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Effect of Hypophysectomy, Thyroidectomy, Castration and Adrenalectomy on Diurnal Food and Water Intake in Rats (40511)

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Rats consume the bulk of their food during the dark phase of the diurnal cycle (1, 2). In a previous report we observed that hypophysectomy altered but did not abolish the rats normal feeding rhythm (2). The question ensued as to which of the several pituitary hormones might be implicated in the partial maintenance of the natural diurnal feeding rhythm. Thyroxine and growth hormone are necessary for typical growth and development. Prolactin has been observed to increase prior to the usual onset of feeding in the rat (3, 4). The gonadotropins have also been reported to influence feeding patterns in rats (5, 6). More recently a large body of literature has considered the possible relationships of corticosterone rhythms and feeding behavior (7–12). Antidiuretic hormone also appears to be important in maintaining the light–dark distribution of drinking in rats (13).

In the present study we wanted to discern if thyroidectomy, adrenalectomy or castration would mimic the effects of hypophysectomy on modifying the normal diurnal feeding rhythm of rats or alter the animals natural drinking rhythm.

Materials and methods. Experiment 1. Male hypophysectomized and nonoperated Sprague-Dawley rats were purchased from Simonsen Laboratories Inc. Gilroy, CA. All animals were born on the same day and the hypophysectomies were performed when the animals were 40 days old. On arrival the hypophysectomized rats were injected with 2 μg of triiodothyronine and 1 mg of corticosterone. The corticosterone injections were continued for 2 days. The rats were housed individually under a light–dark (L:D) ratio of 12:12 with lights on at 0700 hr for 13 days before the start of the measurement period. Throughout the study the animals were given Purina Rat Chow and water *ad libitum*. To prevent disturbance of the rats, entry to the animal room was restricted to a few minutes each day for routine maintenance. At the start of the measurement period the rats were 56 days old and weighed 137.3 ± 3.0 g (hypox) and 245.1 ± 4.6 g (controls). During the six day measurement period food intake, spillage and water consumptions were recorded at 0700 hr and 1900 hr. This was accomplished by exchanging preweighed food cups and water bottles for those on the cages. Thus the amount of food (corrected for spillage) and water consumed during the dark and light phases of the diurnal cycle could be ascertained.

For statistical comparisons the experimental animals were considered hypophysectomized if they did not show weight gains (weight 3 days after arrival 139.2 ± 3.0 g and at end of the study 138.2 ± 2.0 g) and after histological examination showed no pituitary remants in the sella turcica. This yielded a population of 13 hypophysectomized and 14 control animals.

Experiment 2. Male Sprague-Dawley rats that were born on the same day, were again purchased from Simonsen and housed and cared for under the conditions noted in *Experiment 1.* The animals were randomly divided into four groups. The first group was thyroidectomized by Simonsen Laboratories at 31 days of age. After their arrival the remaining animals were operated on at 42 days of age. The second group of rats was bilaterally castrated. The third group received bilateral adrenalectomies. This group was then given 1% saline drinking water and injected with 1 mg corticosterone for three days. The final group was sham operated with one third receiving sham thyroidectomies, another third sham castrations and the remainder sham adrenalectomies. The 6-day measurement period commenced when the animals were 58 days old. At this time the shams

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who weighed 287.9 ± 7.6 g were significantly [F(3,40) = 122.5, $P < 0.001$] heavier than the thyroidectomized (127.7 ± 3.8 g $P < 0.001$), castrated (252.9 ± 7.4 g, $P < 0.01$) or adrenalectomized (236.0 ± 6.0 g, $P < 0.001$) animals. Diurnal food consumption was measured as outlined in *Experiment 1* while cyclic water intake was computed with the use of Wahmann graduated water bottles. Thyroidectomies were verified if the animals showed no significant weight gains over a 10-day period (127.7 ± 3.8 g vs 132.7 ± 4.6 g). Adrenalectomies were considered complete if the animals died within 15 days of being given tap water to drink (average day of death after switch 7.3 ± 0.4 days) and after autopsy showed no remnants of adrenal tissue. These criteria yielded a population of 12 thyroidectomized, 10 castrated, 8 adrenalectomized and 14 sham operated rats.

The data from both experiments were analyzed using Student's *t* test, ANOVA, Duncan's multiple range test, Krusal-Wallis test, Mann Whitney-*U* test and Chi-square.

