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CONTROL OF THE CIRCADIAN RHYTHM OF LOCOMOTION
IN THE ROUGH-SKINNED NEWT,
TARICHA GRANULOSA

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TABLE OF CONTENTS

INTRODUCTION	1
MATERIALS AND METHODS	3
ANALYTICAL PROCEDURE	5
EXPERIMENTAL TREATMENT OF ANIMALS	6
DESCRIPTION OF LIGHTING	7
RESULTS	7
ENTRAINMENT OF THE LOCOMOTOR RHYTHM TO ENVIRONMENTAL LIGHT CYCLES	10
ENTRAINMENT OF THE LOCOMOTOR RHYTHM TO ENVIRONMENTAL TEMPERATURE CYCLES	11
THE ROLE OF EXTRAOCULAR PHOTORECEPTION IN LIGHT ENTRAINMENT	14
DISCUSSION AND CONCLUSIONS	17
ACKNOWLEDGEMENTS	21
LITERATURE CITED	23

CONTROL OF THE CIRCADIAN RHYTHM OF LOCOMOTION
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Jeffrey L. Briggs

ABSTRACT — Over 51,000 hours of locomotor activity of *Taricha granulosa* were recorded using two types of low cylindrical plastic monitoring chambers. This species shows distinct rhythms of activity under normal day-night cycles (LD), shortened day-night cycles, constant darkness (DD) and constant light (LL). The rough-skinned newt is day active and crepuscular. Animals tested under various LD conditions show peak activity within 8 (62%) to 12 (71%) hours after lights-on. The activity peaks are random with respect to the lights-off. In addition they follow "Aschoff's Rule" for circadian rhythms as it applies to day-active animals: (1) they show a longer period under DD (mean = 24.73 hours) than LL (mean = 23.7 hours) and (2) the period decreases with the increasing intensity of light under LL (130-454 lux). However, the number of animals which show arrhythmia increases with increasing intensity.

Entrainment to the light-dark cycle is evident and the activity cycle can be phase-shifted by changing the timing of the light cue. Although newts can entrain to temperature pulses (cold from 9° C to 4° C) under constant light, they entrain to the light cycle when given a choice between light and temperature. Activity generally ceases below 6° C regardless of the state of the light-dark cycle.

There is no apparent difference between eyeless and intact newts in the ability to use light cues in entrainment.

INTRODUCTION

The time sense in animals and plants has been investigated by many people over the past half century, but most intensely in the past twenty-five years (Pittendrigh and Bruce, 1957). In every animal studied to date an inherent rhythm of some sort has been found. Internal clocks are known from such diverse groups as amphipods, isopods, insects, fish, amphibians, reptiles, birds and mammals (reviewed in Aschoff, 1965). The purpose of this investigation is to characterize the locomotor rhythm of the rough-skinned newt, *Taricha granulosa* Skilton.

One of the earliest studies of locomotor rhythms in salamanders (Kalmus, 1940) showed a daily activity rhythm in the "Axolotl larva" with a nocturnal peak. Van Bergeijk (1967) discovered a daily rhythm in the bullfrog (*Rana catesbeiana*). They also reached peak activity at night (0200 hours). Adler (1969) used special techniques to show that the slimy salamander (*Plethodon glutinosus*) could be entrained to a light cycle and that the locomotor rhythm could be phase-shifted by manipulation of the light cue though the rhythm was lost quickly under free-run conditions. He also demonstrated that enucleated animals performed as well as those with eyes and suggested that the photoreceptor was extraocular. Adler (1971) demonstrated that the frog *Rana clamitans* lost its ability to entrain locomotion to light cycles when the frontal organ was removed even though the pineal (epiphysis cerebri) remained intact.

Apparently all salamanders lack a frontal organ. However, the work of Kelly (1963) on the newt *Taricha torosa* showed that there are well developed sensory cells with outer segments in the pineal body. These cells are generally recognized as photoreceptive (Wurtman, Axelrod and Kelly, 1968) and photosensitive cells have been demonstrated to occur in other pineal derivatives of fish, anurans and reptiles. Eakin (1970) believes that the parietal eye of lizards, along with other light-sensitive organs, including the pineal gland and perhaps certain regions of the brain, transduce the external light stimulus into internal hormonal stimuli.

Adler (1970) reviewed the role of extraocular photoreception in amphibian rhythms and orientation. That amphibians perceive light without eyes has been known for many years (Willem, 1891; Pearse, 1910) and the whole area of extraocular photoreception ("dermal" light sense) was reviewed by Steven (1963).

Extraocular photoreception is also involved in orientation (Landreth and Ferguson, 1967a and b) and pigmentation changes (Bogenschutz, 1965; Oshima and Gorbman, 1969). Adler (1970) suggests an additional role in the possible receipt of photic cues for photoperiodic responses such as gonadal development and water drive (breeding drive).

The ability to use a sun compass in orientation has been demonstrated in numerous species of amphibians, including *Taricha granulosa*. The use of a sun compass demands a time sense phased to local time to compensate for the apparent movement of the sun across the sky. Landreth and Ferguson (1967b) showed that eyeless *T. granulosa* can orient to a light cue or the sun and that the orientation direction can be shifted about 90° by a six-hour phase shift in the photoperiod. The suspected site of the photoreceptor is the optic tectum (Landreth and Ferguson, 1967b).

