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Extraretinal Light Perception: Entrainment of the Biological Clock Controlling Lizard Locomotor Activity

Abstract. *The circadian activity rhythm of the iguanid lizard* Sceloporus olivaceus *can be entrained by light cycles whether or not the animals have eyes. Removal of the pineal organ and parietal eye in blinded lizards does not prevent entrainment. Our data demonstrate the existence of an extraretinal photoreceptor which can mediate entrainment of a biological clock in reptiles.*

In the absence of any external cues, activity rhythms of many vertebrates exhibit periodicities which are approximately, but rarely exactly, 24 hours. Such circadian rhythms reflect the functioning of the animal's endogenous biological clock. Without exception, circadian rhythms can be readily entrained (synchronized) to light cycles with periods of exactly 24 hours. Recently several investigators have demonstrated that the photoreceptors involved in mediating entrainment of activity rhythms in at least two classes of vertebrates are, in part, extraretinal. The perching activity of the house sparrow *Passer domesticus* can be entrained via extraretinal receptors located in the brain (*1*). The locomotor rhythms of both the slimy salamander *Plethodon glutinosus* and the green frog *Rana clamitans* can be entrained by light cycles after the removal of the eyes (*2*). Extraretinal receptors also participate in time-compensated celestial orientation of the southern cricket frog *Acris gryllus* and thus by inference in the entrainment of its biological clock (*3*). Data are presented here which show that the activity rhythms of a third class of vertebrates, the reptiles, can be entrained by light cycles in the absence of all known photoreceptive structures.

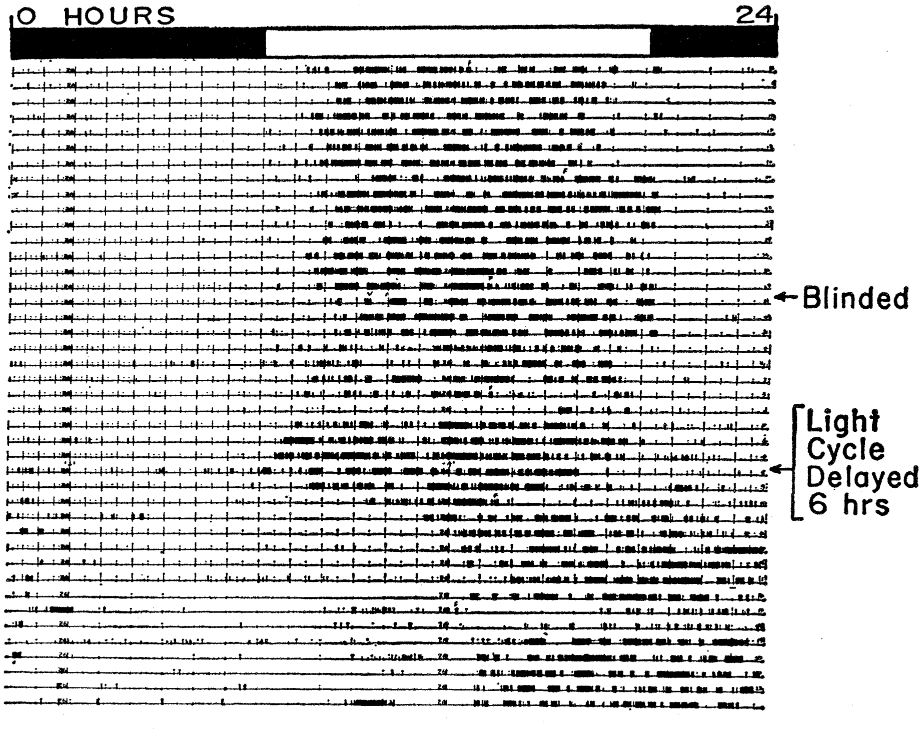

Fig. 1. Entrainment of a lizard after removal of the lateral eyes. The initial lighting regimen is diagramed at the top of the record [solid black, darkness; white, white light (30 lux)]. The lizard was blinded on the 16th day of the record. Eleven days days later the light cycle was delayed by 6 hours. Note entrainment to the new light cycle after several days of transients. Hour zero is at midnight Central Standard Time.