Results. Experiment 1. The controls (Table I) ate 87.0 ± 0.6% (6-day average) and the hypophysectomized rats 77.2 ± 1.5% of their food (Z = 5.27, $P < 0.001$) during the dark phase of the diurnal cycle. The controls (Table I) also drank a significantly greater percentage of their water during the dark period compared to the hypophysectomized subjects, (6-day average, 86.3 ± 1.5% vs 74.2 ± 7.3%, Z = 5.13, $P < 0.001$). It should be noted that while hypophysectomy modified the normal diurnal feeding cycle of the rats the rhythm was still strongly influenced by the photoperiod. The control group demonstrated little day to day variation in the percentage of food they consumed during the dark and light periods of the diurnal cycle. However, the hypophysectomized animals displayed a great deal of day to day variation (summed group data, Dark phase: controls, $\chi^2 = 30.4$, $P > 0.99$; hypophysectomized $\chi^2 = 172.9$ $P < 0.001$).

Experiment 2. There was a significant difference [H(3) = 84.8, $P < 0.001$] in the percentage of daily food consumed during the dark phase by the four groups (Table II). The thyroidectomized (THX) rats ate a significantly lesser percentage of their food in the dark period compared to the shams (6 day average, THX 79.5 ± 1.8% vs SHAMS, 92.1 ± 0.9%, Z = 6.70, $P < 0.001$). On the other hand the adrenalectomized (ADX) animals consumed a slightly larger percentage of their food during the dark phase when contrasted to the shams (6-day average, 96.8 ± 0.7%, Z = 3.52, $P < 0.001$). The castrates did not differ significantly from the controls (6-day average, 91.1 ± 0.8%).

The shams, adrenalectomized (ADX) and castrated (CAST) animals had very little day to day variation in the percentage of daily food they consumed during the dark period (summed group data, Dark phase: shams, $\chi^2 = 52.4$, $P > 0.99$; ADX, $\chi^2 = 13.5$, $P > 0.99$; CAST, $\chi^2 = 22.1$, $P > 0.99$). However, the thyroidectomized rats manifested a large

TABLE I. PERCENTAGE OF FOOD AND WATER CONSUMED DURING THE DARK PHASE OF THE DIURNAL CYCLE AND AVERAGE DAILY FOOD (GRAMS) AND WATER (ML) INTAKE.

	Percentage of consumption in the dark phase					
	Food			Water		
	Hypox ($n = 13$)	Control ($n = 14$)	*P*	Hypox ($n = 13$)	Control ($n = 14$)	*P*
Day 1	84.6 ± 3.1	91.5 ± 1.5	<0.05	86.1 ± 2.1	92.1 ± 1.6	<0.05
Day 2	81.3 ± 3.9	85.7 ± 0.9	N.S.	74.2 ± 2.7	85.7 ± 1.2	<0.001
Day 3	77.3 ± 4.6	86.6 ± 1.8	≤0.07	58.6 ± 7.0	76.3 ± 4.6	<0.05
Day 4	75.9 ± 2.9	86.8 ± 0.9	<0.001	96.0 ± 1.7	99.8 ± 0.4	N.S.
Day 5	71.9 ± 2.9	85.9 ± 1.5	<0.001	69.4 ± 2.8	87.3 ± 1.2	<0.001
Day 6	72.3 ± 3.4	85.1 ± 1.9	<0.01	61.5 ± 6.0	76.3 ± 5.6	N.S.
6 Day average	77.2 ± 1.5	87.0 ± 0.6	<0.001	74.2 ± 2.3	86.3 ± 1.5	<0.001
	Average food consumption (g)			Average water consumption (ml)		
6 Day average 24 hr consumption	10.0 ± 0.2	23.7 ± 0.3	<0.001	27.8 ± 0.8	36.7 ± 1.1	<0.001

TABLE II. PERCENTAGE OF FOOD AND WATER CONSUMED DURING DARK PHASE OF THE DIURNAL CYCLE.

