segment grew in the normal way, by the addition of cells at the retinal margin (*12*). Therefore, the translocated segment may be considered to be a polyclone derived from founder cells that had moved into the retinal rudiment from the opposite side of the presumptive forebrain. Wedge-shaped segments of pigmented retinal cells have also been observed in allophenic mice (*13*), in mosaic pigmented retina of axolotls (*14*), and in *Xenopus* in which marked cells have been grafted into the retina at embryonic stages (*15*). In those cases, too, the wedge shape of the marked polyclone was thought to show that the founder cells were initially close to the optic nerve head and that the clone grew by addition of cells at the retinal periphery. To our knowledge, however, no previous evidence has been published of reciprocal translocation of prospective retinal cells across the midline or of any contribution of cells to the retina from the opposite side of the embryo. We suggest that the translocated cells establish an incipient chiasma before the outgrowth of nerve fibers from the retina. If a similar translocation occurs in the mammalian embryo, the question arises whether the anomaly of crossing of optic axons at the chiasma found in albino mammals (*16*) may be due to an anomaly of the translocated retinal segment.

MARCUS JACOBSON
GIRO HIROSE
Department of Anatomy, College of Medicine, University of Utah, Salt Lake City 84132

References and Notes

1. G. B. Lapashov and O. G. Stroeva, *Development of the Eye* (Israel Program for Scientific Translations, Jerusalem, 1963).
2. H. Petersen, *Ergeb. Anat. Entwicklungsgesch.* **24**, 327 (1923); H. Woerdemann, *Arch. Entwicklungsmech. Org.* **116**, 220 (1929); C.-O. Jacobson, *J. Embryol. Exp. Morphol.* **7**, 1 (1959).
3. E. Manchot, *Arch. Entwicklungsmech. Org.* **116**, 689 (1929); O. Mangold, *Ergeb. Biol.* **7**, 193 (1931).
4. C. R. Stockard, *Am. J. Anat.* **15**, 253 (1913); H. B. Adelmann, *Q. Rev. Biol.* **11**, 161 and 284 (1936).
5. M. Fischberg, *Genetica* **24**, 213 (1949); *J. Embryol. Exp. Morphol.* **6**, 393 (1958).
6. G. Fankhauser, *Q. J. Exp. Biol.* **20**, 20 (1945).
7. P. D. Nieuwkoop and J. Faber, *Normal Table of* Xenopus laevis *(Daudin)* (North-Holland, Amsterdam, ed. 2, 1967).
8. R. C. Graham and M. J. Karnovsky, *J. Histochem. Cytochem.* **14**, 271 (1966).
9. I. Simpson, B. Rose, W. R. Loewenstein, *Science* **195**, 294 (1977).
10. G. Hirose and M. Jacobson, in preparation.
11. G. Fankhauser and T. Humphrey, *Proc. Natl. Acad. Sci. U.S.A.* **29**, 341 (1943).
12. K. Straznicky and R. M. Gaze, *J. Embryol. Exp. Morphol.* **26**, 67 (1971); M. Jacobson, *Brain Res.* **103**, 541 (1976).
13. B. Mintz and S. Sanyal, *Genetics* **64** (Suppl.), 43 (1970).
14. E. van Deusen, *Dev. Biol.* **34**, 135 (1973).
15. R. K. Hunt, personal communication.
16. R. W. Guillery, *Brain Res.* **14**, 739 (1969); *Sci. Am.* **230** (No. 5), 44 (1974).
17. Supported by grant BNS-13910A from the National Science Foundation.

