; Blaustein, Note 3). Since the meal pattern is altered primarily on the night of proestrus, and since ovariectomy has opposite effects and estradiol reverses ovariectomy's effects, these results are consistent with the conclusion that the decrease in meal size at proestrus is due to estradiol.

The primary effect of OVX and EB replacement could be to modify intermeal interval rather than meal size. This is highly unlikely since during the dynamic stage following EB treatment meal size decreases significantly, before meal frequency compensates. Unfortunately, the immediate effects of ovariectomy cannot be directly observed, because of the consequent surgical trauma.

Wade (1972, 1975b; Wade & Zucker, 1970) has suggested that estradiol may act on the ventromedial hypothalamus (VMH) to depress food intake in the intact female rat. This suggestion derives from the fact that in female rats estradiol implants in the VMH, but not other hypothalamic regions, depress food intake and body weight (Beatty, O'Briant & Vilberg, 1974; Jankowiak & Stern, 1974; Wade & Zucker, 1970). That the hormone implants are acting physiologically is supported by studies that have found a high concentration of ³H-estradiol uptake in the VMH of ovariectomized rats (Pfaff

& Keiner, 1973). Additional support came from similarities in the meal pattern of OVX and VMH-lesioned female rats but until now, direct comparisons of the long-term effects were not possible. Teitelbaum and Campbell (1958) examined the meal patterns of VMH-lesioned female rats in the dynamic and static stages of body weight gain. In their experiment that used a free-feeding situation, dynamic VMH rats ate meals twice the size of static stage VMH rats. By the static stage the size of individual meals nearly returned to preoperative levels, indicating that the altered meal size as well as the alteration in total food intake was transient. In a more detailed examination, Becker and Kissileff (1974) demonstrated that in VMH-lesioned female rats that became obese, meal size increased during the first three days and then declined until the static stage when meal size was only slightly elevated above baseline. The results of the present study on the meal patterns of ovariectomized rats are clearly at odds with the preceding description of the meal pattern of VMH-lesioned female rats. Whereas in VMH-lesioned rats meal size returns nearly to normal, following ovariectomy meal size remains permanently elevated. This discrepancy could be most easily explained if, as Becker and Kissileff (1974) suggest, the VMH or its connections exert a restraint on meal size, as well as affecting other micro-regulatory parameters. These controls may be neurologically distinct, and estradiol could influence but one of these.

It has been suggested that estradiol's entire effect is upon a long-term weight regulation system, not directly upon the "mechanisms which mobilize or inhibit feeding" (Redick et al., 1973, page 546). An extensive body of data indicates that there is, indeed, an influence on body weight regulation (e.g., Wade, 1975b). On the other hand, the present experiments suggest that there is a direct effect, at least on short-term food intake that is independent of -- but perhaps overlapping with -- controls of body weight regulation.

There is a great deal of evidence for some intestinal contribution to the termination of short-term food intake (Ehman, Albert & Jamieson, 1971; Snowden, 1975; Young, Gibbs, Antin, Holt & Smith, 1974). Because OVX and EB replacement each result in a permanent modification of meal size, this suggests that a correlate of meal termination is being changed. Among the possibilities is an alteration in the secretion of or sensitivity to cholecystokinin (CCK) which has been suggested as an important factor in the termination of short-term food intake (Gibbs, Young & Smith, 1973a, 1973b; Smith, Gibbs & Young, 1974). Recent data however, suggest that a change in sensitivity to CCK is not involved since the C-terminal octapeptide of CCK suppressed short-term food intake in EB-treated OVX rats and oil-treated OVX rats to the same extent (Blaustein, Note 4). Likely candidates that could affect the putative intestinal satiety mechanisms are an alteration in gastrointestinal motility or

stomach emptying. Two surgical manipulations that result in decreased meal size are vagotomy with rats on liquid diet (Davis & Booth, 1974; Snowdon & Epstein, 1970) and lateral hypothalamic (LH) lesions (Kissileff, 1970; Snowdon & Wampler, 1974; DeCastro, Note 2). Vagotomized rats' short meals are accompanied by an acceleration in gastric emptying (Snowdon, 1970); the LH-lesioned rats' shortened meals are accompanied by an acceleration in gastrointestinal transit (Ralph & Sawchenko, Note 5). Conversely, the increases in meal size which follow VMH lesions (Teitelbaum & Campbell, 1958) are accompanied by decreases in gastrointestinal transit (Sawchenko, Ralph & Balagura, 1975). The speculation that these types of changes may accompany changes in meal pattern over the estrous cycle and after ovariectomy or EB replacement is supported by the fact that in the human female, gastric emptying varies over the menstrual cycle (Crean, 1963).

These studies shed some new light on eating through the estrous cycle. The results of ovariectomy demonstrate that in the absence of ovarian hormones, an alteration in one parameter of food intake (meal size) occurs that is not dependent on changes in total food intake. Because estradiol replacement reverses this modification, estradiol is probably the hormone responsible for the cyclic alteration of meal size in the intact female rat. Furthermore, in OVX and EB-treated rats, this alteration in meal size is

permanent and does not revert to control levels when food intake returns to control levels. In summary, it is likely that the meal size modifications are due to a direct effect of estradiol on some component of the mechanisms that normally terminate short-term food intake and are not secondary to changes in the level of total daily food intake.

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