mins, brewer's yeast, and zinc oxide ranked the diets as 3 > 2 > 1. These nearly isocaloric diets (≅ 4 kcal/g) were composed of the following (grams of ingredient per kilogram of diet 1, 2, and 3, respectively): casein, vitamin-free test, 200.0, 280.0, and 350.0; cornstarch, 260.8, 192.6, and 157.6; sucrose, 260.8, 192.6, and 157.6; corn oil (Mazola), 135.0, 135.0, and 135.0; nonnutritive fiber, 56.4, 51.9, and 40.0; salt mixture (U.S. Pharmacopoeia XIV), 60.0, 102.0, and 110.0; vitamin mixture, 23.0, 39.0, and 42.2; brewer's yeast, 4.0, 6.9, and 7.4; zinc oxide, 0.05, 0.08, and 0.10. The B10C3F$_1$ control mice were fed diet 1, ≅ 40 g/week [7 g daily on Monday through Thursday and 12 g on Friday (≅ 160 kcal/week)]. For the first month of restriction the B10C3F$_1$ mice received diet 2, about 29 g/week [6 g daily on Monday through Wednesday and 11 g on Friday (115 kcal/week)]; thereafter they received diet 3, about 22 g/week [6 g on Mondays and Wednesdays and 10 g on Fridays (90 kcal/week)]. The B6 control mice were fed diet 1, about 27 g/week [4 g daily on Monday through Thursday and 11 g on Friday (110 kcal/week)]. For the first month of restriction the B6 mice received diet 2, about 23 g/week [5 g daily on Monday through Wednesday and 8 g on Friday (90 kcal/week)]; thereafter they received diet 3, about 20 g/week [6 g on Monday and Wednesday and 8 g on Friday (80 kcal/week)]. Casein, fiber, salt mixture, vitamin mixture, and brewer's yeast were purchased from ICN Pharmaceuticals (Cleveland, Ohio); the other ingredients were bought locally.

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## Alterations in Precision of the Crossed Retinotectal Projection During Chick Development

Abstract. *Chick embryos received partial eye lesions. Examination of the embryos early in development showed the partial retina projecting across the entire tectum. At later stages the partial retina projected only to the appropriate portion of the tectum. The results suggest that the retina initially projects diffusely to the tectum and that the topographically aberrant projections are eliminated during subsequent development.*

Retinal ganglion cells in the vertebrate eye project to visual nuclei in the brain, such as the optic tectum, in an orderly topographical fashion. A major problem in developmental neurobiology is to determine how these orderly projections develop. Since highly ordered optic pathways and terminations have been observed in a number of adult animals (*1*), it has been suggested that optic axons grow from the eye in an orderly fashion and maintain this order to the terminal nuclei (*2*). Another possibility, however, is that optic axons grow to the terminal nuclei in a diffuse manner and interactions in the terminal field somehow refine the projection into the ordered pattern of the adult. The two possibilities have not been adequately tested.

Crossland *et al.* (*3*) addressed this problem in the developing retinotectal pathway of the chick embryo. Partial ablation of an optic cup during the third day of incubation resulted in the absence of a retinal projection to a portion of the contralateral tectum at 18 days. The region of the tectum lacking innervation corresponded topographically to the ablated region of the eye. Since projections from the remaining portion of the eye were not found in inappropriate tectal regions, it was concluded that at least quadrantal topographic order was maintained in the developing retinotectal system. This experiment did not, however, rule out the possibility that transient projections of broad distribution or not conforming to the topographic order were present before day 18 and subsequently disappeared. In the present experiment, chick embryos with partial retinal ablations were examined at earlier developmental stages to determine whether the projection pattern of retinal ganglion cell axons to the tectum is initially broad and subsequently refined to the mature pattern or whether the projection forms a tightly ordered map from the beginning.