The activity rhythm of the iguanid lizard *Sceloporus olivaceus* is entrained by a cycle of 12 hours of fluorescent light (30 lux) and 12 hours of dark (*LD 12*:12) after complete surgical removal of both eyes (Fig. 1) (*4*). All blind lizards which gave measurable amounts of activity (17 cases) clearly entrained to this stimulus.

In addition to their eyes, many lizards possess two organs embryologically derived from the roof of the diencephalon, which, on the basis of ultrastructural and electrophysiological evidence, are thought to be photoreceptive—the parietal eye and the pineal organ (*5, 6*). Among the lizards these two organs are diverse in morphology and location. Some lizards do not possess a parietal eye at all. Electron microscopy has shown that the pineal organ (or epiphysis proper) often contains cells with modified photoreceptive ultrastructure (*5*). The parietal eye contains well-organized photoreceptors which resemble the cones of the lateral eye (*5–7*). Electrophysiological responses to illumination have been recorded from the parietal eye of several lizard species, including members of the iguanids, and from the pineal organ of lacertid and iguanid lizards after removal of the parietal eye (*8*).

In spite of the strong evidence for a photoreceptive function of both the parietal eye and the pineal organ in some species of lizards, complete removal of both these organs in previously blinded animals did not prevent entrainment to a fluorescent light cycle in any of the *S. olivaceus* tested (Fig. 2) (*9*). Nine lizards survived blinding and removal of the parietal eye and pineal organ and in addition gave activity records which were adequate to assay entrainment. All of these entrained to the light cycle subsequent to the surgery. Two other blind lizards entrained after removal of the parietal eye but died before pinealectomy. Records of several lizards showed activity too sparse for interpretation, but in no case did a lizard with an interpretable record fail to entrain after any of the surgical procedures described. Although all fluorescent bulbs were surrounded by water jackets (*4*), a control experiment was performed which eliminated the possibility that the blind lizards were entrained by a temperature cycle rather than by the light cycle (Fig. 3).

Lizards were also tested for their ability to entrain to a dim green light source of approximately 0.1 lux (Sylvania Panelescent Nite-Lite). Of nine lizards with eyes intact, three entrained to this stimulus. Two of the six that failed to entrain showed relative coordination to the light cycle (*10*). The rest free-ran through the light cycle (expressed their endogenous circadian rhythms in its presence). These data indicate that 0.1 lux of green light is near the threshold for entrainment of normal lizards. Those lizards which entrained to the dim light cycle began free-running immediately after removal of their eyes (Fig. 4), which suggests that the eyes are somehow involved in the entrainment process in intact lizards. However, the relative roles of the eyes and the extraretinal receptor or receptors in entrainment are unknown.

In other vertebrate classes the anatomical site or sites of the extraretinal receptors involved in entrainment are, for the most part, not known. There is evidence that evaginations of the roof of the diencephalon in members of the other lower vertebrate classes [fish (including cyclostomes) and amphibians (*5, 6, 11*)] can probably function as photoreceptors, but in only one case is there direct evidence that they are involved in entrainment. Adler has demonstrated that removal of the frontal organ [which is considered by some to be homologous to the parietal eye of lizards (*6*)] of blinded green frogs will cause them to free-run in the presence of a light cycle (*2*). In house sparrows the extraretinal receptor or receptors responsible for entrainment is located in the brain. The pineal organ is probably not involved and is certainly not the only extraretinal photoreceptor (*1*). In adult mammals there is no evidence that extraretinal receptors are involved in entrainment. Blind adult rats and mice will not entrain to light cycles (*12*). In juvenile rats, however, an extraretinal photoreceptor has been implicated in the control of the circadian rhythm of the amount of serotonin in the pineal organ (*13, 14*). Removal of the Harderian gland, a gland found within the orbit of many vertebrates, abolishes the effect of light on the amount of serotonin in the pineal