It is apparent that light can control the locomotor rhythm of amphibians. It is also known to be involved in the control of the locomotor rhythms of mammals, birds, and reptiles as well, but how the cue is transduced into internal factors such as a daily cycle of hormone level is not understood. One of the major internal factors now under investigation is the role of melatonin production by the pineal. The cyclic appearance of melatonin has been most thoroughly studied in the laboratory rat (reviewed by Wurtman, Axelrod and Kelly, 1968). Though the pineal in mammals is thought to be strictly secretory, the synthesis and release of melatonin by the pineal is controlled by environmental lighting. The light is received by the eyes and the pineal is stimulated to produce melatonin by sympathetic nerve impulses arriving from the retina via the superior cervical ganglia. Axelrod and Wurtman (1965) have suggested that the pineal in mammals also functions as the transducer of periodic light cues into hormonal rhythms (exogenously controlled) to which numerous other endogenous self-sustaining rhythms, such as wheel running activity in rodents, are entrained. Wong and Whiteside (1968) found that injections of melatonin inhibited wheel running activity in rats but further investigation of the exact role of the pineal on this activity rhythm is needed. Taylor (1971) has preliminary evidence that daily administration of melatonin via a water flow-through system can serve to rephase the rhythm of free-running *Ambystoma tigrinum* larvae. He used sun compass orientation as his assay of "clock" changes rather than locomotor rhythm.

Axelrod, Wurtman and Winget (1964) found that light could control melatonin and HIOMT (the melatonin-forming enzyme,

hydroxyindole-O-methyl transferase) levels in the pineal of the chicken and that continuous light caused increased HIOMT activity. The reverse occurs in rats as continuous light leads to decreased HIOMT and melatonin levels (Axelrod, Wurtman and Snyder, 1965; and Moore, Heller, Wurtman and Axelrod, 1967). In the house sparrow (*Passer domesticus*), Menaker (1968) found that the eyes were not necessary for maintenance of a circadian locomotor rhythm under constant conditions, though the birds could still entrain to 24-hour cycles of light and dark without a pineal (Gaston and Menaker, 1968). The pinealectomized birds, with eyes intact, expressed an unexplained arrhythmicity in locomotor rhythm similar to that expressed by a majority of intact birds under constant bright light. Johnson (1939) similarly found that the deer mouse (*Peromyscus maniculatus*) showed arrhythmia in locomotor cycles under bright constant light.

Quay (1965) summarized the distribution of HIOMT in the lower vertebrates noting that it is not exclusively confined to the pineal as in mammals. He found that HIOMT is commonly found in the eyes of fish, reptiles and amphibians as well as in the pineal. Axelrod, Quay and Baker (1965), found HIOMT with equal activities in the pineal area, the optic tectum and the hypothalamus in anurans.

Melatonin cycles in amphibians are thought to be related to the blanching reaction of some adult and larval anurans in the dark (Bagnara, 1965). However, the relationship between blanching behavior and cyclic melatonin patterns is not clear.

MATERIALS AND METHODS

All *Taricha granulosa* used in this study were captured at Cronemiller Lake in McDonald Forest, 12.5 km N of Corvallis, Benton County, Oregon. Most animals were captured just prior to use with an L-shaped drift fence with drop cans as they migrated into the pond. Some animals were collected from the lake with a dip net. The animals were brought directly to the laboratory where they were used in a variety of experiments.

A cylindrical activity chamber (Figure 1A) was used to monitor locomotor activity. The chamber was mounted by ball bearings on a yoke and gimbals so that tilting in two directions could be registered. This chamber (Type A) was in the form of a covered circular track 25 cm in outside diameter, 6 cm wide and 5 cm deep. A circular chamber was used to reduce the tendency of the

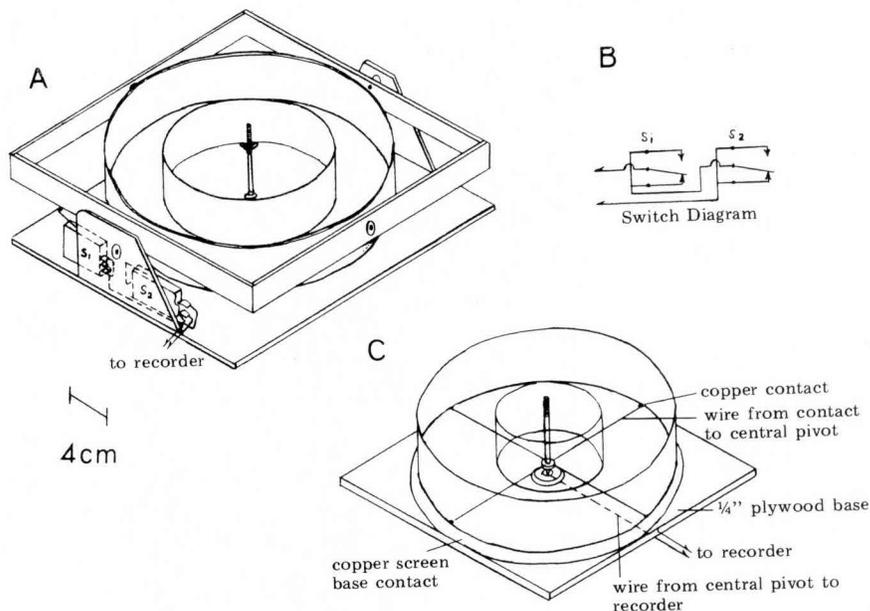


FIGURE 1A

Isometric drawing of the yoke and gimbals type activity chamber. The cylindrical chamber can tip in any direction activating either switch 1 or switch 2 every time an axis is crossed.

FIGURE 1B

Wiring diagram for microswitches in the Type A chamber. An "off" pulse was recorded on an Esterline-Angus event recorder as a vertical mark. S_1 and S_2 refer to switches 1 and 2.

