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Effects of Thyroxine and Thyroparathyroidectomy on Circadian Wheel Running in Rats

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McEACHRON, D. L., C. L. LAUCHLAN AND D. E. MIDGLEY. *Effects of thyroxine and thyroparathyroidectomy on circadian wheel running in rats.* PHARMACOL BIOCHEM BEHAV **46**(1) 243–249, 1993.—Thyroparathyroidectomized (TPX) and thyroidectomized male rats display shorter free-running activity periods and enhanced activity levels. These experiments were designed to determine whether this effect is due to the loss of thyroid hormones. The running wheel activity of 36 male rats, 19 TPX and 17 sham operated, was studied. The animals were kept in constant conditions for 7 weeks to obtain baseline data. Half the rats were then injected SC with capsules containing T4, while the other half were injected with blanks. All animals were then allowed to free-run undisturbed for another 8–9 weeks. TPX rats displayed significantly shorter baseline periods (average difference: 0.26 h) and heightened activity. Thyroxine treatment significantly lengthened TPX animals' cycles (average increase: 0.28 h) but did not affect intact rats' circadian rhythms. Thyroxine did, however, significantly decreased the activity levels of both TPX and sham-operated rats. These findings indicate that changes in TPX rats' activity cycles are caused by a reduction in thyroid hormones and that thyroxine acts on activity rhythms and levels by different mechanisms.

Circadian rhythm Thyroid Thyroxine Activity Rat Thyroparathyroidectomy
Depression Affective disorder

A number of neuroendocrine factors have been shown to modify behavioral circadian rhythms in mammals. Prominent among these factors are estrogens, pineal melatonin, and thyroidectomy. While the hormones estradiol and melatonin have been conclusively demonstrated to influence biological cycles (2,9), such has not been the case for the thyroid hormones, thyroxine (T4) or triiodothyronine (T3). The experiments that have demonstrated altered circadian rhythms in rats, for example, have relied on surgical thyroidectomy or thyroparathyroidectomy to induce these alterations (11,12). This procedure leaves open the question of whether the loss of calcitonin, thyroid hormones, or both is the causal factor. The objective of these experiments was to determine if the effects of thyroparathyroidectomy (TPX) on rat activity rhythms could be reversed by chronic T4 treatment alone.

Previous experiments have demonstrated three main effects of TPX surgery. First, male TPX rats show a significantly shorter free-running activity cycle when compared with intact male controls. Second, the pattern of activity appeared to be more consolidated and coherent in TPX animals compared to controls. Third, TPX male rats are often significantly more active than sham-operated but intact males (11,12). Results with females were considerably less clear-cut. In the one published report on TPX females, no reliable differences were found in either period or activity level between intact and TPX females or, for that matter, between either group of females and TPX males (12). Given the observation that intact females were themselves more active with significantly shorter free-running activity periods, a common mechanism for the effects of estradiol and TPX surgery has been suggested (8). Insofar as the most robust alterations in activity rhythms have been observed in male TPX rats, these experiments focussed exclusively on the effects of T4 treatment in male animals.

As stated above, the main objective of these experiments was to determine if a lack of thyroid hormones was responsible for the observed changes in activity rhythms and levels displayed by TPX rats. However, the original motivation for examining rhythms in TPX animals were observations that associated hypothyroidism and abnormal circadian cycles with affective disease in humans (4–6,17). There are reports that also associated hyperthyroidism with certain affective symptoms or stages (3,18). Therefore, it was decided to investigate chronic T4 treatment in intact as well as TPX animals.

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METHOD

Two replications were undertaken, the first lasting from December, 1990 to April, 1991, and the second running from July, 1991 to November, 1991. The protocols and procedures for each replication were essentially identical.

