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OPTIC NERVE REGENERATION WITH RETURN OF VISION IN ANURANS

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THE RECOVERY of normal vision after regeneration of the optic nerve demonstrated in adult urodele amphibians (8, 9) requires that the ingrowing optic fibers reëstablish in the brain centers discriminative functional associations which are differentially suited to the diverse retinal points from which the optic fibers arise. If the relationships between retinal field and brain centers formed in regeneration were disorderly or undifferentiated, normal vision involving discrete perception of small objects and their accurate localization in space would be impossible.

Conceivably, the central reflex relations as reëstablished after regeneration might not be suitably arranged at first, but become properly adjusted only later through experience by a process of trial and error or other means of functional adaptation. Or the adequacy of the functional effect might somehow operate during the process of regeneration to regulate the formation of appropriate central connections. Both these theoretical possibilities, however, have been ruled out in the case of the newt, *Triturus viridescens* (8), in which it has been found that the restoration of normal vision after optic nerve regeneration is quite independent of functional adaptation.

When severance of the optic nerve in this animal is combined with 180 degree rotation of the eyeball on its optic axis, visual perception after recovery is systematically reversed about the optic axis corresponding to the rotated position of the retinal field. Reversed optokinetic reactions, erroneous spatial localization of small objects, and other clear indices of reversed vision are displayed consistently and without later adjustment, just as in animals in which the eyeball has been rotated with the optic nerve left intact (7). Thus in *Triturus* reëstablishment of reflex relations in the visual centers is apparently predetermined in an orderly manner by growth factors regardless of the suitability of the functional effect for the animal.

Exactly how linkages between retina and brain centers are systematically restored by growth processes remains to be demonstrated. The work on *Triturus* suggested certain possible interpretations, however, and because of their important bearing on problems of broader significance concerning the developmental differentiation and integrative action of the nervous system it became strongly desirable to make sure that these results were not due merely to peculiarities of this one species.

The present paper deals accordingly with an extension of the previous experiments on the newt to several species of the distantly related and, so far as the visual system is concerned, more highly developed anuran amphibians, the frogs and toads. Included also are the results of some attempts at further analysis of the problem; namely, the effects on vision of localized lesions placed in the optic lobes of the brain before and after optic nerve regeneration.

PROCEDURE AND MATERIALS

Plan of the experiments. The general procedure was similar to that used previously in the case of *Triturus*. Severance of the optic nerve was combined with 180 degree rotation of the eyeball on its optic axis. With eye rotation the character of visual perception after regeneration of nerve connections between retina and brain centers might be (i) normal, (ii) reversed about the optic axis corresponding to the reversal of the retinal field, or (iii) randomly blurred. If the recovered vision turned out to be normal in quality, despite the reversed position of the retinal field, it would be strong indication that function is of primary importance in regulating establishment of the central connections. Recovery of reversed vision, on the other hand, would show that the original retino-central relations are systematically restored in a predetermined manner regardless of functional effect. If visual perception on recovery should prove to be neither normal nor systematically reversed but instead a blurred confusion, it would indicate that redistribution and termination of the regenerating fibers is disorderly and nonselective, as in peripheral nerve regeneration (14).

The optic nerve was purposely pulled and teased apart in a rough manner in all cases rather than cut cleanly in order to prevent any neat approximation of the ends of individual fibers. All operations were performed under ether anesthesia with aid of a dissecting microscope with magnification of 21 times. After operation the tadpoles were kept at room temperature in 7-liter aquaria, and the frogs and toads in moist terraria of the same size.

Animals. Six species from three different families of anurans (*Bufo terrestris, Hyla cinerea, H. crucifer, H. squirella, Rana clamitans,* and *R. pipiens*) were used in the experiments.[1] All the animals were gathered in northeastern Florida. The specimens of *R. clamitans* were undergoing metamorphosis when brought into the laboratory and were operated upon shortly after. All the other adult animals had attained full size at the time they were gathered during their respective breeding seasons. The tadpoles were operated on in mid-larval stages. Although not identified with certainty, they were very probably all *H. crucifer*. In general the differences in the results obtained on different species were not of sufficient importance for the essential problems concerned to warrant burdening the reader throughout with the species name of each animal mentioned. Hence only the species and numbers included under each main treatment will be indicated and the species name of individual cases will be stated only where the results gave reason to believe that there might exist some significant species difference.

Criteria of vision. The ability to localize small objects in space was the principal index of visual perception used in testing recovery in the adult animals. The accuracy with which frogs, particularly the tree frogs, gauge distance and direction in leaping for prey is quite remarkable (3, 5). *H. cinerea* was frequently observed in the course of this study to capture with a single leap houseflies walking at a distance of 35 cm., a comparatively easy feat in view of some reports. This is mentioned only to furnish some indication of the efficiency of the anuran visual system. No attempt was made in the present experiments to determine the limits of such performances. The experimental tests were generally made at distances between 5 and 15 cm. Discrete localization of small objects in different sectors of the visual field furnishes of course an excellent index of the functional properties or "local signs" of different retinal areas.