FIGURE 1C

Isometric drawing of the Type B chamber. Four contacts around the edge were wired to the central pivot which rested on a copper screen base. When the copper screening was contacted a circuit was completed making the pen mark.

newts to curl up in corners. Two microswitches were mounted to record tilting in two planes and were connected to an Esterline-Angus, 20 channel, spring driven event recorder. The switches were wired to produce an "off pulse" whenever the animal crossed one of the axes in either direction (Figure 1B). Each off pulse was recorded as a single vertical mark of the pen on a slowly moving (45.7 cm/day) chart. A total of six chambers were constructed with clear plexiglas and installed in separate 46 x 60 cm boxes with 30 cm walls and open tops. These were kept in a

temperature and light controlled room. The recorder was kept outside the room so that the output of the chambers could be inspected at any time without entering the room. Since the floor of each chamber was covered with filter paper and 15 to 20 ml of water, the relative humidity remained high and probably fairly constant though the humidity in the room varied from 25% to 45%.

In addition, five Type B chambers were developed so that activity could be monitored using a different principle (Figure 1C). The output of the Type A and Type B chambers could then be compared to look for monitoring biases. Each 22 cm diameter chamber was mounted on a pointed 1 cm central pivot. Four contacts were mounted around the outside edge and were wired to the central pivot to complete a circuit to the event recorder whenever one of them touched the copper screen base. The records produced by the Type B chambers were qualitatively similar to those produced by the Type A. However, more than one pen mark frequently occurred as the sensitive contacts rocked back and forth over the copper screen when the animal moved. Observations showed that marks occurred only when the animal was moving but that the number of marks per half hour was not equal to the number from Type A chambers. For this reason periodogram analysis (Enright, 1965a and b) was carried out only on the data from Type A chambers. The results of these statistical analyses were then used to help interpret by comparison, the records obtained simultaneously from the Type B chambers.

ANALYTICAL PROCEDURE

Actograms were prepared for each animal by cutting the activity records into 24-hour segments. Each succeeding day's record was glued on a piece of cardboard under the preceding day's record. Thus the progression of time was read from upper left to lower right. The actograms from the Type A chambers were quantified by counting the number of pen marks in each 30-minute period. The data were then punched sequentially on IBM cards and analyzed using a periodogram program obtained from Enright (1968). The method of analysis has been explained in detail by Enright (1965a and b). The program searched the data for periods between 18 and 28 hours by calculating and printing the root-mean-square amplitude (A_p) for each form estimate for periods at 0.1-hour intervals. The period with the highest A_p was regarded as the peak period. The data were then run again using a second program which I call PROFILE. PROFILE essentially

tabulated the data as if a form estimate were to be made for the period detected by the periodogram analysis. Instead of a form estimate, each one-half hour interval was summed and divided by the grand total of all activity units (pen marks) to get the relative frequency of activity in that one-half hour period.

EXPERIMENTAL TREATMENT OF ANIMALS

Animals were blinded by bilateral enucleation without anesthesia (anesthesia may affect activity rhythm). The eyeball was freed from the lids with small, curved blunt forceps. The curved forceps were then used to protrude the eyeball from the orbit while micro-scissors were used to cut the muscles, remaining connective tissue and the optic nerve. Very little bleeding occurred. In early experiments no antiseptic was used but in later experiments Furicin powder (Nitrofurazone, Eaton Lab.) was packed into each orbit. If the eye did not come out intact, the animal was not used.

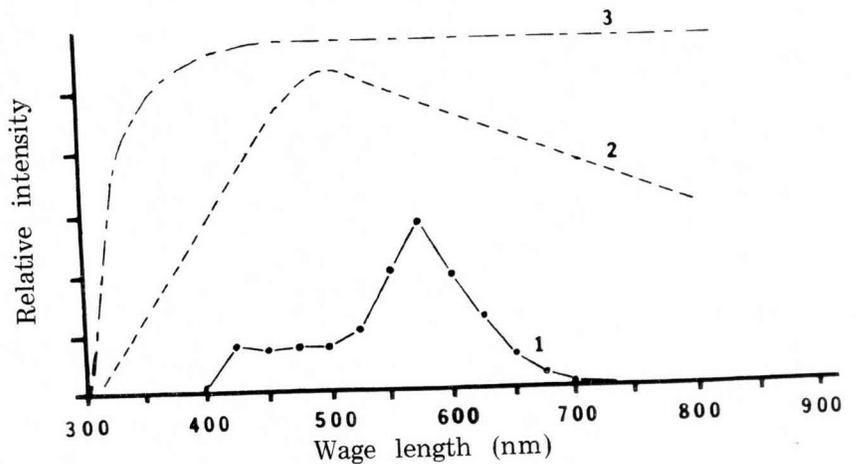


FIGURE 2

The lower curve (1) shows the relative intensities of the different wavelengths of the fluorescent lights (with diffuser in place) used in these experiments. The middle line (2) shows the relative intensities in natural sunlight and the top line (3) is the response curve of the YSI Kettering Model 65 spectroradiometer used to obtain the 2 light spectra. The response of the meter is nearly constant in the area of interest (visible light, 400 to 750 nm).

(Table 1). This is significantly ($P > .95$) different from both the LD and DD light regimes. The mean period of the activity rhythm of the remaining 20 newts was 23.7 hours but was highly variable (coefficient of variation = 6.89) with a large range (22.1 to 27.1