14 July 1978; revised 5 September 1978

Eye-Specific Termination Bands in Tecta of Three-Eyed Frogs

Abstract. *An extra eye primordium was implanted into the forebrain region of embryonic* Rana pipiens. *During development both normal and supernumerary optic tracts terminated within a single, previously uninnervated tectal lobe. Autoradiographic tracing of either the normal or supernumerary eye's projection revealed distinct, eye-specific bands of radioactivity running rostrocaudally through the dually innervated tectum. Interactions among axons of retinal ganglion cells, possibly mediated through tectal neurons, must be invoked to explain this stereotyped disruption of the normally continuous retinal termination pattern.*

During development, ganglion cell axons from the vertebrate retina terminate in a highly ordered pattern that maps the retina, and thus visual space, within the tectal lobes of the midbrain. The stereotypy of this projection and the ability of ganglion cells in lower vertebrates to reestablish the pattern during axonal regeneration suggest that highly specific pre- to postsynaptic cell interactions may be involved (*1*).

This theory of rigid retinal ganglion cell to tectal cell chemoaffinities has recently been challenged. Expansion and compression of the visual field projection following retinal or tectal ablations indicate a more plastic system (*2–5*). In a modification of the chemoaffinity hypothesis, it is proposed that graded affinities between cells of one part of the retinal field for one part of the tectal field serve to orient the projection along topographic axes. Competition between retinal ganglion cell axons could then organize the presynaptic terminals over the available postsynaptic space (*4*, *6*, *7*).

We present here anatomical results in a preparation designed to test the importance of optic fiber to optic fiber interactions during the development of the anuran retinotectal map. The three-eyed *Rana pipiens* used in these experiments have two complete retinal projections innervating a single previously uninnervated (that is, naive) tectal lobe from early development.

The animals were produced by implanting either a right or left eye primordium in the forebrain region of embryos with minimal dorsoventral rotation of the transplant and without disturbing the two normal optic evaginations. All operations were performed between Shumway stages 17 to 19, before retinal axons penetrate the brain but after the retinal axes have been determined (*8*). In

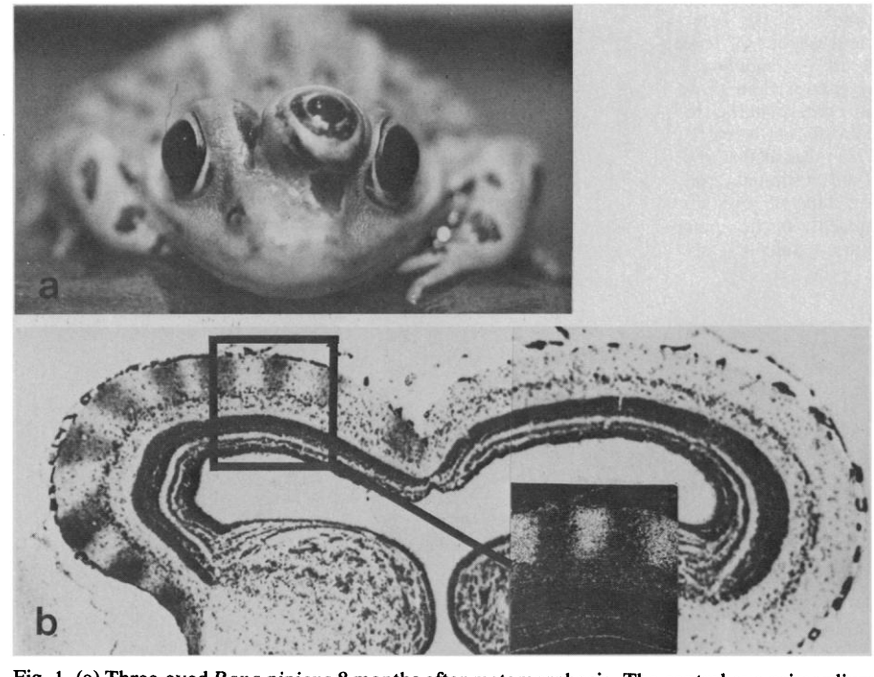

Fig. 1. (a) Three-eyed *Rana pipiens* 8 months after metamorphosis. The central eye primordium was implanted at Shumway stage 17 from a similarly staged donor. The supernumerary eye has externally normal dimensions, but lacks a pupillary response. (b) Autoradiographic distributions of grain densities in the optic tectum of a 3-month postmetamorphic three-eyed frog after injection of 10 μCi of [^{3}H]proline into the vitreous body of the normal eye. (Inset) Dark-field enlargment showing the pronounced segregation of labeled and unlabeled regions of the tectal neuropil.