Subjects

In each replication, 18 surgically altered male Sprague-Dawley rats, 60 days old and weighing approximately 300 g, were obtained from Zivic-Miller Laboratories (Zelienople, PA). Nine rats were thyroparathyroidectomized (TPX) while 9 animals received sham operations (note that in the second replication, one 'sham' animal was later identified as a TPX). Upon arrival, the animals were communally housed for 20 days in wire cages containing three or four animals each. During this time, they were kept on a 16L : 8D cycle and supplied with Purina Laboratory Chow ad lib. All animals were provided with a 2% calcium lactate (by weight) water solution ad lib.

Protocol

After 20 days, each rat was placed in an individual Wahmann running wheel cages that were housed in groups of six in sound-attenuating and light-proof cabinets. Fans provided air circulation and a mild white masking noise. The cabinets were themselves located in a special light-tight facility with interior lighting provided by 7.5-W red safelights (<0.1 lux). Microswitches attached to each cage signaled activity to an IBM PC-XT computer in an adjacent room. Activity data were collected continuously but were recorded in 15-min blocks for later analysis. For the rest of the experiment, the animals were fed and given calcium lactate water ad lib. Water bottles were changed and food replenished every 2–3 days. The bedding under the cages was replaced every 7–10 days. Animal care was conducted according to a randomized schedule to avoid providing the animals with temporal cues. The dates and times of animal maintenance were recorded to ensure a randomized schedule and monitor the effect of human contact. All procedures conformed to N.I.H animal care guidelines and were reviewed by the University of Pennsylvania's Institutional Animal Care and Use Committee.

Once in the cabinets, the rats were maintained on a 12L : 12D cycle (lights on at 6 a.m. EST) for 1 month to ensure normal entrainment and activity. Fifteen-watt incandescent bulbs providing 10–15 lux were used and controlled by a microprocessor-based timer (Chrontrol, Lindberg Enterprises, San Diego, CA) located in the adjacent computer room. After entrainment was achieved, all animals were exposed to constant dim red light (<0.2 lux) for the remainder of the experiment.

The rats were maintained under these conditions for 7 weeks to obtain sufficient data to determine the baseline free-running periods. At the start of the eighth week, each animal was removed from his cage and SC injected with either T4-containing or blank pellets (Vivo-Trial, Endocon, Walpole, MA) while under methoxyflurane (Metofane, Pittman-Moore, NJ) anesthesia. A Harman Injector (Mark I) was used and the procedure required less than 5 min per animal. At no time was any animal exposed to any illumination other than red light at <0.2 lux. After the injection, the rats were returned to their cages in the cabinets and monitored for another 8–9 weeks.

In the first replication, it was noted that rats receiving T4 pellets drank considerably more water than animals injected with blanks. In both replications, water consumption was monitored both to ensure an adequate supply and to record any changes associated with T4 treatment.

Animals were weighed three times during the course of each experiment: 1) prior to initially being placed in the activity cages; 2) when removed from those cages for injection of the pellets; and 3) at the end of the experiment. Comparison of the weights was used to monitor changes in both TPX and sham animals associated with T4 treatment.

After the period of free-running activity following the injections, the animals were removed from the activity cages and sacrificed by carbon dioxide inhalation. Approximately 5 cc of blood was obtained from each rat by cardiac puncture for analysis of serum T3 and T4 levels. The blood was spun in a centrifuge at 5000 rpm for 20 min, the serum removed by pipette and frozen at −40°C until analyzed. In the second replication, the rats were also decapitated and the brains removed and frozen at −70°C for quantitative receptor autoradiography (16).

Period Determination

Period estimations were made by two methods. Two individuals blind to each animal's condition were asked to draw the best-fitting line on computer-generated actograms for both baseline and treatment periods. The angle of the line was converted to a period and the two independent estimates were averaged. The second method was based upon chi-square periodograms and utilized the TAU software package created by Dr. Jonathan Schull (MiniMitter, Sunriver, OR). The manual and computer-generated estimates were then averaged to give a single period for each animal and phase of the experiment. Previous results have shown that averaging manual and computer estimations reduces group variances, thus providing a more precise measurement (11,12). The TAU program also provided activity levels, wave-forms, and amplitudes for each animal before and after injection. As a measure of rhythm consolidation, the mean percentage of time during each cycle when activity was recorded was calculated for each rat.

