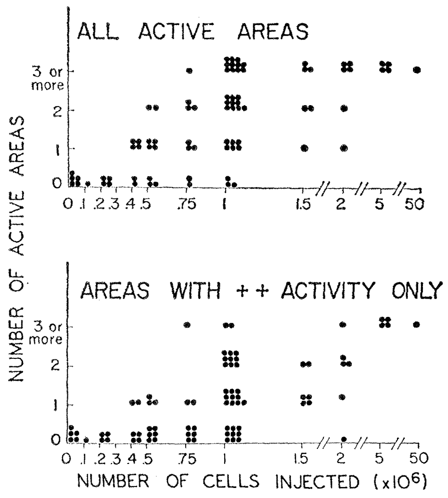

Fig. 1. Distribution of antibody against sheep erythrocytes in 81 spleens from mice receiving various numbers of normal spleen cells.

cells, per million injected spleen cells, is clearly a lower limit, since not all the injected cells can be expected to settle in the spleen. The fraction which does can be estimated by reinjecting the whole or a part of a spleen whose content of precursor cells is already known. For example, in one experiment 50 × 10^6 normal spleen cells were injected into four irradiated mice, and 1 day later their spleens were pooled and reinjected into another eight irradiated mice, which were then immunized and assayed as usual. As Table 2 shows, a considerable reduction in activity resulted from the transfer, and therefore presumably from any such transfer, so that only about 4 percent of the injected precursor cells settle in the spleen. Thus the two active areas of antibody against sheep erythrocytes normally derived from a million spleen cells represent about 50 precursor cells in the inoculum, which is equivalent to 1 per 20,000 cells, or about 5000 per normal spleen.

A major question at this stage is whether the active areas discussed above contain all the antibody activity in the irradiated spleen, or only the activity against sheep erythrocytes. To investigate this, the response to a second, noncross-reacting antigen was simultaneously measured. The mice were immunized with 2 × 10^8 pig erythrocytes at the same time as with the sheep erythrocytes. For the assay a second agar overlay, containing pig erythrocytes, was poured on top of the first, which contained the sheep cells (or vice versa). The layer in which lysis occurred could be identified by focusing up and down with the stereomicroscope, or by peeling off the top layer to reveal the lower. In nonirradiated mice injected with sheep cells or pig cells or both and assayed on double-overlay plates, lysis consistently developed in the appropriate layer or layers, regardless of which layer was on top; no cross-reaction was detectable under these conditions. In the nonirradiated, doubly immunized animals, killed on the 4th day after immunization, essentially every piece of the spleen white pulp (300 to 400 pieces) produced lysis of both types of erythrocyte. In 20 irradiated animals given normal spleen cells and then doubly immunized, activity against both sheep and pig erythrocytes was detectable, but in different areas (Table 3). With the larger spleen-cell doses there was occasional overlapping, but maximum activity against both antigens was never found in the same piece. Five spleens had active areas against pig cells only; two had activity only against sheep cells.

We conclude that if single precursor cells are in fact responsible for the areas of localized antibody formation, which are apparently of a single specificity, the progeny of each precursor must be restricted to forming exclusively, or predominantly, a single type of antibody. Whether this restriction is imposed by contact with the antigen, or whether it is inherent in the precursor cell itself, remains to be demonstrated.

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Interocular Transfer in Goldfish: Color Easier than Pattern

Abstract. *Goldfish were trained to monocularly discriminate different patterns that also differed in color. When tested with reversed combinations of color and pattern cues, the fish responded on the basis of pattern while using the trained eye but on the basis of color while using the untrained eye. Interhemispheric transfer of color information was therefore more effective than that of pattern.*

Binocular integration of visual behavior has special application among those vertebrates whose optic pathways are completely crossed. In the fish, visual input is projected from each eye only to the contralateral optic tectum (*1*). However, visual information must reach the ipsilateral half of the brain, because fish trained to discriminate colors or shapes monocularly can discriminate effectively when tested while using the untrained eye alone (*2*). The phenomenon of interocular transfer implies that each half of the brain has access to visual information that reaches it by way of two separate channels: (i) the direct retinotectal input and (ii) information arriving by way of certain unspecified commissural pathways. The function of these commissures is analogous to that of the corpus callosum of mammals, which relays visual information between the cerebral hemispheres of cat and monkey (*3*).

The intact fish apparently behaves much like a mammal with a split optic chiasma, in which visual input travels to only one-half of the brain. Like mammals prepared in this way (*3*), goldfish show definite limits to interocular transfer (*4*) and also can learn to discriminate opposing shapes by way of opposite eyes (*5*). Neither in fish nor in mammals do the commissural pathways transmit an identical copy of the visual world as viewed by the directly stimulated half of the brain. For these reasons, new paradigms developed for analysis of interocular equivalence in fish should prove useful in exploring the functions of the more complex mammalian brain.

