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.

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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-

ments, the donor rats were first given 8 to 10 days of bar-press training, during which each bar-press response was reinforced with a food pellet; next the animals were given 3 days of extinction training, during which no food was given no matter how often the animal pressed the lever; then the rats were given 3 days of repeated acquisition training, during which each bar press again yielded the rat a food pellet. During the first part of the training, the rats learn to press the bar more and more often within a given period of time; during the second stage of training (extinction), the lack of reward causes the animals to reduce their rate of bar pressing; and during the final stage, the rats show a very rapid reacquisition of the original response. A second group of donor animals served as controls for handling and sensitization. After the training sessions were over, the donors were killed and homogenates prepared from their brains were injected intraperitoneally into untrained recipient rats. "Memory transfer" effects were consistently reported for animals that had received material from trained donor animals.

Late in 1968 we began what was designed to be little more than a replication of this prior work by Dyal and his associates. However, the results of our first experiment were so unanticipated that we subsequently undertook several additional studies to confirm our findings. In all the experiments reported here, we used 60- to 90-day-old Sprague-Dawley male rats maintained on a 22.5-hour food-deprivation schedule (*7*). Grason-Stadler operant chambers were modified so that the lever was moved to the side of the chamber opposite the cup into which the food pellets dropped. Lever presses were automatically cumulated on counters.

In our first experiment, 12 donor animals were given eight 30-minute sessions of bar-press training in which each bar press was reinforced by a 45-mg Noyes food pellet delivered into the food cup. Twelve additional animals served as controls. These animals were handled each day but were not trained. The 8 days of bar-press training given the experimental donor animals were followed by three daily extinction sessions in which bar presses were not reinforced with food, which were followed in turn by three daily sessions of bar-press reacquisition training. All sessions were 30 minutes in duration.

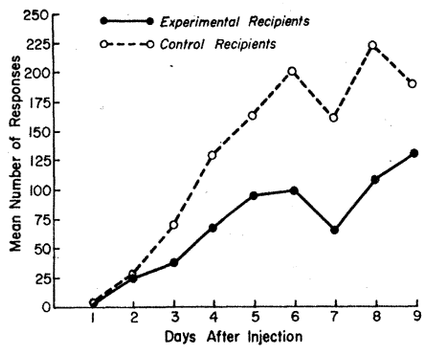


Fig. 1. Mean number of bar presses on each of nine daily sessions for recipient animals injected with brain homogenate either from the experimental or from the control donor groups.

Within 15 minutes after the final training sessions, both experimental and control donor animals were killed by decapitation. The brains, excluding the olfactory bulbs and cerebellum, were removed, frozen on Dry Ice, and stored at −20°C. These brains were then assigned code numbers, and the remainder of the experiment was performed with "blind" procedures. Whole-tissue homogenates were prepared by adding 1.0 ml of 0.154*M* NaCl per brain and homogenizing gently with 15 strokes with a motor-driven pestle and with an ice bath surrounding the homogenizer. Recipient animals were each injected intraperitoneally with 3.2 ml of the homogenate.

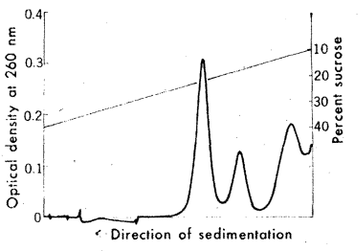
Recipient animals were placed on a 22.5 hour food-deprivation schedule beginning 6 days prior to injection; they had been food-deprived for 23 hours at the time of injection. Twenty-four hours after injection, all recipient animals were given the first of nine daily (30-minute) bar-pressing sessions in which each bar press was reinforced with one 45-mg Noyes food pellet. Bar-press responses were automatically cumulated on counters.

Figure 1 presents the mean number of bar presses emitted by the experimental and the control recipients on each of the 9 days of training in the first experiment. The differences between these two groups on the final 2 days of training are statistically reliable by the Mann-Whitney U test ($P = .02$). Thus, the control animals (injected with homogenate from untrained brains) were reliably superior to the experimental recipients (injected with homogenate from trained brains), as measured by bar pressing. This result was entirely unexpected because in our previous replication of the Dyal paradigm (*6*) the opposite results were found.

There was, however, one major procedural difference between this study and our previous experiment. In this study, because of difficulty in obtaining recipient animals, we discontinued the training of the experimental donors for 1 week after the 3 days of extinction, so that recipient animals would be available at the exact time that the donors were killed. During this 7-day rest period, the donors were maintained on the deprivation schedule but were not trained. It occurred to us that the 7-day rest period with no training could conceivably have served the function of "incubating" the training experience just preceding it (that is, the extinction training), so that the learned tendency "not to press the lever" was perhaps the most likely aspect of the training to "transfer." We decided to test this notion by a second experiment in which we interposed a rest period at various points during the donor training regimen (*8*). Since in the past we had often obtained what appeared to be more reliable results when we used an RNA-rich extract instead of whole-brain homogenates, we decided to extract RNA from the donor brains and to inject it directly into the brains of the recipients.

