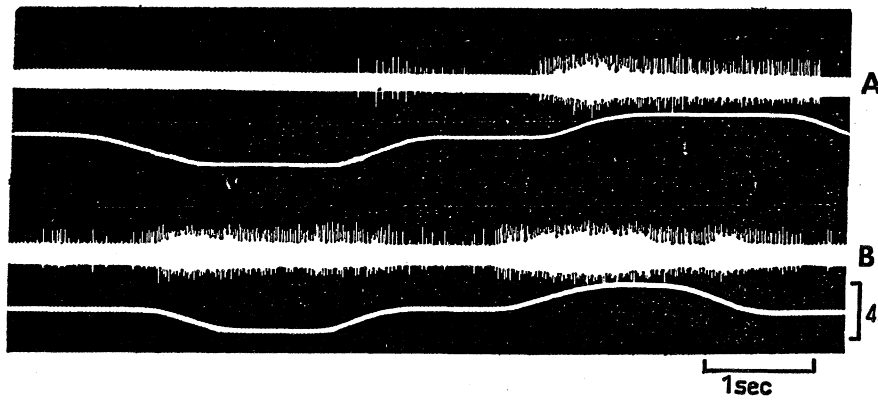

Fig. 2. Records of impulse traffic in the inferior oblique nerve trunk of the fish whose eye movements are illustrated in Fig. 1. (A) Normal right eye. Top trace, nerve recording; bottom trace, position of fish; head-up movements signaled by depression of the trace; head-down movements signaled by elevation of the trace. Note that the normal inferior oblique motoneurons only fire as the head is lowered and only discharge tonically when the fish is held head down. (B) Reinnervated left eye. The nerve record shows fibers that discharge both when the head is up and when it is down. Continued discharge when the head is up is the response pattern normally seen in records from the superior oblique nerve.

oblique muscles in the same eye would have reduced or abolished the normal counterrotation of the eye. Figure 1 shows that this was not so. When the head was up movements of the operated and control eyes were equal.

It is clear that nerves can grow into foreign muscles and retain their characteristic reflex behavior. The selectivity on which coordination depends is therefore peripheral, between nerve and muscle. Inappropriate nerves either do not form terminals, or those formed do not work. We favor the latter view because of the results of electron-microscopic studies of cross-innervated and doubly innervated eye muscles. Only morphologically normal nerve endings were seen when it was clear from the reflexes that nerves that once worked had just been suppressed (*2*). So far there seems to be no reason to think that foreign nerves establish themselves in these multiply innervated muscles except by the formation of the usual terminal synaptic apparatus. No unusual endings have been seen in the present muscles. However, whether or not multineuronal innervation is allowed, a subtle but strong selectivity must operate to block transmission of excitation from foreign nerves as long as the correctly matched nerves are present. Perhaps the developmental mechanisms for neuromuscular connection permit the formation of inappropriate synapses but regulate transmission precisely. The morphological and physiological correlates of suppressed transmission are not known.

The mechanism of coordination in reinnervated muscles recalls the resonance theory of Weiss (*4*), who suggested that nerves broadcast messages for several muscles, each of which could decode the correct command and ignore all the others. After regeneration, nerves of one kind are no longer segregated in trunks and reinnervated muscles receive a mixture of commands for many muscles but, through peripheral filtering, respond only to the correct ones. Presumably they do so, not because they resonate to the right message, as Weiss thought in 1924, but because some competitive process of chemical recognition has made ill-matched synapses ineffective.

If the regulatory mechanisms of neuronal growth tolerate and maintain the terminals of active motoneurons in a muscle, even when they are prevented from transmitting, we wonder whether a similar mutual repression of synapses might control interneuronal connections in the central nervous system. A repressive process that depended on the activity of a synapse would provide a learning mechanism based on the faculties of intercellular recognition that are important in brain development (*5*).

