Eye Dominance Columns from an Isogenic Double-Nasal Frog Eye

Abstract. *Removing the posterior (temporal) two-thirds of the* Xenopus *eye bud produces a remaining fragment, which becomes round and grows to a normal adult size eye. Electrophysiological and anatomical analyses showed that each of the two halves of this eye projected across the entire optic tectum in mirror image (double-nasal) fashion, and that fibers from each half-eye sorted out to form eye dominance stripes on the tectum. That both halves of the mirror-symmetric map were derived from only one animal, and from only one side of the head, rules out global markers such as right versus left and histocompatibility differences as causing the formation of these stripes.*

The lower vertebrate visual system has been a model for studies of patterning of nerve connections. The neuronal projection from the retina to the optic tectum (the retinotectal projection) is a two-dimensionally ordered map in which each point on the retina has a corresponding termination site in the tectum. Recent work on the neuroanatomy of frogs and goldfish, in which the visual system has been experimentally altered, has demonstrated that doubly innervating a single optic tectum (with two eyes) results in alternating regions of terminations from the eyes (*1*). Similar results have recently been obtained from innervating the tectum with a compound eye made up of right and left half-eyes (*2*). Thus, fibers from equivalent locations in the two retinas (or two half-retinas) project together to the same region of a single tectum, only to sort out and synapse in bands according to their eye of origin. These results have been of interest because the stripes that are formed resemble the ocular dominance columns seen in higher vertebrates (*3*). Perhaps of more importance is that these ocular dominance stripes (in lower vertebrates) may yield some new insight into the fiber-fiber and fiber-target interactions that may work together to form the orderly retinotectal projection.

The experiments performed on the formation of ocular dominance stripes in lower vertebrates have resulted in several proposed mechanisms for stripe formation. The signals or labels that form the basis of these proposed fiber segregation mechanisms range from local labels that only identify cells that are neighbors in the retina, to more global labels that mark the entire eye of origin for each fiber. Local labels would provide information concerning the proximity of the cell bodies (in the retina) to optic nerve fiber terminals in the optic tectum. Any one optic nerve fiber will have neighbors in only one retina; thus, if fibers were to sort on the basis of such local labels, segregation into ocular dominance territories would be expected. Conversely, the fibers could segregate from one another by sorting themselves on the basis of some global chemospecificity label that differs slightly for the two eyes innervating the same tectum. This label could be a left-right marker or some histocompatibility marker that varies from animal to animal. The experiments performed to date have not addressed this possibility, since they have all used the left and right eyes of the same animal or the eyes of two different animals to doubly innervate the optic tectum.

In an attempt to test this possibility, we produced isogenic duplicated eyes in *Xenopus* frogs. These were produced by removing the temporal two-thirds portion of the stage 31–32 eye bud, leaving a nasal-1/3 fragment in situ. This fragment heals to produce a normal-appearing eye in the postmetamorphic adult that maps in a mirror-image fashion to the optic tectum (*4*). That is, each locale in the optic tectum receives input from two regions of the retina, symmetrically positioned with respect to the dorsal-ventral axis of the eye (a double-nasal map) (Fig. 1). Thus, the tectum was innervated by two overlapping projections, as it is when two eyes (*1*) or two half-eyes (*2*) innervate the same tectum together. However, these two projections arise from the same embryonic eye rudiment, eliminating the possible right-left and animal-animal differences of previous experiments. If ocular dominance stripes are obtained from this preparation, then global chemical differences (right-left or animal-animal) would become unlikely to have caused stripe formation.