Fertilized White Leghorn chicken eggs were incubated in a forced-draft incubator at 37°C. On the third day of incubation the eggs were removed from the shell and transferred to an egg culture chamber (*4*). At this time the superior or inferior nasal quadrant of the right eye of experimental embryos was burned to the point of bleaching with a fine-tipped electrocautery. Care was taken not to make a hole in the eye, since this results in developmental retardation of the entire retinotectal projection (*5*). The embryos were maintained in a forced-draft tissue culture incubator at 37°C, 95 percent humidity, and 1 percent $CO_2$ for the duration of the experiment.

Horseradish peroxidase (HRP) was used as an anterograde tracer to map the retinotectal projection. On day 10, 12, 14, or 16 of development the embryos were injected in the right eye with 0.5 to 2 μl of 30 percent HRP (Boehringer Mannheim) in 2 percent dimethyl sulfoxide and saline. After 12 hours the embryos were perfused through the left ventricle with 0.5 percent glutaraldehyde in phosphate buffer followed by 2 percent buffered glutaraldehyde. The brains were removed and cut into 40-μm serial sections. The sections were reacted with tetramethylbenzidine and hydrogen peroxide and counterstained with neutral red (*6*), and the tecta were reconstructed with a drawing tube attached to a microscope. The blue reaction product was plotted to show the distribution of the retinal projection. Normal embryos without lesions were treated in a similar manner to serve as controls. There were 52 experimental embryos and 43 control embryos.

The distribution of HRP reaction product revealed a heavy projection from the injected eye to the contralateral tectum. In 10-day control embryos this projection covered all but about the caudal quarter of the tectum (Figs. 1A and 2A). In 90 percent of the 12-day control embryos and all the 14- and 16-day control embryos the projection covered the entire tectal surface. This pattern is consistent with the results of previous developmental studies (*5*, *7*).

In experimental embryos killed on day

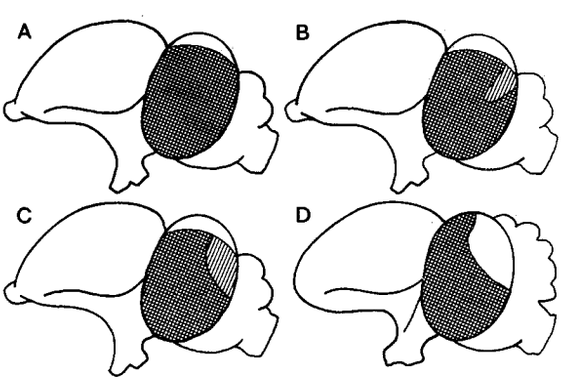


Fig. 1. Diagrams showing retinal innervation of the tectum, as reconstructed from stained serial sections. Cross-hatching indicates normal retinal input; hatching, reduced retinal input; and white, no retinal input. (A) Innervation in a normal embryo on embryonic day 10. (B) Reduced retinal input to the caudal-inferior tectum in a 10-day embryo given a superior nasal retinal lesion on day 8. (C) Similarly reduced input resulting from a lesion on day 3. (D) Lack of retinal projection to the caudal-inferior tectum in a 16-day embryo given a superior nasal lesion on day 3.


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10 or 12 the heaviest projections extended to portions of the tectum corresponding topographically to the intact portion of the retina. However, a sparse projection was also found in the tectal quadrant corresponding to the ablated retinal quadrant (Figs. 1C and 2B). Embryos that received a superior nasal retinal lesion during early development had a substantial projection to the rostral and caudal-superior tectum. Scattered reaction product was found in the caudal-inferior tectum, which corresponded to the ablated portion of the eye. Similarly, in embryos with inferior nasal retinal lesions, all of the tectum received a heavy retinal projection except the caudal-superior region, which received a sparse projection. However, embryos killed on days 14 or 16 had no identifiable retinal projection to tectal areas corresponding to the retinal quadrant ablated on day 3 (Figs. 1D and 2C). In embryos with superior nasal retinal lesions, no projection was found in the caudal-inferior tectum, and in embryos with inferior nasal retinal lesions, no projection was found in the caudal-superior tectum. The remainder of the tectum appeared to have normal retinal innervation, presumably arising from the intact portions of the retina.