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Harderian Gland: Influence on Pineal Hydroxyindole-O-Methyltransferase Activity in Neonatal Rats

Abstract. *A circadian rhythm has been found in hydroxyindole-*O*-methyltransferase activity of the pineal gland of blinded 12-day-old rats. Five additional hours of lighting can partly prevent the nocturnal increase in pineal hydroxyindole-*O*-methyltransferase activity in such rats. Removal of the Harderian gland abolishes this response to light in 12-day-old blinded animals, giving further support to the suggestion that this gland may function as an extraretinal photosensitive organ influencing the pineal gland in blinded suckling rats.*

The concentration of serotonin in the pineal gland of the rat undergoes a rhythmic variation, reaching a maximum at about the midpoint of the light cycle, and falling rapidly after the onset of darkness. Extending the lighting period prevented the serotonin fall in intact, but not in blinded mature rats. In immature rats, however, extended lighting prevented the pineal serotonin fall in both intact and blinded animals (*1*).

In a previous study (*2*), we confirmed the persistence of the pineal serotonin rhythm in immature blinded rats and the effect of additional light on that rhythm. We also reported that the removal of the Harderian gland abolished the effect of light on the pineal serotonin levels of the blinded 12-day-old rat and suggested that the Harderian gland may act as an extraretinal photoreceptor influencing the pineal serotonin rhythm in immature rats.

Pineal hydroxyindole-*O*-methyltransferase (HIOMT) activity also undergoes a circadian rhythm. In intact rats maintained on a 7 a.m. to 7 p.m. light-dark schedule, HIOMT activity reached a peak at about midnight and a nadir near the end of the light period (*3*). This normal increase in HIOMT activity during the 7 p.m. to midnight period did not occur in blinded adult rats maintained under these lighting conditions (*4*). The influence of light conditions on pineal HIOMT activity in blinded immature rats has not been reported.

The present study was designed to investigate the influence of light on HIOMT activity in blinded suckling rats and to examine the possible connection between the Harderian gland and this aspect of pineal activity.

Long-Evans rat pups were kept with their mothers in plastic cages under a 6 a.m. to 6 p.m. lighting schedule in a constant-temperature room for at least 4 days prior to the experiment. At 9 days of age, all rats were blinded by complete bilateral enucleation or by bilateral enucleation combined with complete removal of the Harderian gland. The operation was carried out under light ether anesthesia. Animals were blinded by pressing the edges of a curved eye forceps on either side of the eye to force open the eyelids and push the eye forward. The forceps were closed about the base of the eye, and the eye and the attached optic nerve were pulled forward. By pushing the forceps somewhat deeper into the optic cavity, the eye and the Harderian gland could be removed simultaneously. After removal of the Harderian gland, the contents of the eye cavity were also gently aspirated. Bleeding was not profuse and soon stopped. The completeness of removal of the Harderian gland was determined by examining the eye cavity for fluorescence at 366 nm after the rats were killed.

The operated rats were returned to their mothers after the operation and maintained under diurnal lighting conditions until they were 12 days old. Five hours before being killed they were removed from their mothers and distributed into four treatment groups of four rats each (Fig. 1). At the appropriate times the rats were decapitated and the pineals were quickly removed and weighed. We determined HIOMT activity by following the procedure of Axelrod *et al.* (*4*). The identity of the [^{14}C]melatonin formed enzymatically in the pineal homogenates was established by thin-layer chromatography (*5*).

Forty-eight male and female rats were utilized. Sixteen rats were used in each experiment and the experiment was replicated in three separate trials with similar results.

As seen in Fig. 1, pineal HIOMT activity was higher in blinded rats killed at 11 p.m. than in those killed at 1 p.m. (group B to A; $P < .05$), whereas activity in animals maintained in light until 11 p.m. was intermediate (group D). These results indicate that there is a rhythm of HIOMT activity in pineal glands of 12-day-old enucleated rats that is influenced by light, even after enucleation. This effect of light on the HIOMT rhythm was abolished by removal of the Harderian gland (group C). The HIOMT activity in the pineals of animals both enucleated and harderianectomized and maintained in light was identical to the enzyme activity in blinded animals maintained in darkness (group C versus B) and significantly higher than those blinded animals kept in light (group C versus D; $P < .05$).