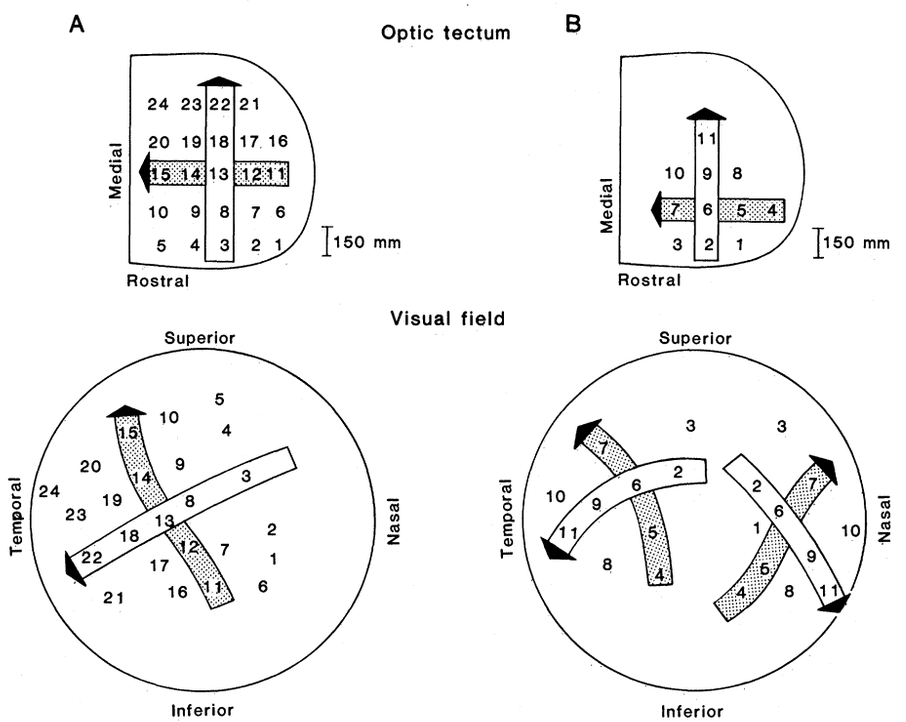

Fig. 1. (A) The retinotectal map (right eye to left tectum) of an intact control animal (sibling of the nasal-1/3 animals). Each number of the tectal outline depicts an electrode position; the corresponding number in the visual field represents the stimulus position that optimally evoked responses at the electrode tip (*10*). For each electrode position, there is one corresponding position in the visual field. The set of these positions demonstrates the one-to-one correspondence typical of the normal retinotectal map. (B) The projection from a nasal-1/3 animal. The retinotectal map shows a mirror-image duplication. For each electrode position, two regions of the visual field evoked responses at the electrode tip, as though two eyes were innervating the tectum. In these duplicated maps, one of the two responsive visual field areas was always stronger than another, but only in very rare cases was there a complete absence of response from one half of the duplicate map. This demonstrates that either (i) the electrodes are sensitive to neuronal activity as far as 100 μm distant (consistent with the receptive fields typically obtained) or (ii) the segregation of the terminals into dominance stripes is not complete, so that each location on the tectum receives some limited input from both halves of the duplicate map.

The pattern of the retinotectal projection was assayed by the typical extracellular electrophysiologic techniques. The retinotectal map built by this procedure was the typical double-nasal pattern (Fig. 1). By recording responses in the ipsilateral tectum, both halves of the map were shown to be functionally connected to the tectal cells. Ispilateral responses were mediated by the contralateral tectal cells, and were therefore present only if the contralateral connections were functional. Individual electrode positions were many times more responsive to stimulation in one half of the duplicated projection than in the other, perhaps reflecting the segregation of the terminals into ocular dominance columns; no systematic analysis of these variations was performed. The animals were then revived for later use in anatomical axon-tracing experiments.

The distribution of optic nerve fibers on the surface of the optic tectum was analyzed by autoradiography. Both control and duplicated eyes were injected with 10 to 20 μCi of tritiated proline, and the animal was allowed to survive for about 18 hours, at which time the brain was fixed (in alcohol, acetic acid, and Formalin), embedded in paraffin, and sectioned at 10 to 20 μm. The sections were dipped in NTB-2 emulsion and exposed at 4°C for 2 weeks. When the entire eye (either double nasal or normal) was labeled, the tectum displayed a uniform grain density much like that of a normal frog (three animals, Fig. 2A). To determine whether the two halves of the double-nasal map segregated from one another to form stripes, it was necessary to allow only half of the retina (one of the duplicate maps) to transport the radiolabel. A semicircular electrolytic lesion was made near the head of the optic nerve by inserting an electrode through the sclera and passing current (*5*). This procedure destroyed the retina in that region and the fibers of passage from more peripheral retina, so the only surviving axons were on the side of the eye without a lesion. By correctly positioning the lesion (using the ventral fissure as landmark) the temporal or nasal half of the retina could be selectively labeled. The size and placement of the lesions were checked after fixation by dissecting away the cornea and the lens to reveal the extent of remaining healthy retina. The radioactively labeled proline was injected immediately after the lesion was made. This procedure will be referred to as "half-labeling."