0036-8075/78/1110-0639$00.50/0

most cases the supernumerary eye primordium developed into an externally normal eye (Fig. 1a). Ten animals with externally normal supernumerary eyes were used in this study. These ranged in age from Taylor and Kollros (T & K) (*9*) stage V to 3 months after metamorphosis. Two to 5 days before animals were killed, [^{3}H]proline (5 to 20 μCi in 0.5 to 2 μl) (specific activity, 60 to 115 mCi/mmole, New England Nuclear) was injected into the vitreous body of either the supernumerary eye (eight animals) or the dually innervating normal eye (two animals). In all cases, upon dissection, three distinct optic nerves were found to penetrate the brain at the level of the diencephalon. Tectal projections from the injected eyes were subsequently traced autoradiographically in transverse paraffin sections (*10*).

Our results demonstrate that the optic tract of neither the normal nor the supernumerary eye was able to form a continuous projection along the dually innervated tectal surface. Instead, the termination pattern within the superficial tectal layers appeared as labeled clumps of neuropil. This innervation pattern was consistent in all ten animals examined. In all cases discontinuities in grain density produced labeled and unlabeled bands that alternate across the mediolateral extent of the tectum (Fig. 1b).

Grain counts suggest that few, if any, axons from the labeled retina extend into the unlabeled bands. In Fig. 2 grain densities within the superficial tectal layers (level A) are compared to those at corresponding deep positions in efferent layer 7 (level B). This animal is a T & K stage XIII tadpole whose supernumerary eye had been injected 7 days before death. Since retinal ganglion cell axons do not terminate within level B (*11*), grain counts at this level were assumed to be (local) tissue background. All counts were performed at equivalent depths within the tectum at the approximate center of the unlabeled and labeled bands. Grain densities in the superficial unlabeled eye bands are not significantly higher than the tissue background. Similar grain counts were obtained from the dually innervated tectum of a postmetamorphic frog whose normal eye had been injected.

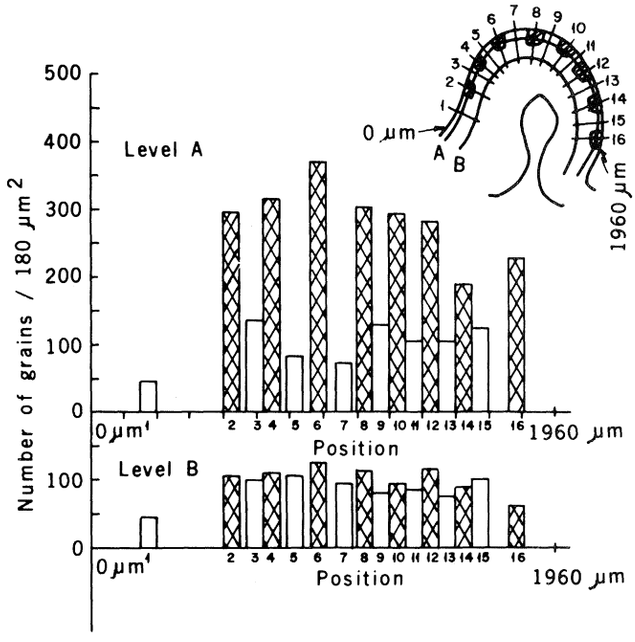

Fig. 2. Histograms comparing grain counts of the band and interband regions of the superficial tectal neuropil (*A*) and in corresponding deep tectal layer 7 (*B*). This animal was killed at T & K stage XIII 7 days after 10 μCi of [^{3}H]-proline was injected into the vitreous body of the supernumerary eye. Grain counts in the interband regions of level A are not significantly greater than those at corresponding locations in level B. This suggests that few, if any, terminals from the labeled eye are present in the interband regions.

