Return of vision in larval eyes exchanged between Amblystoma punctatum and the cave salamander, Typhlotriton spelaeus

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Right eyes were exchanged and normally oriented between larvae of 13 Typhlotriton spelaeus and 13 Amblystoma punctatum. At operation the Typhlotriton ranged from 31 mm. to 92 mm. in length, a wide larval age span. The Amblystoma larvae at operation varied from 28 mm. to 34 mm. in length. Reciprocal hosts were usually sacrificed at the same time to compare grafts with normal donor left eyes. Some Amblystoma hosts were approaching metamorphosis when sacrificed. Return of vision was demonstrated in 5 Amblystoma and 4 Typhlotriton transplants including both grafts in 3 reciprocal pairs with good retinas connected with the brain by regenerated optic nerves. Vision failed to return in 3 Typhlotriton and 5 Amblystoma eye grafts because of the lack of optic nerve connections. Some hosts, difficult to test, gave no visual responses although the grafts possessed good retinas with slender optic nerves leading to the brain. By some selective mechanism the optic nerve fibers from the retinas of these two different species of salamanders reach the proper centers of the optic tectum, even though in one of them, the cave salamander, the eye normally undergoes degeneration in the late life of the host.

In a series of studies on the eyes of cave salamanders, a detailed study of the morphology and function of the eyes of larval and adult Typhlotriton spelaeus has recently been recorded by me in which a review is given of what little is known about these animals. Although the bodies of these salamanders are light flesh-colored, their eyes are small and pigmented.

Vision is present in the larvae over a wide age span although some of the oldest ones still with good retinas and optic nerve connections with the brain demonstrate poor vision or none at all. As they become older they appear to depend less upon vision than upon other senses. Although a few adults still have some vision until their eyes become covered by the lids and skin invades the cornea, it is usually after metamorphosis when the eyes undergo marked degeneration and lose connection with the brain. Degeneration spreads first over the retina in the ganglion and receptor cell layers as the optic nerve degenerates.

It has been recorded by me in a group of experiments that the retina and other structures survive and appear histologically normal when the larval Typhlotriton spelaeus eye is excised and reimplanted in its normal position. The optic nerve regenerates and normal vision is re-estab-
lished when the nerve reaches the brain. This is quite remarkable for an eye which normally later undergoes degeneration. It is similar to that found in transplanted larval eyes of other species of salamanders9-5 where the neural retina also survives, thus preserving the ganglion cell fibers from which the new optic nerve can immediately regenerate. The eye of these larvae can also regenerate a lost lens from the dorsal iris the same as has been shown in other salamanders.7-11

If the neural retina is lost, as in the case of a transplanted eye of adult salamanders, it is replaced by regeneration from the surviving retina pigment cells.12-14 Because of the fate of the eyes of Typhlotriton spelaeus after metamorphosis, it is doubtful that the retinal pigment in the eyes of the adult of this species possesses this regenerative capacity.

The following experiments were devised to determine how successfully the eyes could be exchanged between these cave animals and other species of salamanders.

**Materials and methods**

The Typhlotriton spelaeus larvae used in these experiments were collected from caves in the Ozark regions of the state of Missouri by two friends, Dr. O'Dell W. Henson and Dr. Gary Meyers, to whom I wish to express my thanks for making these rare salamanders available for my experiments now in progress on cave-dwelling urodèles. The Amblystoma punctatum larvae which are part of these studies were reared in the laboratory from eggs obtained from ponds in the vicinity of New Haven, Conn.

It was found previously1 that dechlorination of the water was not required for the cave salamanders. All of the experimental animals survived in perfect health when kept in the laboratory at room temperatures of 70 to 80°F. in separate wire-covered finger bowl aquariums containing fresh tap water changed every other day. Although the commonly cultured white worms, Enchytraeus, were occasionally used, the chief supply of food was small pieces of beef liver fed three times a week. This is an ideal diet for all species of salamanders which I have kept in my laboratory for many years.