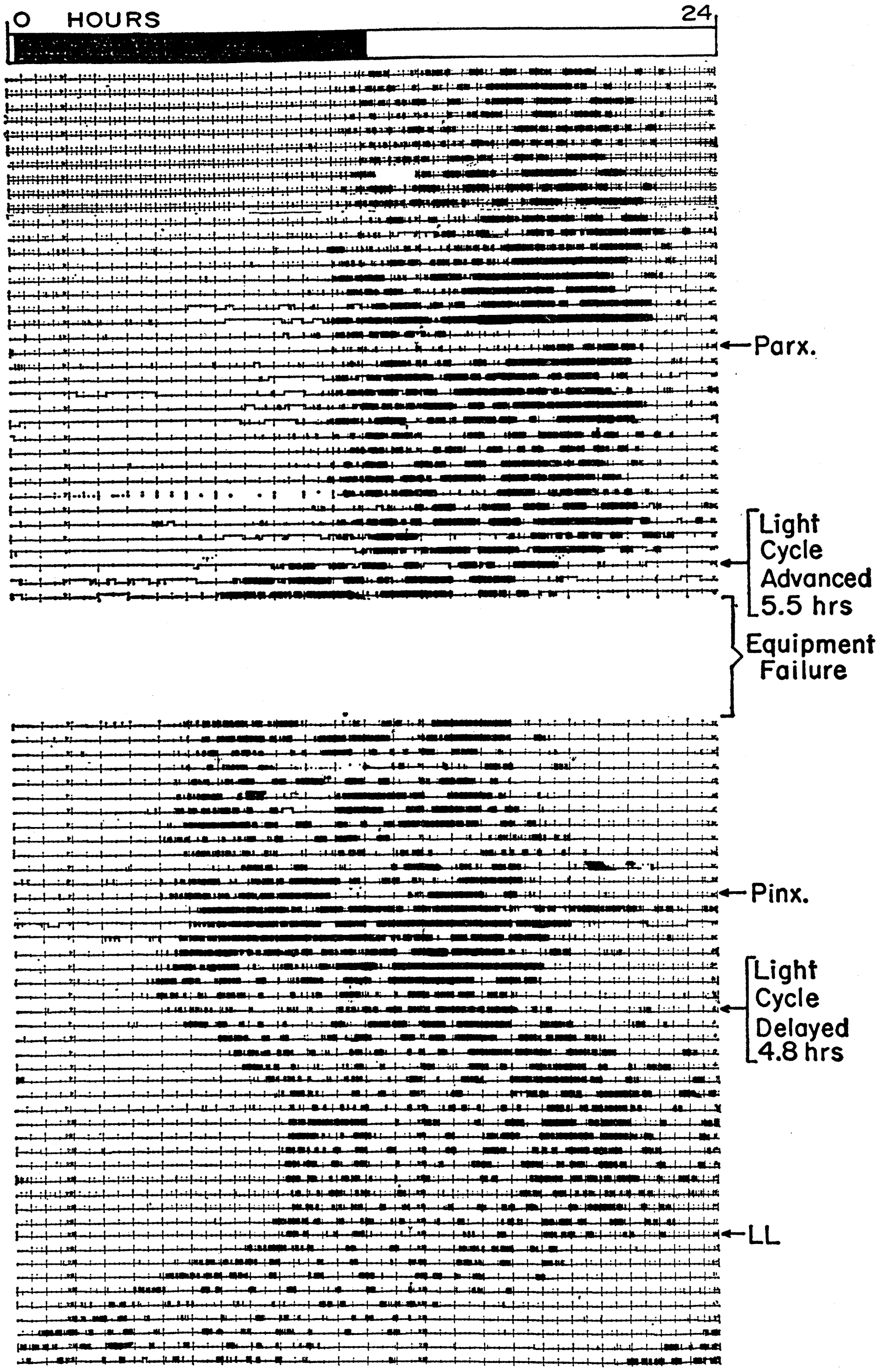

Fig. 2. Entrainment of a lizard after removal of the lateral eyes, parietal eye, and pineal organ. The blind lizard was initially entrained to the light cycle diagrammed at the top of the record. After removal of the parietal eye (*Parx.*) the lizard entrained to a new light cycle, the onset of which was advanced 5.5 hours from the onset of the initial light cycle. The pineal organ was then removed (*Pinx.*), and the light cycle was delayed 4.8 hours. The lizard was finally put under continuous dim green light (*LL*, 0.1 lux) in which it free-ran with a period of 22.8 hours.

