Table 2. Approximate concentrations of arsenic in wash water. The results were calculated from the concentration of arsenic in dry detergent and the manufacturer's directions, assuming no arsenic from any other source than the stated detergent. All values are given in parts per billion.

Detergent	Container			
	10-gallon washer	30-gallon washer	60-gallon washer	1-gallon presoak basin
A	50–60	20	10	130–150
B	30–40	30	20	
C	10	10	5	
D	20	20	10	
E	50	40	20–30	
F	10	2–4	1–2	20–30
G*	2.5	1.5	0.5–1.0	
H	70–100	25–35	10–20	170–250

* Lower limits of detection.

Table 3. Arsenic concentration (ppb) in water in Lawrence, Kansas. The U.S. Public Health Service mandatory maximum is 50 ppb; the recommended maximum is 10 ppb.

Sample	Average	Range
Input Lawrence water plant	3.1	2.6–3.6
Lawrence tap water*	0.4	0.4–0.5
Raw sewage (Lawrence plant) input	2.7	2.0–3.4
Treated sewage (Lawrence plant) output to river	1.8	1.5–2.1
Kansas River at Lawrence	3.3†	
Kansas River at Topeka‡	8.0	

* Lawrence water treatment includes "cold-lime softening"; the value of 0.4 ppb is at the lower limit of detection. † Single determination. ‡ (8).

the seven samples studied were sent to four independent laboratories, and arsenic analyses were requested. While the results were not duplicated exactly, the general pattern of findings was not altered.

For an internal check on our method, several samples were run a sufficient number of times to obtain an estimate of the coefficient of variation, which we determined by the range method (*7*). The precision of samples —individually sampled and wet ashed —was within that given for the silver-diethyldithiocarbamate method for intralaboratory results (*1*). Even with variations in sampling and ashing, the precision was well within that given for interlaboratory results by Standard Methods (*1*). There was also good agreement between the silver-diethyldithiocarbamate and emission-spectrography data.

The treatment processes now used in many sewage or waste effluent plants do not remove the arsenic. However, two common water-treatment methods were tested on a laboratory scale. At an initial arsenic concentration of 200 ppb, these limited tests indicate an arsenic removal factor of 85 percent for cold-lime softening treatment and 70 percent for charcoal filtration treatment. Nevertheless, in river systems in which the water is heavily reused (for example, the Ohio River), a potential danger does exist and warrants further study. The present concentration of 2 to 8 ppb of arsenic in one river system is too close to the recommended 10 ppb.

E. E. ANGINO
State Geological Survey of Kansas and
Department of Civil Engineering,
University of Kansas, Lawrence 66044
L. M. MAGNUSON, T. C. WAUGH
O. K. GALLE, J. BREDFELDT
State Geological Survey of Kansas,
University of Kansas

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Extraoptic Celestial Orientation in the Southern Cricket Frog Acris gryllus

Abstract. *Celestial orientation and setting of the biological clock in the southern cricket frog* Acris gryllus *can be cued by light stimuli received by extraoptic receptors in the brain. These extraoptic photoreceptors may also be used in learning new orientational directions. A mechanism for a light-activated biological clock is discussed.*

Frogs and salamanders can use celestial cues to orient correctly on a compass course (*1*). Such compass-course orientation require a learned component, that is, a particular shoreline, and a celestial cue in conjunction with a timing mechanism phased to local time. Several workers have shown that blinded amphibians may continue to exhibit normal motor and physiological responses to light cues. Blinded newts *Taricha rivularis* are able to home over relatively great distances (*2*). Although the mechanism by which these blind animals homed was attributed to olfactory cues, it has since been shown that rough-skinned newts *Taricha granulosa* can home by use of extraoptic photoreceptors (EOP) (*1*). In situations which precluded the use of olfactory cues, blinded *T. granulosa* continued to orient correctly on a compass course. Eyeless slimy salamanders *Plethodon glutinosus* are able to phase-shift circadian locomotor rhythms in response to changes in light-dark (LD) cycles (*3*).

These and other experiments have shown that light reception in a number of animals may be mediated by EOP as well as with the eyes (*4, 5*). The ability of salamanders to home and orient correctly and to shift circadian locomotor rhythms in response to LD cycle changes indicates that EOP-mediated cues can be used as direction-finding mechanisms and to reset the biological clock.