The operations were performed under a compound dissecting microscope by means of fine needle-pointed forceps and iridectomy scissors sharpened under the microscope. The larvae were anesthetized in a 0.02 per cent solution of MS 222 (tricane methanesulphonate, Sandoz) containing the commonly used amphibian salt solution of Holtfreter. During the operation a reciprocal pair of larvae, one Typhlotriton and one Amblystoma, was laid on cotton in this solution so that the right side of the head and the eye of each individual was not covered by fluid. An incision was made around each eye in the skin near the cornea and then the tissue attachments to the orbit were severed to separate the eye from the orbit. When this was completed the two eyes were exchanged, arranged in normal orientation, and pressed gently into the orbit.

The deeper Amblystoma orbit gave ample room for the smaller Typhlotriton graft (Fig. 1). However, the larger Amblystoma eye in the more shallow Typhlotriton orbit always bulged prominently from the side of the host's head (Figs. 3 and 13). Within a few minutes the transplanted eye adhered tightly to the tissues in the new orbit. There was very little or no bleeding. Each larva was then gently submerged in fresh cool tap water in its labeled fingerbowl aquarium. Air was bubbled through the water of the aquarium for a half hour, during which time the larva began swimming normally again. None of the eyes became detached. The host skin united with the cut edges of the graft, and healing with return of circulation to the graft was complete within a few days.

Two Amblystoma hosts died by accident on the forty-fourth day after operation. All others survived until they were finally sacrificed and fixed in Zenker's solution. All heads were prepared in serial sections by the usual paraffin methods and stained in hematoxylin and Erythrocin for histologic studies.

The principal test for vision employed a rotating drum, 23 cm. in diameter, lined with vertical alternating black and white stripes 2 cm. in width. The speed of the drum was usually one revolution in 27 seconds. In an otherwise dark room, the source of light was a 200 watt Macbeth lamp with a diffusion glass daylight filter. Conditions are ideal for beginning the tests if the animal is in the center of the dish and quiet before the drum begins rotating, clockwise for a left eye, counterclockwise for a right eye.15

In salamanders which show good vision the head turns in the direction of the moving drum until the fore part of the body also turns in a C-shaped position. The animal often reorients and repeats this performance and occasionally follows by walking in the direction in which the drum is moving. Sometimes as the tests are continued the older cave salamanders become excited and swim rapidly about, even jumping out of the aquarium if it is not covered. This often happens.
with cave salamanders which respond only occasionally to the vision tests.

Another method employed a moving lure, a dark bead impaled on the end of a white wire. The Amblystoma larvae usually followed the lure as it was moved around the outside of the aquarium. The cave salamanders with good vision occasionally followed the lure, especially when it was moved above the surface of the water of the aquarium. These were the larvae which often came to the surface of the water to await the insertion of small pieces of liver at feeding time.

Experimental results

_Ptygnotriton spelaeus_ eyes transplanted to _Amblystoma punctatum_. There were 13 pairs of larvae in which the right eyes were exchanged between _Ptygnotriton spelaeus_ and _Amblystoma punctatum_ (Table I). A reciprocal pair is shown in Figs. 1 and 3, 55 days after operation. At the time of operation the Ptygnotriton larvae varied from 31 mm. to 90 mm. in length and the Amblystoma ranged from 26 mm. to 34 mm. in length. The larvae of this species metamorphoses between 40 and 50 mm. in length. All grafts were normally oriented and healed well in place. The smaller Ptygnotriton eye fitted well in the larger Amblystoma orbit (Fig. 1) while the larger Amblystoma eye bulged prominently from the surface of the head as it lay in the shallow new orbit (Figs. 3, 4).

All the Amblystoma hosts with the exception of Cases 1 and 2 grew 10 mm. or more in length during the period of the experiments. Several of them were attaining their maximum larval growth. In fact, Case 5, 40 mm. in length at the time of sacrifice, was entering metamorphosis. Its body was becoming darkly pigmented and the gills were very short, 54 days after operation.