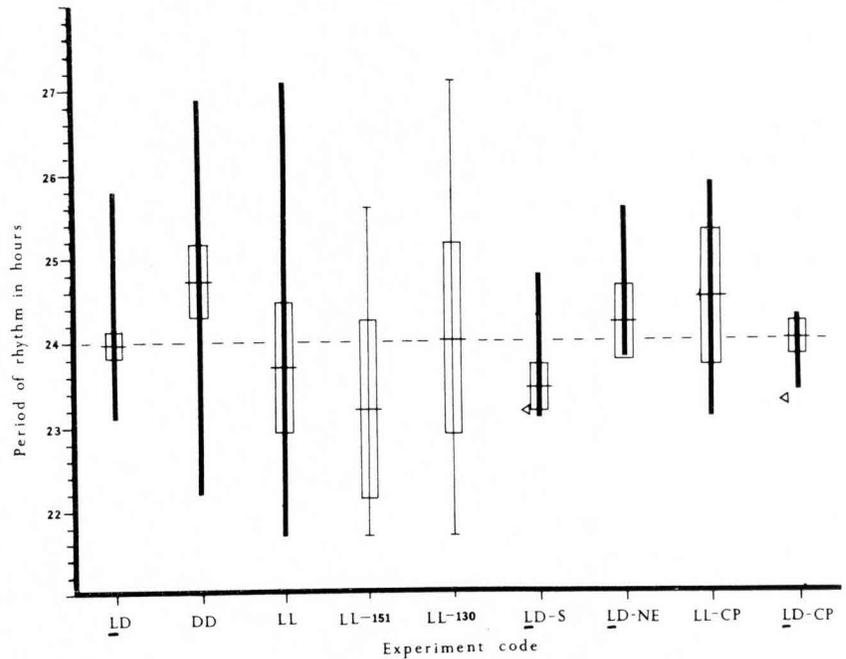


FIGURE 3

Summary of the periods (Tau) of the activity rhythms in *T. granulosa* under different light and temperature conditions. The horizontal mark indicates the mean while the box indicates t (student's t) standard errors on either side of the mean, i.e., 95% confidence limits. The vertical line indicates the range of observed values. Experiment codes: LD: entrainment under various 24 hour cycles of lights-on, lights-off and under constant temperatures (all data combined); DD: Free-run under constant darkness (0 lux) and constant temperatures; LL: Free-run under constant light of various intensities (151 and 130 lux combined) and constant temperature; LL-151: Free-run under constant light (151 lux) and constant temperatures; LL-130: Free-run under constant light (130 lux) and constant temperatures; LD-S: entrainment under a short light-dark cycle (23.2 hour mean indicated by triangle) and constant temperature; LD-NE: entrainment of blinded (bilateral enucleation) animals under a 24-hour light cycle and constant temperature; LL-CP: entrainment to a 4-hour pulse of cold from 9°C to 4°C with a 24-hour period under constant light; LD-CP: entrainment of *T. granulosa* under the influence of two different Zeitgeber regimes, a 24-hour light-dark cycle and a 23.28-hour (mean) cold pulse (9°C to 4°C) cycle. The triangle marks the period of the cold pulse.

TABLE 1

Summary of the locomotor activity of *Taricha granulosa* under a variety of experimental conditions. Experiment codes are the same as Figure 3. Dark period is zero lux in all cases. Cold pulse Zeitgeber (drop from 9° to 4°C) lasted about 4 hours. The period length of the cold pulse was 9°: 4°C (20:4) under LL and 9°: 4°C (19.28: 4) under LD.

Light Cond.	Intensity (lux)	Mean Period (τ)	Imposed Photo Period	St. Error of Mean	Coef. of Variation	95% Conf. Limits	No Rhythm	No Activity	Number Used	% With Rhythm	95% Conf.
<u>LD</u>	Various As LL	23.98 hr.	<u>8:16 to 16:8</u>	0.078	1.75	23.82 — 24.14	10	2	41	70.7%	±13.9%
DD	0	24.73 hr.	—	0.208	4.29	24.30 — 25.16	2	1	29	89.6%	±10.9%
LL	Combined	23.70 hr.	—	0.365	6.89	22.94 — 24.47	27	0	47	42.5%	±14.1%
LL	454	—	—	—	—	— —	8	0	8	0.0%	—
LL	151	23.20 hr.	—	0.457	5.57	22.15 — 24.25	11	0	19	42.1%	±22.2%
LL	130	24.04 hr.	—	0.519	7.48	22.91 — 25.17	8	0	20	60.0%	±21.5%
<u>LD-S</u>	<u>130:0</u>	23.45 hr.	<u>17.2:6</u>	0.117	1.59	23.19 — 23.71	0	1	11	91.0%	±17.0%
<u>LD-NE</u>	<u>151:0</u>	24.21 hr.	<u>12:12 to 18:6</u>	0.195	2.41	23.77 — 24.65	3	3	15	60.0%	±24.8%
LL-CP	130	24.51 hr.	—	0.338	3.65	23.71 — 25.31	3	0	10	70.0%	±28.4%
<u>LD-CP</u>	<u>130:0</u>	24.02 hr.	<u>20:4</u>	0.086	1.08	23.83 — 24.22	1	0	10	90.0%	±18.6%

hours). If the behavior is examined by light intensity (Table 1) under LL it is apparent that more animals became rhythmic as the intensity was lowered from 151-130 lux. Also τ (tau) tended to lengthen as the intensity dropped, although there is no significant difference between the two mean periods.

Under constant darkness (DD = zero lux) the mean period was 24.73 hours (coefficient of variation = 4.29). The 95% confidence interval for the combined DD data does not overlap that of the LD experiments. Typical variability of tau under DD is shown in the four actograms of Figure 4.