Statistical Analyses

The two replications used a total of 36 animals, 17 sham operated and 19 TPXs (one sham was determined to actually be a TPX after sacrifice). A two-way ANOVA with repeated measures was used to analyze period data as well as weight changes. Drinking was analyzed by a simple two-way ANOVA. Activity levels suffered from noncorrectable heterogeneity of group variances and were analyzed by nonparametric tests. Scheffe F-tests were used for post hoc evaluations (13). One TPX animal died before an adequate postinjection period or activity level could be ascertained.

RESULTS

As previously reported (11,12), TPX male rats displayed significantly shorter periods when compared to sham-operated control animals, $F(1, 31) = 5.96$, $p < 0.05$. The interaction between surgery (TPX vs. sham), pellet (T4 vs. blank), and replication (before vs. after implantation) was significant, $F(1, 31) = 5.17$, $p < 0.05$. Post hoc tests established that thyroxine significantly lengthened TPX animals' circadian activity periods (Scheffe $F = 4.37$, $p < 0.05$), while T4 treatment left shams' cycles unchanged (Scheffe $F = 0.048$, NS). The average period difference postinjection minus baseline was 0.047 h for TPX and 0.010 h for sham-operated rats injected with

blank pellets compared with 0.014 h for sham-operated and 0.28 h for TPX rats injected with T4 containing pellets. Post hoc comparison of T4-treated TPX animals vs. sham-operated rats implanted with blank pellets indicated that the groups were not significantly different (Scheffe $F = 0.12$, NS). These results are displayed in Fig. 1.

Also, as previously reported (12), the activity levels of the TPX animals were significantly greater than those displayed by sham-operated control animals (Mann-Whitney $U = 304$, $p < 0.01$). To examine the effect of T4 replacement, the difference in mean activity per cycle before and after injection was calculated as a percentage of the original preinjection mean activity and analyzed by a Kruskal-Wallis test. There was a significant difference between the groups, $\chi^2(3) = 12.84$, $p < 0.01$. Post hoc comparisons indicated that T4-treated animals, both TPX and sham (Scheffe $F = 8.4$, $p < 0.05$ and 13.3, $p < 0.05$, respectively) were significantly less active after implantation. The activity of rats given blank pellets was not significantly altered (Scheffe $F = 0.36$, NS for TPXs, and $F = 3.9$, NS for shams). TPX rats treated with T4 were not significantly different from shams implanted with blank pellets (Scheffe $F = 1.5$, NS). All post hoc tests utilized base 10 logarithms. These results are displayed in Fig. 2.

In contrast, the consolidation measures did not show significant changes associated with surgery or T4 replacement. The percentage of each cycle during which animals were active was 38 ± 4% for shams and 35 ± 5% for TPX animals, a difference that did not quite reach significance ($t = 1.39$, $p < 0.09$, one-tailed). Both TPX and sham animals became slightly more consolidated when injected with blank pellets (37% for shams and 33% for TPXs), while T4-injected animals became slightly less consolidated (39% for shams and 38% for TPXs). These trends also did not reach significance.

