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Abstract approved: _____
(Robert M. Storm)

Over 56,000 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, shortened day-night cycles, constant darkness (DD) and constant light (LL). The rough-skinned newt is day active and crepuscular. I found that 62% of the animals tested show their peak activity within 8 hours of the light-on cue and 71% within 12 hours. The activity peaks are random with respect to the light-off cue. 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 = 24.72 hours) than LL (mean = 23.7 hours) and (2) the period decreased with increasing intensity of light under LL. However, the number of animals which show arrhythmia

increased with increasing intensity.

Entrainment to the light-dark cycle is evident and the activity cycle could be phase-shifted by changing the phase relationship of the light cue. Although newts can entrain to temperature pulses (cold from $9^{\circ}C$ to $4^{\circ}C$) under constant light, they entrain to the light cycle when given a choice between light and temperature. Activity generally ceases below $6^{\circ}C$ 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. The site of the receptor was narrowed only to the dorsal midbrain.

An hypothesis relating conflicting behavior in the field and the laboratory is presented. Also, an hypothetical model is presented of the interrelationship between exogenous and endogenous factors in locomotor rhythm control.

Investigation of the Circadian Rhythm of Locomotion in the Rough-skinned Newt, Taricha granulosa

by

Jeffrey Lawrence Briggs

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Professor of Zoology

in charge of major

Redacted for Privacy

Head of Department of Zoology

Redacted for Privacy

Dean of Graduate School

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

INTRODUCTION

The time sense in animals as well as plants has been investigated by many people over the past half century, but this field has come under most intense study in the past fifteen 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, amphibia, 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.

One of the earliest studies of locomotor rhythms in salamanders was done by Kalmus (1940). He 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 (1969a) 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. To localize the site of extraoptic photoreception, parts of the dorsal surface of the heads of eyeless animals were painted with black airplane dope. The time to initiate the fright reaction when the room lights were turned on was recorded. With this technique he was able to localize the receptive area to the forebrain and/or midbrain. This assumes that the receptors for the fright reaction are the same as those used in the entrainment of the locomotor rhythm which may or may not be true. Using the traditional techniques of actogram construction and periodogram analysis, I showed that Taricha granulosa can entrain to light-dark cycles and that the site of the extraoptic reception is probably the dorsal-anterior brain.

In the frog Rana clamitans, the site of the receptor for entrainment has been narrowed to the pineal end organ. Adler (1969b) demonstrated that this frog lost its ability to entrain locomotion to light cycles when the pineal end organ was removed even though the pineal (epiphysis cerebri) remained intact.

T. granulosa has no pineal end organ, however the work of Kelly (1963) on the close relative T. torosa showed that there are well developed sensory cells with outer segments in the pineal. 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, transduces the external light stimulus into internal hormonal stimuli.

Adler (1969b) has recently reviewed the role of extraoptic photoreception in amphibian rhythms and orientation. The ability of this class to perceive light without eyes has been known for many years (Willem, 1891; Pearse, 1910) and the whole area of extraoptic photoreception ("dermal" light sense) was reviewed recently by Steven (1963).

In addition to Adler's work on rhythms, extraoptic photoreception has been shown to be involved in orientation (Landreth and Ferguson, 1967a and b) and pigmentation changes (Bogenschütz, 1965; Oshima and Gorbman, 1969). Adler also suggests that it may play a role in 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 by Ferguson, Landreth and others. 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^{o} by a six-hour phase shift in the photoperiod. The suspected

site of the photoreceptor is the optic tectum. The ability of Taricha to perceive light cues used in orientation naturally suggests it for use in the study of the use of light cues in entrainment of locomotor rhythm. If entrainment cues can be perceived without eyes, are they perceived by the optic tectum also?

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 is the cue transduced into internal factors such as a daily cycle of hormone level. 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 light zeitgebers 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.

Axelrod, Wurtman and Winget (1964) found that light could control melatonin and HIOMT (a melatonin forming enzyme, Hydroxyindole-O-methyl transferase) levels in the pineal of the chicken and that continuous light causes increased HIOMT activity. This is exactly opposite to the case in rats where 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 normal entrainment of the circadian locomotor rhythm. Gaston and Menaker (1968) found that the pineal is 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. 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.

The distribution of HIOMT in the lower vertebrates was summarized by Quay (1965) and he found that it is not exclusively confined

to the pineal as is the case in mammals. He found that HIOMT is commonly found in the eyes of fish, reptiles and amphibians as well as in the pineal. In a study by Axelrod, Quay and Baker (1965) HIOMT was found with equal activities in the pineal area, the optic tectum and the hypothalmus in anura.

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 other cyclic behavior 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 north of Corvallis, Benton County, Oregon. Most animals were captured by an L-shaped drift fence with drop cans as they migrated into the pond just prior to use. The aquatic stage animals were collected from the lake by 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 gimbal 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 newts to curl up in corners and become inactive. Two microswitches were mounted to record rocking 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 plexiglass 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%.

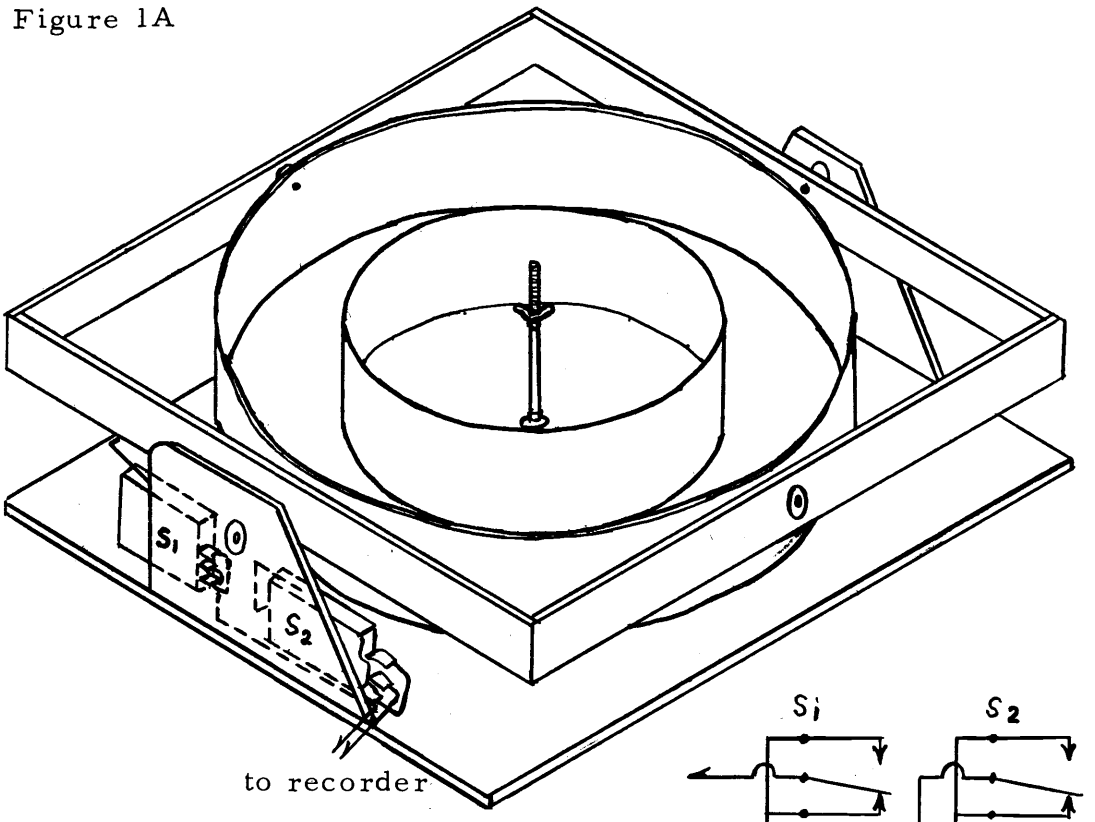
In addition, five new Type B chambers were developed so that activity could be monitored using a different principle (Figure 1c). The output of the Type A and Type B chambers could then be compared to look for monitoring biases. Each 22 cm diameter chamber was mounted on a pointed 1 cm central pivot. 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, 1965 a and b) was carried out only on the data from Type A chambers. The results of these

Figure 1A. Isometric drawing of the yoke and gimbal 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 generated every time the Esterline-Angus event recorder to make 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.

Figure 1A



Switch Diagram

Figure 1B

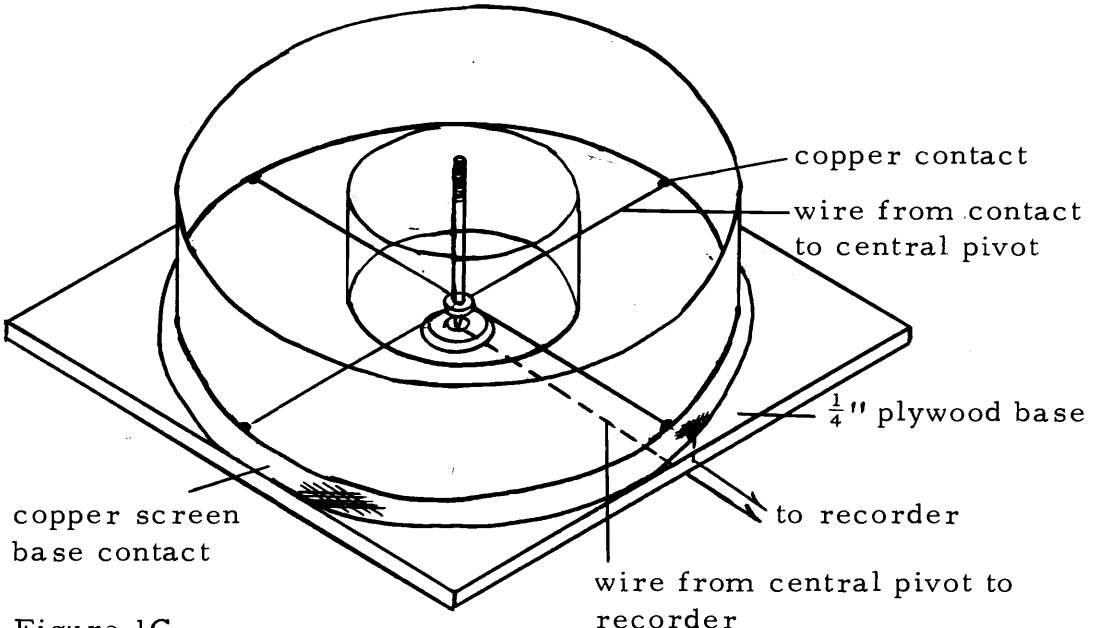


Figure 1C

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 preceeding 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 analysed using a periodogram program obtained from Enright (1968). The program was modified for use on Oregon State University's CDC 3300 computer (Appendix I). The method of analysis has been explained in detail by Enright (1965a and b). The program searches 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 these periods at 0.1-hour intervals. The period with the highest A_p is regarded as the peak period. The data were then run again using a second program which I call PROFILE (Appendix II). 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.

The results were divided into two seasons. The first includes 1 August through 31 January. During this time the animals leave their terrestrial summer areas and migrate to the breeding ponds. The migrations begin with the first rains of the fall when the males start moving, usually entering the ponds before the females, which start arriving in mid-December. The second season includes the late breeding season from 1 February through the peak of egg laying from late April into early June to migration away from the ponds (Pimentel, 1952).

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 and in many cases none. In early experiments no antiseptic was used but in later experiments Furicin powder (furazolium chloride) was packed into each orbit. If the eye did not come out intact, the animal was not used.

In some experiments, clear or black opaque plastic (Visqueen) was implanted under the skin at the top of the head. After sponging with 70% ethanol, a transverse slit was made in the skin near the base of the skull (Figure 2) and the skin freed anteriorly from the skull with a sterilized micro-scalpel. The plastic pieces were boiled previously for 20 minutes and placed in 70% ethanol with sterile forceps. The plastic was then slipped into the pocket thus formed and the wound dressed with Furacin powder. The actual positions and sizes of the plastic pieces is shown in Figure 13.