Proof of return of vision was obtained in 4 Ptygnotriton grafts (Cases 4, 5, 6, and 13, Table I). It may also have been present in the graft of Case 10. Two of the Amblystoma hosts (Cases 9 and 11) with good grafts were found dead on the forty-fourth day. The remaining 11 hosts were sacrificed 17 to 55 days after operation.

Since the reciprocal pairs of larvae were sacrificed at the same time in most cases, the size of the right transplanted eye can
Table I. Comparison of eye and lens size in grafted and normal control eyes of Typhlotriton based on number of 10 μ sections passing through structures. Length of animal in millimeters.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Length in millimeters</th>
<th>Days after operation</th>
<th>Diameter in microns</th>
<th>Vision proved</th>
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<td>A. punctatum right eye graft on T. spelaeus host</td>
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*Graft resorbed.  
†Animals died on forty-fourth day.

be compared with the normal left eye of the appropriate donor (Table I). In only a few cases was there much difference between them.

The structures of the grafted Typhlotriton eye on the Amblystoma hosts survived well including the retina and the lens. There were 3 cases (2, 3, and 12) with good retinas, but the optic nerves did not reach the brain. In 3 cases (1, 7, and 8) there were very slender optic nerves connecting well-developed retinas with the brain but no vision responses were obtained in these animals through the graft. The remaining 4 cases (4, 5, 6, and 13, Table I) had good retinas connected by optic nerves to the brain and all showed vision in the transplanted eyes.

Fig. 1 shows the gross appearance of a right transplanted Typhlotriton eye on an Amblystoma larval host. This larva was a late Amblystoma 44 mm. in length at the time it was sacrificed, 55 days after operation. The histologic appearance of the eye is shown in Fig. 2. Its structures are well defined. The retina is slightly thicker than that in the normal eye of a Typhlotriton larva. There was vision in this eye which was connected with the brain by an optic nerve. This eye was 740 μ in diameter and is slightly larger when compared with the left control eye (700 μ in diameter) of the Typhlotriton donor (Case 4, Table I). In other cases (Table I) the Typhlotriton graft is slightly smaller. However, this does not necessarily indicate that it is an effect of the new environment, for it has already been pointed out† that the Typhlotriton eyes in a single larva may differ in size.

Fig. 4 gives the gross appearance of a 40 mm. Amblystoma larva with a small, darkly pigmented Typhlotriton eye graft 55 days after operation. Its histologic appearance is shown in Fig. 5. It compares well with the normal control left eye of the Typhlotriton donor (Case 13, Fig. 7). There was a good optic nerve connecting it with the Amblystoma brain, indicated by an arrow in Fig. 6. The other larger left optic nerve from the large normal left eye of the Amblystoma host is also shown. Vision was demonstrated in this eye (Fig. 5) as well as in the donor control (Fig. 7). There was only a slight difference in the size of these two eyes and their lenses (Table I).

Fig. 8 also shows the histologic appear-
spelaeus and Amblystoma punctatum larvae between which right eyes were exchanged. Size is and records of vision are also given

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Length in millimeters</th>
<th>Days after operation</th>
<th>Diameter in microns</th>
<th>Vision proved</th>
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Fig. 4. Gross appearance of a 40 mm. Amblystoma larva (Case 13) with a transplanted right Typhlotriton eye, 55 days after operation. Vision was proved in this eye as well as in the reciprocal graft (Figs. 13 and 14). (×4.5.)

Fig. 5. Photomicrograph of the retina of the functional Typhlotriton graft shown in Fig. 4. (×65.)

Fig. 6. Photomicrograph showing optic nerve, indicated by arrow, of Typhlotriton graft (Fig. 5) entering brain of salamander illustrated in Fig. 4. (×65.)

ance of a transplanted right Typhlotriton eye on a 40 mm. Amblystoma larva 55 days after operation (Case 5, Table 1). Although the structures of the eye are crowded and contracted in the section, the features of the retina and other elements of the eye are complete. There is a contrast, however, when compared with the normal control
left eye of the Typhlotriton donor (Fig. 9). The Amblystoma host for this Typhlotriton graft (Fig. 8) was beginning metamorphosis at the time the animal was sacrificed. This may be associated with the histologic appearance of the eye.