Weight changes were analyzed by calculating the amount gained or lost from the beginning of the experiment to the time of injection and comparing those with the changes observed from injection time to sacrifice by a two-way ANOVA with repeated measures. TPX surgery significantly reduced

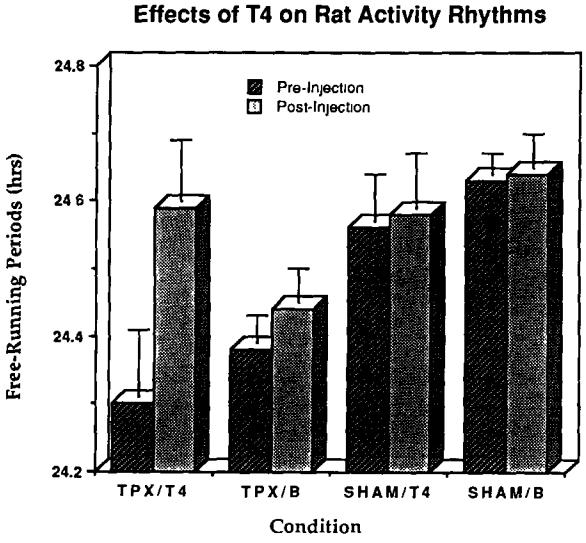


FIG. 1. The effects of thyroparathyroidectomy (TPX) and thyroxine (T4) on free-running circadian activity periods in male rats. The 'B' indicates implantation with a blank pellet. Error bars indicate standard error of the mean.

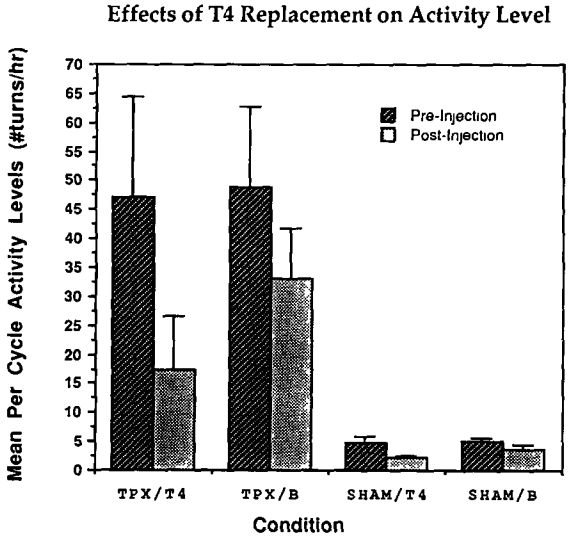


FIG. 2. The effects of thyroparathyroidectomy (TPX) and thyroxine (T4) on levels of wheel-running activity in male rats. The 'B' indicates implantation with a blank pellet. Error bars indicate SEM.

the amount of weight gained compared with controls, $F(1, 31) = 18.77$, $p < 0.01$. There was also a significant replication (before vs. after injection) effect, $F(1, 31) = 42.35$, $p < 0.01$, as well as significant interactions between surgery and type of pellet (T4 vs. blank), $F(1, 31) = 13.55$, $p < 0.01$, between surgery and replication, $F(1, 31) = 90.44$, $p < 0.01$, and between surgery, pellet type, and replication, $F(1, 31) = 16.35$, $p < 0.01$. In essence, TPX animals gained less weight than sham-operated animals except when injected with T4-containing pellets. The effect of T4 replacement was to significantly increase the rate of weight gain in TPX animals, the reverse of the overall trend, while causing the sham-operated animals to actually loose weight. These results are displayed in Fig. 3.

The amount of fluid consumed per 24 h was measured after implantation only. The resulting two-way ANOVA indicated that injection with T4 significantly increased the amount drunk in both shams and TPXs (from an average of 58 ml/day to 110 ml/day) $F(1, 30) = 58$, $p < 0.01$. There was no interaction with surgery nor did the surgery itself significantly alter drinking. These results are shown in Fig. 4.

Insofar as T4 replacement significantly altered both activity periods and activity levels, the changes in period were correlated with the changes in mean activity levels in TPXs injected with T4 pellets. The correlation was -0.46, indicating that the greater the loss of activity, the greater the lengthening of the animal's free-running period. However, the trend was not significant. We also attempted to determine if changes in the period of T4-treated TPXs were associated with their baseline periods. This trend was also not significant ($r = -0.22$, NS).