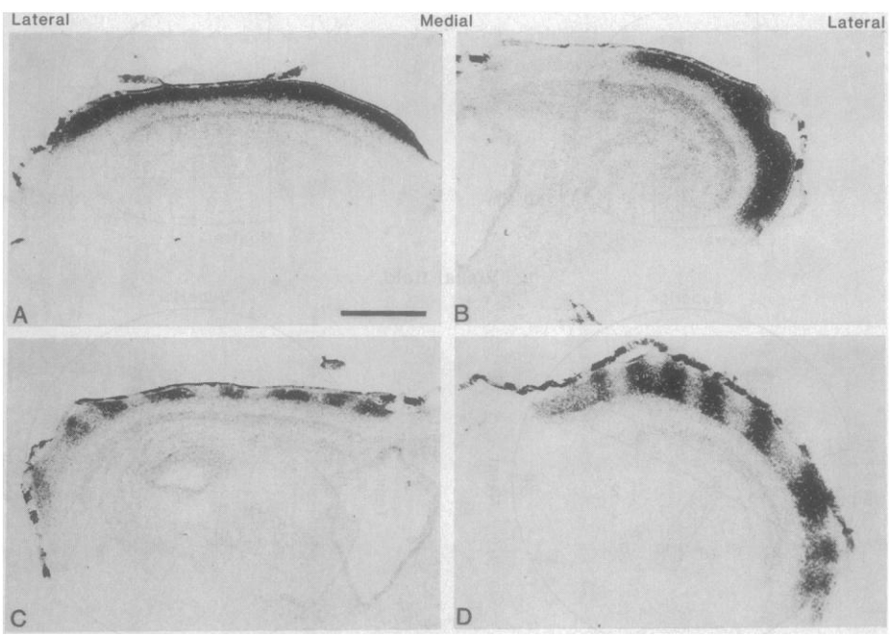

Fig. 2. Projection pattern of retinal ganglion cells in the optic tectum assayed by autoradiographic techniques. In these light-field photographs, the radioactivity (indicating the presence of optic nerve fibers) appears as black grains. (A) Injecting a nasal-1/3 (duplicated) eye produced a solid band of label over the full extent of the tectum. (B) Half-labeling a normal eye by placing a lesion in one half of the retina before injecting the isotope produced a solid band of label covering only a portion of the tectum. That portion depended on the region of the retina lesioned (nasal half-lesion pictured). (C) Half-labeling the nasal or temporal half-retina of an isogenic double-nasal (nasal-1/3) eye from the animal whose other eye is shown in (B). The clusters of the label are stripes cut in cross section. (D) A similar striped distribution of label seen in three-eyed frogs after one eye was injected with isotope. An extra eye was inserted into the right orbit of a stage 39–40 embryo, and one of the two "right" eyes received the isotope injection as an adult. Slightly different angles of section and rostro-caudal levels of the tectal cross section account for the slight differences in the shapes of the tectal cross sections, the distribution of label, and the apparent differences in the thickness of the optic neuropil. All animals were approximately 8 months beyond metamorphosis at the time of the experiment. Bar, 250 μm.

In normal frogs, half-labeling yielded a grain pattern expected from the known ordering of the retinotectal projection (nine retinas) (Fig. 2B). Temporal half-labeling (lesion in the nasal half) produced grains over the anterior tectum that stopped abruptly near the middle of the tectum; no grains were seen in the posterior tectum. Nasal half-labeling produced grains over the posterior tectum, with only light labeling in the anterior tectum, produced by the fibers of passage on their way to the posterior tectum. The exact position of the grain boundary varied slightly from animal to animal by a few hundred micrometers, reflecting both the variability in the placement of the lesions and variability in the plane of histological section. In contrast, half-labeling of animals with double-nasal retinotectal projections demonstrated that each half of the retina projected over the entire anterior-posterior extent of the tectum in agreement with the electrophysiologic evidence (five retinas). Furthermore, the grains were not uniformly distributed across the lateral-medial extent of the tectum but were instead clustered into stripes (Fig. 2). These stripes were 100 to 125 μm wide and stretched almost the entire anterior-posterior extent of the tectum as have been observed when two eyes (*1*) or two half-eyes (*2*) innervate the same tectum. The stripes were less noticeable at the extreme posterior end of the tectum, perhaps reflecting the developmental immaturity of this region.