The site of EOP has been a point of interest for some time. Although a dermal light sense has been shown in all the major metazoan phyla, its function appears to be primarily involved with phototactic responses (*5*). Recently the pineal complex (pineal-parietal organs in reptiles and fish; pineal-frontal organs in frogs and toads) has received increasing attention as the site of EOP in lower vertebrates. Cytological and electron micrograph studies of amphibian pineal complexes have shown both photoreceptive and secretory cells in the pineal and frontal organs (*6*). Workers have also shown that both the frontal organ and the pineal stalk are photosensitive (*7*).

Here we report that blinded southern cricket frogs *Acris gryllus* can use EOP-mediated photic cues to respond to changes in LD cycles, to orient on a correct compass course, and to learn a new shoreline (*y*-axis). This last point is of singular importance since learning involves complex neural relationships which may or may not be present in simple responses to light cues.

Five groups of frogs were used in each of the three different phases of this study. Group 1, normal vision and a transverse incision made in the skin of the dorsal surface of the head 3 to 5 mm posterior to the eyes; group 2, normal vision and a sheet of opaque Teflon inserted underneath the skin of the head, covering the pineal and brain; group 3, blinded with a clear sheet of plastic inserted under the skin so as to cover the pineal and brain; group 4, only the eyes removed; and group 5, blinded with a sheet of opaque Teflon inserted under the skin so as to cover the pineal and brain. All tests were made in a vinyl aquatic arena (3 m in diameter) on the roof of a four-story building from which only the sky was visible. Ten frogs were tested at a time.

To determine the relationship of EOP to maintenance of the biological clock, we placed frogs representing the five groups, from a known shoreline, in an environmental chamber for 7 days under constant temperature (80°F) and humidity (77 percent). The LD cycle was advanced 6 hours, compared with ambient, thus leading to an approximate 90 degree counterclockwise shift in the directional responses of those frogs able to perceive the new LD regime. Frogs from all groups except group 5 (blinded—EOP covered with opaque Teflon) exhibited such a shift in orientation (Fig. 1, *a–d*) when tested in the aquatic arena; group 5 frogs scored randomly Fig. (1*e*). The responses in groups 2 and 3 reflect the relationship of EOP and optically perceived photic cues in controlling the orientational mechanism in *A. gryllus*. Note that frogs in group 2 (normal vision—EOP covered with opaque Teflon) and group 3 (blinded—EOP covered with clear plastic) responded similarly to the new LD regime.

These data suggest that incoming photic cues, whether received by the eyes or by EOP, are capable of resetting the biological clock in *A. gryllus*. The experiments, however, do not preclude the possibility that group 5 frogs might have become out of phase with local time and hence scored incorrectly. If frogs from a known shoreline were collected, operated on, then tested the same day, the chance of their becoming out of phase would be minimized. Further, a random score from group 5 frogs and correct scoring in the remaining groups when tested the same day would strongly implicate reception of photic cues by EOP in orientational responses. Such a test was performed and only frogs from group 5 (blinded—EOP covered with opaque Teflon) scored randomly (Fig. 1, *f–j*). The similar responses shown by groups 2 and 3 indicate that light information received either through the eyes or by EOP sites can be used to orient correctly on a compass course. The distance of the test arena from the collection area, wind movement within the arena (*1*), and the fact that group 5 frogs scored randomly appear to rule out the use of olfactory cues in