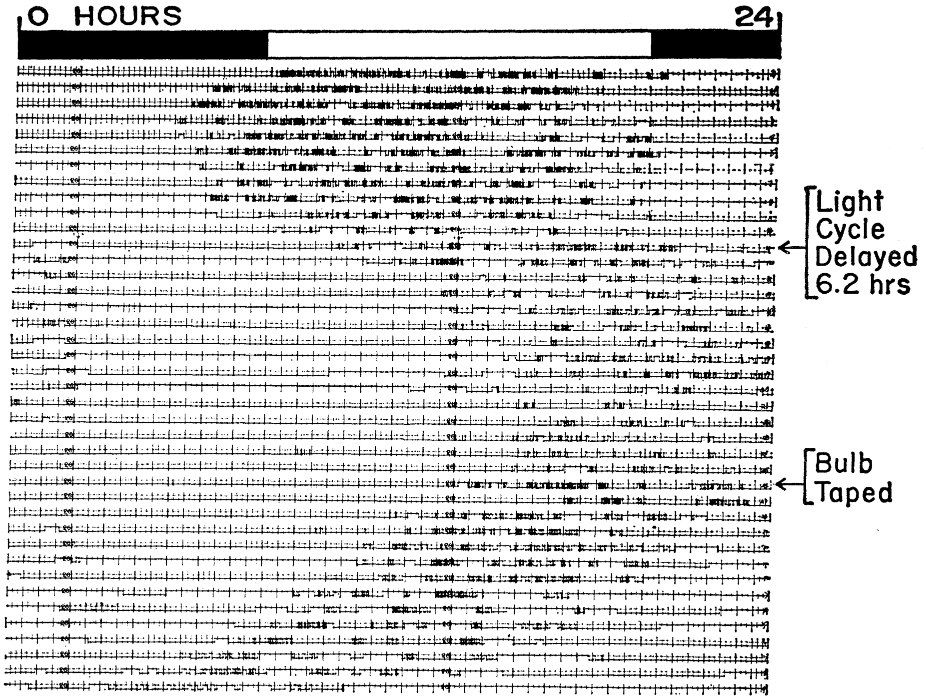

Fig. 3. A control experiment performed to eliminate the possibility of entrainment by low-amplitude temperature cycles. The lizard was blinded and the pineal organ and parietal eye were removed before the beginning of the record. Note that the lizard entrains to the light cycles when they are present. On the day marked *Bulb Taped*, a fluorescent bulb which had been painted black and covered with black tape over its entire surface was substituted for the normal bulb in the water jacket. The taped bulb was on the same "light" cycle as the previous bulb. This procedure eliminates the light cycle but preserves any low-amplitude temperature cycle which may be present. The lizard free-runs when exposed to the taped bulb, showing that the light cycle is the true entraining agent.

