

# The Effects of Thyroxine and Estradiol Benzoate on Wheel Running Activity in Female Rats<sup>1</sup>

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STERN, J. J. AND M. MURPHY. *The effects of thyroxine and estradiol benzoate on wheel running activity in female rats.* PHYSIOL. BEHAV. 9 (1) 79-82, 1972.—The administration of 30–50 µg thyroxine to female rats for 21 days disrupted wheel running activity and cyclic vaginal cornification. Thyroxine produced a decrease in spontaneous activity and the number of activity peaks, and resulted in prolonged vaginal cornification. Following ovariectomy, which reduced activity further, 15 µg/day estradiol benzoate increased overall activity. The data demonstrate that the activity changes produced by thyroxine administration are similar to those observed by others after thyroid removal. In addition, both procedures appear to produce their effects by altering ovarian functioning.

Wheel running activity      Activity      Thyroxine      Estradiol benzoate

THYROIDECTOMY reduces the overall activity and eliminates the 4–5 day activity cycles of female rats [7, 8]. Like thyroid removal, thyroid administration produces a disturbance in spontaneous activity [7]. The present study re-examines the activity changes produced by thyroxine administration. In addition, the experiment investigates the mechanism by which high levels of thyroxine alter activity. Low levels of thyroxine do not significantly reduce activity if ovarian steroid levels are artificially maintained [8]. This finding suggests that the changes in running consequent to thyroidectomy are not a direct result of thyroid removal but an indirect result mediated by altered ovarian function. Thus the present study investigates whether the activity changes produced by thyroxine administration are a direct result of high thyroxine levels or an indirect result mediated by ovarian dysfunction.

## METHOD

### Animals

The animals were 54 virgin Sprague-Dawley female rats reared in our laboratory. At the start of the experiment the females ranged in age from 91–175 days and weighed 173–237 g.

### Maintenance

The females were housed in individual activity wheels with attached living cages. The living cage (8 × 4 × 4 in.) allowed the rat to feed and lie down but gave no room for running. The activity drums (14 in. dia; 4 in. width) were precalibrated for torque according to the method of Lacey [5]. The opening between the activity wheel and living cage was kept small so that the rats were unable to leave the drum without first stopping it. Total daily activity (revolutions) was recorded each day at 9.00 a.m.

Rat chow and water were available ad lib. The laboratory was on a natural light cycle with testing taking place from May to October, 1970. A thermostat maintained the temperature at approximately 74°F.

### Procedure

The 54 females were randomly assigned to one of 6 groups (Table 1). After an adjustment period of 3 weeks, activity records were taken. Starting on recorded Day 22 and continuing throughout the experiment, the animals in Groups 2–6 had L-thyroxine ( $T_4$ ) administered daily according to the schedule shown in Table 1. The females in Group 1 had oil administered for Days 22–42 at which point they were eliminated from study. On Day 43, 5 or 6 animals from each of Groups 2–6 were ovariectomized (ov-x) while 4 animals were sham ov-x. The operations were conducted under pentobarbital anesthesia supplemented with ether and atropine sulphate (1/100 grain). Starting on Day 57 and continuing for 14 days, 3 of the ov-x animals from each group were given 15 µg estradiol benzoate (EB) daily; the remaining ov-x females were given oil. All injections were administered sc at approximately 10.00 a.m. The sham operated animals were eliminated from study on Day 57.

For Days 1–42 vaginal smears were taken by lavage nightly at approximately 10.00 p.m. The smears were classified according to the method outlined by Zarrow, Yochim, and McCarthy [11].

## RESULTS

### Pretreatment (Days 1–21)

Prior to  $T_4$  administration (Day 22) the females showed 4–5 day activity cycles; the females had an activity peak every 4–5 days (Table 1). An activity peak is operationally

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defined as an increase in activity of at least 50% over that on the preceding day.

As depicted in Fig. 1, most (58%) of the non peak activity prior to treatment was between 1000 and 2999 revolutions/day. The vaginal smears taken during this period were cornified every 4–5 days. The cornified stage was coincident with an activity peak 96% of the time.