Pinealectomy was performed under a 3X binocular dissecting scope on animals immobilized by cooling in an ice bath. A flap was sawed in the top of the skull (Figure 2) with a 4mm diameter Dremel circular saw, with its standard blade ground as thin as possible. The flap was carefully lifted and held back with a retractor. The meninges were then cut in the area of the pineal, being careful not to cut the larger blood vessels, especially near the extensive network of the paraphysis. The pineal was then destroyed in place using a Hyfrecator (The Birtcher Corporation, L. A., California) set at the second intensity level. A tiny wire probe was inserted into the pineal area and the electrical charge coagulated all the cells in the area of the probe. In this way the pineal was destroyed and the thin underlying habenular commisure, though also coagulated, was not ruptured. The meninges were replaced but not sewn and the flap was lowered

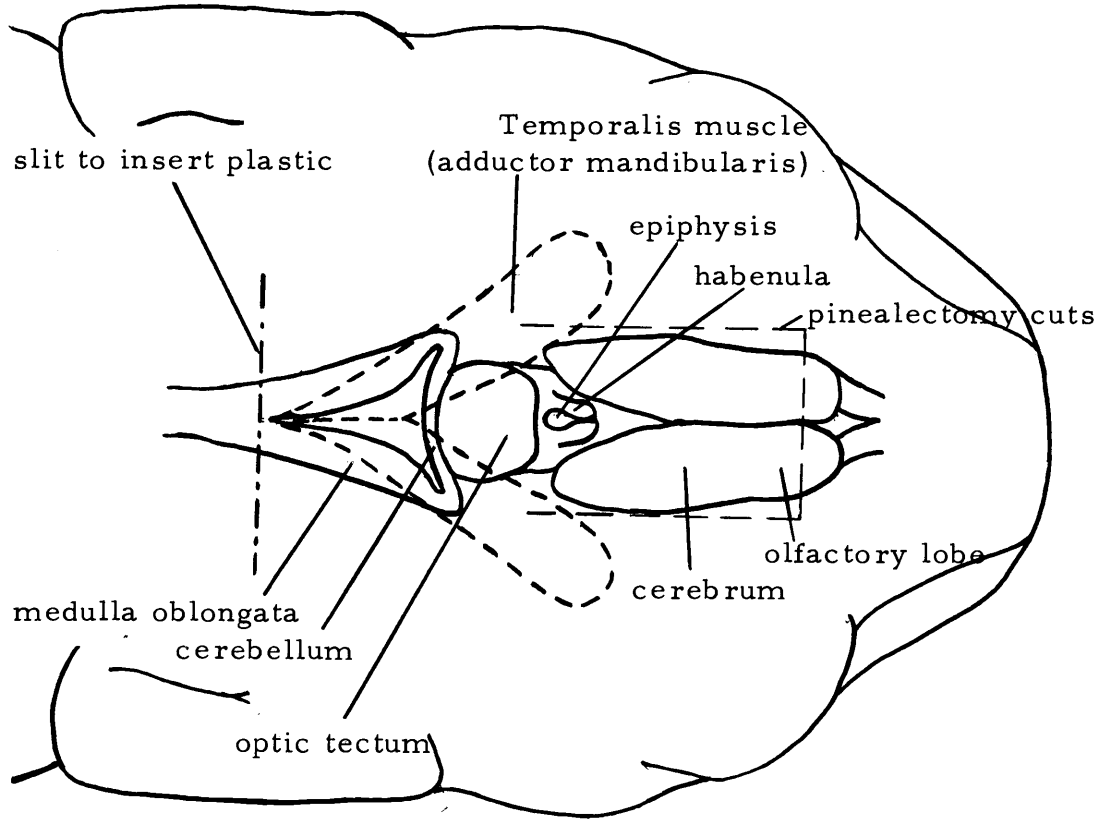


Figure 2. Dorsal view of the head of Taricha granulosa showing the relative positions of the various parts of the brain, the saw cuts for pinealectomy, and the skin slit for insertion of plastic under the skin.

into place. It was not sewn either, but generally healed nicely within 1 week. The animals withstood the operations well and none were lost. Two practice animals were kept to check survival. One had a 1 cm gash in the left cerebrum and neither had good clean operations but survived over one year.

After the experiments the animals were fixed in 10% formalin. The brains were then removed and imbedded in paraplast, sectioned, and stained with eosin-hematoxalin to examine the exact nature of the damage done in the pinealectomy.

Description of Lighting

The light source for all experiments was a bank of double fluorescent tube fixtures (4 tubes in all). Each of the two double fixtures was covered by a translucent plastic diffuser producing the spectrum shown in Figure 3. This spectrum was measured using a YST Kettering Model 65 spectroradiometer. It shows a peak intensity at 575 mu as opposed to open sunlight measured on the roof of the OSU Oceanography building (500 mu). The intensity was reduced by placing layers of gray plastic window screening inside the translucent diffuser. These acted as a neutral density filter and had no effect on the relative spectral intensities. The overall light intensity was measured with a KAHLSICO 268 WA 620 light meter and recorded in lux.

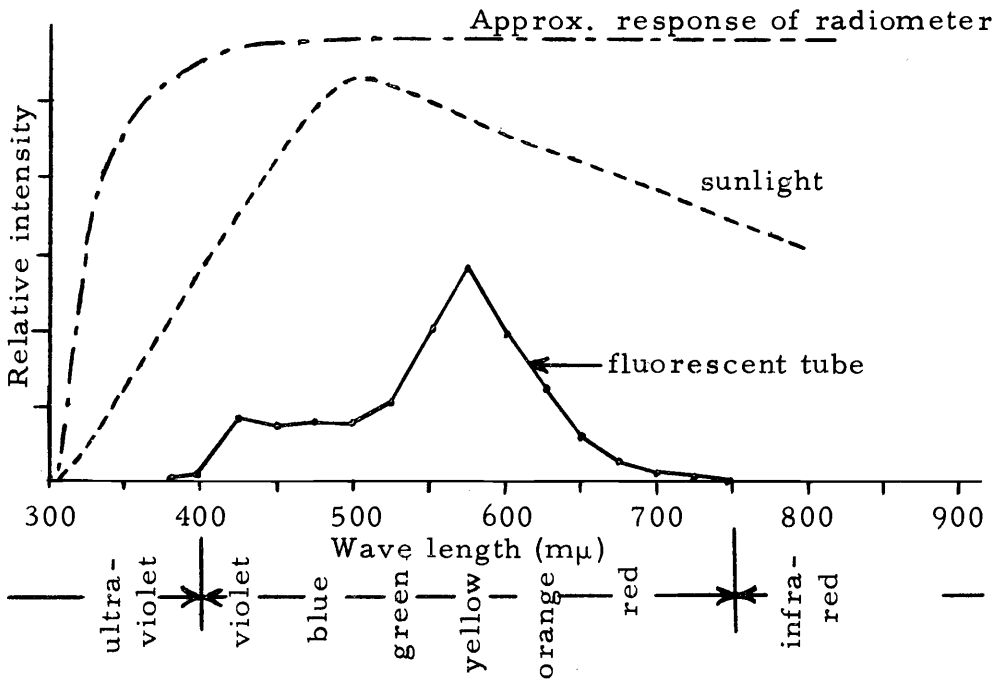


Figure 3. The lower curve shows the relative intensities of the different wavelengths of the fluorescent lights (with diffuser in place) used in these experiments. The middle line shows the relative intensities in natural sunlight and the top line is the response curve of the YSI Kettering Model 65 Spectroradiometer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

RESULTS

Characterization of the Circadian Rhythm of Locomotion

Taricha granulosa does have an inherent rhythm of activity which is close to 24 hours in period under free run conditions. The rhythm can be entrained to a light-dark cycle in the laboratory using the fluorescent lighting previously described.

The results were separated into two categories based on the time of year when the experiments were initiated. This was to check for seasonal differences in behavior that might be related to stage in the yearly breeding cycle. The results of the experiments under entrainment to light-dark cycles (LD) and free runs under constant light (LL) and constant darkness (DD) are summarized in Table 1 for the first season and Table 2 for the second. The combined results for both seasons are presented in Table 3 and Figure 4.

Entrainment was evident in 70.7% of those animals tested under various LD regimes and constant temperatures between 8 and 10°C. The 95% confidence limits for all relative frequencies presented were calculated using the normal approximation to the binomial (Cochran, 1966) and are ±13.9% for 70.7%. The two mean periods for the separate seasons are well within this range so there appears to be no seasonal variation in the ability to entrain to a light cue. The mean period length for the LD experiments is 24.01 hours. The

Table 1. Summary of the locomotor activity of T. granulosa with eyes from late summer through early winter (August 1 through January 31). Each experiment lists the period of the rhythm separately for activities in type A and type B chambers.

	Exper. Number	Light Conditions	Period (T) A	Period (T) B	Mean Period	No Rhythm	No Activity	Total No. Used	% with Rhythm
	2 & 3	8:16	24.0	None	24.35	0	0	6	100
		454 lux	24.0		SE .30				
			24.3						
			25.8						
LD			23.9						
			24.1						
	25	12:12	24.05	24.0	24.01	2	0	7	71
		151 lux		24.1	SE .05				
				23.8					
				24.1					
					24.20	2	0	13	85
					SE .16				
	29	151 lux	25.6	22.9	23.74	3	0	8	62
LL			22.2	24.2	SE .58				
			23.8						
	8	454 lux	0	None		4	0	4	0
					23.74	7	0	12	42
					SE .58				

continued

Table 1. (continued)

	Exper. Number	Light Conditions	Period (T) A	Period (T) B	Mean Period	No Rhythm	No. Activity	Total No. Used	% with Rhythm
	5	0 lux	25.1 24.9 25.6 25.1 24.7 24.8		25.03 SE .13	0	0	6	100
DD	7	0 lux	26.1 26.4 24.3 23.9		25.18 SE .63	2	0	6	67
	24	0 lux	22.9 24.2	25.9 24.6 24.4 24.1 24.6	24.39 SE .33	0	0	7	100
					24.80 SE .21	2	0	19	89

Table 2. Summary of the locomotor activity of T. granulosa with eyes from mid-winter through late summer (February 1 through July 31). Each experiment lists the period of the rhythm separately for activities in type A or type B chambers.