	Food				Water			
	THX ($n = 12$)	Cast ($n = 10$)	ADX ($n = 8$)	Sham ($n = 14$)	THX ($n = 12$)	Cast ($n = 10$)	ADX ($n = 8$)	Sham ($n = 14$)
Day 1	86.6 ± 5.3	93.0 ± 2.0	97.2 ± 1.9	96.2 ± 1.2	92.5 ± 3.9	98.8 ± 0.8	92.8 ± 3.0	98.2 ± 0.4
P[a]	<0.02	N.S.[b]	N.S.		N.S.	N.S.	<0.05	
Day 2	76.4 ± 3.2	91.1 ± 2.6	96.4 ± 1.4	93.2 ± 2.1	87.9 ± 4.7	89.0 ± 5.8	88.9 ± 4.0	97.9 ± 1.2
P	<0.001	N.S.	N.S.		<0.05	N.S.	<0.01	
Day 3	72.5 ± 7.0	90.5 ± 2.0	97.4 ± 1.7	90.2 ± 2.3	89.2 ± 2.5	96.3 ± 1.6	90.3 ± 3.8	95.9 ± 1.1
P	<0.02	N.S.	N.S.		<0.05	N.S.	N.S.	
Day 4	79.3 ± 3.1	90.9 ± 1.9	96.1 ± 2.1	90.1 ± 2.5	93.5 ± 3.0	95.4 ± 1.9	89.5 ± 2.6	94.8 ± 1.8
P	<0.02	N.S.	N.S.		N.S.	N.S.	N.S.	
Day 4	86.7 ± 3.0	89.8 ± 1.9	95.7 ± 2.0	91.2 ± 1.7	91.8 ± 2.0	96.1 ± 1.0	87.9 ± 3.1	93.8 ± 1.5
P	N.S.	N.S.	N.S.		N.S.	N.S.		
Day 6	80.8 ± 2.8	92.0 ± 1.6	98.2 ± 1.1	92.2 ± 2.0	93.0 ± 3.1	98.8 ± 0.6	92.0 ± 1.3	97.8 ± 0.8
P	<0.01	N.S.	N.S.		N.S.	N.S.	<0.001	
6 Day average	79.5 ± 1.8	91.1 ± 0.8	96.8 ± 0.7	92.1 ± 0.9	91.1 ± 1.4	95.5 ± 1.2	90.2 ± 1.2	96.4 ± 0.5
	<0.001	N.S.	<0.001		<0.001	N.S.	<0.001	

[a] Statistical comparison made against shams only.

[b] NS = non-significant.

day to day variation (Dark phase, $\chi^2 = 87.2$, $P < 0.025$).

The average daily food intake of all three experimental groups was significantly attenuated [F(3) = 398, $P < 0.001$] when contrasted to the sham animals (6-day average, shams, 25.2 ± 0.4 g; THX, 9.6 ± 0.2 g, $P < 0.001$; ADX, 19.3 ± 0.4 g, $P < 0.01$; CAST, 22.3 ± 0.4 g, $P < 0.05$). Interestingly while the food intake of the three experimental groups was diminished in comparison to the shams only the thyroidectomized animals displayed an altered diurnal feeding rhythm.

While the percentages of daily water consumed during the dark phase differed significantly in the four groups [H(3) = 19.0, $P < 0.001$] the differences were slight (Table II). The thyroidectomized rats drank (6-day average) 91.1 ± 1.4% and the shams 96.4 ± 0.5% ($Z = 3.12$, $P < 0.001$) of their water during the dark phase. The adrenalectomized animals also consumed a lower percentage of their saline water during the dark phase compared to the controls (6-day average, 90.2 ± 1.2%, $Z = 4.50$, $P < 0.001$). However, it should be noted that such a comparison between controls and adrenalectomized rats may not be entirely valid since the controls were given water and the adrenalectomized rats saline to drink. The castrates again did not differ significantly from the shams (95. ± 1.2%).

Average daily water consumption of the four groups also differed [F(3) = 373.7; $P < 0.001$]. The adrenalectomized rats drank more (6-day average, 54.4 ± 1.2 ml, $P < 0.001$) and the thyroidectomized less (6-day average, 15.1 ± 0.4 ml, $P < 0.001$) than the shams (6-day average, 33.5 ± 0.5 ml). The water consumption of the castrated group did not depart significantly from the shams (6-day average, 30.1 ± 1.1 mls).

Discussion. The feeding data of the first experiment are in good agreement with our previous findings (2) in that hypophysectomy attenuates but does not abolish the natural diurnal feeding rhythm of rats. Our data are also in accord with the observations of Stephan and Zucker (14) who used hypophysectomized-ovariectomized rats. However, their data (14) indicated a more pronounced attenuation of the feeding cycle. The explanation for the magnitude differences of the feeding rhythms of the present study and that of Stephan and Zucker (14) might be attributed to either procedure, sex dissimilarity, strain or to the fact that their animals had hypophysectomies combined with ovariectomies. In contrast to the observed modification of the diurnal feeding rhythm noted in the present study, our earlier work (2) and that of Stephan and Zucker (14) are the findings of Jouhaneau-Bowers (15) who observed no alteration in the diurnal feeding rhythm of rats following hypophysectomy. The reason for these discrepancies (2, 14, 15) are unclear but may be the result of procedural differences. The attenuated diurnal drinking rhythm observed by Stephan and Zucker (14) and in the present study were of the same extent.