In the second experiment, we used the procedures outlined previously to give 12 donor rats eight sessions of bar-press training, three sessions of extinction training, and then three sessions of reacquisition training. Seven of these animals received a 7-day rest period immediately after they had completed their initial acquisition training but prior to extinction training (Acq-Rest-Ext-Reacq); the remaining five animals received a 7-day rest period after extinction but prior to bar-press reacquisition training (Acq-Ext-Rest-Reacq). We hypothesized that, if rest periods did indeed serve an incubatory function of consolidating the learning experience just preceding them, recipient animals receiving brain material from donors given their incubation period after acquisition training (Acq-Rest-Ext-Reacq) should be superior in their rate of learning the bar-press response to recipient animals injected with material from donors in group Acq-Ext-Rest-Reacq. A third group of five animals served as untrained donors.

After the final day of donor training, all donor animals were killed by



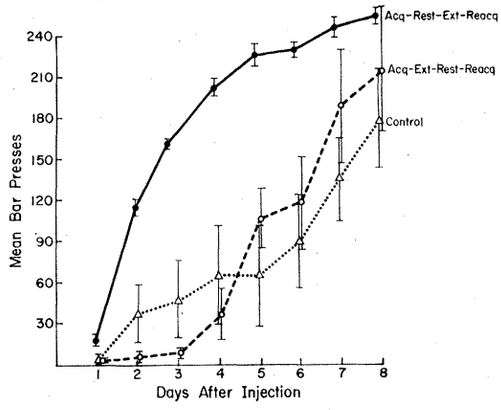


Fig. 2 (above). Density gradient sedimentation profile (10 to 40 percent linear sucrose) of 1/5 brain equivalent of RNA-rich extract. Fig. 3 (right). Mean number of responses emitted by the recipient groups on each of 8 days following injection of RNA-rich brain extract. The vertical bars represent one standard error of the mean.

decapitation, and an RNA-rich extract was prepared from their brains. To avoid introducing experimenter bias, "blind" procedures were used in these steps and in the injection and testing of the recipient animals.

RNA was obtained by a cold phenol extraction procedure (*9*). The product derived from this isolation procedure has been evaluated by several methods (*10*). The extract contains RNA as well as negligible amounts of DNA and includes protein and polysaccharide contaminants. A complete quantitative analysis of the extract awaits further study. The yield of product from one brain reserved for assay was dissolved in 10.0 ml of gradient buffer containing 0.01*M* sodium acetate, *p*H 5.1, $10^{-3}M$ sodium ethylenediamine tetraacetate, and 0.1*M* NaCl. Two milliliters of the solution was layered on a 28-ml linear gradient of 10 to 40 percent ribonuclease-free sucrose in the gradient buffer and centrifuged at 25,000 rev/min for 15 hours at 0°C in an SW25.1 rotor of a Spinco L2-65B ultracentrifuge. The gradient was analyzed for the distribution of 260-nm–absorbing material with an LKB quartz flow cell with a 4-mm light path modified for use in a Beckman DU spectrophotometer with Gilford components and continuous recording on a Sargent recorder.

The density gradient optical density profile (Fig. 2) indicated the presence of both 28*S* and 18*S* ribosomal RNA and transfer RNA. The ribosomal RNA appeared to be undegraded, for the 28*S* : 18*S* peak area ratio was greater than 2.0. No tests of the integrity of the other components of the extract were performed.

Twelve rats served as recipients and were given subdural intracranial injections of the RNA extract (*11*). Four animals were injected with the extract obtained from the brains of the untrained donor animals, four with extract from the brains of the Acq-Rest-Ext-Reacq group donors, and four with extract from the brains of the Acq-Ext-Rest-Reacq donors. One of the recipients injected with RNA from group Acq-Rest-Ext-Reacq died shortly after being injected; no data are reported for that animal.

Twenty-four hours after these injections, the recipient animals were given the first of eight daily (30-minute) bar-press sessions, during which each bar press was reinforced by a Noyes pellet. Figure 3 presents the mean number of bar presses emitted by each of the recipient groups during each of the eight sessions. The means during the initial 3 days of training were submitted to an unweighted-means solution analysis of variance (*12*). The results of this analysis indicated that the groups were significantly different in their mean rate of bar pressing ($P < .05$) and that there was a significant interaction effect between sessions and treatment ($P < .05$). Individual comparisons between the three groups indicated that recipients injected with extract from group Acq-Rest-Ext-Reacq donors were significantly superior to either of the other groups ($P < .05$) and that the recipients injected with extract from untrained donors were reliably superior to animals injected with extract from Acq-Ext-Rest-Reacq donors in mean number of responses over the first 3 days of training ($P < .05$).