DISCUSSION

Thyroxine (T4) treatment reversed the shortening of TPX animals' circadian activity periods, resulting in a mean period for the T4-treated TPX animals indistinguishable from normal values. T4 treatment had no effect, however, on the period of intact animals' wheel-running rhythms, despite serum

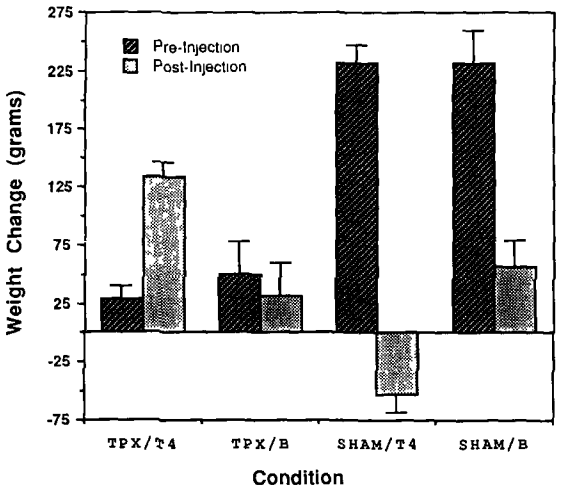


FIG. 3. The effects of thyroparathyroidectomy (TPX) and thyroxine (T4) on weight in male rats. The 'B' indicates implantation with a blank pellet. Error bars indicate SEM.

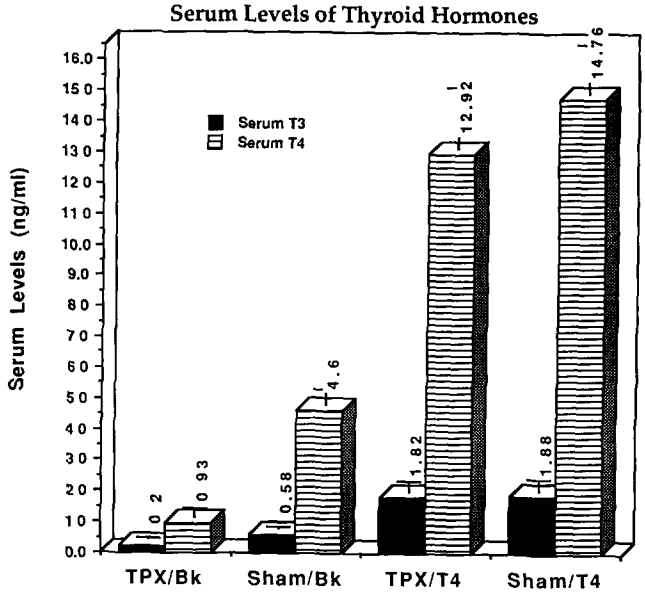


FIG. 5. Serum thyroxine (T4) and triiodothryonine (T3) from T4-treated and untreated (Bk) sham-operated and thyroparathyroidectomized (TPX) rats. Actual levels are shown and error bars indicate SEM.

T4 levels in these animals of almost four times normal (Fig. 5). This indicates that T4 is reversing an effect unique to thyroidectomized animals and not simply acting on the circadian system in a dose-response manner. One possible explanation is that levels of thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH) are the causal factors. Levels of these hormones are regulated to a great extent by physiological concentrations of thyroid hormones (10,14,15,19). This hypothesis requires that reductions of TSH and/or TRH below normal levels (i.e., in T4-treated shams) are without significant effect, but that increased concentrations of one or both peptides (in TPXs) accelerates activity rhythms. The role of thyroid state would then be secondary, operating through changes in the regulatory peptides