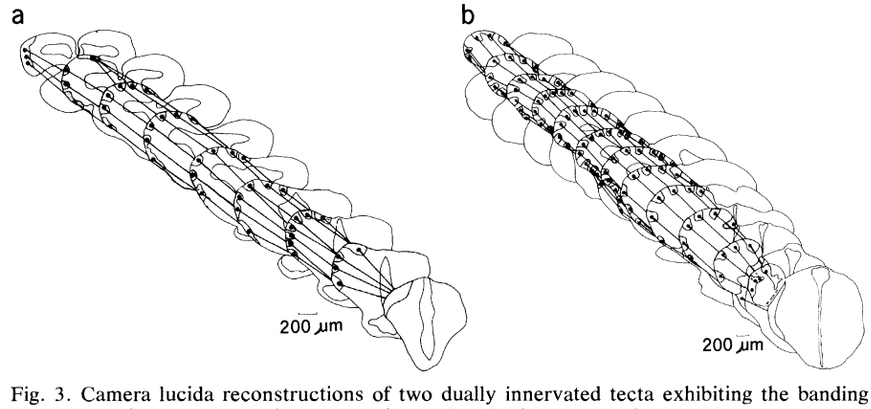

Fig. 3. Camera lucida reconstructions of two dually innervated tecta exhibiting the banding pattern. Sections were traced at 100-μm intervals. (a) The pattern of a T & K stage V tadpole after an intraocular injection of [^{3}H]proline into its supernumerary eye. The more caudal sections pass through relatively undifferentiated tectal regions and contain diffuse label in the lateral regions of the tectum. (b) The pattern of a 3-month postmetamorphic frog whose normal dually innervating eye had been injected with [^{3}H]proline.

Figure 3a shows a typical reconstruction of the midbrain in a T & K stage V tadpole whose supernumerary eye was injected with [^{3}H]proline. A reconstruction from the normal eye which shared the dually innervated tectum of a postmetamorphic frog is illustrated in Fig. 3b. In both cases the labeled bands are cross sections of continuous slabs that run throughout the rostrocaudal extent of the tectum with relatively few fusions or divisions (*12*). The pattern is strikingly similar to the ocular dominance columns observed by using similar techniques in the superior colliculus and striate cortical layer IV of the monkey and cat (*13*).

The posterior sections of Fig. 3a show no evidence of clumping. This is the region of the tectum that was undergoing differentiation at the time of death. Continuous labeling in newly generated tectal regions was seen in all of the tadpoles. This absence of bands in the caudal tectum of tadpoles is the only qualitative difference in termination pattern with age. However, there appears to be a gradual increase in the number of labeled supernumerary eye bands with increasing developmental age. For example, at comparable tectal levels T & K stage V tadpoles had four to five labeled bands while a T & K stage XIII animal had ten labeled bands.

Initial electrophysiological recordings in the superficial layers of these tecta have shown that (i) activity can be visually elicited by stimulating either the normal or supernumerary eye and (ii) the four quadrants of the visual field are projected to the appropriate four quadrants of the tectal lobe by at least the normal eye (*14*). In addition, observations on postmetamorphic three-eyed animals suggest that the normal eye's projection to the dually innervated tectum is functional. The frogs are capable of localizing prey in the portions of the visual field subserved only by the dually innervated tectum.

In most previous experiments in which

dually innervated tectal lobes have been produced, all or part of the projection normally destined for the ipsilateral tectal lobe has been diverted onto the contralateral one in adult animals (*2, 5, 15–18*). Thus, Levine and Jacobson demonstrated an interdigitating termination pattern in parts of dually innervated goldfish tecta that appears somewhat similar to the one we report (*16*). However, in their preparation distinctly labeled tracts could be seen in bands for the unlabeled eye when high-power microscopy was used. Electrophysiological maps of these goldfish were highly variable, with large binocular areas dispersed among single eye zones. The regular alternating eye bands predicted by the autoradiography were not seen electrophysiologically. A number of other investigators, however, using longer survival times but essentially the same preparation, have obtained electrophysiological evidence of eye-specific terminal clumping (*2, 17*). Schmidt has also demonstrated autoradiographically that clumping occurs in the same regions of the tectum where it was detected electrophysiologically (*5*). Similar clumping has also been observed following unilateral tectal damage in fetal (and neonatal) rodents (*7, 19*).