#### Thyroxine Treatment (Days 22–42)

The animals given oil for Days 22–42 (Group 1) continued to show 4–5 day activity cycles, high levels of activity (Table 1) and vaginal cornification on the days of peak activity. The animals given 20–50 µg T<sub>4</sub> (Groups 3–6) had disrupted cyclic activity: the 4–5 day cycles which characterized the females prior to Day 22 disappeared. Instead the females displayed irregular cycles of 3–16 days ( $\bar{x}=8.1$ ; Table 1). There did not appear to be any relation between the dose of T<sub>4</sub> and the amount of irregularity.

Taken as a whole, the females that received T<sub>4</sub> (Groups 2–6) showed a significant reduction in non peak activity ( $p<0.01$ ; Wilcoxon Z test for matched samples). As seen in Fig. 1 there was a marked shift in activity; 65% of the activity now fell between 1 and 1999 wheel turns/day. In addition no activity fell between 4000–4999 revolutions/day; prior to T<sub>4</sub> administration 13% of the activity was this high. Taking the groups individually there was a reduction in

activity for Groups 4, 5 and 6. Groups 2 and 3 (10 and 20 µg T<sub>4</sub>) did not show a reduction in activity.

T<sub>4</sub> administration upset vaginal cyclicity. For Days 22–43 Groups 3–6 displayed vaginal estrus 58% of the time ( $p<0.001$ ;  $\chi^2$ ). Group 2 (10 µg T<sub>4</sub>) was in estrus more often than controls but significantly less than Groups 3–6 (Fig. 2). There were no intergroup differences between Groups 3–6.

#### Ovariectomy (Days 43–56)

Those females ov-x on Day 43 showed a significant ( $p<0.01$ ) reduction in activity when compared to their own activity for Days 22–42 (Wilcoxon Z test for matched samples) and when compared to the activity of their sham controls for Days 43–56 (Mann Whitney U tests; Table 1). Taken individually all groups showed a reduction in non peak activity in comparison to Days 22–42; Groups 2–5 also showed a reduction in comparison to their sham controls. The activity of Group 6 was not lower than their controls; in fact they were 280 revolutions/day greater (NS). It should be noted that Group 6 females had been receiving the largest dose of T<sub>4</sub> (50 µg) and had shown the greatest reduction in activity prior to ovariectomy.

Ovariectomy almost totally eliminated the appearance of activity peaks. For Days 43–56 the ov-x animals had a total of 3 peaks (1 Group 4; 2 Group 6) while the sham operated animals had 42 peaks ( $p<0.001$ ).

TABLE 1  
ACTIVITY (REVOLUTION/DAY) OF THE FEMALES DURING THE VARIOUS PHASES OF THE EXPERIMENT

Group	N	Treatment*			Days		57–70
			1–21	22–42	43–56		
2	10	oil	(a)	2688	2931	ov-x	EB†
			(b)	5438	5841		
			(c)	4.3	4.4		
3	10	10 µg T <sub>4</sub>	(a)	2185	2368	915	1667
			(b)	4971	5100	—	3213
			(c)	4.5	4.3	—	—‡
4	9	20 µg T <sub>4</sub>	(a)	3165	2511	1003	2766
			(b)	5991	5030	—	—
			(c)	4.6	7.0	—	—
5	10	30 µg T <sub>4</sub>	(a)	2444	1600	809	1481
			(b)	4830	3216	1900	3413
			(c)	4.2	6.3	—‡	5.9
6	10	40 µg T <sub>4</sub>	(a)	3385	1481	883	1211
			(b)	5800	2651	—	2366
			(c)	4.6	9.5	—	8.6
		50 µg T <sub>4</sub>	(a)	2990	1011	1283	1003
			(b)	4533	2385	1755	2036
			(c)	4.5	8.3	8	1973

\* µg T<sub>4</sub>/day.

† Estradiol Benzoate, 15 µg/day.