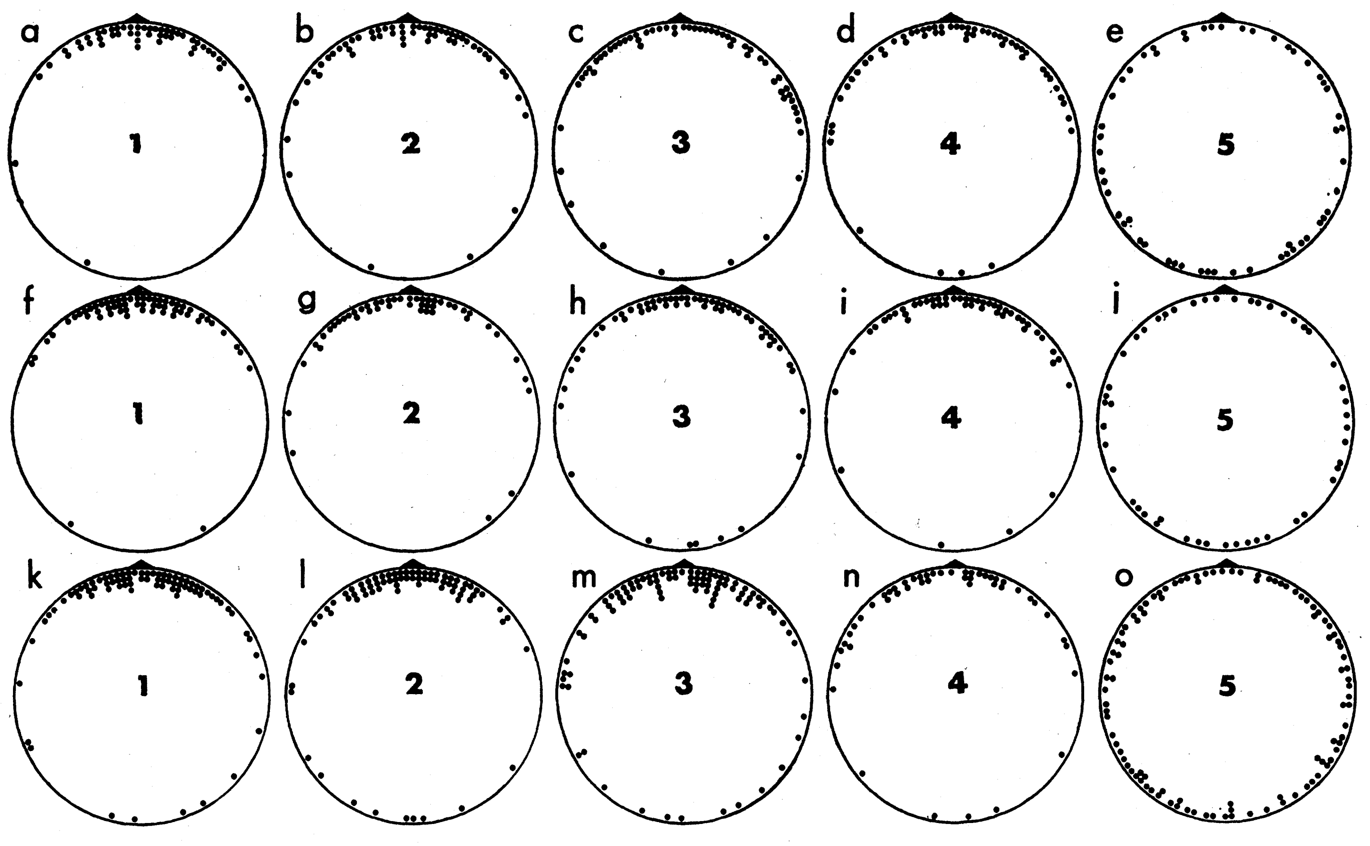

Fig. 1. Directional responses of southern cricket frogs; pointer refers to expected direction; all *y*-axes have been rotated to a common direction; black dots represent positions of frogs after testing; numbers indicate test groups. Tests *a* to *e*, frogs subjected to a 6-hour advance in LD cycle. Tests *f* to *j*, frogs collected, operated on, and tested the same day. Tests *k* to *o*, frogs which had been operated on and trained to an artificial shoreline. Group 1, normal vision, EOP site unblocked; group 2, normal vision, EOP site covered with opaque Teflon; group 3, blinded, EOP site covered with clear plastic; group 4, blind, EOP site unblocked; group 5 blinded, EOP site covered with opaque Teflon.

sun-compass orientation in *Acris gryllus*.