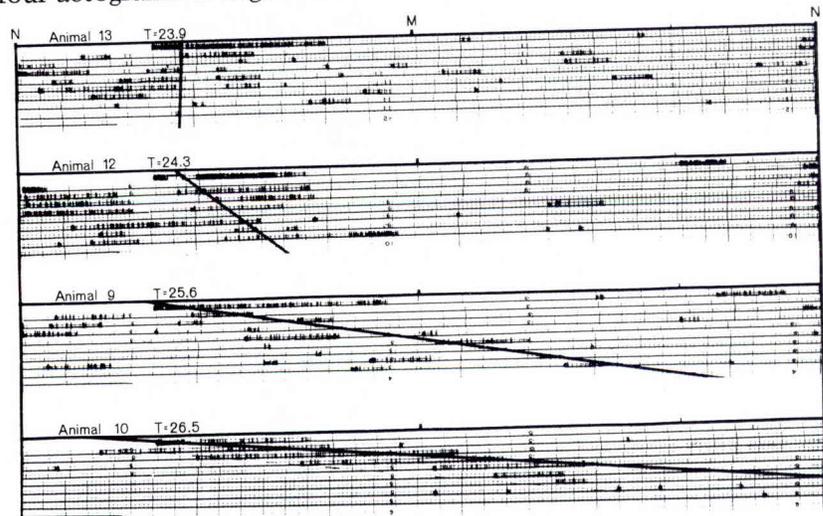


FIGURE 4

Actogram of animals 9, 10, 12, and 13 showing variability of the free-run rhythm under DD and constant temperature. The periods are indicated by the sloped lines. The mean period (Tau) is 25.2 hours (SE = 0.6455 hours).

ENTRAINMENT OF THE LOCOMOTOR RHYTHM TO ENVIRONMENTAL LIGHT CYCLES

Entrainment is the synchronization of one rhythmic phenomenon, such as an endogenous clock controlling locomotor activity, with another rhythmic phenomenon, such as the environmental cycling of light and dark, so that both rhythms have the same period. The forcing rhythm in the entrainment process is called the Zeitgeber. The most important environmental entraining rhythms in other animals are those of light or temperature or both. The ability to entrain locomotion to light cycles under constant temperature has been discussed above. In

addition, a short period entrainment experiment was performed. Eleven newts were entrained to a photoperiod of LD 17.2:6, that is, a 23.2-hour day. Tau during entrainment averaged 23.45 hours (Table 1), which is significantly different from 24 hours.

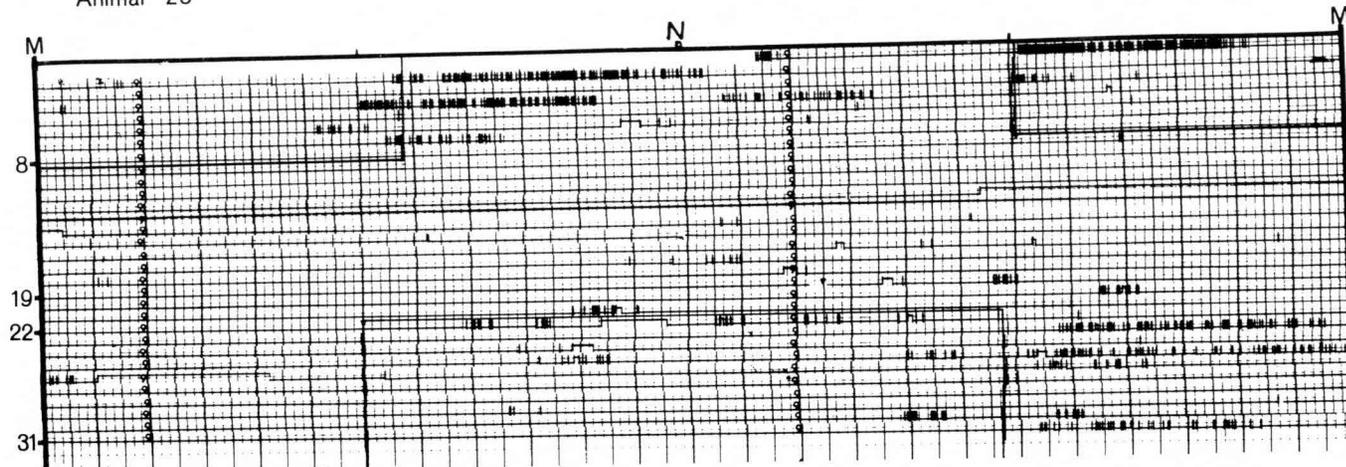
The locomotor rhythm can also be phase shifted by manipulation of the light regime. The phase shifting of 12 hours in animal 23 is presented in Figure 5. Normal entrainment to LD 12:12 (151:0 lux) occurred for the first eight days with lights-on at 0700 hours and lights-off at 1800 hours. A profile analysis of the 23.5-hour rhythm showed that 73% of the activity occurred between the lights-on cue and five hours into the period, with the peak at 3.5 hours. The newt then free-ran under LL through day 21. During this time there was no clear rhythm, as is common under LL (151 lux), but the activity which did occur usually came during the subjective day. Starting on day 22 the light was turned out at 0600 and on at 1750 or phase shifted about 12 hours from the original LD cycle. Under this reversed light regime about 70% of the activity occurred between 1830 and 2330 hours with the peak at 1830 or 1.75 hours after lights-on.

In animal 23 the environmental cue is apparently lights-on. In the 20 additional animals monitored in Type A chambers under LD, 62% showed peak activity within the first 8 hours and 71% within the first 12 hours after the lights-on cue. The peaks of activity were randomly distributed throughout the day when measured from the lights-off cue (Table 2). The high correspondence of peak activity with the lights-on cue and the 62% showing peak activity during light hours suggests that newts are day active, at least when in the activity chamber. This confirms that *T. granulosa* locomotor rhythms conform to "Aschoff's Rule" which states that day-active animals show a longer circadian period under DD than when under LL, and that for light-active animals the period of the circadian rhythm decreases with increasing light intensity. My evidence (Table 1) suggests that newts show a day active response to both free run conditions and light intensity. The rhythm of locomotion thus appears to be truly circadian in that it has a period close to 24 hours under constant conditions of light and temperature, and can be entrained to the environmental light cycle.