For comparison, three-eyed frogs were made by placing an additional eye bud from a second animal immediately posterior to one of the host's eye buds at stage 39–40. After injection of the host or donor eye and autoradiographic processing, stripes like those reported in *Rana* (*1*) were observed (two animals) (Fig. 2D). The orientation and spacing of the stripes were similar to those of the isogenic double-nasal animals described above.

The presence of ocular dominance stripes in our frogs with double-nasal retinotectal projections makes it extremely unlikely that the cause of stripe formation is some global marker in the two retinas (right versus left or animal versus animal differences), because the two halves of the retina that make up the double-nasal projection are from the

same side of the same animal. Instead, the stripes are likely to be produced by some interaction that allows neighboring fibers in the retina to recognize one another in the tectum. If the fibers attempted to maintain maximum contact with their neighbors from the retina, periodic patterns, like the observed stripes, would probably result (*6*). Two good candidates for such neighbor recognition mechanisms are biochemical interactions (*7*) and neuronal activity (*8*). For example, nerve activity has been suggested to play a role in the patterning of nerve connections as a result of the observation that neighboring fibers have similar activity patterns, even in the absence of visual stimuli. If the fibers followed the rule, "fibers that fire together stay together," segregation on the basis of the eye of origin would be expected. Near neighbors in the same retina will have a much greater synchrony of firing than fibers originating from another eye but innervating the same area of the tectum. Biochemical oscillations could play a role similar to nerve activity, serving to keep near-neighboring fibers in the retina as near neighbors in the tectum. Recent experiments that block nerve activity with neurotoxin demonstrate an absence of fiber segregation into ocular dominance columns or stripes (*9*). Our experiments, taken with those that block nerve activity, implicate coincident nerve activity as a likely factor in the formation of ocular dominance stripes.

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References and Notes

1. M. I. Law and M. Constantine-Paton, *J. Neurosci.* **1**, 741 (1981); R. L. Meyer, *J. Comp. Neurol.* **183**, 883 (1979); in *Developmental Biology, Pattern Formation, Gene Regulation*, D. McMahon and C. F. Fox, Eds.(Benjamin, Menlo Park, Calif., 1975), pp. 257–275; R. L. Levine and M. Jacobson, *Brain Res.* **98**, 172 (1975).
2. J. W. Fawcett and D. J. Willshaw, *Nature (London)* **296**, 350 (1982).
3. D. H. Hubel and T. N. Wiesel, *ibid.* **221**, 747 (1969); S. Le Vay, D. H. Hubel, T. N. Wiesel, *J. Comp. Neurol.* **159**, 559 (1975); T. N. Wiesel, D. H. Hubel, D. M. K. Lam, *Brain Res.* **79**, 273 (1974).
4. C. F. Ide, B. E. Kosofsky, R. K. Hunt, *Dev. Biol.* **69**, 337 (1979); N. McDonald, "Appendix," in J. Feldman and R. M. Gaze, *J. Comp. Neurol.* **162**, 13 (1975).
5. R. L. Meyer, *J. Comp. Neurol.* **189**, 273 (1980).
6. H. Meinhardt, *Models of Biological Pattern Formation* (Academic Press, New York, 1982); N. V. Swindale, *Trends Neurosci.* **4**, 102 (1981).
7. C. Von der Malsburg, *Biol. Cybern.* **32**, 49 (1979); D. J. Willshaw and C. Von der Malsburg, *Philos. Trans. R. Soc. London Ser. B* **287**, 203 (1979).
8. D. O. Hebb, *Organization of Behavior* (Wiley, New York, 1949); G. S. Stent, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 997 (1973); D. J. Willshaw and C. Von der Malsburg, *Philos. Trans. R. Soc. London Ser. B* **194**, 431 (1976); V. A. Whitelaw and J. D. Cowan, *J. Neurosci.* **1**, 1369 (1981).
9. R. L. Meyer, *Curr. Topics Dev. Biol.* **17**, 101 (1982); *Science* **218**, 589 (1982).
10. Retinotectal mapping was accomplished by typical extracellular electrophysiologic techniques. The animals were anesthetized with Finquel (MS-222, Ayerst) and then paralyzed with a small injection of curare (tubocurarine). After limited craniotomy to expose the optic tecta, a platinum-tipped platinum-iridium microelectrode (5 Mohm before tipping) was lowered into the superficial neuropil of the optic tectum. The signals recorded by the electrode were amplified (×1000), filtered (100 Hz to 3 kHz bandpass; 60 Hz rejection), and monitored on an oscilloscope and loudspeaker. For each electrode position, the regions of visual space that would elicit activity at the electrode tip were determined through the use of an Aimark projection perimeter as a stimulus.
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Immunochemical Localization of NADP-Specific Isocitrate Dehydrogenase in *Escherichia coli*