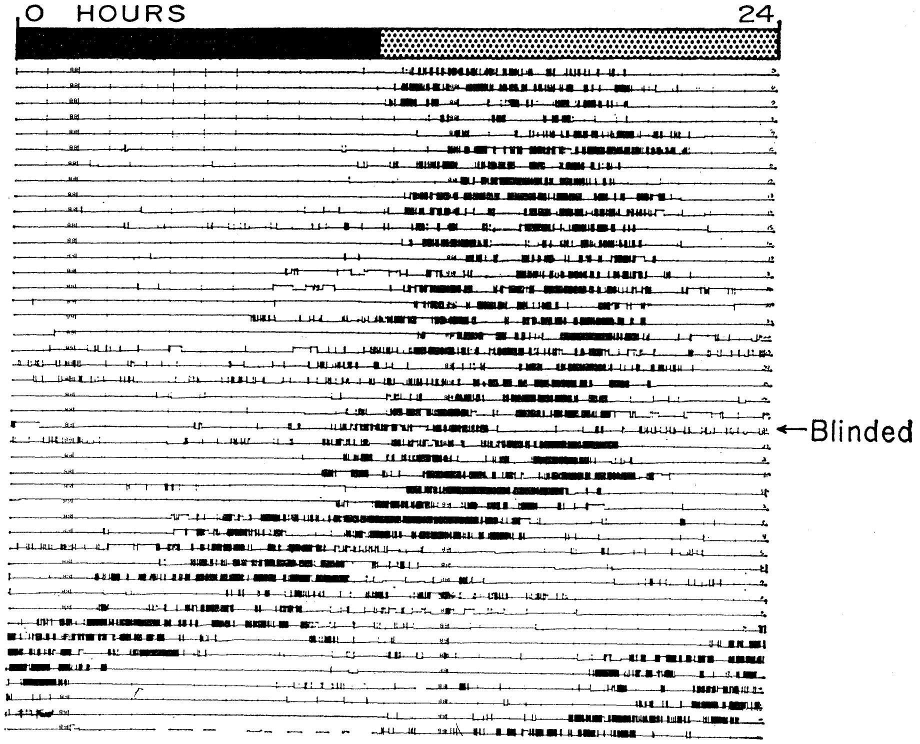

Fig. 4. Contribution of the lateral eyes to perception of an entraining light cycle. The normal (unoperated) lizard was entrained to a light cycle in which the light (indicated by stippling in the diagram) was approximately 0.1 lux (green light). After removal of the lateral eyes the lizard free-ran through the light cycle with a circadian period of about 23 hours.

glands of blinded suckling rats. Wetterberg *et al.* suggest that this gland may be an extraretinal photoreceptor (*14*). The fact that removal of the Harderian glands did not prevent entrainment to fluorescent light cycles by several blinded *S. olivaceus* with pineal organ and parietal eye removed suggests that this gland is probably not involved in entrainment of the lizard activity rhythm.

Our data show that both the eyes and an extraretinal receptor, other than the pineal organ or parietal eye, are involved in the entrainment of lizards. In the light of other recent findings these data add strength to the hypothesis that all vertebrates may rely on cues perceived extraretinally to regulate biological clocks as well as certain other physiological responses (*15*).

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4. Lizards were individually housed in wire cages inside light-tight wooden boxes which in turn were located in Hotpack environmental chambers held at a constant 29°C (± 0.5). Each box had a single fluorescent light source (Ken Rad F4T5/cw) housed in a glass water jacket through which tap water was continuously circulated. It was necessary to use water jackets to insure that the lizards were entraining to visible light rather than to the heat produced by the fluorescent bulbs. Control of temperature cycles is particularly important in view of Hoffmann's data which show that lacertid lizards can be entrained to temperature cycles with amplitudes as low as 0.9°C [K. Hoffmann, *Z. Vergl. Physiol.* **58**, 225 (1968)]. Continuous temperature recordings of individual boxes were made by a Speedomax 12-channel temperature recorder (Leeds and Northrup). No differences (within a resolution of 0.2°C) in ambient temperature between a lights on or off condition were observed at the level of the lizards. Each box also held a single dim green light source (Sylvania Panelescent Nite-Lite). Heat produced by the Panelescent light was negligible. The wire cage was bolted to a jiggle platform. Any motion of the lizard broke an electrical contact, and the event was recorded on a single channel of an Esterline-Angus operations recorder. Lizards were blinded by complete removal of both eyes. Blind lizards were force fed 2 ml of finely ground mealworms by stomach tubing once a week. Sighted lizards were fed live mealworms.
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9. The parietal eye is easily removed, and complete removal can be determined by visual inspection under a dissecting microscope. Complete removal of the pineal organ was confirmed histologically in five of the nine pinealectomized lizards. One lizard showed some pineal tissue remaining after pinealectomy. Three of the lizards died before the brains could be taken for histology. The paraphysis was often removed along with the pineal organ, and damage to adjacent nervous tissue was sometimes evident. All operations were performed on lizards immobilized by cooling and embedded in crushed ice.
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Reinnervated Eye Muscles Do Not Respond to Impulses in Foreign Nerves

Abstract. *Normal movements return to carp eyes after section and regeneration of the IIIrd and IVth nerve trunks. Two months after reinnervation, records of impulses in the inferior oblique nerve during tilting of the body show activity of the normal motoneurons to that muscle, together with discharge patterns characteristic of the antagonistic superior oblique and some of the rectus muscles. These axons must have found their way into the inferior oblique trunk during sprouting at the lesion and must be maintained after reinnervation. Impulses from foreign axons are without detectable effect on eye movement and therefore must be blocked at their termination in the muscle. Previous study of cross-innervated and doubly innervated fish eye muscles revealed only structurally normal neuromuscular junctions. Transmission from foreign junctions in multiply innervated muscle is blocked by competitive molecular recognition and control mechanisms that do not cause degeneration.*