	Exper. Number	Light Conditions	Period (T) A	Period (T) B	Mean Period	No Rhythm	No Activity	Total No. Used	% with Rhythm
	10	11:13	23.1	None	23.60	1	0	4	75
		454 lux	24.2		SE .32				
			23.5						
	12	12:12	24.0	None	23.95	2	0	4	50
		151 lux	23.9		SE .05				
	15	18:6	23.5	None	23.85	1	0	5	80
		151 lux	24.0		SE .13				
LD			23.8						
			24.1						
	16	18:6	24.0	None	24.0	0	0	2	100
		151 lux	24.0						
	18	14:10	23.9	None	23.9	1	0	2	50
		151 lux							
	32C	6:18	24.85	24.0	24.05	1	2	9	67
		130 lux	23.85	24.0	SE .17				
			23.95	23.68					
	19	14:10		None		2	0	2	
		151 lux							
					23.91	8	2	28	64
					SE .08				

continued

Table 2. (continued)

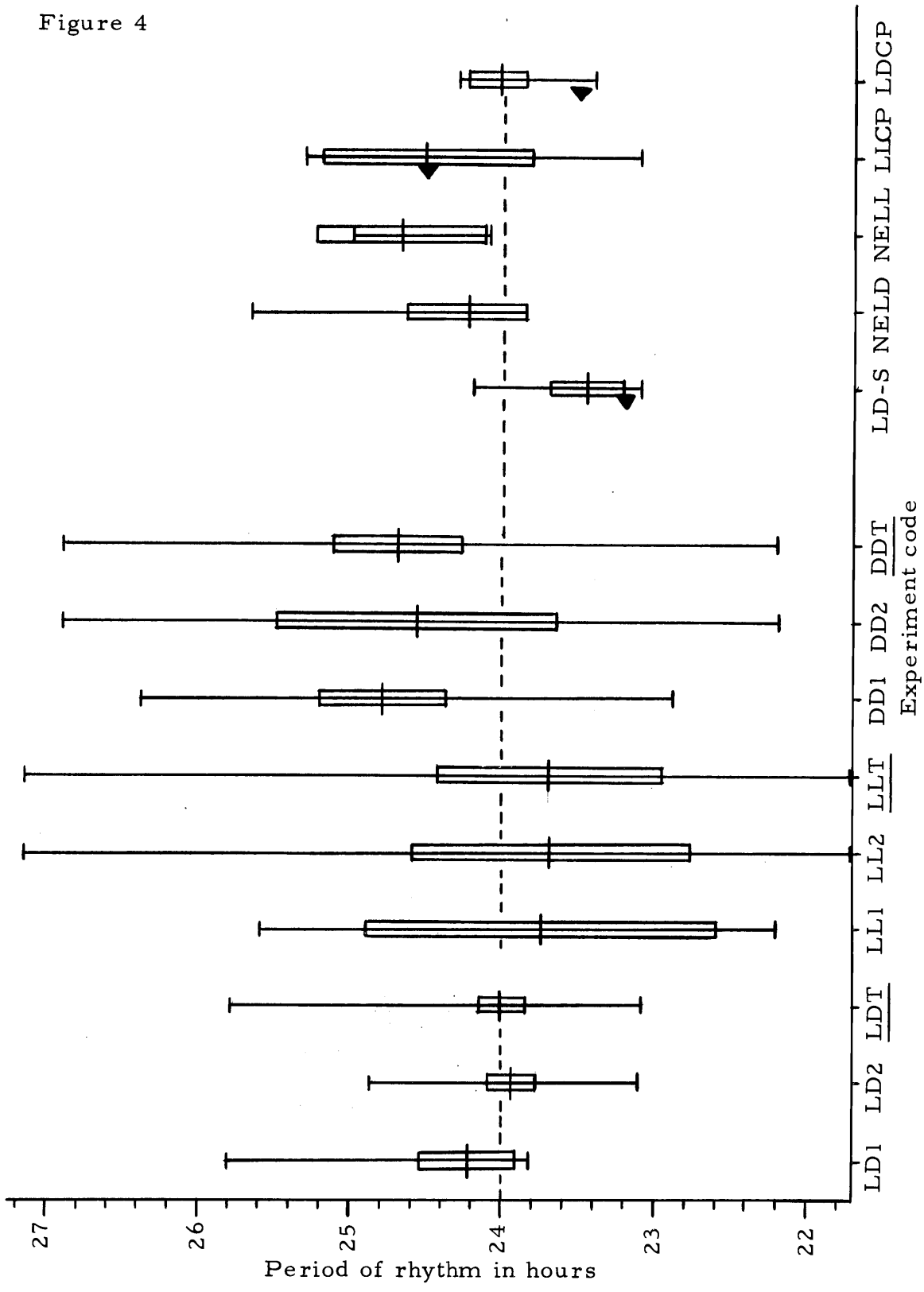
	Exper. Number	Light Conditions	Period (T) A	Period (T) B	Mean Period	No Rhythm	No Activity	Total No. Used	% with Rhythm
	17	151 lux	0	None	--	2	0	2	0
	8	454 lux	0	None	--	4	0	4	0
	11	151 lux	0	None	--	4	0	4	0
	14	151 lux	23.1	None	22.30	2	0	5	60
			22.1		SE .42				
			21.7						
LL	31A	130 lux	25.8	22.4	23.91	4	0	10	60
			26.3	24.24	SE .75				
			22.1						
			22.6						
	31D	130 lux	23.3	24.1	24.18	4	0	10	60
			27.1	23.17	SE .79				
			25.7						
			21.7						
					23.69	20	0	35	43
					SE .46				
	32A	0 lux	26.1	24.3	24.58	0	1	10	90
			24.0	24.26	SE .47				
			22.2	23.5					
DD			26.9	25.5					
				24.48					
					24.58	0	1	10	90
					SE .47				

Table 3. Summary of the locomotor activity of T. granulosa with eyes during all seasons of the year under constant temperature (10 - 12°C).

Light Cond.	Mean Period	Standard Deviation	St. Error of Mean	Coef. of Variation	No Rhythm	No Activity	Number Used	% with Rhythm	95% Conf.
LD	24.01 hr	0.45	0.08	1.874	10	2	41	70.7	±13.9%
DD	24.72 hr	1.06	0.21	4.308	2	1	29	89.6	±10.9%
LL	23.70 hr	1.64	0.37	6.898	27	0	47	42.5	±14.1%
454 lux	--	--	--	--	0	-	8	0	±0
151 lux	23.20 hr	1.29	0.46	5.55	11	-	19	42.1	±22.2%
130 lux	24.04 hr	1.80	0.52	7.49	8	-	20	60.0	±21.5%

Figure 4. Summary of the periods of the activity rhythms in T. granulosa under different light and temperature conditions. The long horizontal line indicates the mean while the box indicates two standard errors on either side of the mean. The vertical line indicates the range. Experiment codes: LD1: entrainment under 24-hour light cycles and constant temperature during early migration (Table 1); LD2: entrainment under 24-hour light cycles and constant temperature during late migration (Table 2); LDT: entrainment data from LD1 and LD2 combined; LL1: Free-run under constant light (151 or 130 lux) and constant temperature during early migration; LL2: Free-run under constant light (151 lux) and constant temperature during late migration; LLT: Free-run data from LL1 and LL2 combined; DD1: Free-run under constant darkness (0 lux) and constant temperature during late migration; DDT: Free-run data from DD1 and DD2 combined; LD-S: entrainment under a short day light cycle (23.2 hour mean) and constant temperature. Black triangle at entraining period; NE-LD: entrainment of blinded (bilateral enucleation) T. granulosa under a 24-hour light cycle and constant temperature. Data are from all seasons. NE-LL: Free-run of blinded T. granulosa under constant light (151 lux) and constant temperature during July; LL-CP: entrainment to a cold pulse of four hours duration (11^{o}:6^{o}C) with a 24-hour period under constant light. Black triangle marks the period of the cold pulse. LD-CP: entrainment of T. granulosa under two distinct zeitgeber regimes, a 24-hour light-dark cycle and a 23.28-hour cold pulse (11^{o}:6^{o}C) cycle. The black triangle marks the period of the cold pulse.

Figure 4


LD1
LD2
LDT
LL1
LL2
LLT
DD1
DD2
DDT
LD-S
NELD
NELL
LLCP
LDCP
Experiment code
27
26
25
24
23
22
Period of rhythm in hours

variability was extremely low (coefficient of variation = 1.874), though there were a few extreme periods (range = 23.1 to 25.8 hours).

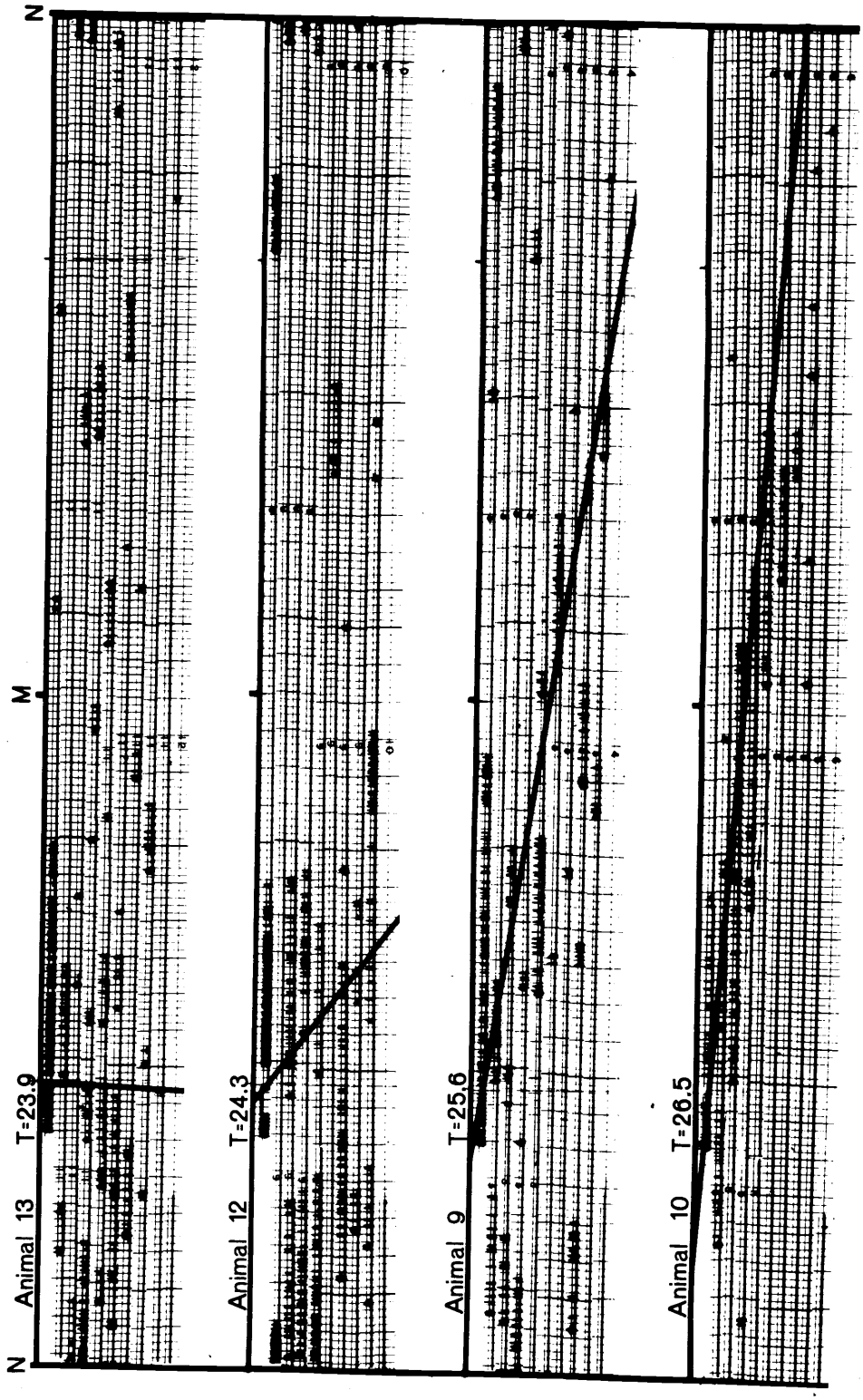
I found that a large proportion of animals showed activity under constant light but manifested no distinct rhythm. Of the 47 newts tested under LL, only 42.5% showed a distinct rhythm (Table 3). This is significantly ($p > .95$) different from both the LD and DD light regimes. The mean period of the activity rhythm of the remaining 20 newts was 23.7 hours but was highly variable (coefficient of variation = 6.898) with a large range (22.1 to 27.1 hours). If the behavior is examined by intensity (Table 3) under LL it is apparent that more animals become rhythmic as the intensity is lowered and the period tends to lengthen although there is no significant difference between the two mean periods.

Under constant darkness (DD = zero lux) the mean period was 24.72 hours (coefficient of variation = 4.308). The 95% confidence interval for the combined DD data did not overlap that of the LD experiments. Typical variability of period (T) under DD is shown in the four actograms of Figure 5.