In this study, then, as in our previous study on pineal serotonin (*2*), the Harderian gland may be functioning as an extraretinal photosensitive organ involved in some circadian rhythms influencing indoleamine metabolism in neonatal rats. We cannot, however, exclude the possibility that the Harderian gland is required for the maintenance of photosensitivity in some other organ. In addition, contributions to this sensitivity from other tissues within the orbital cavity also cannot be completely excluded at this time. Further, removal of the Harderian gland may impose an additional stress on this experimental group as compared to those enucleated alone, and, although it is unlikely, this could conceivably contribute to the difference in photic response of the two groups. Finally, as pointed out by Klein and Lines (*6*) and Zweig and Snyder (*6*), HIOMT activity in the pineal is increasing very rapidly in rats of the age used in this study. It is possible that the Harderian gland may affect the rate at which the activity of this enzyme increases and what, in our study, appears to be a change in response to light may be the result of a developmental change.

In their study, Klein and Lines (*6*) observed only minimal diurnal changes in pineal HIOMT activities in intact rats under 25 days of age. These results are quantitatively different from the larger fluctuations observed by us. One possible explanation for these discrepant results could be that intact animals, used by Klein and Lines, behave differently from enucleated animals, used in our study. More likely, the difference resides in the handling of the infant animals. In the study by Klein and Lines, infants were kept with their mothers until the time of killing, whereas in our studies they were removed from the mothers 5 hours before being killed. Prior removal of the infants from the maternal nest could impose a stress upon the infants which could contribute to the differences observed. Perhaps more important, however, infant suckling animals tend to burrow beneath the mother and thereby avoid continuous exposure to light. These animals cannot, then, be truly considered equally light exposed. Indeed, burrowing behavior occurs even in animals isolated from their mothers, so that animals in groups should perhaps be continuously redistributed or kept in individual compartments to obtain more uniform exposure to light.

In his classic studies on porphyrins, Kluver (*7*) raised the possibility that glands of the orbit may serve some

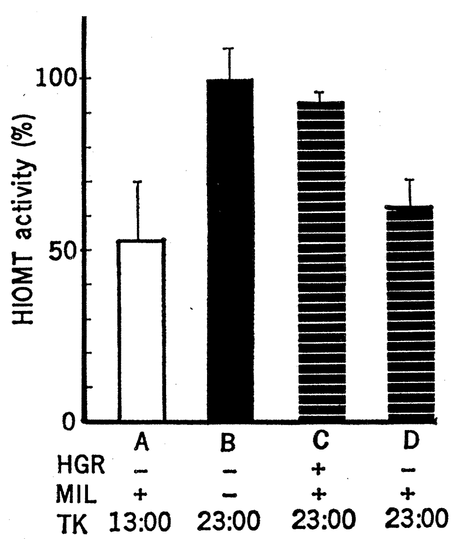

Fig. 1. Effect of light and dark, and of removal of the Harderian gland, on the hydroxyindole-*O*-methyltransferase (HIOMT) activity of the pineal gland in response to light in 12-day-old blinded rats treated as described below. Each group contained 12 rats from three separate replicates. Within each replicate the enzymic activity was calculated as percent of the mean activity of the enucleated animals at 11 p.m. The overall mean of the activity of the enucleated animals at 11 p.m. was 54 pmole of [^{14}C]melatonin formed per hour per milligram of pineal. Vertical bars are the standard errors of the calculated percentages for the three replicates. *HGR*, Harderian gland removed (–, no; +, yes); *MIL*, maintained in light; *TK*, time killed.

function in visual reception and suggested the involvement of porphyrins which are in high concentration in the rat Harderian gland. Although our results do not specifically implicate the porphyrins of the Harderian gland in the enzymic response to light, they do lend some support to Kluver's notion of photoreception by orbital glands.

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Cardiac Responses on the Visual Cliff in Prelocomotor Human Infants

Abstract. *Human infants younger than crawling age yielded reliable cardiac decelerations when placed directly atop the deep side of a visual cliff and generally nonsignificant changes when atop the shallow side. Distress was elicited less frequently on the deep side than on the shallow at these ages, in contrast to the behavior of older infants and other species. Prelocomotor infants thus can discriminate the two sides of the cliff, but not by means of distress at loss of optical support.*

The visual cliff, an apparatus for investigating depth perception in the older human infant, can also be used for testing discrimination capacities in prelocomotor infants. Visual cliff studies have up to now been limited to older infants by relying, as an index of depth perception, on the infant's avoidance of crawling over the "deep" side of the cliff. Only the absence of a suitable dependent variable has prevented the testing of younger infants on the apparatus. Previous studies (*1*) reporting marked emotionality in animals placed directly over the visual cliff's deep side suggested that the autonomic responses of infants placed directly over either side of the cliff might serve to discriminate the deep from the shallow sides, even at ages when the infant is much too young to crawl.