These results demonstrate that a transient retinal projection is present in topographically inappropriate regions of the tectum after partial retinal ablations made early in development. This aberrant projection appears to be eliminated between 12 and 14 days of incubation.

To determine whether the presence of retinal fibers in inappropriate tectal regions at 10 and 12 days represents a "sprouting" phenomenon of neighboring intact axons induced by the early lesions, partial retinal lesions were made in embryos later in development. On day 8, the superior or inferior nasal retinal quadrants were ablated with a fine ophthalmic scalpel inserted into the eye at the limbus. The eyes were injected with HRP 0, 12, 24, or 48 hours later, and on day 10 or 12 the brains were removed and processed for HRP histochemistry. Operated eyes were processed histologically to verify placement of the retinal lesions.

The results were similar to those obtained with retinal ablations performed during early development. In embryos with superior nasal lesions there was a sparse projection to the caudal-inferior tectum, and in embryos with inferior nasal lesions there was a sparse projection to the caudal-superior tectum (Fig. 1B). The sparsely innervated region of the tectum was usually smaller than that seen after the early lesions, but this may have been a result of the relative size of the lesions.

This study suggests that during the early development of the chick visual system, at least some areas of the retina project diffusely to inappropriate regions of the tectum. The results from the ablations made just before an HRP injection suggest that these diffuse projections are present normally and are not lesion-induced. The retinal projection appears to be refined during subsequent development, leaving a tightly ordered retinotopic map on the tectum. Previous examinations of stained fibers on the developing tectum suggested an apparent meandering of fibers, which then became progressively more organized as development proceeded (*5*). It is difficult to reconcile the apparent diffuseness in the developing pathway with the theory that an orderly ingrowth of optic axons to the tectum is the sole mechanism for generating the orderly projection. It seems necessary to invoke mechanisms involving elimination of the inappropriate projections. It should be pointed out that the bulk of the retinotectal projection does appear to be organized, at least at a quadrantal level. This order might be accounted for by passive mechanisms, such as mechanical guidance (*8*).

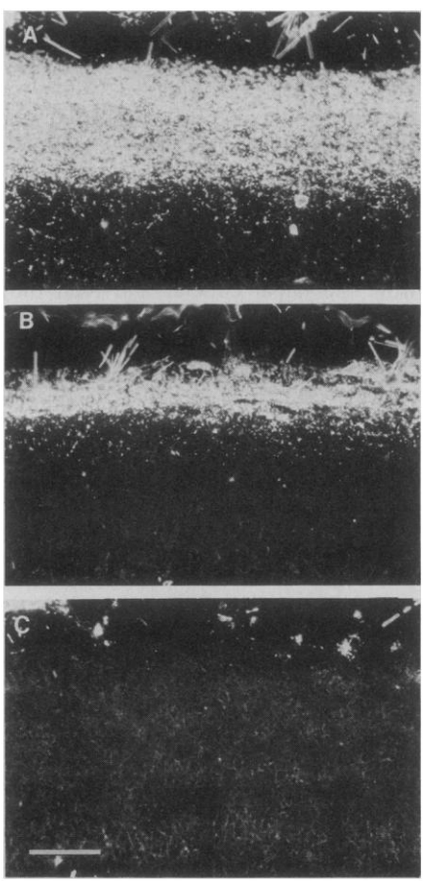


Fig. 2. Dark-field micrographs of HRP (white grains) in the stratum opticum of the caudal-inferior tectum. (A) Retinal projection in a 10-day normal embryo. (B) Reduced retinal input in a 10-day embryo given a superior nasal lesion in the contralateral eye on day 3. (C) Absence of retinal input in a 14-day embryo given a superior nasal lesion in the contralateral eye on day 3. HRP was injected into the eye contralateral to the tecta shown in these micrographs. Scale bar, 50 μm.