Entrainment of the Locomotor Rhythm to Environmental Light Cycles

How is the circadian rhythm of locomotion kept in phase with environmental rhythms? What acts as the daily zeitgeber? The

Figure 5. 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 is 25.2 hours (SE = 0.6455 hours).


N
M
N
Animal 13
T=23.9
Animal 12
T=24.3
Animal 9
T=25.6
Animal 10
T=26.5

most important environmental entraining rhythms in other animals are those of light and/or temperature. The ability to entrain locomotion to light cycles under constant temperature has been discussed above. In addition, a short period entrainment experiment was performed. In experiment 32C, eleven newts were entrained to a LD photoperiod of 17.2:6, that is, a 23.2-hour day. The period during entrainment averaged 23.45 hours (Table 4).

The locomotor rhythm can also be phase shifted by manipulation of the light regime. The phase shifting of 12 hours in animal 23 in experiment 12 is presented in Figure 6. Normal entrainment to a 12:12 (151:0 lux) LD cycle 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 light-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, but the activity which did occur came during the subjective day. Starting on day 22 the light was turned out at 0600 and on at 1745 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 light-on.

In animal 23 the environmental cue is apparently light-on. In

Table 4. Summary of locomotor activity of T. granulosa under various experimental conditions. Each experiment lists the period of the rhythm separately for activities in type A and type B chambers.

Special Conditions	Season	Exper. Number	Light Condition	Period (T) A	Period (T) B	Mean Period	No Rhythm	No Activity	Total No. Used	% with Rhythm
	Dec.	26	12:12 151 lux	--	23.9	--	1	0	2	50
No eyes LD	June	16	18:6 151 lux	24.1	None	--	1	1	3	33
	July	18	14:10 151 lux	25.65 23.9	None	24.77 SE .87	1	0	3	67
	July	19	14:10 151 lux	24.7	None	--	0	0	1	
		32D	18:6	24.0 23.95 23.85	24.0	23.95 SE .04	0	2	6	67
						24.23 SE .20	3	3	15	60
No eyes LL	July	17	151 lux	25.0 24.9 24.1	None	24.67 SE .28	0	0	3	100

continued

Table 4. (continued)

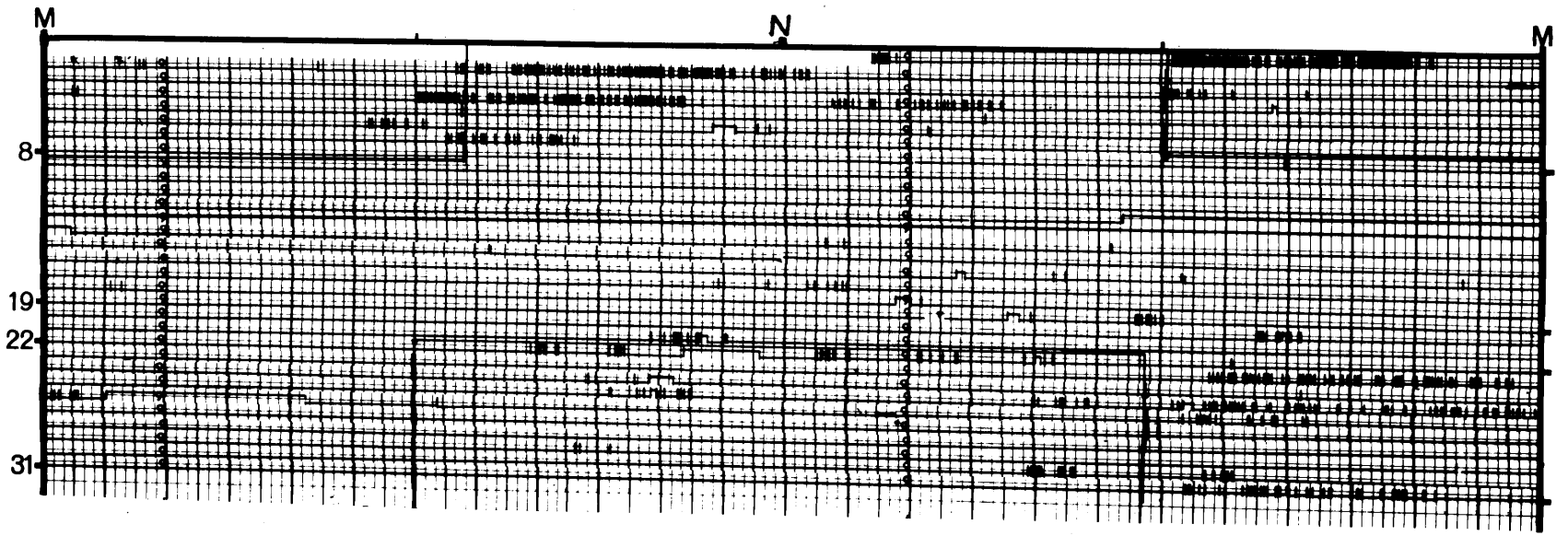
Special Conditions	Season	Exper. Number	Light Condition	Period (T) A	Period (T) B	Mean Period	No Rhythm	No Activity	Total No. Used	% with Rhythm
No eyes or pineal	July	19	14:10 151 lux	25.7	None		1	0	2	50
With eyes & black plast.	Jan.	26	12:12 151 lux	24.4 24.4	24.0 24.1	24.23 SE .10	1	0	5	80
No eyes & black plast.	Dec.	27	"	23.9	None		0	1	2	50
	Dec.	28	DD	24.8	None		0	1	2	50
With eyes & clear plast.	Dec.	27	"	24.1 25.0	24.2 24.0 24.0	24.26 SE .19	0	0	5	100
	Dec.	28	DD	24.8	None		0	1	2	50
Eyes with short day 23.2 hr = $\bar{r}$	Apr.	32B	17.2:6	23.1 24.2 23.7 23.8 23.7	23.2 23.2 23.2 23.2 23.2	23.45 SE .12	0	1	11	91
4 hrs cold (4°C) each day in LL	Feb.	31B	LL cold 4:20	23.1 25.9 24.2	24.5 25.3 24.5 24.1	24.51 SE .34	3	0	10	70
LD 20:4 & Cold 4:17.28	Mar.	31F	cold= 23.28	24.2 24.2 23.4 24.1	24.0 24.0 24.0 24.0 24.33	24.03 SE .09	1	0	10	90

Table 5. Summary of entrainment of T. granulosa to light-on and light-off cues based on peak activity as indicated by profile analysis (Appendix II). 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).

	Light-On Cue				Light-Off Cue			
Hours Since Light 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		

Figure 6. Actogram of animal 23.
Day 1 - 8: Entrainment to light-dark of 11 hours light and 13 hours dark with the lights on at 0650; LD = 11:13 @ 0650.
Day 8 - 22: Free-run under constant light (LL). T is unclear. Chamber cleaned on day 19.
Day 22 - 31: Reentrainment to LD = 12:12 @ 1800 resulting in a photoperiod reversed from the control period. T = 23.9 hours.

Animal 23



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 light-on cue. The peaks of activity were randomly distributed throughout the day when measured from the light-off cue (Table 5). The high correspondence of peak activity with the light-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 the first part of "Aschoff's Rule" which states that day-active animals show a longer circadian period under DD than when under LL, whereas the opposite is true for dark-active animals. The second part of Aschoff's Rule states that for light-active animals the period of the circadian rhythm decreases with increasing light intensity, while the opposite is true of dark active animals. My evidence (Table 3) suggests that newts show a day active response to 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 by the environmental light cycle.

Entrainment of the Locomotor Rhythm to Environmental Temperature Cycles

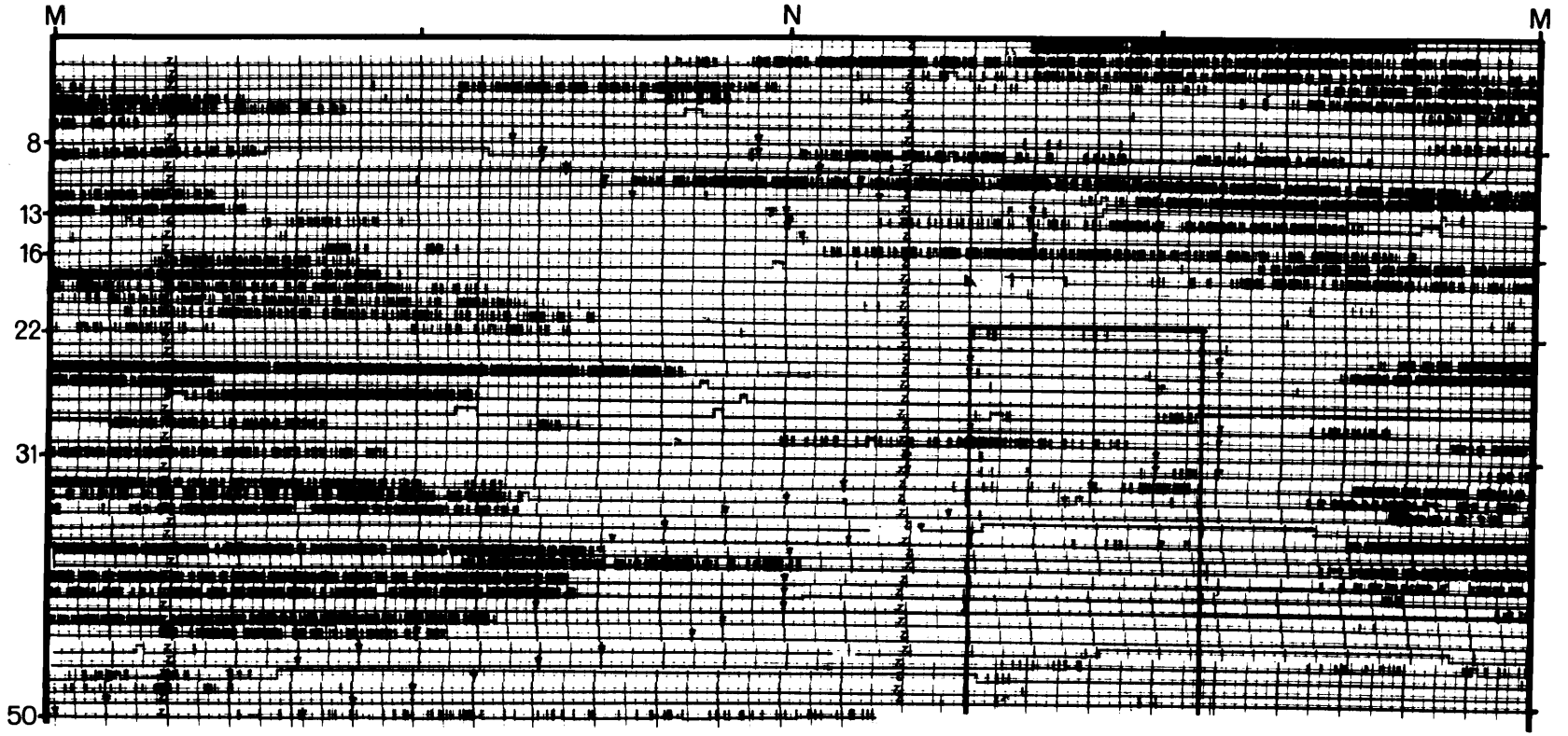
The second major environmental cue is temperature. The results of the experiments are summarized in Table 5 and Figure 4

with an actogram of animal 65 presented in Figure 7 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). The period of the rhythm was 26.3 hours; however, the mean of six animals was 23.91 hours (experiment 32A in Table 2). 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 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 on the light period but curtailed its activity as the cold pulse advanced into its normal activity time.

Figure 4 (LL-CP) shows that newts can entrain to a temperature pulse while under constant light. When given a choice between light and temperature cues (Figure 4, LD-CP), newts show an overwhelming preference for the light cue.