To further assess the significance of cues received by EOP and those received for pattern vision and to study possible relationships between the two with respect to learning, we designed the following test. Frogs representing the five groups were taken from a known shoreline and placed in aquatic training pens. These pens were on the roof of a four-story building, and each had an artificial shoreline rotated 90 degrees relative to the site of collection. After 7 days the frogs were removed and tested in the previously described aquatic arena. Group 5 frogs scored randomly and thus apparently did not learn the new land-water relationship (Fig. 1*o*). The other four groups responded correctly to the new *y*-axis (Fig. 1, *k–n*). These data show the similarity of orientational responses of frogs receiving photic cues from two separate receptor sites, that is, eyes (Fig. 1*l*) and EOP (Fig. 1*m*) and agree with implications of regions of the dorsal diencephalon and associated structures as the EOP "organ" (*3*, *7*). That a new *y*-axis can be learned by cues from either site suggests that such cues are being channeled into common areas for biological clock entrainment and correlation for interpretation and action.

In amphibians the diencephalon is a transitional sector of the brain interpolated between the olfactory field anteriorly and all other sensorimotor fields posteriorly, and combines functions of the sensory, intermediate, and motor zones (*8*). The pineal organ, which can act as a photoreceptor or can be influenced by some other photoreceptive structure (*3*, *9*), develops as an evagination of the dorsal wall of the diencephalon and remains attached to it by the pineal peduncle (*8*, *10*). Research in birds and mammals has implicated the pineal in the control of biological clocks and testicular recrudescence (*11*). Although the mechanism of stimulation may differ (*11*, *12*), control seems to be exerted through the cyclic production of melatonin (5-methoxy-*N*-acetyltryptamine) which is synthesized from the *O*-methylation of *N*-acetylserotonin by acetylserotonin methyltransferase (E.C.2.1.1.4) in response to light (*11*). This enzyme system has been found in all classes of vertebrates (*11*, *13*) and a control function is indicated in lower vertebrates, since the blanching response of amphibians which have been kept in constant darkness can be alleviated by administration of melatonin or prevented by pinealectomy (*6*, *14*).

Additional evidence for an oscillating enzyme system in amphibians similar to that of birds and mammals was found by Adler (*15*)—removal of the frontal organ in blind green frogs *Rana clamitans* led to the loss of their ability to make circadian locomotor rhythm changes in response to varied LD cycles. This result was probably due to nonactivation of either the pineal or another part of the diencephalon by photic cues from EOP sites in the frontal organ. His data indicate that the frontal organ in *R. clamitans* acts as an EOP site, at least insofar as entraining circadian locomotor rhythms.

Our data demonstrate that there are extraretinal areas in the brain of *Acris gryllus* capable of receiving light for entrainment of biological clocks but also capable of receiving directional information to be used for orientation.

DOUGLAS H. TAYLOR
DENZEL E. FERGUSON
Department of Zoology, Mississippi State University, State College 39762

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Incubation Effects in Behavior Induction in Rats

Abstract. *Incubation (rest) periods interposed during donor training regimens significantly enhance the "memory transfer" effect reported by some investigators. When extracts from the brains of donor rats given interpolated rest during acquisition training were injected into recipient animals, statistically reliable and experimentally reproducible "memory transfer" effects were found.*

In 1961 and 1962, the Planarian Research Group in this laboratory reported that if trained flatworms were fed to untrained cannibalistic flatworms, the cannibal planarians appeared to acquire part of the training by ingestion (*1*). In 1965, investigators in four other laboratories (*2*) reported transfer of experiential information from trained to untrained mammals by injecting untrained animals with material extracted from the brains of trained animals. These successful reports of "memory transfer" were followed by a series of negative reports (*3*) that did not substantiate the validity of the effect. Since that time, experiments in more than 23 laboratories would seem to suggest that an effect of some sort does exist in mammals (*4*). Yet, in light of the apparent difficulty in replicating these experiments in some laboratories, it appears that the basic phenomenon may be highly elusive. In the present experiments we attempted to define more adequately one of the important factors—the strength of donor training—which we believe must be controlled for the effect to occur.

We used the same basic paradigm that was first reported by Dyal and his associates (*5*) and replicated successfully in our laboratory (*6*). In these experiments, training of the donor animals takes place in an operant chamber (Skinner box) in which the animal is trained to press a lever or bar in order to obtain a reward (reinforcement) of a food pellet. In the Dyal experi-