There was excellent vision in the eye which was connected with the brain by a good optic nerve indicated by an arrow in Fig. 10. The large optic nerve from the normal left Amblystoma eye can be seen entering the brain at this level. It may be said that at the time of sacrifice the normal control eye (Fig. 9) also showed good vision in its 81.5 mm. Typhlotriton donor.

Fig. 11 (Case 10, Table I) gives the histologic appearance of another transplanted Typhlotriton right eye on a 37 mm. Amblystoma larva 49 days after operation. The eye is well developed. Except for a thinning and a loss of a few cells in the ganglion layer, the retina was still well defined. There was a slender optic nerve connected with the brain. It was not possible to test vision in this larva for there was an air bubble trapped in the abdominal cavity which kept the animal floating ventral side up at the surface of the water during much of the time.

The retina of another Typhlotriton graft on a 37 mm. Amblystoma host is shown in Fig. 12 (Case 12) 49 days after operation. The ganglion cell layer is thin in some areas. The outer reticular zone was lost. The rod and cone cells in most places have been lost. There is only a short optic nerve stump protruding from the eye. Vision tests had already indicated that the animal was blind in this eye.

On the whole the Typhlotriton eye survived well on the Amblystoma host. There was no incompatibility or host response to foreign tissue. In 3 cases, 2, 4, and 8, it was noticed that the host skin was invading the periphery of the cornea. However, this is common after metamorphosis in Typhlotriton adults.

Amblystoma punctatum eyes transplanted to Typhlotriton spelaeus. This is the other group of the reciprocal pairs of
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Fig. 10. Photomicrograph showing optic nerve, indicated by arrow, of Typhlotriton graft (Fig. 8). (×65.)

Fig. 11. Photomicrograph of Typhlotriton graft on a 37 mm. Amblystoma (Case 10) 49 days after operation. (×65.)

Fig. 12. Photomicrograph of partially degenerated retina of graft from an old Typhlotriton donor (Case 12), 49 days after operation. (×130.)

larvae between which the eyes were exchanged (Table I). It therefore consists of 13 Typhlotriton larval hosts, each with a right eye transplant which came from the Amblystoma hosts just described. The Typhlotriton larvae ranged from 31 mm. to 90 mm. in length, a wide spread in larval age. One Typhlotriton larva (Case 1) was observed for 157 days and during that time it grew from 38 mm. to 70 mm. in length. The larva which was sacrificed 62 days after operation grew from 31 mm. to 39 mm. in length. Unfortunately their transplanted right eyes resorbed, but these data give some idea of the rate at which well-fed Typhlotriton spelaeus larvae grow.

The remaining 11 Typhlotriton hosts (Table I) were preserved from 49 to 55 days after operation. During this time the older larvae (Cases 3, 4, 11, 12, and 13) did not increase in size. Other older larvae (Cases 5, 6, 7, 8, 9, and 10) increased only between 1 mm. and 2 mm. in length. It appears from these data that in late larval life these salamanders grow very slowly.

The technical procedures in the operation and treatment of the hosts were the same as those described for the preceding group. The grafts were also normally oriented and, although they were large for the shallow Typhlotriton orbit, and always bulged from the side of the head (Figs. 3 and 4), they were never lost by detachment.

Return of vision was demonstrated in 5 of the Amblystoma grafts (Table I) and in 3 of these cases (5, 6, and 13) the reciprocal Typhlotriton grafts in these pairs also demonstrated a return of vision. The Amblystoma grafts survived very well in all cases except the two (Cases 1 and 2) already mentioned where they resorbed.