Regrowth of cut motor nerves in some lower vertebrates restores full coordinated movement to the reinnervated muscles (*1*). When the superior oblique muscle of the carp eye is innervated first by the nerve to the antagonist muscle and then superinnervated by the correct nerve, contractions caused by the antagonist innervation cease as soon as the response to the original nerve appears. Foreign nerves of proven ability stop working when the original nerve grows back. No ultrastructural signs of degeneration of neuromuscular junctions accompanies the repression of the first innervation. Either foreign motoneurons no longer bring impulses to the muscle or neuromuscular transmission is blocked (*2*). We have distinguished between these two possibilities by the following experiments.

In two carp the IIIrd and IVth nerves to one eye were cut where they run together in the cranium. The eye was paralyzed except for flick movements from the posterior rectus muscle which is innervated by the VIth nerve. Nineteen days later other movements began to return, weakly at first but appropriate in direction and timing as indicated by the conjugate behavior of the intact eye. After two more months movements of both eyes were coordinated and equal. Figure 1 shows the rotation of both eyes in response to tilting the whole body up and down. The difference between the curves is no greater than normal (*3*).

The fish were then anesthetized with MS 222 and paralyzed with gallamine. The eyes and extraocular muscles were removed, leaving the nerve stumps free. The animals were mounted in an apparatus which could be tilted up or down and rotated to the left or right. An electrode was placed successively on the cut ends of the inferior oblique nerves of the normal and the reinnervated sides. Figure 2 shows the responses to tilting. Normally the inferior oblique neurons discharge when the head is down and stop firing if the head moves up. Neurons of the superior oblique nerve discharge for movements in the opposite direction. On the reinnervated side there were fibers in the inferior oblique trunk which increased their discharge when the head was down and others which discharged when the head was up—reflex behavior which usually identifies motoneurons of the superior oblique. Other maneuvers revealed the discharge patterns of motoneurons from the rectus muscles in the same nerve.

Therefore, sprouting and regrowth of the IIIrd and IVth nerves must have been haphazard, and axons from different kinds of motoneurons must have entered channels which eventually led them out into the inferior oblique branch of the IIIrd nerve in the orbit. Two months later they were still there and they showed normal reflex responses. Nevertheless, the movements of the eye produced by the inferior oblique muscle corresponded not to the totality of the impulses in the trunk but only to those delivered by fibers of the original inferior oblique nerve. Thus, when the head is held up, superior oblique neurons should discharge to pull the eye around toward the horizontal while the inferior oblique motoneurons should stop. The neurogram from the operated side (Fig. 2) shows a strong burst of superior oblique neurons in the inferior oblique nerve. If this burst had been transmitted to the muscle, coinnervation of the two

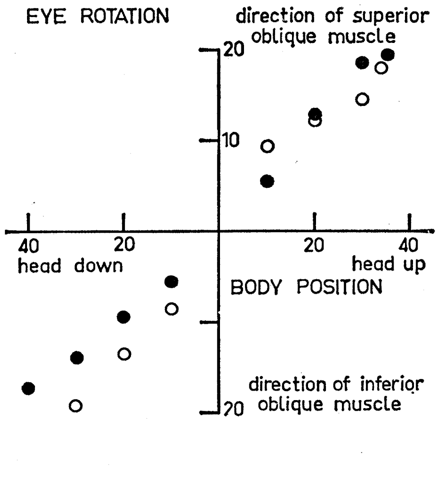

Fig. 1. Graph of the wheellike rotation of the carp eye about the optic axes when the body of the fish is tilted up and down from the normal horizontal position. The reflex movements, mainly of labyrinthine origin, tend to keep the eye horizontal. Rotation when the head is up is almost entirely due to contraction of the superior oblique muscle; rotation when the head is down is due to the inferior oblique muscle [see (*5*) for further details]. Left operated eye, filled circles; right control eye, open circles. Measurements were made 2 months after reinnervation of the left eye muscles. Both eyes show normal reflex movements, and the difference between them is no greater than that in normal fish. Axes in degrees from horizontal.