ENTRAINMENT OF THE LOCOMOTOR RHYTHM TO ENVIRONMENTAL TEMPERATURE CYCLES

The second major environmental cue is temperature. The results of the experiments are summarized in Table 1 and Figure 3 with

Animal 23



12

FIGURE 5

Actogram of animal 23

Days 1-8: Entrainment to light-dark cycles of 11 hours light and 13 hours dark with the lights on at 0650; LD 11:13 at 0650 (151:0 lux).

Days 8-22: Free-run under continuous light; LL 151 lux. Tau is unclear. Chamber cleaned on day 19.

Days 22-31: Re-entrainment to LD 12:12 (151:0 lux) at 1800 resulting in a photoperiod reversed from the control period. Tau = 23.9 hours.

TABLE 2

Summary of entrainment of *T. granulosa* to light-on and light-off cues based on peak activity as indicated by profile analysis. The frequency tallies are separated into a group with peak activity in the light and a group with peak activity in the dark for both cues. The relative frequencies (rf) and 95% confidence limits are calculated using the normal approximation to the binomial (Cochran, 1966).

Hours Since Light Cue	Light-On Cue				Light-Off Cue			
	Light Act.	Dark Act.	8-Hour Freq.	12-Hour Freq.	Light Act.	Dark Act.	8-Hour Freq.	12-Hour Freq.
0	3	0			0	0		
1	2	0			0	0		
2	1	0			0	0		
3	2	0	13	15	0	2	7	10
4	2	0	rf = .619	rf = .714	0	0	rf = .333	rf = .476
5	1	0	± .207	± .193	0	0	± .202	± .214
6	1	0			1	0		
7	1	0			1	1		
8	0	0			0	2		
9	0	0			1	0		
10	0	0			0	0		
11	0	2	4		0	1	7	
12	0	0	rf = .190		0	1	rf = .333	
13	0	0	± .168		1	0	± .202	
14	0	2			1	1		
15	0	0			0	0		
16	0	0			1	0		
17	0	1			2	0		
18	0	0			2	0		
19	0	1	4	6	2	0	7	11
20	0	1	rf = .190	rf = .286	0	0	rf = .333	rf = .524
21	0	0	± .168	± .193	0	0	± .202	± .214
22	0	0			0	0		
23	0	1			1	0		
24	0	0			0	0		
Totals	13	8			13	8		

an actogram of animal 65 presented in Figure 6 as an example of the response to the temperature experiments. Here the first eight days were spent under free-run conditions (LL = 130 lux at 9° C). Tau of animal 65 was 26.3 hours; however, the mean of six animals was 23.91 hours. Profile analysis revealed that the peak of activity came fourteen hours into the period starting at midnight on day one. A four-hour cold pulse down to 4° C with a period of 24.5 hours was used as an entraining Zeitgeber under LL from day 8 through 13. The peak of the 25.9-hour activity rhythm came 12.5 hours into each period. This is a phase advance of 16 hours or delay of 8 hours from the previous free-run and is closely associated with the end of the cold pulse, suggesting entrainment. After a brief free-run under LL, there were nine days of exposure to LD 20:4 and four cold pulses (CP) simultaneous with the four hours of darkness. The dark pulse was then maintained on a 24-hour period while the cold pulse was advanced on a mean 23.28-hour period. The animal entrained to the light period but curtailed its activity as the cold pulse advanced into its normal activity time.

Newts can entrain to a temperature pulse while under constant light (Figure 3, LL-CP). When given a choice between light and temperature cues (Figure 3, LD-CP), newts show a preference for the light cue. This is similar to data obtained by Adler (1968) for entrainment in the salamander *Plethodon glutinosus*.

THE ROLE OF EXTRAOCULAR PHOTORECEPTION IN LIGHT ENTRAINMENT

Light cycles play an important role in the entrainment of activity rhythms in the rough-skinned newt. Since extraocular photoreception is common in amphibians and known to play a part in orientation to light cues in *T. granulosa* specifically (Landreth and Ferguson, 1967a), the final phase of this study was to investigate the role of the extraocular photoreceptors (EOP) in the light entrainment of activity rhythms.

Fifteen animals were tested to establish if blinded animals could entrain to LD photoperiods. The results (Figure 3, LD-NE, and Table 1) confirm that retinal photoreception is not necessary for entrainment to light, since the mean period of 15 animals was 24.21 hours.

The actogram of animal 31 (Figure 7) is presented as evidence of entrainment. The animal was blinded 4 days prior to day 1 and

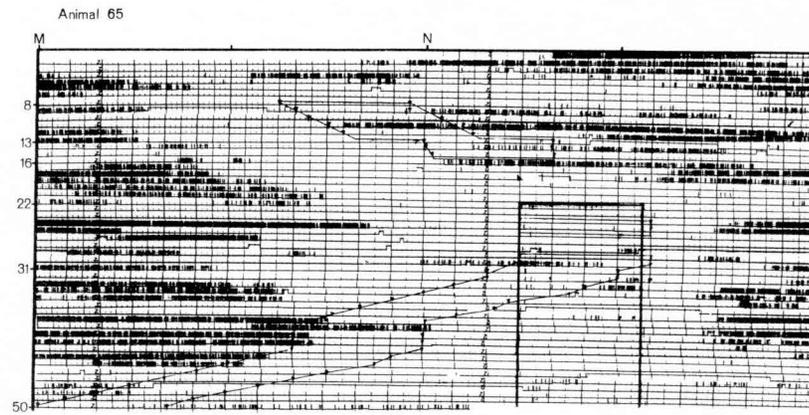


FIGURE 6

Actogram of animal 65. This is a center-pivot Type B chamber actogram and the periods are estimated by inspection.

Day 1: Escape reaction after animal placed in chamber at 1550.

Days 2-7: Free-run under LL (130 lux) and constant temperature of 9°C. Tau = 26.5 hours (approx.).