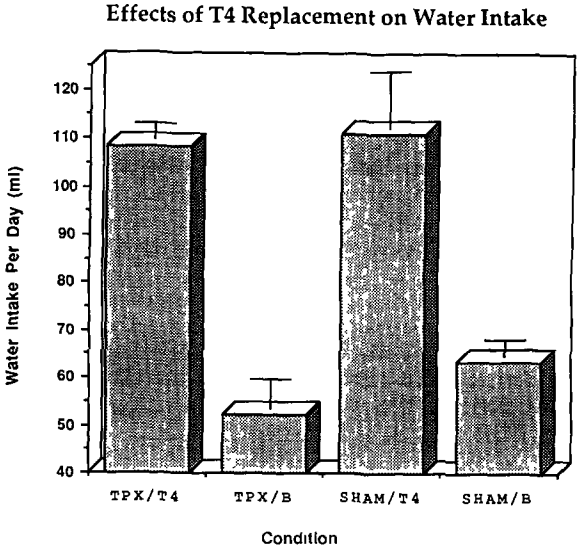
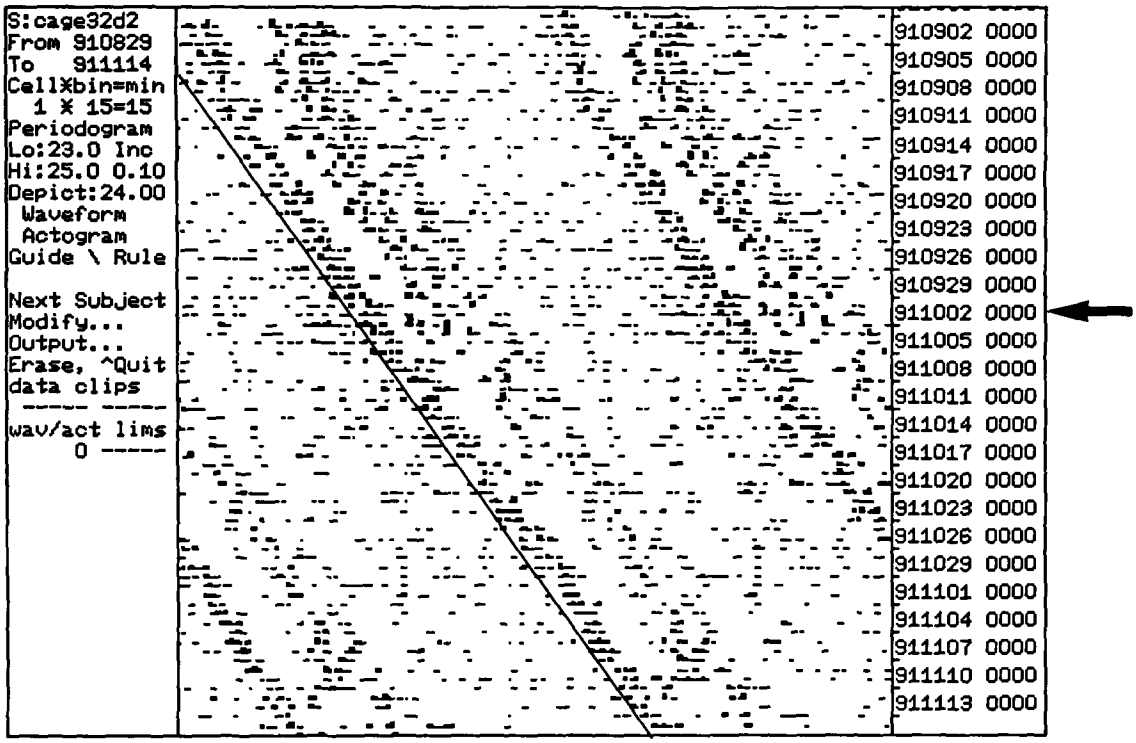