Most investigations of interhemispheric relations in various mammals have been concerned with measurement of interocular transfer after various midline surgical transections. The extent of transfer varies with the method of training and with the specific stimuli employed. The relation between stimulus parameters and success in transfer is an interesting problem because of the possibility that commissures throughout the neuraxis may transmit information underlying different dimensions of visual experience. For instance, cats with transections of both optic chiasma and corpus callosum can transfer discrimination in brightness but not in pattern when trained by a method entailing rewards of food (*6*). Furthermore, Downer (see *7*) concluded that monkeys with split chiasmata had acquired bilateral memories of discrimination in color but not in pattern.

No investigator has excluded the alternative possibility that the superiority of mammals in transferring discrimination in color or brightness simply reflects their greater ease in learning and recognizing these particular sets of stimuli. There is good evidence that the inherent "difficulty" in a given discrimination critically affects the extent of interhemispheric transfer. Myers (*8*) inferred that cats, after transection of the optic chiasma, acquired bilateral memories of an easy, but not of a difficult, discrimination in pattern. Muntz (*9*) found support for the same relation from his own studies of interocular transfer in the octopus and from Schulte (*2*) with carp and Menkhaus (*10*) with chickens.

I now report on the relative extent of interocular transfer of discrimination of color and pattern of goldfish. A feature of my experiment is a test procedure that circumvents usual problems in deciding the "relative difficulty" of two separate discriminations. When fish are trained to discriminate two stimuli that differ in dimensions of color and of pattern, the "discriminabilities" of the two sets of cues can be directly compared by testing the fish with reversed combinations of colors and patterns. That is, as the formerly positive color appears with the negative pattern (and vice versa for the second stimulus), the fish must choose between potentially conflicting cues, or else respond in a confused manner. When the fish responds on the basis of color cues despite the presence of the opposing pattern cues, one may infer that the color pair was the more discriminable under the experimental conditions. In considering the processes underlying interhemispheric transfer, one should ask whether the rank order of discriminability is ever reversed during transfer tests by way of the untrained eye. If this were ever so, it would demonstrate that factors other than difficulty determine the extent of visual transfer across the commissures.

Table 1. Responses by nine fish during test sessions, of six trials each, with potentially conflicting patterns and colors, after monocular training with colored patterns.

Stimulus		Response	
Positive	Dominant cue	1st test	2nd test
Group 1, tested on trained eye			
Red-random	Pattern	6/6	No test
Red-random	Pattern	5/6	6/6
Red-horizontal	Pattern	5/6	4/6
Green-random	Pattern	5/6	5/6
Green-horizontal	No response	3/6	3/6
Mean score: 79 percent pattern			
Group 2, tested on untrained eye			
Red-random	Color	6/6	6/6
Red-random	Color	6/6	5/6
Red-horizontal	Color	5/6	6/6
Green-random	Color	6/6	6/6
Mean score: 96 percent color			

Nine goldfish, about 15 cm long, were trained to discriminate colored patterns by a procedure involving avoidance of shock (*2*). The fish learned to escape from a starting box, through an opaque swinging door, into an identical goal box in order to avoid delivery of shock at the end of the 10-second presentation of the positive stimulus. Each fish was trained to suppress this avoidance response during the 10-second presentation of the negative ("no-go") stimulus. During training sessions the escape door was locked from behind during negative trials, so that the subject collided with the door during an inappropriate response by avoidance; this punishment proved more effective than the customary procedure of shocking the subject for incorrect reactions.

Stimuli consisted of pieces of red or green matte tape affixed to 3-cm white matte squares. The "horizontal" pattern consisted of three colored stripes laid at the top, middle, and bottom of the square, leaving two intervening white stripes of equal width. The opposing "random" pattern was made from small irregular clippings from identical stripes, which were scattered over the white background in a random but fairly homogeneous array. Each stimulus was presented at the end of a transparent lucite rod. Stimuli were lowered into the aquarium to a position directly lateral to and 10 cm from one eye. During tests of interocular transfer, the stimuli were presented in the corresponding position within the lateral field of the untrained eye. Use of a flat-grey background inside the aquarium prevented color reflections of stimuli from reaching the untrained eye during conditioning. During training, positive and negative trials were alternated irregularly.

When each fish had performed nine or ten consecutive trials without error, the first "conflict test" was given with the new color-pattern combinations. For example, a subject trained to avoid green-horizontal but not red-random would be tested with red-horizontal and green-random stimuli. Each test session consisted of trials in A-B-A-B-B-A or B-A-B-A-A-B order, where stimulus A included the formerly positive pattern and stimulus B the corresponding negative pattern. Subjects were then retrained on the original stimuli to the criterion of nine errorless trials and retested once again with the potentially conflicting cues. The order of trials was reversed between the two test sessions for each subject. During both test sessions no punishment followed failure to avoid, and the escape door was unlocked during all trials. The five fish of group 1, using only the trained eye, received both test sessions, whereas the four fish of group 2 were tested for interocular transfer with the conflicting stimuli. In this way, the relative dominance of color cues or pattern cues could be compared between the two halves of the brain.