Figure 7. Actogram of animal 65. This is a center-pivot type chamber actogram and the periods are estimated by eye.
Day 1 - 8: Free-run under LL and constant temperature. T = 26.5 hours (approx.)
Day 8 - 13: Entrainment under LL to a 4-hour temperature cold pulse (9° to 4°C) with T of the zeitgeber equaling 24.5 hours. Cold pulse bracketed by black triangles.
Day 13 - 16: Entrainment under LL to a 4-hour temperature pulse with T = 24.0 hours and phase delayed 2.5 hours.
Day 16 - 22: Free-run under LL and constant temperature.
Day 22 - 31: Entrainment to LD = 20 : 4 @ 1500 and to a simultaneous cold pulse of 4 of the 9 days (pulse controller failed on three days).
Day 31 - 50: Entrainment to LD = 20 : 4 @ 1500 and a 5°C cold of 4 hours with a period of 23 hours. (Held at 24 hours on days 40 and 41). Activity entrained on the light pulse though activity was generally curtailed during the cold pulse as it advanced through the active period.

Animal 65



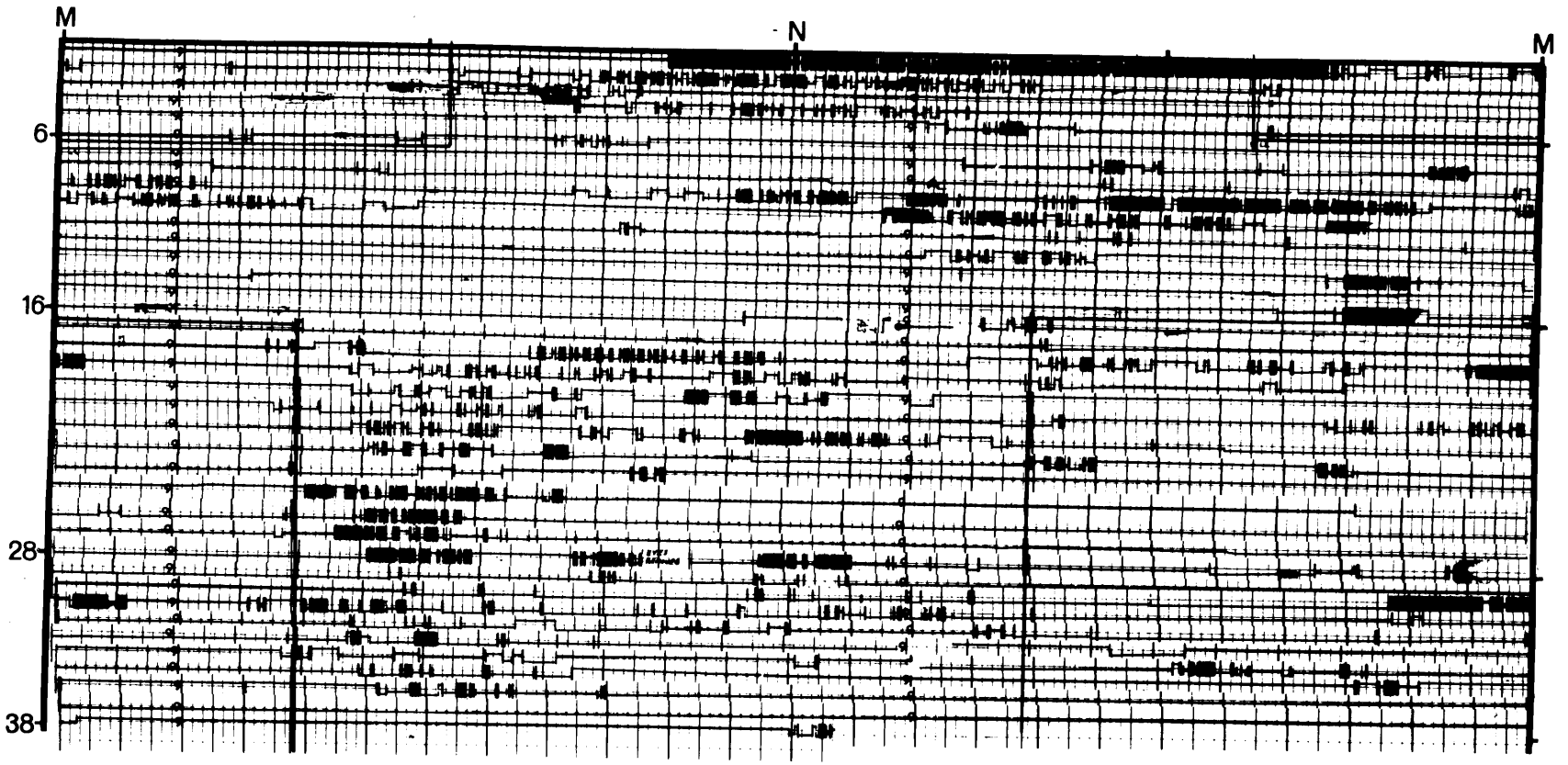
The Role of Extraoptic Photoreception in Light Entrainment

Light cycles play an important role in the entrainment of activity rhythms in the rough-skinned newt. Since extraoptic photoreception (EOP) 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 EOP in the light entrainment of activity rhythms.

Initial experiments were performed to establish that blinded (bilateral optic enucleation) animals could entrain to LD photoperiods. The results (Figure 4, NE-LD, and Table 5) confirm that retinal photoreception is not necessary for entrainment to light, since the mean period of 15 animals was 24.23 ± .4 hours. The actogram of animal 43 is an exemplification of entrainment in blinded newts (Figure 8). A 12:12 (151:0 lux) LD entrainment was initiated on day 16 after a free-run in LL. The entrainment continued until enucleation at 0940 on day 28 as a preoperative control period. The entrainment to light-on is evident and there is no apparent difference in phase or period between the control rhythm and the blinded rhythm. Only three eyeless newts were tested under LL and the results are not conclusive but the high variability of period length previously demonstrated in intact LL newts is suggested (Table 5 and Figure 4, NE-LL).

Figure 8. Actogram of animal 43.
Day 1 - 6: Entrainment under LD = 13:11 @ 0630 and constant temperature (9°C).
Day 6 - 16: Free-run under LL.
Day 16 - 28: Reentrainment under LD = 12:12 @ 0400 (phase shifted ahead 2.5 hours).
Day 28: Animal enucleated at 1030.
Day 28 - 38: Continued entrainment of eyeless animal under LD = 12:12 @ 0400.

Animal 43



The expected response in activity rhythm to enucleation would be a free-run in constant darkness (T > 24 hours). The free-run response does not occur. The experiments represented by Figures 9 through 12 were initiated with a free run in DD to act as a control period. The animals were then entrained under LD = 12:12 for the rest of the experiment. On day 18 surgery was performed. Figures 9 and 10 show the resulting activity when black plastic was implanted under the skin on the top of the head of animals with eyes.

Animal 51 responded initially by ceasing activity but by day 27 activity again began. This pattern of activity suggests light entrainment though night activity was also increased. Animal 53 did not show the post-operative reduction in activity of animal 51, however, the same pattern of basic light entrainment with increased random night activity persisted.

On day 33 the black plastic was replaced with clear plastic. Again 51 reduced activity post-operatively but both 51 and 53 show good entrainment through day 56. Under DD the periods shifted as expected.

Figures 11 and 12 resulted from the same regime as Figures 9 and 10, except that on day 18 the newts were both enucleated. Animal 52 reduced its activity until day 27 when a vague day-active rhythm became evident. Animal 54 showed an immediate vague day-active rhythm. On day 33 black plastic was inserted under the skin

Figure 9. Actogram of animal 51.
Day 1 - 9: Free-run under DD and constant temperature ($9^{o}C$). T = 24.6 hours (approx.)
Day 9 - 18: Reentrainment to LD = 12:12 @ 0745.
Day 18: At 1630 a piece of opaque black plastic was implanted under the skin on the dorsal part of the head. The eyes were left intact.
Day 18 - 33: Continue entrainment under LD = 12:12 @ 0745.
Day 33: Black plastic removed and replaced with clear plastic at 1600.
Day 33 - 56: Continue entrainment under LD = 12:12 @ 0745.
Day 56 - 64: Free-run under DD and constant temperature. T = 24.2 hours (broken line: T = 23.6 hours).

Animal 51

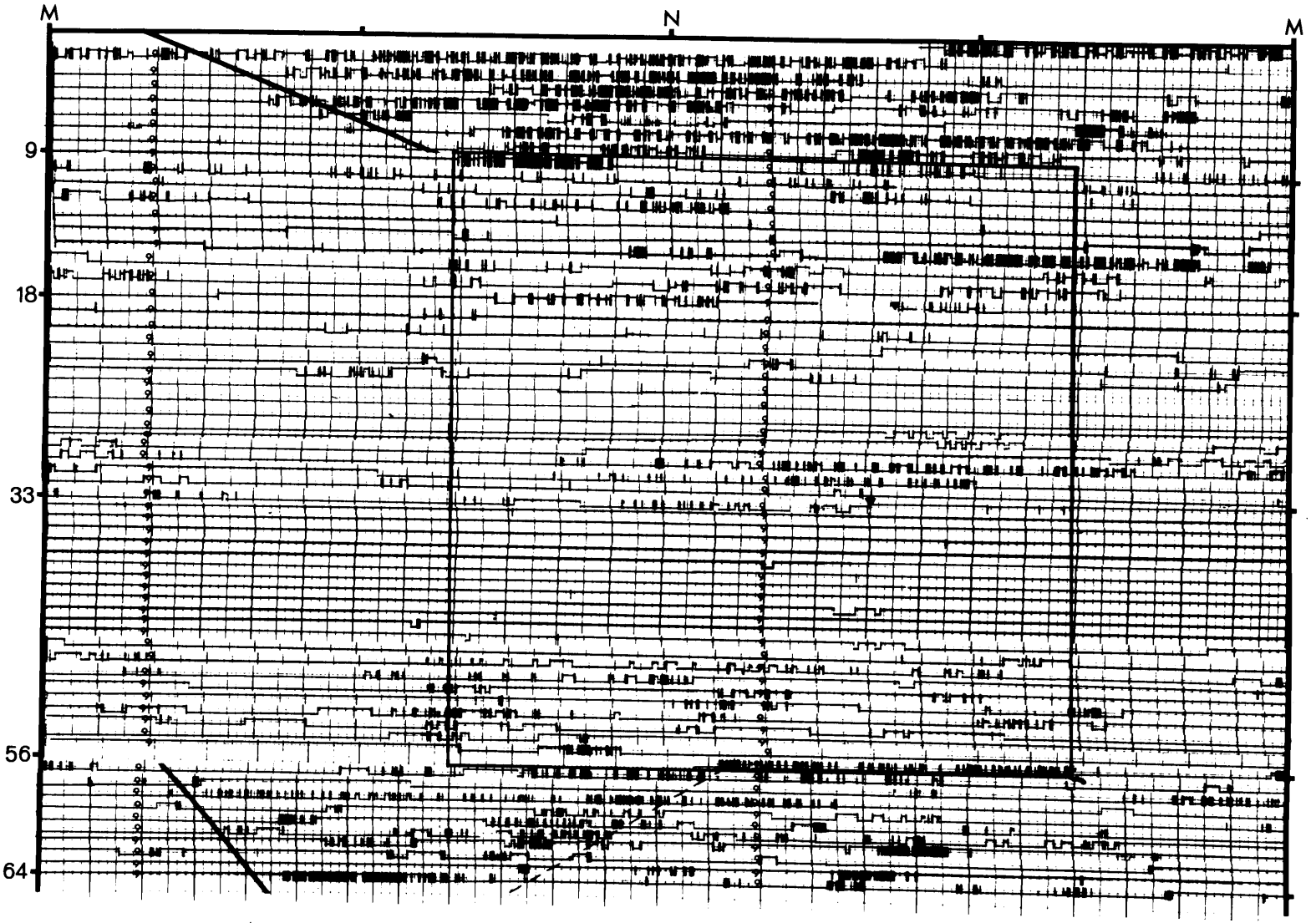


Figure 10. Actogram of animal 53 under the same experimental conditions as animal 51.
Day 1 - 9: T = 24.1 hours (approx.).
Day 56 - 64: T = 24.1 hours (approx.)