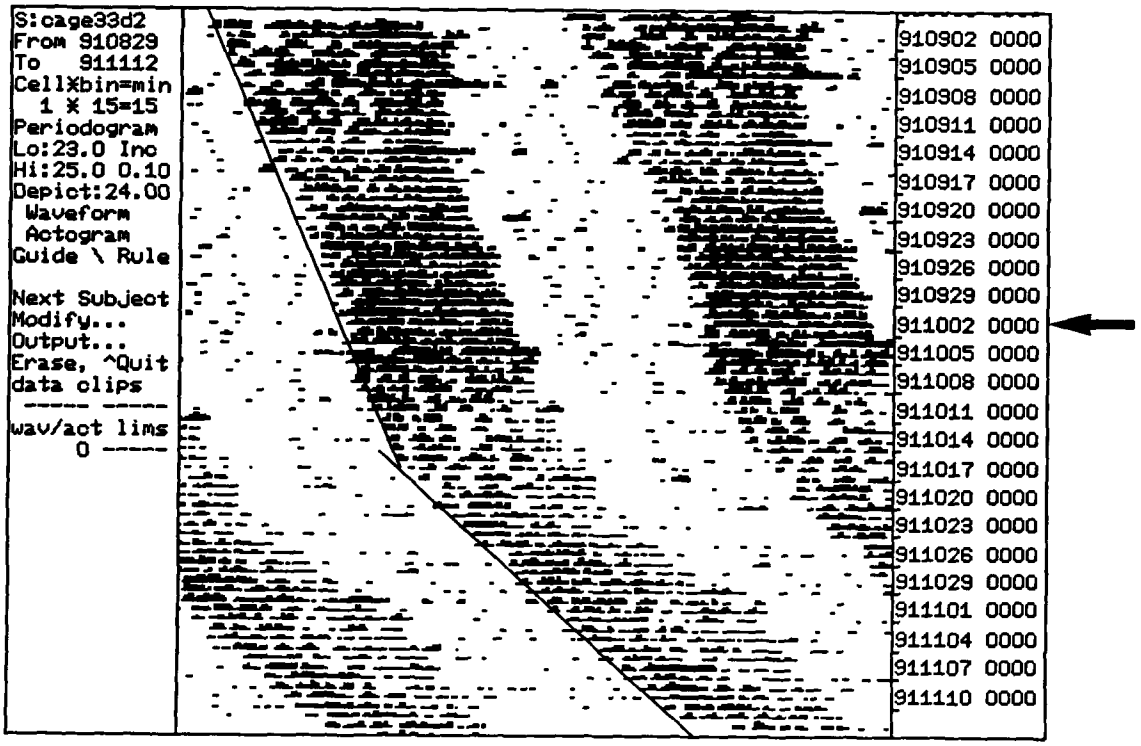
FIG. 4. The effects of thyroparathyroidectomy (TPX) and thyroxine (T4) on water consumption in male rats. The 'B' indicates implantation with a blank pellet. Error bars indicate SEM.

Thyroxine treatment also reversed the heightened activity observed in TPX rats, significantly reducing activity levels to the point where TPX animals' activity were statistically indistinguishable from normal values. Figure 2, however, does not seem to reflect this statistical result—activity in T4-treated TPXs still seems much greater than that observed in either group of shams. To satisfy ourselves that T4 did indeed eliminate the difference between TPXs and untreated shams, a Mann-Whitney test was run on the T4-treated TPXs' and blank-injected shams' postimplantation activity levels. Insofar as this is not a post hoc test, the result could not be considered legitimate if a significant difference was uncovered. On the other hand, if such a difference were not found, then we could be reasonably certain that a significant difference is not being disguised through the use of a post hoc test. The result was not significant (U = 51, NS), thus supporting the conclusion obtained through use of the Scheffe test.