There were 3 Typhlotriton hosts (Cases 8, 11, and 12, Table I) in which no vision responses were obtained through the Amblystoma transplants. Histologic examination showed that in each of these eyes there were injuries to the central portions of the retina and that no optic nerves left the eye. These eyes were definitely smaller than their normal left eye controls on the donors.
There were two larvae (Cases 7 and 9, Table I) in which there was a beautiful large Amblystoma graft, but these Typhlotriton hosts also gave no visual responses through the transplanted eyes. Histologic examination revealed that the optic nerve in each case was only a short stump attached to the eye. However, the retinas in these cases appeared the same as in the grafts which showed good vision.

Figs. 3 and 13 show the gross appearance of the larger Amblystoma right eye on Typhlotriton hosts, 76 mm. (Case 4) and 90 mm. (Case 13), respectively, in length, 55 days after operation. The size of these grafts and their lenses compare well with that of the normal left control eye of the Amblystoma donor (Table I). There is no great reduction in size of the graft.

The graft shown in Fig. 3 (Case 4) had the appearance of a normal Amblystoma eye with a good optic nerve connecting it with the brain. However, no convincing visual response was obtained. As has been noted before, large Typhlotriton larvae are often quite restless and difficult to test for vision. Perhaps much of the time they are not dependent upon vision.

The other older Typhlotriton larva (Case 13) shown in Fig. 13 demonstrated good visual response through the Amblystoma graft. The histologic appearance of the retina is shown in Fig. 14. Except for a loss of a few ganglion cells in a normal single layer, the retina has the same appearance of a normal Amblystoma larval eye. It has been pointed out before that an Amblystoma larval eye showing function often loses a few ganglion cells after transplantation. The slender optic nerve of this eye (Fig. 14) is indicated by an arrow in Fig. 15. This is one of the reciprocal pairs (Case 13, Table I) in which return of vision was demonstrated in the two exchanged eyes.

There was another typical large Typhlotriton larval host (Case 5, Table I), 81.5 mm. in length, which possessed a large normal-appearing Amblystoma graft 54 days after operation. The normal control Amblystoma donor eye measured 1,180 μ in diameter with a 500 μ lens. This Ambly-
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Fig. 16. Photomicrograph of Amblystoma eye on 81.5 mm. Typhlotriton (Case 5) 54 days after operation. Vision returned in this eye as in reciprocal graft (Fig. 8). (x65.)

Fig. 17. Photomicrograph of optic nerve, indicated by arrow, from Amblystoma graft entering Typhlotriton brain. (x65.)

Fig. 18. Photomicrograph of Amblystoma eye on a 62 mm. Typhlotriton (Case 6) 54 days after operation. Vision returned in this graft. (x65.)

Amblystoma graft measured 1,200 μ in diameter with a 490 μ lens. Therefore, in growth this graft proceeded at the rate it would have if it had been left on the donor.

Its histologic appearance is quite normal and is shown in Fig. 16. Its optic nerve although slender reached the brain and is indicated by an arrow in Fig. 17. This Typhlotriton host is another one of a pair in which each of the exchanged eyes demonstrated a return of vision (Table I). The reciprocal host of this pair (Case 5) is the Amblystoma donor which was entering metamorphosis at the time this pair was sacrificed.

Case 6 (Table I) is another Typhlotriton larva, 62 mm. in length, of a reciprocal pair in which the exchanged eyes had a return of vision 54 days after operation. Fig. 18 shows the retina of the Amblystoma graft on the Typhlotriton host. It has the same appearance as the one shown in Fig. 14 which also demonstrated a return of vision. There was a good optic nerve from this eye connecting it with the brain as in the case shown in Fig. 15.

From the results of these experiments there is proof that the eyes can be exchanged successfully between the larvae of these two different species in which their eyes after metamorphosis have a different fate.

Discussion

It was shown in a previous study of normal larvae and adults that vision can be demonstrated in many of these cave salamanders although sometimes it is not as good in some animals as it is in others. Also it was pointed out that in some individuals only one eye gives evidence of vision in the tests.