Animal 53

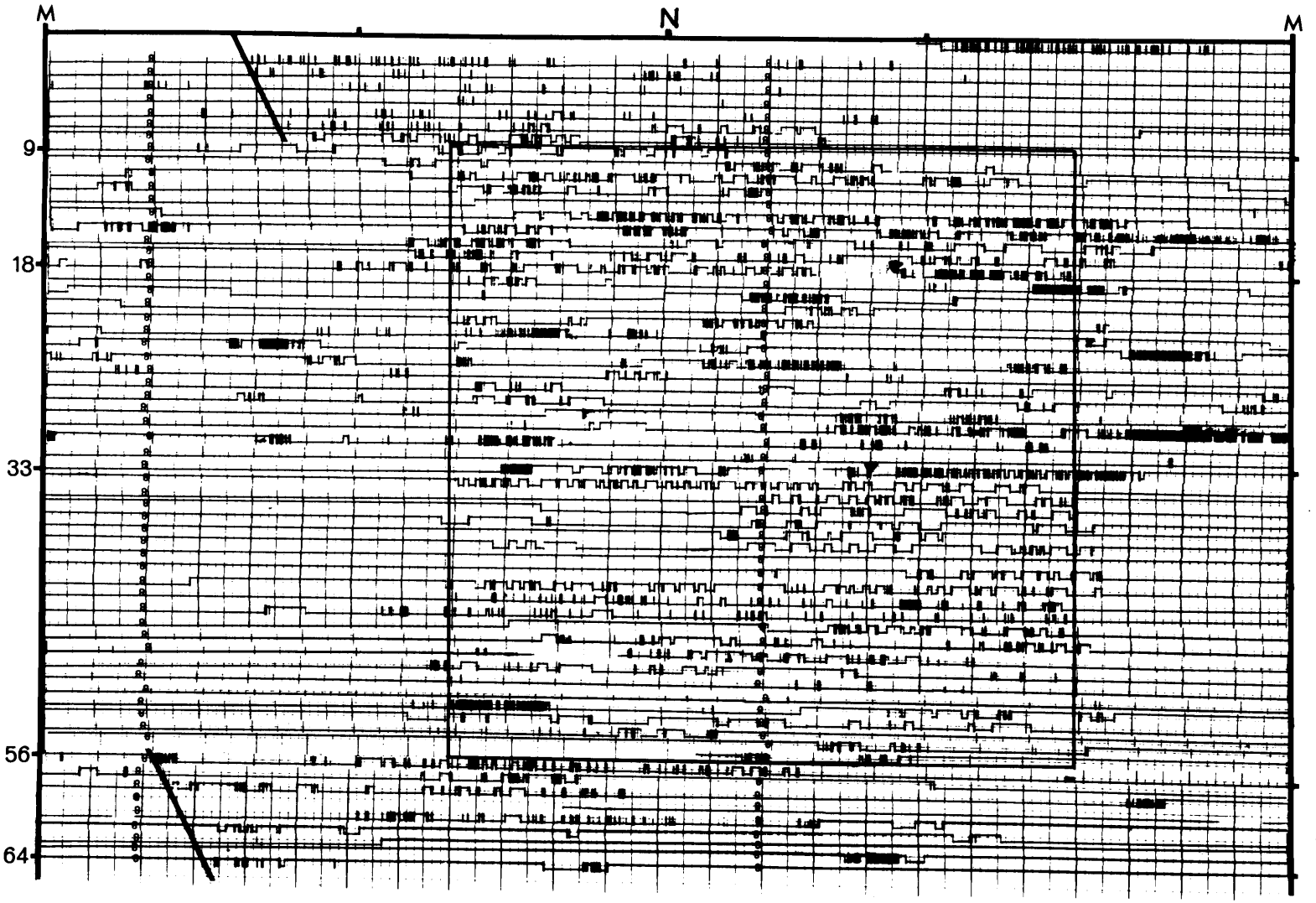


Figure 11. Actogram of animal 52 under the same conditions as animal 51 except that on Day 18 the animal was enucleated and on Day 33 opaque black plastic was implanted under the skin on the top of the head.
Day 1 - 9: T = 24.4 hours (approx.).
Day 56 - 64: Continued low level arrhythmic activity.

Animal 52

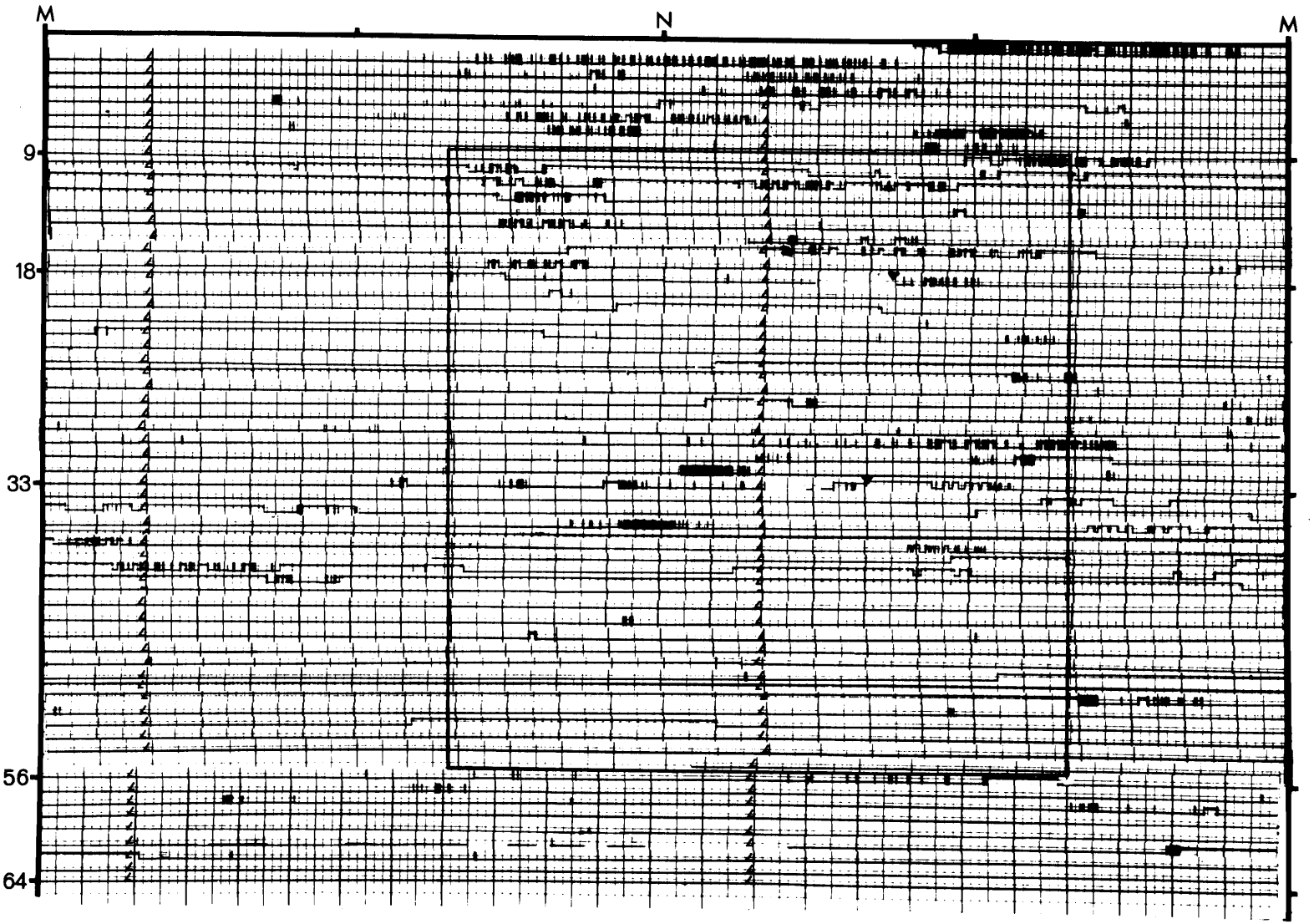
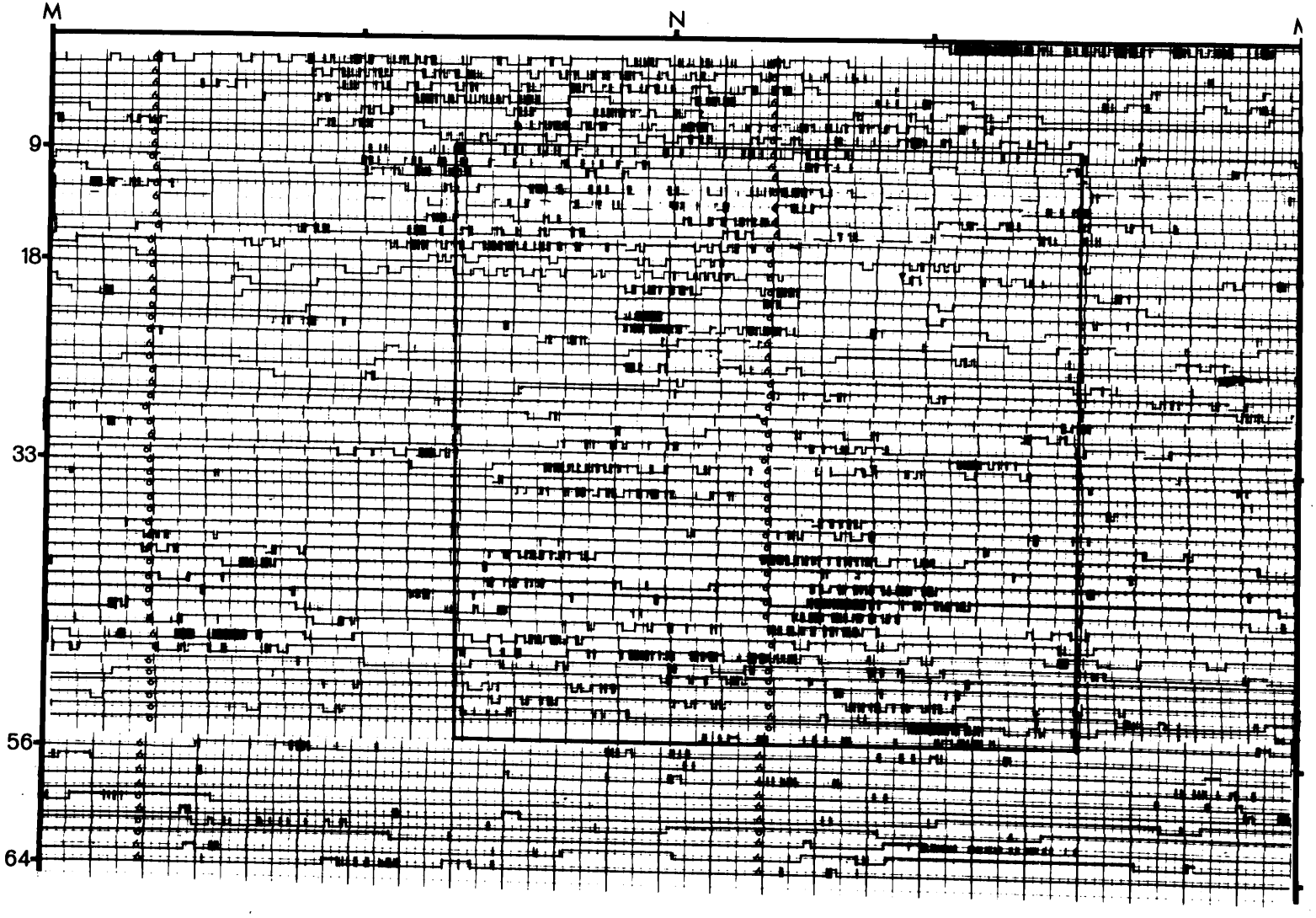


Figure 12. Actogram of animal 54 under the same experimental conditions as animal 52.
Day 1 - 9: T = 24.2 hours (approx.)
Day 56 - 65: Continued arrhythmic activity.