Abstract. *The intracellular localization of isocitrate dehydrogenase was determined by immunochemical techniques with ultrathin sections of* Escherichia coli. *The thin sections, which were obtained by ultracryomicrotomy, were incubated first with antiserum specific for the enzyme and then with a protein A–gold complex. Transmission electron microscopy showed that the gold label was dispersed mainly in the cytoplasm.*

Intracellular antigenic sites have been located by electron microscopy in ultrathin sections of eukaryotic cells prepared by cryomicrotomy (*1–4*). Beesley and Adlam (*5*) reported, however, that immunolabeling when used with bacterial cryosection systems lacks specificity compared to its use with methacrylate preparations.

Tokuyasu (*6*) succeeded in using ultracryomicrotomy to prepare tissue for electron microscopy, and subsequently Painter *et al.* (*7*) used horse spleen ferritin as an electron-dense immunolabel to locate pancreatic ribonuclease by electron microscopy on frozen thin sections. Iron-dextran (Imposil) antibody conjugate was also reported to be a suitable immunolabel for transmission electron microscopy (*8*). Another electron-dense particle that has been used as an immunolabel is colloidal gold (*9, 10*) complexed with protein A, which specifically binds with the F_c portion of immunoglobin G (IgG) (*11*).

We used ultracryomicrotomy and labeling with the protein A–gold complex to investigate the localization of isocitrate dehydrogenase (E.C. 1.1.1.42) in *Escherichia coli*. This enzyme occupies a prominent position in the tricarboxylic acid cycle and has an absolute requirement for nicotinamide adenine dinucleotide phosphate (NADP). Although the enzyme has generally been assumed to be cytoplasmic, this has never been demonstrated directly.

Antibody specific for isocitrate dehydrogenase was raised in rabbits (*12*) injected with enzyme that had been purified by immunoaffinity in a column containing protein A coupled to IgG (*13*). The serum was collected, and the crude immunoglobulin fraction was precipitated with 33 percent $(NH_4)_2SO_4$. The precipitate was dissolved in 0.02*M* K_2HPO_4, *p*H 8.0, and desalted by passage through a Sephadex G-25 column equilibrated in the same buffer. The immunoglobulin was then applied to a DEAE–Affi-gel blue column equilibrated in the K_2HPO_4 solution, and the purified IgG was eluted by washing the column with the same solution. The purified IgG used in control experiments was obtained as above from serum from the same rabbits before they were immunized.

Antibodies specific for isocitrate dehydrogenase were then purified from the IgG fraction by affinity chromatography on Affi-gel 15 to which purified isocitrate dehydrogenase had been covalently bound (*14*). The IgG was applied to the column, which was then washed with 1.5*M* NaCl to remove all nonspecific IgG. The antibodies were eluted with 3*M* NaCNS, *p*H 6.6, and immediately desalted by passage through a Sephadex G-25 column equilibrated in phosphate-buffered saline at *p*H 7.2.

To determine the effect of glutaraldehyde fixative on the antigen, we incubated purified isocitrate dehydrogenase at room temperature with increasing concentrations of the aldehyde for different time periods. A sample from each tube was then tested for its capacity to agglu-