Four of the five fish in group 1 responded on the basis of the familiar pattern differences (Table 1); therefore these particular patterns were more discriminable than the red and green cues. Only one fish failed to respond at all during test sessions; since it had performed without error during the interpolated retraining session, color and pattern cues were probably about equally discriminable, and this led to conflict. On the other hand, all four subjects of group 2 (tested with the untrained eye) responded unambiguously on the basis of the color differences. There was no overlap in performance between these two groups, tested while using the trained or untrained eye. In order for the goldfish to demonstrate color dominance by way of the untrained eye, color information must be relayed by the commissural system more effectively than is the relevant information on pattern.

These results indicate that two processes of visual coding must underlie the learning of discrimination in this experiment. However, in absence of a specific control for differences in apparent brightness of the red and green colors, I cannot state with finality that hue alone comprised the second coding dimension. When differences in brightness are controlled, goldfish do transfer interocularly a discrimination between red and green stimuli on the basis of hue (*11*). Color probably was the dimension controlling behavior in my study also, since goldfish "generalize" without error from discrimination between solid red and solid green to discrimination between red-on-white and green-on-white patterns, despite the great increase in luminous flux (*12*).

The idea that interocular transfer of color discrimination is generally superior to transfer of an equally discriminable pattern discrimination depends on further exploration with other appropriate color-pattern combinations. Final proof that two visual coding processes (specified by behavioral analysis) in fact utilize different commissural pathways requires evidence obtained either from direct surgical disruption of the pathways, or from electrophysiological recording within the neural substrate of transfer.

Use of the "conflict test" method has proved effective for analyzing interocular transfer of ability to discriminate visually. The fact that the rank order of discriminability of two visual dimensions can be reversed between eye and eye provides a tool for disentangling other dimensions of the visual world. For example, among pattern discriminations that differ along two possible dimensions, interhemispheric transfer may impose a selective bias such that one dimension transfers more readily than the other. Because the commissural systems may sometimes act as "filters," appropriate interocular generalization tests can reveal distinct coding processes which are inextricably mixed during the more familiar binocular generalization paradigms.

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Vocalization Evoked from the Optic Lobe of a Songbird

Abstract. *Vocalizations from songbirds have been evoked by electrical stimulation of the torus. These vocalizations resemble the normal alarm and scolding calls of the species. The torus of the birds appears to correspond functionally and anatomically to the mammalian central gray matter.*

The rostral central gray matter of the midbrain has been found by stimulation of both restrained (*1*) and freely moving (*2*) cats and monkeys to be important in the vocal expression of threat behavior (*3*). Damage to the central gray matter can result in partial or complete loss of emotional vocalization in cats (*4, 5*) and dogs (*6*). That it has "motivational" importance has been shown by passive avoidance of (*7*) and escape from (*8*) stimulation of the central gray matter in cats and by decreases in conditioned avoidance responses to noxious stimuli after lesions in cats (*5*) and rats (*9*). The important role of the central gray matter in emotional vocalization in mammals suggests that it might play a corresponding role in the vocalization of birds. The experiments below delineate an area in the avian midbrain from which vocalizations can be evoked by electrical stimulation. These results, together with physiological and neuroanatomical considerations, suggest that the torus semicircularis of birds corresponds to the central gray matter of mammals.

A species of the suborder Passeres ("songbirds") was chosen because this group has an advanced sound-production apparatus (*10*) and is renowned for its vocalizations. Adult male red-winged blackbirds (*Agelaius phoeniceus*) obtained from the marshes around Rochester, New York, were used. Birds were placed in a restraining device that prevented wing and leg movements from exerting pressure on the head holder. After application of a local anesthetic to the ears and crown, the birds were placed in a stereotaxic instrument in such a way that the bill could be opened and closed freely. Electrodes, 0.2 mm in diameter, were made of nichrome steel insulated with enamel and Insl-X; they were exposed only at the pointed tips. A Wyss stimulator (*11*) was used for monopolar stimulation with the indifferent electrode being a steel screw in the skull anterior to the brain. A frequency of 30 pulses per second was employed, with a biphasic pulse of 3.5-msec rising time and 7.0-msec falling time (negative phase). The electrode was lowered through the brain, and at 1-mm intervals a 500-μa current was applied for 30 seconds. When a responsive area was found, the interval was shortened to 0.1 or 0.2 mm and the vertical extent of the area was usually determined.

Vocalizations were elicited from an area beneath the optic tectum including the torus semicircularis (*12*) and the underlying gray matter (*13*). The responsive area was roughly triangular in cross section at its thickest part, ranging in vertical extent from 3.0 mm at the medial end of the torus to a fraction of a millimeter at its most lateral extent. Calls were obtained from stimulation of almost the entire anteroposterior extent of the torus, about 3.0 mm. In the torus, all of 44 points distributed along seven electrode tracks in five birds yielded vocalizations; no points tested in the torus failed to yield vocalizations. Of all points tested, thresholds were lowest (20 to 30 μa) in the center and lower border of the torus (as in Fig. 1) and increased with more ventral, medial, and lateral positions of the electrodes in the torus or outside it. In the gray matter underlying the torus,