However, in this group all of the Typhlotriton donors of grafts, which were also the recipients of the Amblystoma eyes, had shown good visual responses in both eyes before operation. Therefore, there were no Typhlotriton eyes which were already non-functional at the time they were transplanted. It can therefore be assumed that their histologic appearance was the same as the normal control at that time.
When the histologic appearance of these grafts were later compared with the normal controls, it was quite evident that the structure and size of the transplanted eye in most cases held up well on the host of the new species. This can be said also in the reciprocal experiments.

The studies of these grafts terminated while the hosts were still in the late larval stage. Insofar as the Amblystoma eye is concerned with the preservation of its retina and regeneration of its optic nerve, there was already evidence that if the foreign host tolerated this eye there was a good chance of its success even if the new host metamorphosed. However, the Typhlotriton hosts were still in the late larval stage at the termination of the experiments. Therefore, it is not known what would have happened to the Amblystoma graft if the Typhlotriton host had metamorphosed, a time when its own normal left eye is subject to degeneration. There were not enough salamanders to test this experiment, but when more material is available the fate of similar eye grafts on them will be studied.

The Amblystoma hosts for the Typhlotriton eyes were also in the late larval stage when the experiments terminated. One of the reasons for terminating the experiments at that time was because of the fact that already 2 valuable hosts of the 13 animals had died on the forty-fourth day (Table I). This is not uncommon when operated larvae approach metamorphosis.

As already mentioned, there was one Amblystoma host (Case 5, Table I, and Fig. 11), however, which was beginning metamorphosis. Histologic sections showed that the host skin was invading the cornea just as it does in the normal Typhlotriton eye after metamorphosis. The eyelids had not yet developed. It would have been interesting to have followed the progress of this Typhlotriton eye on the Amblystoma host for sometime after metamorphosis. In this way one can test the effect of metamorphosis of a host of a different species, especially if it can also be compared with what happened to the normal control eye of the Typhlotriton donor when it metamorphoses. Such experiments will be carried out as soon as new material becomes available.

I have already repeatedly shown that the eyes of salamanders can be successfully exchanged between different species with a return of vision when connected with the new brain. The present experiments again point to the well-established fact that the optic nerve fibers of the grafted salamander eye selectively find the proper brain centers of the new brain which must be similarly organized to restore vision. What is interesting here is that the Amblystoma eye, which never normally degenerates, functions so well when connected with an optic tectum normally associated with an eye whose retina and optic nerve eventually degenerate. Since nothing is known about the optic tectum of these cave salamanders, a study of this area of visual mechanism is in progress.

Summary

1. Right eyes were exchanged and normally oriented between 13 Typhlotriton spelaeus and 13 Amblystoma punctatum larvae. At operation the Typhlotriton ranged from 31 mm. to 92 mm. in length, a wide larval age span. The Amblystoma larvae at operation varied from 28 mm. to 34 mm. in length.

2. Reciprocal hosts were usually sacrificed at the same time to facilitate comparison of the grafts with the normal control donor left eyes. Some Amblystoma hosts were approaching metamorphosis at the termination of the experiments.

3. All grafts healed well in place even though the implanted Amblystoma eye was large for the shallow orbit of the Typhlotriton host. Two of them eventually resorbed but all others survived.

4. Return of vision was demonstrated in 5 Amblystoma and 4 Typhlotriton transplants including both grafts in 3 reciprocal pairs with good retinas connected with the brain by regenerated optic nerves.
Vision failed to return in 3 Typhlotriton and 5 Amblystoma grafts because of the lack of optic nerve connections with the brain. Some hosts, for various reasons, were difficult to test, although the transplanted eyes possessed good retinas and slender optic nerves leading to the brain.

6. By some selective mechanism the optic nerve fibers from the retinas of these two different species of salamanders reach the proper centers of the optic tectum, even though in one of them, the cave salamander, the eye normally undergoes degeneration in the late life of the host.

REFERENCES