‡ Only one activity peak, i.e. no interpeak interval.

(a)  $\bar{x}$  non peak activity.

(b)  $\bar{x}$  peak activity.

(c)  $\bar{x}$  interpeak interval (Days).

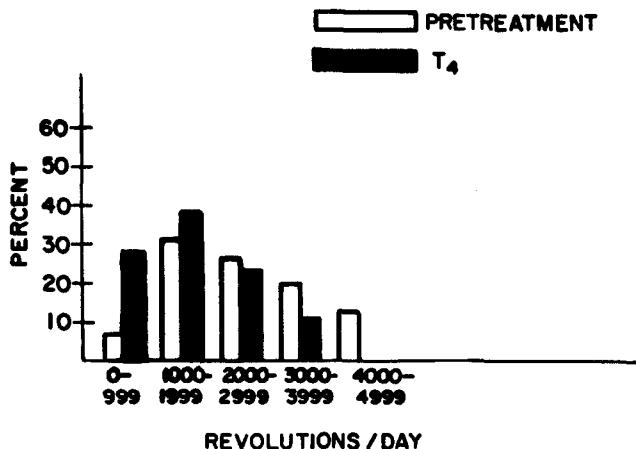


FIG. 1. The percentage of daily non peak activity between 1 and 4999 revolutions/day for Days 1-21 (pretreatment) and Days 22-42 (T<sub>4</sub>- administration).

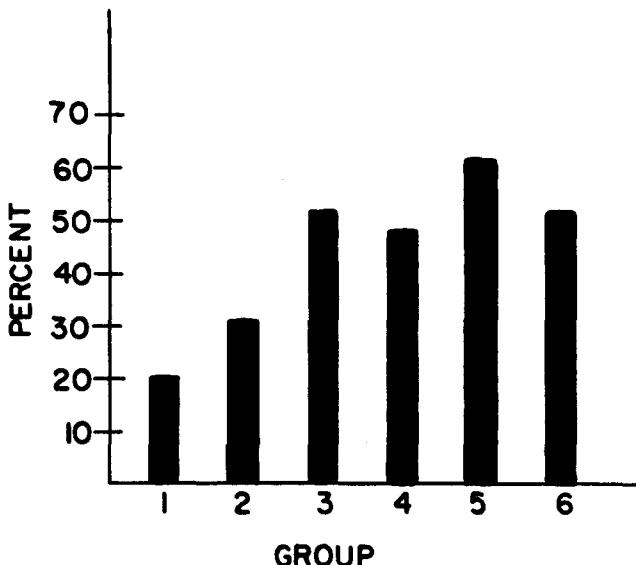


FIG. 2. The percentage of vaginal smears showing cornification for Days 22-42.

Sham ovariectomy was without effect on activity (Fig. 3).

#### Estradiol Benzoate Treatment (Days 57-70)

The females in Groups 2-6 that received oil for Days 57-70 continued to show low activity and no activity peaks (Table 1). Those females given 15 µg EB/day showed an increase in activity over Days 22-42 and over Days 43-56. Their activity was still, however, lower than that for Days 1-21 (Fig. 4; Table 1). Taken individually, all groups showed an increase in activity over Days 43-56; Groups 4-6 also showed an increase over Days 22-42. Remember that Groups 2 and 3 did not show any reduction in activity for Days 22-42. While 15 µg EB/day increased overall activity it did not reinstate cyclicity; during the two weeks of EB treatment there was only one activity peak (Group 2).

#### DISCUSSION

To display cyclic vaginal cornification and activity, the female rat requires restricted levels of thyroid hormone [6, 7, 8]. With low T<sub>4</sub> (e.g. following thyroidectomy [7, 8] or propylthiouracil administration [6]) the intervals between vaginal estrus and the intervals between activity peaks are lengthened. With high T<sub>4</sub> (e.g. during thyroid feeding [6] or T<sub>4</sub> administration) vaginal smears are cornified over half the time and the interval between activity peaks is increased.