The 4- to 5-day estrous induced feeding-activity cycle (5, 6, 16) appear to be directly related to the cyclic secretion of ovarian hormones. There, however, still appears to be a prominent diurnal feeding and drinking rhythm present in ovariectomized rats (14). The current study demonstrates a normal feeding and drinking cycle is also present in castrated male rats.

Corticosterone normally peaks (17) prior to the usual onset of feeding in the rat (1). Recently Krieger (7) and Moberg, Bellinger and Mendel (8) observed that when rats are meal fed during the light phase the corticosterone rhythm shifts in as little as three days such that corticosterone peaks prior to the feeding period. Constant light (18) or lesions that disrupt the natural diurnal feeding rhythm (19–21) also alter the normal corticosterone rhythm (9, 22, 23). In an earlier study (9) we entertained the possibility that in rats the corticosterone rhythm might be associated with ingestive behavior per se, or more probably with arousal brought about in conjunction with, or in anticipation of, the onset of feeding under both natural and imposed feeding situations. The data collected on the adrenalectomized rats of the present study demonstrate that a corticosterone rhythm is not obligatory for the rats to display a diurnal feeding rhythm comparable to the controls. In fact the diurnal feeding rhythm of the adrenalectomized animals, if anything, appeared to be more biased by the light–dark cycle. On the other hand the diurnal drinking rhythm of these rats was slightly modified. However, one might predict such an occurrence considering the large volume of fluid necessarily consumed by these rats in order to maintain body sodium chloride homeostasis.

It is noteworthy that thyroidectomy modified the diurnal feeding rhythm to approximately the same extent as hypophysectomy. Thus it appears that the attenuation of the diurnal feeding rhythm subsequent to hypophysectomy can probably be attributed to a lack of thyroid secretions. How the thyroid acts in conjunction with other factors to influence the diurnal feeding cycle is uncertain. Thyroxine and triiodothyronine are not seemingly released in a circadian fashion in the rat (24), thus their having a diurnal release cannot be responsible for influencing the diurnal feeding rhythm. Also since both thyroxine and triiodothyronine possess long biological half-lives one would not anticipate a cyclic release of the hormones to play an influential role in the maintenance of the feeding cycle.

As expected (25, 26) food consumption and body weight of the thyroidectomized and hypophysectomized rats were significantly reduced compared to the controls. However, we do not feel these reduced parameters are responsible for generating the altered feeding rhythm. Both of these factors were also significantly reduced in the castrated and adrenalectomized rat (27–29) who do not show the attenuated feeding rhythm. Furthermore the altered feeding rhythm does not seem to be correlated with decrease in activity of the hypophysectomized (30) or thyroidectomized (25) rats since castrated (31) and adrenalectomized (32) rats who are also hypoactive do not demonstrate a depressed feeding cycle. Another possibility of why the thyroidectomized animals show an altered feeding rhythm could be due to their reported (33) lowered body temperature. A reduced body temperature could induce the thyroidectomized animals to eat more than controls during the light phase to try and maintain their body temperature. However, this is again probably not the case since the hypophysectomized rats experienced about the same alteration in their feeding rhythm as the thyroidectomized rats yet they have been reported to have a normal body temperature (33). Therefore changes in body temperature are probably not the causative factor.

Since proper thyroid secretion is necessary for normal nervous tissue function (34) it is conceivable that a lack of thyroid hormones could alter the function of some central mechanism [ie., suprachiasmatic nucleus [SCN] (21), ventromedial (19) or dorsomedial (20) hypothalamic nuclei] that generates the ingestive rhythms. However, the likelihood of this possibility is questionable since thyroidectomy attenuated the feeding cycle to a greater degree than the water rhythm. For a lack of thyroid secretion to have a differential effect on some central nervous system mechanism, such as the SCN, one would have to postulate the SCN having two populations of neurons. One population to generate the feeding rhythm and another to generate the drinking rhythm and that the function of each set of these neurons is affected differently by a lack of the thyroid hormone. This has never been shown. Nevertheless, the present data and the earlier findings of Richter (25) suggest that feeding and drinking rhythms may in fact be dissociated.

Interestingly the hypophysectomized animals displayed a greater modification of their drinking rhythm than the thyroidectomized rats. The reason for this may possibly be attributed to an altered antidiuretic hormone secretion since this hormone has been demonstrated to be of importance in the maintenance of the diurnal drinking rhythms of rats (13).