Loss of the aberrant projections during development may be due to withdrawal of axons or collaterals or to elimination of certain ganglion cells. A significant number of chick retinal ganglion cells die during normal development (*9*), and there is a close temporal correlation between this cell death and the loss of the aberrant projections.

Other investigators have reported refinements and corrections in the developing visual system (*10, 11*). These changes primarily involve the relative distribution of axons from the two eyes. During development of the rat visual system, for example, the retina projects across most of the ipsilateral superior colliculus, and during early postnatal life most of this ipsilateral projection disappears in favor of the contralateral projection (*11*).

In many studies, a deficit induced during development has resulted in retention of a projection normally lost. Lund and Lund (*12*) found that removal of one eye in the neonatal rat caused retention of the expanded ipsilateral retinotectal projection. This suggests that competition is important in determining the final distribution of a projection. In a study similar to the one reported here, Frost and Schneider (*13*) found that partial retinal lesions in neonatal hamsters resulted in an expanded retinotectal projection in adults. This too may have been a result of retention of aberrant projections present at birth. It is not clear why the inappropriate projections were not retained after partial retinal lesions in the chick. The expanded retinotectal projection present in rats during development may represent collaterals of normally projecting axons, whereas in chicks the projection may comprise axons that project completely aberrantly.

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## Peptide and Steroid Regulation of Muscle Degeneration in an Insect

Abstract. *Two types of cell death occur in the intersegmental muscles of the giant silkmoth* Antheraea polyphemus. *The first results from a slow atrophy of the fibers, and the second is a rapid, programmed dissolution of the muscle. Both types appear to be mediated by endocrine factors. The slow atrophy is brought about by the decline in the steroid molting hormone 20-hydroxyecdysone and can be prevented with exogenous steroid. The rapid degeneration is triggered by the peptide eclosion hormone, but the sensitivity of the muscle to the peptide depends on the history of exposure of the muscle to 20-hydroxyecdysone.*

Cell death can serve as a required step in the normal differentiation of tissues (*1*) or as the final event in a pathological condition (*2*). The terminal events in cell death have been examined in detail, but few studies have elucidated the factors responsible for its initiation. We now describe a system in which a set of muscles remains healthy, goes through a wasting atrophy, or shows a programmed degeneration, depending on endocrine treatment.

The intersegmental muscles (ISM) of the giant silkmoth *Antheraea polyphemus* have served as a model system for the exploration of events associated with cell death (*3*). These muscles span the fourth through sixth abdominal segments and are essentially the only skeletal muscles in the diapausing pupa. They assist the adult in the emergence (eclosion) from the pupal cuticle, and then they undergo rapid degeneration (*4*).

The degeneration of the ISM is associated with some event that occurs around the time of adult eclosion. Lockshin (*5*) showed that when abdomens were isolated from *A. polyphemus* just before emergence of the adults, the muscles were routinely preserved. By contrast, isolation at or after eclosion resulted in normal breakdown of the ISM. He concluded that a signal from the anterior end of the animal around the time of eclosion was the initiating factor in muscle death. Using muscle weight as an index (*6*), we compared the fate of the ISM in control animals and isolated abdomens (Fig. 1A). The loss of muscle weight in control animals was precipitous; contractility was lost by 20 hours (*7*), and the muscles were reduced to flimsy bags of membranes by 25 hours. In the isolated abdomens, the muscles were preserved well past their normal time of death. The ISM from the isolated preparations underwent a gradual atrophy, as indicated by a loss in dry weight and a reduction in the diameter of individual muscle fibers, but they remained contractile until about 6 days after isolation. This slow atrophy appears to be a continuation of an exponential decline in muscle mass that normally begins 2 days before eclosion (*8*).