The reduction in activity following thyroidectomy or T<sub>4</sub> administration appears to be due to secondary ovarian dysfunction: EB will increase activity in ov-x/thyroidectomized animals [8] or in ov-x/T<sub>4</sub> treated animals (Groups 2-6 Days 57-70); T<sub>4</sub> does not affect activity in ov-x females (8; Days 43-56). Stern [8] has suggested two mechanisms by which the thyroid may affect ovarian functioning. First, the ovary may require a fairly narrow range of T<sub>4</sub> for optimal production of estrogen. With thyroid alterations, ovarian steroid production is directly affected. Second, changes in the

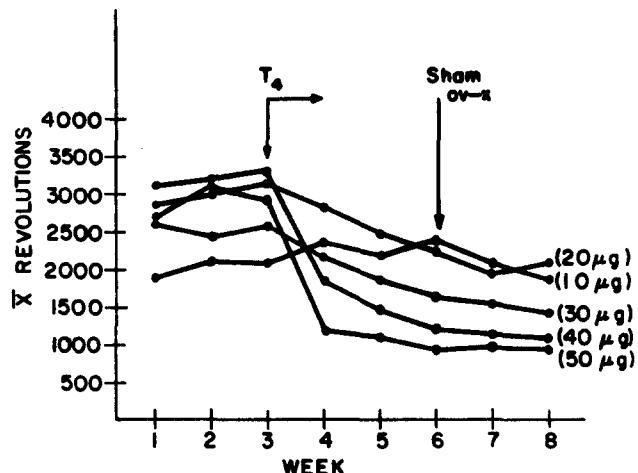


FIG. 3. Mean non peak activity for the sham operated animals for weeks 1-8.

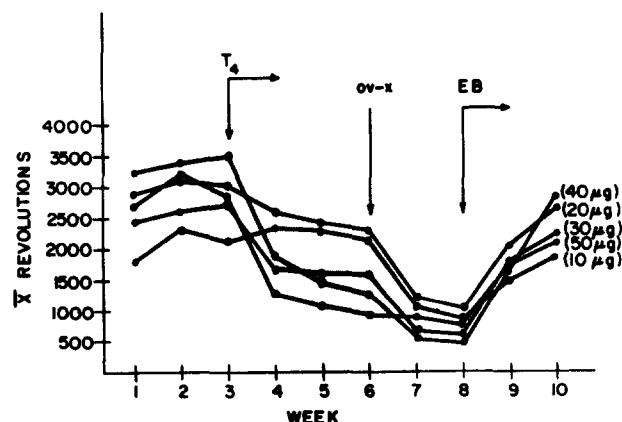


FIG. 4. Mean non peak activity for the ov-x females for weeks 1-10

thyroid may indirectly affect the ovary by altering gonadotropin secretion. Following an increase (decrease) in thyroxine, the pituitary not only decreases (increases) its output of TSH but decreases (increases) its output of gonadotropins. This altered gonadotropin secretion results in ovarian dysfunction which in turn produces the observed reduction in activity.

Previous studies have reported that estrogen increases activity. Implants of estrogen in the hypothalamus along the course of the medial forebrain bundle increase activity in ov-x rats [2]. The administration of estrogen [3] or the sc placement of estrone pellets [10] increase activity in ov-x rats. Subcutaneous implants of estrogen stimulate running activity in castrate male rats [1]. Lastly, progesterone on a background of estrogenic stimulation does not result in more

activity than estrogen alone [4, 9]. The present study also reports an increase in activity due to estrogen administration (Table 1; Fig. 4).

To increase running in ov-x/thyroidectomized females, Stern [8] employed EB (1 µg/day) for 3 days followed by progesterone (0.4 mg) on Day 4. Progesterone is no longer used because with the inclusion of a control group (oil Day 4) it became evident that progesterone was not stimulating activity. As for the change in the dose of EB: with 1 µg/day there was only a brief period of increased activity. After approximately 6 days running returned to prehormone levels; 15 µg/day delays this return. In addition, the higher concentration more reliably produces the initial activity increase.

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