Animal 54

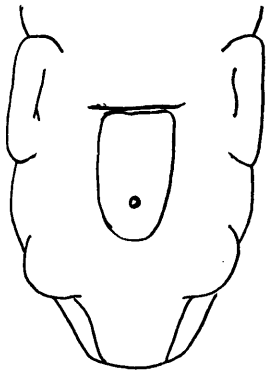


on the top of the heads of animals 52 and 54. Animal 52 showed a decrease in activity and a tendency toward arrhythmia. Animal 54 showed increased activity at night and day as well.

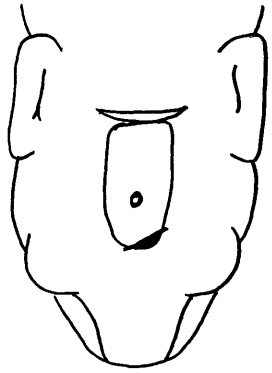
These four animals, as well as the other four used in this experiment, suggest that the EOP site for entrainment is in the dorsal part of the brain (Figure 13). This is the same general site of the EOP used in sun compass orientation. Even with eyes, newts showed decreased ability to distinguish between light and dark in locomotor rhythms when the dorsal brain was blocked from light. If, in addition, the eyes were removed, the locomotor rhythm was practically abolished.

Animals 30 and 31 (Figures 14 and 15) had their eyes removed four days prior to the start of the experiment. Both showed a 24.9-hour period under constant light. After entrainment to a 14:10 light-dark cycle a pinealectomy was performed on day 21. Animal 30 developed a good 25.7-hour rhythm with increased night activity. Animal 31 became completely inactive. Animal 34 (not figured), with eyes intact, was also pinealectomized. It showed excellent entrainment prior to pinealectomy but became inactive afterwards.

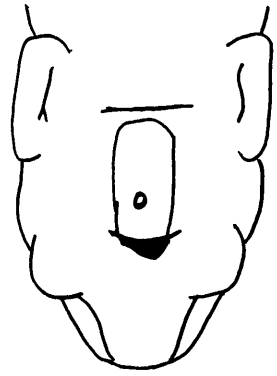
Figure 13. Drawings of the dorsal view of the heads of animals number 51, 53, 52, 54, 30, 31 showing the position of the experimental procedures relative to the pineal area (small circle in the center of the head). Animals 51 - 54 show the position and shape of the plastic inserted under the skin. Animals 30 and 31 show the position of the saw cuts.



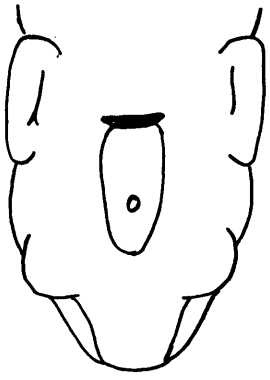
Animal 51



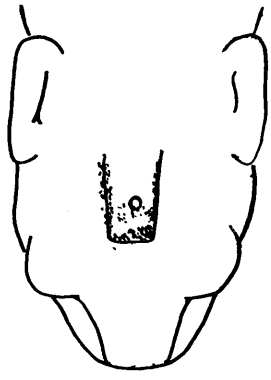
Animal 53



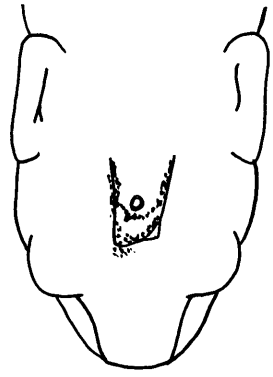
Animal 52



Animal 54



Animal 30



Animal 31

Figure 13

Figure 14. Actogram of animal 30. Animal was enucleated 4 days prior to the start of the experiment.
Day 1 - 7: Free-run under LL with T = 24.9 hours.
Day 7 - 21: Reentrainment under LD = 14:10 @ 0600.
Day 21: Animal was pinealectomized at 1530.
Day 21 - 31: Continue entrainment under LD = 14:10 @ 0600. T = 25.7 hours.

Animal 30

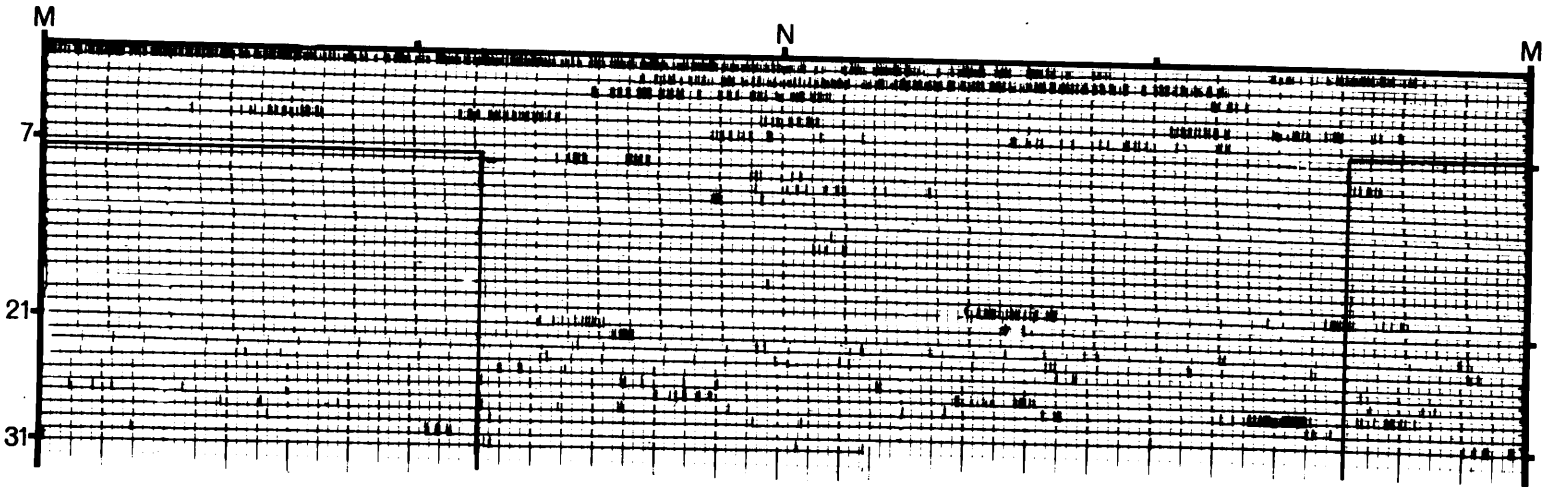
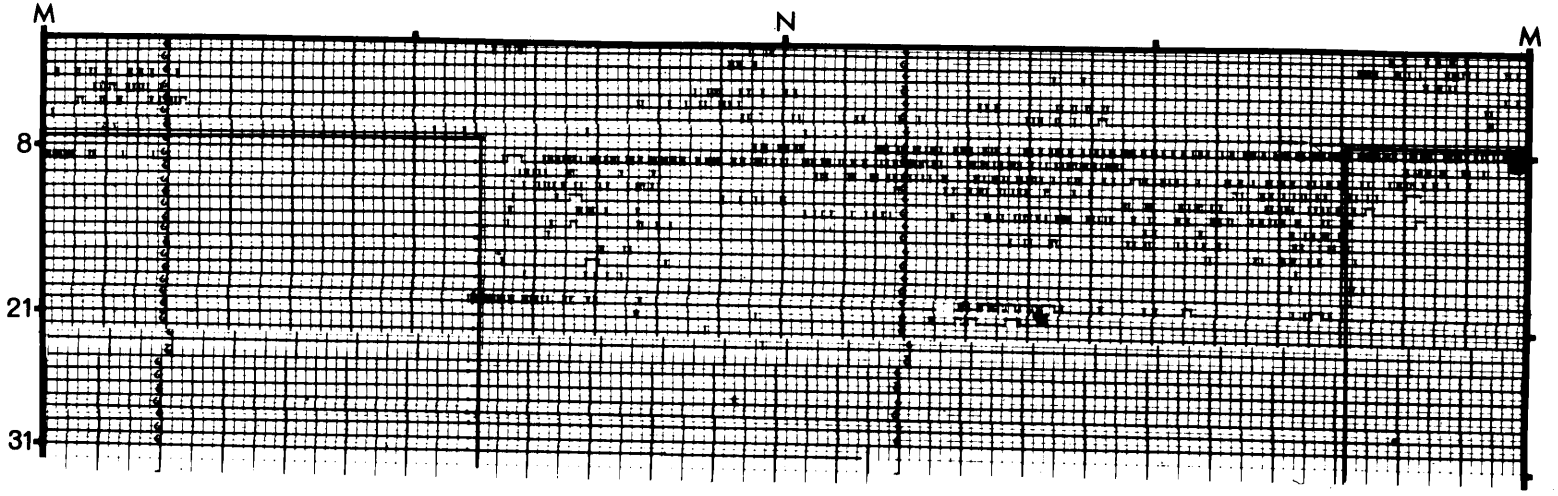


Figure 15. Actogram of animal 31 under the same experimental conditions as animal 30.
Day 1 - 7: T = 24.9 hours.
Day 21 - 31: No activity.

Animal 31



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 and returned. 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^{o} 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 aestivating newts in an aquarium stimulated surface foraging.

Migrations by definition are directed. Landreth and Ferguson (1967b) found that both intact and blinded T. granulosa could use celestial cues in orientation and that heavy cloud cover destroyed this ability. Unfortunately all of their tests were performed during the day or the night but none at twilight. There may be a resolution of this paradox of day-active animals which use celestial cues, moving at night under cloud cover. During most of the day and night cloud cover will obscure and diffuse all celestial cues. Twilight is the only time when a direction is at all clearly evident under cloud cover. I suggest that the newts cue to direction on the sunrise and sunset when clouds obscure the sky and that their mass migration activity starts when they have determined a direction. Much of the migration may occur during the day under the sun in short movements as temperature and humidity permit.

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, however, since temperature cycles and

light cycles are normally highly correlated in natural situations.

Why is there such a high percentage of arrhythmic animals and highly variable periods under bright LL conditions? 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). In the chicken (Axelrod, Wurtman and Winget, 1964), the peak melatonin activity occurred during the light period of the day as in the Japanese quail (Coturnix coturnix japonica), 3 species of African weavers (Steganura paradisaea, Euplectes ater, and Euplectes sp.) (Ralph, Hedlund and Murphy, 1968) and the pigeon (Columba livia) (Quay, 1966). This is opposite to the case in the rat and is possibly related to the fact that rats are nocturnal and these birds are diurnal.

Bagnara (1965) has shown that the nightly blanching reaction of amphibian larvae is mediated by a "pineal hormone" which he suggests is melatonin. Since his paper, Quay (1965) has demonstrated the presence of melatonin in the pineal, brain and eyes of various

amphibians. However, Wurtman and Axelrod (1967) 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 as electrophoretic mobilities of enzyme preparations from the bird, cow and frog differ (Wurtman, Axelrod and Vesell). 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. Is the pineal the "biological clock" as Axelrod and Wurtman (1965) suggest? 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 reestablish a feeding rhythm entrained to 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 and Ambystoma opacum 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 (1965:503) suggests that two systems operate in the blanching reaction of Xenopus larvae: ". . . the lack of light stimulation of the lateral eyes leads to continual release of hypophyseal chromatotropic hormone with consequent potent melanophore expansion." This, in conjunction with the

following system accounts for the fact that if the larvae are left in the dark, the blanching reaction is lost and the animals become very dark.