Adult eclosion in the silkmoths is triggered by a peptide hormone (*9*) released from centers in the head shortly before eclosion. Isolation of the abdomen before this time would prevent contact of the ISM with this peptide. Injection of eclosion hormone (*10*) into abdomens isolated on the day of eclosion, but before the release of the endogenous hormone, resulted in the rapid breakdown of the muscles (Fig. 1A). This degeneration was qualitatively and quantitatively

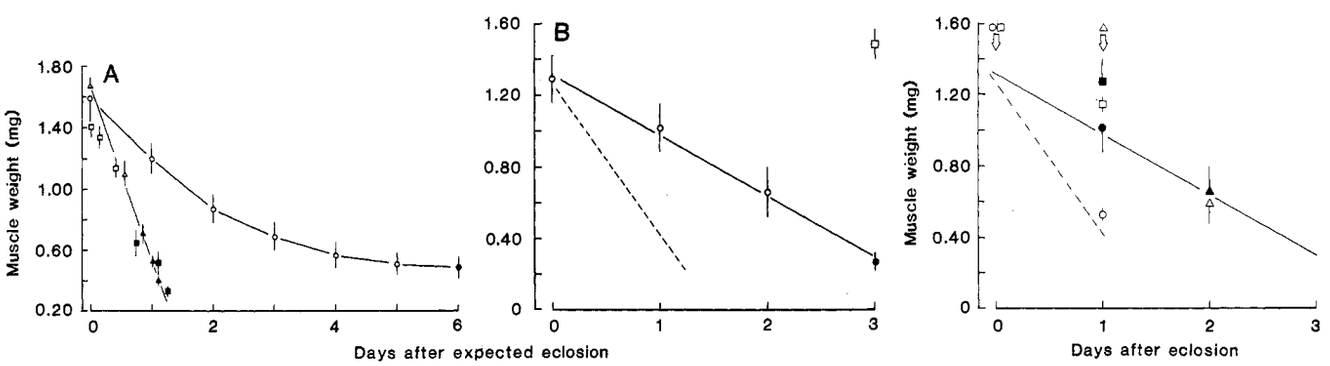


Fig. 1 (left). Effects of endocrine treatments on the degeneration of the intersegmental muscles as quantified by the loss of dry weight. Open symbols represent contractile muscles and closed symbols, noncontractile muscles. (A) Role of eclosion hormone. (□, ■) Intact, naturally emerging adults; (○, ●) abdomens isolated from animals on the day of emergence, but before release of eclosion hormone; and (△, ▲) abdomens injected with 1 unit of eclosion hormone after isolation. A least-squares regression line is drawn for isolated abdomens injected with eclosion hormone. (B) Role of ecdysteroids. (○, ●) Animals receiving an injection of 25 μg of 20-hydroxyecdysone on the day preceding eclosion (day 16). (□) Animals continuously infused with 20-hydroxyecdysone (30 ng/g-hour) beginning 2 days before eclosion (day 15) and continuing until 3 days after expected emergence. The dashed line represents the rate of ISM degeneration found for control animals in (A). Values are means ± S.E. for four or five animals. Fig. 2 (right). Treatment with 20-hydroxyecdysone renders the ISM insensitive to eclosion hormone. The dashed line shows the time course of muscle loss from intact animals or isolated abdomens injected with eclosion hormone (data from Fig. 1A); the solid line shows the weight loss for animals injected with 25 μg of 20-hydroxyecdysone on the day preceding eclosion (data from Fig. 1B). Closed symbols indicate animals injected with Ringer solution; open symbols indicate animals treated with 1 unit of eclosion hormone. (○, ●) Abdomens isolated before eclosion. (□, ■, △, ▲) Intact insects treated with 25 μg of 20-hydroxyecdysone on the day before eclosion (all insects eclosed on schedule). Arrows with associated symbols indicate the time at which various groups were injected with either eclosion hormone or Ringer solution. Values are means ± S.E. for four or five animals.

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