Table 1. Heart rate responses of infants 106 and 55 days old on visual cliff. Results are expressed in beats per minute.

Parameter	Condition		
	Deep	Shallow	Deep minus shallow
	106 days old		
Cardiac highs	−7.80*	−0.90†	−6.90‡
Cardiac lows	−7.40*	−0.80†	−6.60§
Time sample	−6.10*	−0.80†	−5.30§
	55 days old		
Cardiac highs	−5.10‡	+4.80†	−9.90‡
Cardiac lows	−2.80†	+6.80‡	−9.60‡
Time sample	−4.70‡	+5.50†	−10.20*

* $P < .001$. † $P > .05$. ‡ $P < .01$. § $P < .03$.

Heart rate is a sensitive autonomic response in even the youngest human infants (*2, 3*). By measuring heart rate response, we studied visual cliff discrimination in two prelocomotor age samples. The median age of one group (13 males, 7 females) was 106 days (range 75 to 115), and that of the other (seven males, four females) was 55 days (range 44 to 70). Visual cliff model III (*4*, p. 8) was used, with both deep and shallow surfaces consisting of red and white tiles (22 by 22 cm) arranged in checkerboard. Heart rate was recorded on a Grass model 5D polygraph.

The procedure was to assign an infant randomly to one of two conditions, initial deep placement or initial shallow placement. A prestimulus measure of heart rate was obtained before each deep or shallow trial by sitting the infant atop the appropriate side of the visual cliff for 1 minute in the older group, and 30 seconds in the younger group (for whom the longer prestimulus interval often produced crankiness). After the prestimulus period was over, the experimenter placed the infant on his stomach, with his eyes pointed down toward either the deep or the shallow surface of the cliff for the same duration as the prestimulus period. The experimenter had to hold the older infants at a slight angle from the prone position to prevent the child from hitting his head against the glass. The younger infants were placed on foam padding (11 cm thick) to accomplish the same end. Two trials were attempted on each side, except when the infant's state precluded further testing.

Three parameters of heart rate were chosen for study. In one case, heart rate was sampled every 2.5 seconds during the first 30 seconds before the stimulus was administered and during the first 30 seconds of the stimulus period. A simple heart rate difference score was then obtained by subtraction of the mean prestimulus heart rate from the mean stimulus heart rate. The other two parameters, the logic of which is explained by Kagan and Lewis (*2*), were selected to enable comparison with other studies of infant heart rate. One parameter, called "cardiac highs," consisted of sampling the five fastest heartbeats during the 10 seconds just prior to stimulation, and the two fastest beats during each of six succeeding 5-second periods during stimulus presentation. The mean of the cardiac highs during prestimulation was then subtracted from the mean of the highs during stimulation. The analysis of "cardiac lows" was entirely analogous, except for the selection of slowest beats. Regardless of parameter, the index of discrimination of the two sides was simply the heart rate response on the deep side subtracted from that on the shallow.

The hypothesis of the study, that infants would show differential cardiac

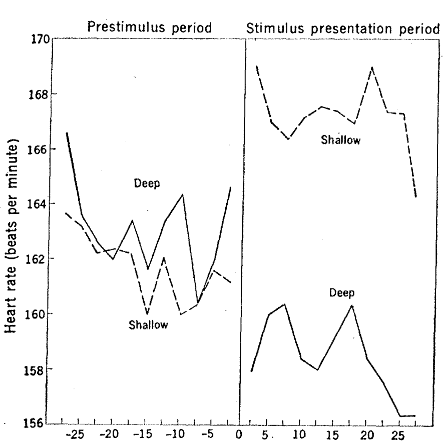

Fig. 1 Heart rates in 55-day-old infants on shallow and deep sides of a visual cliff.