Days 8-12: Entrainment under LL (130 lux) to a 4-hour temperature cold pulse (9° to 4°C) with the T of the Zeitgeber equal to 24.5 hours. Cold pulse bracketed by triangles.

Days 13-15: 4-hour cold pulse starting at 1200 under LL (130 lux). No entrainment evident in this animal.

Days 16-21: Free-run under LL (130 lux) and constant temperature (9°C). Tau = 25 hours (approx.).

Days 22-31: Entrainment to a simultaneous cold pulse and light pulse of 4-hour duration, LD 20:4 (130:0 lux) and 9° to 4°C starting at 1500 each day (cold pulse controller failed on 3 days).

Days 32-50: Entrainment with a choice of 2 Zeitgebers having different period lengths. (1) LD 20:4 (130:0 lux) at 1500 and (2) cold pulse 19:4 (9°C: 4°C). Cold pulse failed to advance on days 40 and 41. Activity entrained to the light pulse with an unexplained phase delay of 6 hours from lights-on. Activity was generally curtailed during the cold pulse as it advanced through the newt's normal active period.

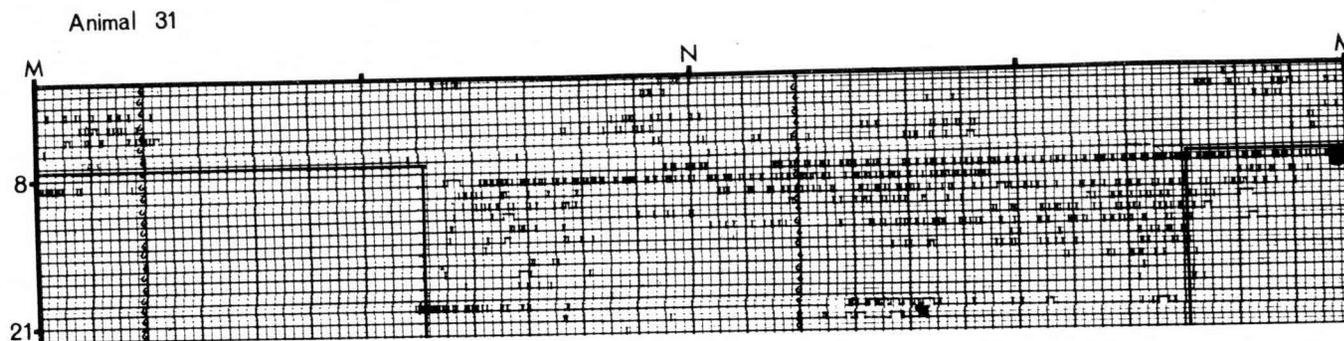


FIGURE 7

Actogram of animal 31. The animal was blinded by bilateral enucleation 4 days prior to the beginning of the experiment.

Days 1-7: Free-run under LL (151 lux). Tau = 24.9 hours. Peak activity occurred 23 hours into the period with a secondary peak at 8 hours.

Days 8-21: Entrainment to LD 14:10 (151:0 lux). Tau = 23.9 hours. The peak of activity has been phase-shifted 7.5 hours forward with activity confined almost entirely to the light period.

held in LL (130 lux) until day 8. During this time a two-peaked activity rhythm ($\tau = 24.9$ hours) was seen with the major activity starting 23 hours into the period. On day 7 the lights were turned out at 2100 hours and LD 14:10 (130:0 lux) imposed through day 21 under constant temperature. Entrainment to lights-on (at 0700) is evident. This represents a phase shift of about 7.5 hours in a blinded animal. The increased activity during the light period is clearly evident. A control animal with eyes intact was run concurrently and showed a similar pattern of activity.

DISCUSSION AND CONCLUSIONS

Pimentel (1952) observed that in Oregon, waves of newt arrivals at breeding ponds were associated with rainfall and warm weather during early winter. During rainy parts of the breeding season some individuals left the pond for as long as 3 days before returning. Further, "most terrestrial movements of *granulosus* (sic) into and out of ponds were found to occur during the night or overcast periods. This, however, may be due to relative humidity or other factors. Movement of *granulosus* is very conspicuous during daylight hours, but it is difficult to ascertain whether this newt is more active during this time" (Pimentel, 1952: 103). These observations combined with the results of this investigation suggest a complex relationship between internal and environmental factors controlling activity. Two important internal factors are the physiological state of the animal in relation to the stage of the yearly breeding cycle and the physiological state of the endogenous clock controlling 24-hour activity cycles. Three environmental factors appear to be important in controlling newt activity: (1) rain or humidity; (2) temperature; and (3) the light cycle.

Under conditions of high humidity and temperatures above 6°C, I believe that normal foraging activity and perhaps short migrational movements occur during the daylight and early evening hours and are under the control of an endogenous clock which is normally entrained to the daily light cycle. Longer range migrational movements probably begin when hormonal levels (prolactin? Grant and Grant, 1958) reach a threshold level and heavy rains occur. Pimentel (*op. cit.*) found that penetration

of water to buried aestivating newts in an aquarium stimulated surface foraging.

The effect of temperature is probably direct as newts generally become inactive below about 6°C. The ability to entrain to cold pulses is not surprising since temperature cycles and light cycles are normally highly correlated in natural situations.

The high percentage of arrhythmic animals and highly variable periods under bright LL conditions may be the result of hormonal interaction. Snyder, Zweig and Axelrod (1964) found that two rhythms of brain hormone appearance were present in rats. Serotonin showed peak activity at 1200 hours and a trough at 2200 while melatonin showed a peak at 2400. The rhythms of these two pineal biochemicals and pineal noradrenalin appear to be controlled by endogenous and exogenous mechanisms. "Diurnal changes in pineal HIOMT activity and noradrenalin content appear to be totally dependent upon environmental illumination, while the pineal serotonin rhythm persists in the absence of lighting cues" (Wurtman, Axelrod and Kelly, 1968:130).