> In the absence of light, through the action of photoreceptors present in either or in both the frontal organ and pineal organ, the pineal is stimulated to release a direct acting melanophore contracting principle, probably melatonin, which quickly causes blanching of larvae. Upon resumption of illumination, release of the melanophore contracting agent ceases, and, gradually, normal metabolic processes reduce the effective quantity of circulating hormone to a level insufficient for melanophore stimulation. As a result, melanophores re-expand because of unchanged hypophyseal influences, and the larva takes on a normal pigmentary state. The blanching reaction is not restricted to Xenopus, it seems to be a general response among amphibian larvae" (Bagnara, op. cit.).

Again the question arises, why do rats, 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 hormone-like factors. The simple model presented (Figure 16), 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 probably a first activity factor which is under the direct exogenous control of light. The production and/or release of this 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

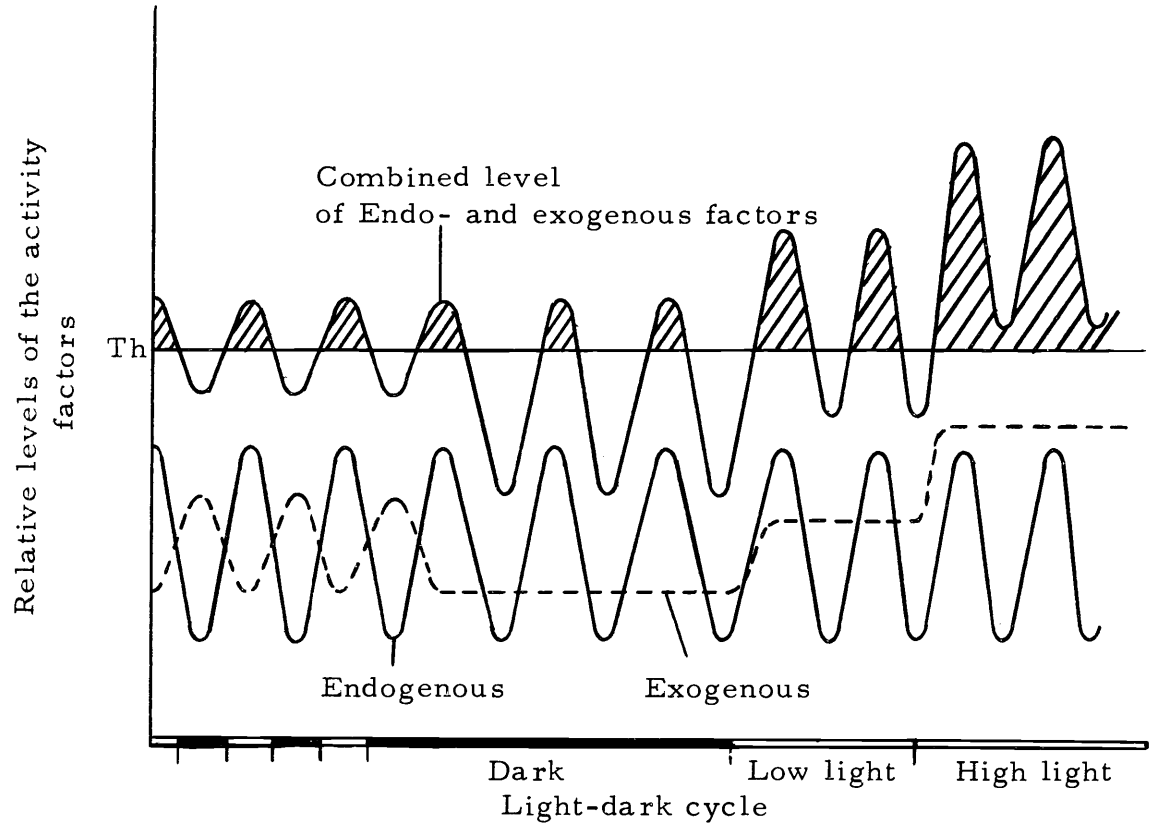


Figure 16. A synergistic model for circadian activity rhythms. This particular example is the simplest hypothetical interactions of hormones that will account for the results observed and is configured for a day-active species. Only two hormone-like factors are used in this example while the actual number 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.

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.

Extraoptic entrainment has been clearly demonstrated in Plethodon glutinosus (Adler, 1969a) and Rana clamitans (Adler, 1969b). Special techniques were used to analyze the data of P. glutinosus since they loose their rhythm rapidly under free-run conditions. Similarly, I have demonstrated that eyes are not necessary for entrainment in the diurnal newt, Taricha granulosa. The exact location of the extraoptic photoreceptor has not been determined for

either orientation or locomotor entrainment. The pineal has been suggested but the size and shape and location make it difficult to remove successfully without disturbing the surrounding areas or rupturing the ventricle. Neither can the optic tectum be easily removed without damage, since it is the roof of the optic lobe. It was therefore not possible to properly remove suspected sites of extraoptic reception.

Microscopic examination of brain serial sections of animal 30 (Figure 14) showed that the habenula was ruptured and there were numerous red blood cells free in the ventrical. Though the damage was general in the area of the habenula and subcommisural organ, the animal showed a distinct rhythm of 25.7 hours. A control animal (34) with eyes intact had a brain condition similar to that of animal 30, however, it became inactive after the operation. Animal 31 (Figure 15) also became inactive after the operation but its brain was in very good condition. The pineal area, however, was a mass of connective tissue, pigment cells and blood cells. The rhythm in animal 30 suggests that areas other than the pineal are involved in photoreception.

The plastic implant experiments definitely suggest that the dorsal brain is involved. Covering the brain with plastic increased activity during the night but a day active rhythm was suggested. Even with eyes removed, an animal with black plastic could still tell when

the lights were on though the ability was reduced. The light reaching the receptor was probably attenuated as it passed through the head from the lateral and ventral surfaces. The presence of sensory cells with retina-like outer segments in the pineal suggests that the pineal is photoreceptive but other areas, such as the optic tectum, can not be ruled out.

Of interest in this respect are the results of Schneider and Pietsch (1968) in their experiments with Ambystoma punctatum larvae. They used five groups of larvae which had undergone eye transplants and/or enucleation: (1) minus--left natural eye removed; (2) triclops--both natural eyes plus a supernumerary eye on the dorsal midline of the head; (3) cyclops--a transplanted eye in the same location as the triclops but lacking both natural eyes; (4) eyeless; and (5) normal. The host region for the transplanted eyes in the triclops and cyclops was the epiphyseal portion of the diencephalon.

The animals were trained in an avoidance paradigm with light as the conditioned stimulus and a mild electric shock as the unconditioned stimulus. The object was to "determine what effect additional or reduced visual input had on initial learning and the maintainance of a learned response. The level of learning achieved increased in an apparent one-to-one fashion in line with the number of eyes the animal possessed. However, the results obtained from

animals possessing only a supernumerary eye suggested that constraints were imposed centrally on the supernumerary eye of a three-eyed animal" (p. 280). The eye of the cyclops had the effect of four additional natural eyes. They do not attempt to explain why an eye positioned in the vicinity of the diencephalon with nervous connections to the area of the epiphysis is more efficient than one positioned lateral to the diencephalon. Perhaps the lateral eyes and associated sight centers are specialized to detect images of environmental factors such as food, mates, predators, etc. while extraoptic photoreception operates in other light intensity and duration related phenomena. With no central nervous system constraints in the absence of lateral eyes, the supernumerary eye may serve as a retinal receptor for the dorsal diencephalon photoreceptive site increasing its efficiency by at least four times.

It is apparent that extraoptic photoreception is important in rhythms and orientation in amphibians. The site and relative importance of the extraoptic photoreceptor in amphibian rhythms must await the development of better microsurgery techniques and microassay methods for rhythm related hormones.

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APPENDIX I

```
      PROGRAM PERIOD
      COMMON X(2000),P(160,75),SUMAV(200),SUMSQ(200),
     1AMPL(200),Z(5)
10    READ (60,750) N,Z
      IF(EOF(60)) CALL EXIT
750   FORMAT (I5,5A8)
      WRITE (61,754) N,Z
754   FORMAT ('1',I5,' DATA POINTS. ',5A8)
      READ (60,751) IEXP,IANIM,(X(I),I=1,24)
751   FORMAT (2A3,2X,24F3.0)
      WRITE (61,753) IEXP,IANIM
753   FORMAT (' EXPERMENT NO.',A5,' ANIMAL NO. ',A5)
      I=25
11    JJ=I+23
      READ (60,755) (X(J),J=I,JJ)
755   FORMAT (8X,24F3.0)
      I=JJ+1
      IF (.NOT. EOF(60)) GO TO 11
      ILIM=101
      ICOR=179
      JLIM=56
      M=5
      L=M*N
      DO 800 I=1,ILIM
      I1=I+ICOR
      JMAX=I1/M
      SUMAV(I)=0.
      SUMSQ(I)=0.
      DO 790 J=1,JMAX
      P(I,J)=0.
      J1=M*J
      KMAX=(L-J1+1)/I1+1
      DO 780 K=1,KMAX
      INDEX=(J1+(K-1)*I1-1)/M+1
780   P(I,J)=P(I,J)+X(INDEX)/FLOAT(KMAX)
      SUMAV(I)=SUMAV(I)+P(I,J)
790   SUMSQ(I)=SUMSQ(I)+P(I,J)**2
800   AMPL(I)=SQRT((SUMSQ(I)-SUMAV(I)**2/
     1FLOAT(JMAX))/FLOAT(JMAX))
      WRITE(61,752) (AMPL(I),I=1,ILIM)
752   FORMAT(10F7.2)
      GO TO 10
      END
```

APPENDIX II

```
      PROGRAM PROFILE
      DIMENSION X(1000),SUM(80),Z(10)
750   FORMAT(I5,10A4,I2,I3)
751   FORMAT(2A3,2X,24F3.0)
752   FORMAT(1H ,6X,' HOUR',6X,'REL. FREQ.')
753   FORMAT(1H ,'EXP NO',A5,'ANIM NO',A5,' PERIOD=',
     1 'F7.3,HOURS---OVER',I3,' PERIODS')
754   FORMAT(1H1,I5,' DATA POINTS',10A4,'START AT',I2)
755   FORMAT(8X,24F3.0)
759   FORMAT(1H ,5X,F7.2,2X,F9.6)
1     READ(60,750)N,Z,ISTART,LIM
      IF(EOFCKF(60).EQ.1) GO TO 99
      WRITE(61,754)N,Z,ISTART
      NN=N/LIM
      TIM=ILIM=LIM
      TIMM=TIM/2.
      READ(60,751)IEXP,IANIM,(X(I),I=1,24)
      WRITE(61,753) IEXP,IANIM,TIMM,NN
      WRITE(61,752)
      I=25
10    MM=I+23
      READ(60,755) (X(LL),LL=I,MM)
      I=MM+1
      IF(EOFCKF(60)-2)11,10,10
11    II=LL=1
      DO 12 IG=1,80
      SUM(IG)=0
12    CONTINUE
      GT=0
      DO 25 LC=1,NN
      M=0
      DO 23 K=II,ILIM
      M=M+1
      SUM(M)=SUM(M)+X(K)
      GT=GT+X(K)
23    CONTINUE
      II=ILIM+1
      ILIM=ILIM+LIM
25    CONTINUE
      DO 35 M=1,LIM
      RF=SUM(M)/GT
      C=M
```

```
      C=C/2
      WRITE(61,759)C,RF
35    CONTINUE
      WRITE(61,760)GT
760   FORMAT(1H-,'TOTAL ACTIVITY UNITS=',F10.0)
      GO TO 1
99    END
```