Reports that injections of TRH increase locomotor activity in rats (1,7) provide support for a hypothesis that the effects of thyroidectomy and T4 on activity levels may be mediated by changes in TRH secretion. As suggested above, TRH may also play a role in the modification of circadian rhythms in TPX animals. In contrast with circadian activity periods, however, T4 also significantly reduced sham-operated animals' activity levels. These data, combined with a nonsignificant correlation between period and activity level changes in T4-treated TPX rats, suggest that alterations in circadian activity periods are not secondary to changes in the amount of activity displayed by the rats. In addition, any hypothesis linking TRH to changes in both activity levels and activity rhythms would require separate mechanisms for these two



A



B

FIG. 6. Activity records of a TPX and sham rat implanted with thyroxine (T4)-containing pellets. The activity records are double-plotted. The arrow at the right indicates the time of implantation. The lines drawn on each actogram indicate an estimate of the free-running period. (A) This record is from a sham (thyroid-intact) animal. (B) This record is from a TPX (thyroparathyroidectomized) animal. These records also demonstrate the shorter periods, higher activity levels, and more consolidated activity patterns of TPX rats compared with shams prior to T4 treatment.

effects, since T4 suppresses intact male rats' activity without altering circadian expression.

An adaptive hypothesis to explain changes in locomotor activity can be suggested in addition to the more mechanistic proposition of modifications in TRH levels. It is possible that activity was being used in these animals for behavioral thermoregulation. Hypothyroid rats should suffer from a decrease in core body temperature. To compensate, these animals become more active. When injected with T4, both TPXs and shams should experience an increase in body temperature that, in turn, leads to a decrease in heat-producing running wheel activity. If this is the case, the activity levels of TPX animals should be controllable by altering environmental temperatures. It would be interesting to determine if temperature cycles have become more powerful Zeitgebers (synchronizers) for TPX rats when compared with intact animals.

The results for consolidation are puzzling. Blind raters show little difficulty in differentiating between TPX and sham-operated rats based upon activity records after being informed that TPXs show clearer and more consolidated rhythms. The trends using the percentage of cycle in which activity is recorded are in the predicted direction but do not reach significance. Further experimentation will be required to resolve this discrepancy and determine if thyroid hormones do play a significant role in activity rhythm consolidation.

Changes in weight and drinking show the metabolic effects of thyroxine treatment. TPX animals eat less than intact animals and therefore gain less weight over time. T4 treatment reverses this effect, leading to an increased rate of weight gain. Intact animals, on the other hand, rapidly gain weight without T4 and actually loose some weight overall with chronic T4 exposure. Both groups of animals significantly (two-fold) increase their intake of water.

The alterations observed in animal weights and water intake might possibly be explained by invoking both indirect and direct effects of T4. Again, the indirect effect involves control of TRH secretion, while the direct effects are concerned with thyroxine's ability to alter basal metabolic rate. Injections of TRH have been shown to inhibit both food and water intake in animals (7). The TPX animals may show a decrease in rate of weight gain compared to shams due to high TRH levels—when T4 is provided, TRH is suppressed and food intake (and weight gain) increases. In intact rats, the role of T4 in suppressing TRH is apparently secondary to direct increases in metabolic rate. The increased metabolism leads to mobilization of energy stores and reduces the animals' weights. The situation appears a bit different when considering water intake—T4 has similar effects in both TPX and shams. In this case, the enhancement in basal metabolic rate and the resulting requirement for increased water may be the overriding factor for both groups of animals. On the other hand, if T4-induced TRH suppression is the causal factor, then water intake is similar to activity levels insofar as suppression below normal concentrations exerts a measurable effect. This again contrasts with T4's influence on circadian activity periods, where only TPX animals show a significant susceptibility. In any case, discovering the true explanation for the observed changes is likely to involve a complex multivariate approach.

In summary, T4 reverses two effects linked with thyroparathyroidectomy in male rats, shortening of the circadian period and increased levels of running wheel activity. Thyroxine also suppresses activity in intact males but does not alter the circadian activity period in these animals. This evidence supports the hypothesis that thyroid state affects activity levels and rhythms in male rats by different mechanisms.

ACKNOWLEDGEMENTS

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