Finally both hypophysectomy and thyroidectomy lower the metabolic rate of rats (33) and thus alter their metabolism greatly. These generalized effects on metabolism may modify the feeding rhythm.

Summary. Hypophysectomy altered but did not abolish the rats' normal diurnal feeding rhythm. The diurnal ingestive rhythm of castrated male rats was comparable to the shams. Adrenalectomy did not attenuate the normal diurnal feeding rhythm but did slightly depress the normal water intake rhythm. Thyroidectomy in a magnitude comparable to hypophysectomy modified the animals' feeding rhythm. While thyroidectomy also altered the diurnal water intake it did so to a lesser degree than hypophysectomy. These data suggest that thyroid secretions may be *partially* responsible for the maintenance of the diurnal feeding rhythm and to a lesser extent the diurnal water intake rhythm in rats.

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1. LeMagnen, J., and Devos, M., Physiol. Behav. **5**, 805 (1970).
2. Bellinger, L., and Mendel, V., Proc. Soc. Exp. Biol. Med. **159**, 80 (1978).
3. Bellinger, L., Mendel, V., and Moberg, G., Horm. Metabol. Res. **7**, 132 (1975).
4. Martin, R., Wangsness, P., and Gahagan, J., Horm. Metabol. Res. **10**, 187 (1978).
5. Slonaker, J., Amer. J. Physiol. **71**, 362 (1924).
6. Wade, G., Physiol. Behav. **8**, 523 (1972).
7. Krieger, D., Endocrinology **95**, 1195 (1974).
8. Moberg, G., Bellinger, L., and Mendel, V., Neuroendocrinology **19**, 160 (1975).
9. Bellinger, L., Bernardis, L., and Mendel, V., Neuroendocrinology **22**, 216 (1976).
10. Levine, S., and Coover, G., Physiol. Behav. **17**, 35 (1976).
11. Takahashi, K., Inoue, K., Kobayashi, K., Hayafuji, C., Nakamura, Y., and Takahashi, Y., Neuroendocrinology **23**, 193 (1977).
12. Morimoto, Y., Arisue, K., and Yamamura, Y. Neuroendocrinology **23**, 212 (1977).
13. Stephan, F., and Zucker, I., Neuroendocrinology **14**, 44 (1974).
14. Stephan, F., and Zucker, I., Physiol Behav. **8**, 315 (1972).
15. Jouhaneau-Bowers, M., Physiol. Behav. **20**, 109 (1978).
16. Brobeck, J., Wheatland, M., and Strominger, J., Endocrinology **40**, 65 (1947).
17. Critchlow, V., *in* "Advances in Neuroendocrinology" (A. Nalbandov, ed.), p. 377. Univ. Illinois Press, Urbana (1963).
18. Siegel, P., J. Comp. Physiol. Psychol. **54**, 294 (1961).
19. Kakolewski, J., Deaux, E., Christensen, J., and Case, B., Amer. J. Physiol. **221**, 711 (1971).
20. Bernardis, L., Physiol. Behav. **10**, 855 (1973).
21. Nagai, K., Nishio, T., Nakagawa, H., Nakamura, S., and Fukuda, Y., Brain Res. **142**, 384 (1978).
22. Scheving, L., and Pauly, J., Amer J. Physiol. **210**, 1112 (1966).
23. Moore, R., and Eichler, V., Brain Res. **42**, 201 (1972).
24. Fukuda, H., Greer, M., Roberts, L., Allen, C., Critchlow, V., and Wilson, M., Endocrinology **97**, 1424 (1975).
25. Richter, C., Endocrinology **17**, 73 (1933).
26. Smith, P., J. Amer. Med. Assoc. **88**, 158 (1927).
27. Grossie, J., and Turner, C., Proc. Soc. Exp. Biol. Med. **118**, 25 (1965).
28. Grunt, J., Endocrinology **75**, 446 (1964).
29. Kral, J., and Tisell, L-E., Acta Endocrinologica **81**, 644 (1976).
30. Richter, C., and Wislocki, G., Amer. J. Physiol. **95**, 481 (1930).
31. Richter, C., Endocrinology **17**, 445 (1933).
32. Richter, C., Endocrinology **20**, 657 (1936).
33. Gemmill, C., and Browning, K., Lab. Animal Care **15**, 329 (1965).
34. Eayrs, J. Brit. Med. Bull. **16**, 122 (1960).

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