Our results differ from these previous observations most notably in the consistency of the banding pattern from animal to animal and in the fact that it is continuous across the entire tectal surface. Recent evidence in goldfish suggests that tectal "specificity" markers have a strong dependence on previous innervation patterns (*5*). Consequently, the fact that ours is a developing, rather than an established system may account for the consistency and completeness of the bands we observe.

All previous experiments on dually innervated anuran tecta have indicated a uniform termination pattern (*18*). Dual innervation has generally been produced by unitectal ablation in adult anurans (*18*). Sharma implanted a supernumerary eye above the midbrain in *Rana pipiens* embryos. A few electrode penetrations made in the tecta of four animals showed no evidence of eye-specific clumping (*20*). Hunt and Jacobson reported electrophysiological studies of three-eyed *Xenopus laevis* (*21, 22*). Their maps indicated that each eye projects continuously across the tectum in apparent disregard of the other eye's presence (*23*).

It should be possible to determine whether the anatomically observed partitioning of tectal space in our three-eyed *Rana pipiens* is equally pronounced electrophysiologically. However, the anatomical results presented here indicate that during development ganglion cell axons dramatically alter their normal continuous termination pattern in the presence of a projection from a second retina. Banding may result if afferent fibers from different retinas grow to the tectum in separate bundles and maintain this separation during termination. This seems unlikely in view of the relatively uniform distribution of labeling in the differentiating regions of tadpole tecta (Fig. 3a). Alternatively, innervation of tectal cells by one retina may increase the probability that these cells will accept subsequent innervation from the same eye.

The mechanism of banding is uncertain at present. However, the three-eyed frog preparations provide a unique opportunity to identify and to manipulate the underlying cellular interactions in order to understand their role in the normal development of the retinotectal system. Furthermore, the parallels between our experimentally induced banding and the ocular dominance columns in cat and monkey suggest that the latter termination pattern may be established through interactions which are basic to a wide variety of neuronal projections.