Bagnara (1965) has shown that the nightly blanching reaction of amphibian larvae is mediated by a "pineal hormone" which he suggests is melatonin. Later, Quay (1965) has demonstrated the presence of HIOMT in the pineal, brain and eyes of various amphibians. However, Wurtman, Axelrod, and Kelly (1968) found that continuous light or dark had no effect on the HIOMT activity in the pineal, brain and eyes of *Rana pipiens*. In addition, the protein structure of HIOMT has probably evolved through different forms since electrophoretic mobilities of enzyme preparations from the bird, cow and frog differ (Wurtman, Axelrod and Kelly, 1968). Thus it is possible for different forms of HIOMT to respond differently to light cycles and explanations of responses in rats, birds and amphibians are not necessarily interchangeable.

Axelrod and Wurtman (1965) suggest that the pineal is the "biological clock". Baum (1966) found through denervation experiments that the pineal probably mediates the control of the feeding rhythm in rats by light. However, the rats could eventually re-establish a feeding rhythm entrained to the light even after pineal denervation, so something else must also be involved. In addition, Kelly and Johnson (1963) showed that the blanching rhythm in *T. torosa*, *Ambystoma opacum* and *Rana pipiens* was not entirely dependent on the pineal for its control. There appear

to be no clear-cut theories of the mechanism of the biochemical transduction of light cycles into animal response rhythms. However, Bagnara (1965:505) suggests that the blanching reaction of *Xenopus* larvae “. . . is a result of the interaction of antagonistic pineal and hypophyseal melanophore controlling principles.”

Rats, mice, birds and *T. granulosa* develop arrhythmia in locomotor rhythms under bright constant light. I suggest that the activity rhythm is controlled by the synergistic effects of at least two factors (which may be hormones). The simple model presented (Figure 8), includes only two hormones, which control activity by an additive effect, and is based on the general model of Wever (1965). Though the biochemical basis may vary between groups, there is possibly a first activity factor which is under the direct exogenous control of light. The production and/or release of this

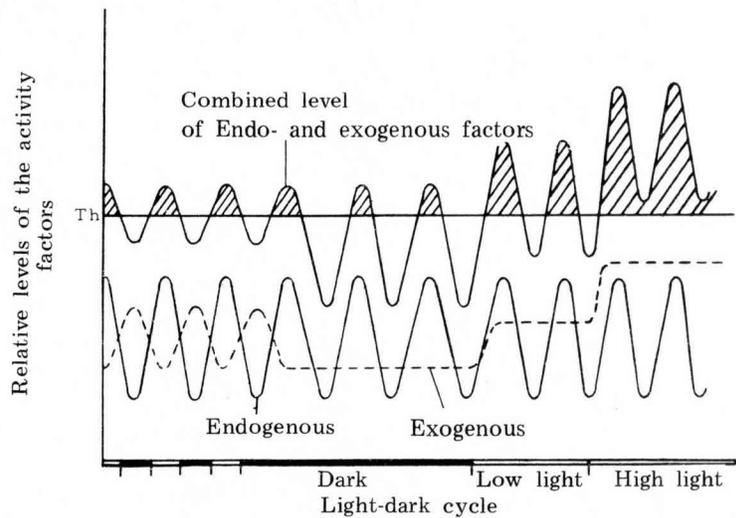


FIGURE 8

A synergistic model for circadian activity rhythms. This particular example is the simplest hypothetical interaction of hormones that will account for the results observed and is configured for a day-active species. Only two hormone-like factors are used in this example while the actual number for any given species may vary. Also a simple additive interaction is used in this example while in reality more complex functions may be involved. The threshold level for the two combined factors (Th) is shown here as fixed while in reality this may vary depending on the season, time of day, age, sex, etc. The cross-hatched areas represent periods of activity when the combined levels of the endogenous and exogenous factors exceed the threshold needed for activity.

factor is a function of intensity and duration of the light. The second hormonal rhythm is under endogenous control and persists under constant conditions. The model presented is based on a simple additive effect of the two factors. Under normal LD conditions the animal becomes active when the added effects of the two factors surpass a threshold level. The endogenous rhythm is kept in phase with the light cycle by the exogenous cycle. Under DD conditions there is little production of the light-induced factor and activity is controlled by the endogenous rhythm. Under constant light of moderate intensity the light-induced factor level is raised but the endogenous rhythm continues to control activity. Under high light intensity the production and release of the exogenously controlled factor is so high that it overrides the control of the endogenous rhythm and activity may occur at any time of the day.

It must be emphasized that the exact nature of the hormones involved is unknown and that the model presented is the simplest that will account for the observed facts. The threshold level of the factors may also be under hormonal control, or there may be more than two factors involved, and the synergistic relationship may be much more complex than a simple additive effect.

Extraocular entrainment has been clearly demonstrated in *Plethodon glutinosus* (Adler, 1969) and *Rana clamitans* (Adler, 1971). Similarly, I have demonstrated that eyes are not necessary for entrainment in the diurnal newt, *Taricha granulosa*. The exact location of the extraoptic photoreceptor in the newt has not been clearly demonstrated but Taylor (1972) suggests that the pineal is the EOP used in the orientation of *Ambystoma tigrinum* while Adler (1971) believes the frontal organ is the EOP used to entrain locomotor rhythms in *Rana clamitans*. Since Landreth and Ferguson (1967b) suggest the optic tectum may function as the EOP in *T. granulosa* it is possible that the derivatives of the roof of the diencephalon may all function in an EOP capacity in the control of biological rhythms.

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