These results suggest that incubation periods may be important in obtaining positive memory transfer effects in certain paradigms. Investigators in at least two other laboratories obtain transfer effects with their paradigms only if their animals are trained 5, but not 7, days a week (*13*). It is therefore possible that the failure of certain investigators (*3*) to replicate reports of memory transfer may reflect their failure to incorporate incubation periods into the training regimens of their donor animals. We do not know why these incubation periods are effective, but we have data in preparation that suggest that incubation periods dramatically reduce the time necessary for donor animals to acquire a particular response in laboratory learning situations.

We do not believe that these incubation periods are critical for memory transfer to occur in all transfer paradigms. When additional treatments, such as extended overtraining or the interpolation of extinction training between acquisition sessions, are introduced into the donor-training phase of transfer paradigms, these incubation periods are probably unnecessary. We do believe, however, that in the absence of the preceding manipulations, incubation periods play an important role in determining whether memory transfer effects occur.

Finally, since the extract used in the second study contained impurities, the present results should not be taken as evidence that RNA is necessarily the active chemical mediating the transfer effect. Enzyme digestion studies currently in progress should provide evidence regarding the role of RNA as mediator for the present effect.

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Illusions and Sampling Environmental Cues

Leibowitz *et al.* (*1*) have demonstrated that the Ponzo illusion may be related to phenomena of size constancy and have suggested an explanation based on differential sampling of environmental cues. However, a closer inspection of their stimulus displays, procedure, and reasoning indicates the need for further inquiry before their conclusions are accepted.

When one inspects the photos for which judgments were obtained, one finds abundant perspective and textural cues. Since neither the degree of texture nor perspective was determined for either photo, it is impossible to conclude that: "perspective is a relatively stronger cue than texture . . ." Clearly, unless cue weights are determined in some fashion (stimulus analysis or scaling studies), their conclusion is unwarranted. Others (*2*) have attempted to untangle this difficult problem.

In attempting to explain the differences between judgments made by Guamanian and Pennsylvanian students, the authors rely solely on an assumed "poverty of experience" theory. Although they state that short vistas, hilly terrain, and the absence of railroads on Guam may be the ecological source of the judgmental differences obtained in their study, the experiences of Guam students with still and motion pictures, streets and roads, telephone and electric wires, rooms, hallways, and so forth were not considered. Nor were possible cross-cultural differences in response bias mentioned.

I think crucial stimulus and subject factors responsible for intra-cultural and cross-cultural differences in estimation of size need further exploration.

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It would be of great value if a system were available which would permit assignment of meaningful quantitative values to various monocular depth cues. However, the absence of such a specification system does not preclude the possibility of an ordinal classification in which one stimulus configuration emphasizes perspective and the other emphasizes texture cues as was the case in our study. We would agree with Schiff that our photographs, as well as any full-tone photographs of natural environments, do not isolate only one cue or the other. In the interest of broadening the base of perceptual research, the environments were selected as particularly rich in monocular depth cues. It would be unnecessarily restrictive to confine one's research to stimuli which have been quantitatively scaled or presented only in their abstract form. As we pointed out in the original paper, no theoretical values were assigned to the functions obtained. Rather, the terms perspective and texture were used to label obvious differences in the particular photographs employed.

The possibility of biased responses due to different interpretations of instructions is a well known problem and every effort was made to avoid it. The instructions, which were designed to be as unequivocal as possible, were identical throughout the study. There was no language problem as instruction at the University of Guam is in English. Intellectually and educationally, the subjects were essentially equal. In addition, a scale was used to assess the extent to which subjects might desire to put themselves in a socially desirable light and thus please the experimenter. The group means and variances were the same for the Guamanian as for Pennsylvanian students (*1*).

We do not suggest that Guamanians have no experience with perspective, but rather that familiarity with these cues in three-dimensional real-life situations is richer for the Pennsylvanian than for the Guamanian subjects. There are no railroad tracks on Guam, most roads are winding with the telephone wires following the roads, the buildings, which are constructed to take advantage of the tropical climate, do not have long hallways. There is, of course, no control over experience with perspective in two-dimensional situations.

We would strongly agree that cross-cultural research presents special methodological problems. Our choice has been to take every precaution with the objective of determining the extent to which results of abstract laboratory studies are applicable to the more familiar natural situations which provide the basis for the majority of our perceptual experiences.

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2 March 1970

Flare Identification Associated with Coronal Disturbances

It has come to my attention that an incorrect identification has been made concerning the causal nature of the Pioneer 6 Faraday rotation events observed by Levy *et al.* (*1*). They observed three large-scale transient phenomena and associated these with type III dekametric solar radio bursts. Table