MARTHA CONSTANTINE-PATON
MARGARET I. LAW

Department of Biology,
Princeton University,
Princeton, New Jersey 08540

References and Notes

1. M. Jacobson and R. M. Gaze, *Exp. Neurol.* **13**, 418 (1965); R. L. Meyer and R. W. Sperry, *ibid.* **40**, 525 (1973).
2. M. G. Yoon, *ibid.* **33**, 395 (1971).
3. J. E. Cook and T. J. Horder, *J. Physiol. (London)* **241**, 89P (1974); J. D. Feldman, M. J. Keating, R. M. Gaze, *Nature (London)* **253**, 445 (1975); J. T. Schmidt and S. S. Easter, *Exp. Brain. Res.* **31**, 155 (1978); S. Udin, *J. Comp. Neurol.* **173**, 561 (1977).
4. R. D. Lund and J. S. Lund, *J. Comp. Neurol.* **169**, 133 (1976).
5. J. T. Schmidt, *ibid.* **177**, 279 (1978).
6. M. C. Prestige and D. J. Willshaw, *Proc. R. Soc. London Ser. B* **190**, 77 (1975); R. A. Hope B. J. Hammond, R. M. Gaze, *ibid.* **194**, 447 (1976); C. Von Der Malburg and D. J. Willshaw, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5176 (1977).
7. K. So, G. E. Schneider, D. O. Frost, *Brain Res.* **147**, 277 (1978).
8. W. Shumway, *Anat. Rec.* **83**, 309 (1940); S. C. Sharma and J. G. Hollyfield, *J. Comp. Neurol.* **155**, 395 (1974).
9. A. C. Taylor and J. J. Kollros, *Anat. Rec.* **94**, 7 (1946).
10. M. Constantine-Paton and R. Capranica, *J. Comp. Neurol.* **170**, 17 (1976).
11. F. Scalia, in *Frog Neurobiology: A Handbook*, R. Llinás and W. Precht, Eds. (Springer-Verlag, New York, 1976), p. 386; G. Székely and G. Lázár, in *ibid.*, p. 407.
12. It is possible that the rostrocaudal direction of these slabs reflects the pathways through which bundles of fibers from each eye enter the tectum [H. D. Potter, *J. Comp. Neurol.* **144**, 269 (1972)]. However, both the low grain density in the unlabeled regions and the large transverse dimensions of the labeled regions (about 250 μm) implies that the terminal arborizations are also largely restricted to the bands.
13. D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **195**, 215 (1968); *J. Comp. Neurol.* **146**, 421 (1972); T. N. Wiesel, D. H. Hubel, D. M. K. Lam, *Brain Res.* **79**, 273 (1974); A. M. Graybiel, *ibid.* **96**, 1 (1975); P. Rakic, *Phil. Trans. R. Soc. London Ser. B* **278**, 245 (1977).
14. We have not performed the fine-grained electrophysiological mapping which will be necessary to determine whether discontinuities are present in the visual-field representation of either the normal or the supernumerary eye.
15. S. C. Sharma, *Exp. Neurol.* **41**, 661 (1973).
16. R. L. Levine and M. Jacobson, *Brain Res.* **98**, 172 (1975).
17. J. R. Cronly-Dillon and B. Glaizner, *Nature (London)* **251**, 505 (1974); R. L. Meyer, *Developmental Biology: Pattern Formation and Gene Regulation* (Benjamin, New York, 1975), pp. 257–275.
18. D. Ingle, *Science* **181**, 1053 (1973); E. Kicliter, L. J. Misantone, D. J. Stelzner, *Brain Res.* **82**, 293 (1974); L. J. Misantone and D. J. Stelzner, *Exp. Neurol.* **45**, 364 (1974).
19. B. F. Miller and R. D. Lund, *Brain Res.* **91**, 119 (1975); K. F. So and G. E. Schneider, *Anat. Rec.* **184**, 535 (1976).
20. S. C. Sharma, *Nature (London) New Biol.* **238**, 286 (1972).
21. M. Jacobson and R. K. Hunt, *Sci. Am.*, **228**, 26 (February 1973).
22. R. K. Hunt and M. Jacobson, *Curr. Top. Dev. Biol.* **8**, 203 (1974).
23. It is possibly significant that the embryonic surgery used by Hunt and Jacobson (*22*) to generate three-eyed *Xenopus* was different from ours. Our attempts to produce three-eyed *Xenopus* by the procedure that was successful in *Rana* resulted in complete resorption of the supernumerary eye primordia in approximately 60 embryos.
24. We thank P. Ferrari Eastman for technical assistance. Supported by NIH research award EYO 1872, NIH service award T32 GM07312 to the Training Program in Cellular and Molecular Biology, and a Whitehall Foundation grant to the Princeton Biology Department.

17 April 1978; revised 1 August 1978

Drosophila Egg Chambers Develop to Mature Eggs When Cultured in vivo

Abstract. *Egg chambers were injected into the abdomen of adult* Drosophila. *When cultured in this manner, even the earliest detectable developmental stage developed into fully mature eggs. Both isolated egg chambers and those still associated with ovarian structures developed equally well. Maturation occurred within host flies of both sexes in the absence of any hormone treatment.*

Since Clancy and Beadle's (*1*) original publication, several investigators have transplanted *Drosophila* ovaries. Frequently, larval ovaries have been transplanted into larval hosts. Bodenstein (*2*), moreover, has observed that ovaries from both late pupal and newly emerged flies are able to develop after transplantation into adult hosts. King and Bodenstein (*3*) used ovarian trans-

0036-8075/78/1110-0641$00.50/0