In the tadpoles the optokinetic response to rotation of the visual field served as the chief criterion of visual function. Although perhaps not indicative of so great a degree of specialization in retino-central associations as is the spatial localization of small objects, the optokinetic reaction is nevertheless dependent on a certain systematic differentiation of central reflex relations. Therefore, its recovery after optic nerve regeneration, like the recovery of spatial localization, requires an orderly reëstablishment of specific functional linkages between periphery and central nervous system which was the main concern of the present experiments.

[1] For aid in identification of the animals acknowledgment is due Charles M. Bogert of The American Museum of Natural History.

Experiments on Tadpoles

The regenerative capacities of anurans being less great than those of urodeles, it was not certain at the start of the experiments to what extent visual function might be recovered, if at all, after section of the optic nerve. The experiments were therefore begun on tadpole stages in which chances of recovery would be greater than in adults.

Operations. In ten control cases (5 unilateral and 5 bilateral) in which the eye was left in normal position, the optic nerve with its sheaths was broken with jewelers' forceps. The stumps of the broken nerve floated rather freely in the fluid of the orbit and although some attempt was made to bring the ends close together before coagulation occurred, the broken ends in the majority of cases remained separated by a distance greater than the diameter of the nerve. Seventeen experimental cases were also prepared (7 unilateral and 10 bilateral) in which all ocular muscles were severed and the eyeball was rotated on its optic axis through 180 degrees. Several days later the nerve of the rotated eye was sectioned as in the control cases. A dorsal approach through a longitudinal incision over the eye was used both in sectioning the nerve and in rotating the eyeball. The unoperated eye of the unilateral cases in both the control and experimental groups was excised.

Recovery. During the first week after operation no optokinetic reactions could be elicited in any of the animals. The first definite signs of recovery appeared on the average 13 days after nerve section but the recovery intervals varied in different cases from 11 to 23 days. One unilateral control case never recovered vision and two bilateral experimental cases recovered vision on only one side. Histological examination revealed that in these three exceptional instances the regenerating optic nerve had not succeeded in reaching the chiasma. Surgical readjustment of the eyeball, which had slipped out of its intended position, was necessary in two experimental cases.

Tests of vision. The tadpoles in water in a fingerbowl were placed on a stationary platform inside a revolving upright cylindrical drum 31 cm. in diameter with opaque vertical black and white stripes on the inner wall. The stripes varied randomly in width from 2 to 7 cm. The optokinetic reaction was found to be readily and consistently elicited with this apparatus. After recovery was well established the animals were tested regularly over a five-day period and at weekly intervals thereafter. The 9 control cases and 16 experimental cases in which vision was successfully recovered displayed good optokinetic responses conforming with the following descriptions.

Results with unrotated eye. Reactions of the bilateral control cases after recovery were quite like those of normal animals. The optokinetic response, consisting of an alternate beating of the tail with the strong beats in one direction, turned the head and body in that direction in which the visual field was revolving. Sometimes the animals turned in small circles in a stationary position and at other times they swam in larger arcs and circles, always in the direction of drum rotation.

The responses of the unilateral control cases were like those of normal animals from which one eye had been excised. Reactions when drum rotation was toward the blind side were normal but those with drum rotation toward the seeing side consisted usually of only a slow sustained flexion of the tail not strong or sudden enough to cause any movement of the head and body.

Results with rotated eye. The reactions of the tadpoles with rotated eyes after recovery were essentially like those of normal and control animals except that the direction of the responses was reversed. Instead of moving the head in the same direction in which the visual field moved, the animals turned in the opposite direction. The unilateral cases responded normally toward the blind side and made only a slow, sustained tail flexion toward the seeing side just as did the unilateral control animals, but the direction of drum rotation which elicited these responses was the opposite from that which was effective in evoking the same responses in the control group.

Reversed vision was also indicated in these animals by spontaneous circus locomotion. The bilateral cases swam in circles either clockwise or counterclockwise depending on how they happened to start. The unilateral cases circled with the blind side toward the center of the arc or circle. This tendency to swim in circles was comparable to that shown by *Triturus* after rotation of the eye (7). Both the reversed optokinetic responses and the spontaneous circling movements had been displayed in similar form by the bilateral cases during the few days immediately following eye rotation prior to optic nerve section. The results show that in these anuran larvae just as in adult *Triturus* the central reflex relations are recovered in orderly form and their reëstablishment is strictly determined by anatomical factors regardless of functional suitability.

Effect of experience. The tadpoles with rotated eyes were kept at least 1 month after recovery; four cases were retained nearly 2 months by which time the forelimbs had emerged. The reversed optokinetic reactions and circus locomotion persisted in all cases, and in no instance was any correction of the reversed responses noted.

Experiments on Adult Frogs and Toads

Operations. In 8 control cases (all bilateral) the optic nerves were sectioned without disturbance of the eyeball or ocular muscles. In 19 experimental cases (all unilateral) severance of the optic nerve was accompanied by rotation of the eyeball. The optic nerve was sectioned with jewelers' forceps through an incision in the roof of the mouth. The inner nerve sheath was completely severed in all cases, but in most animals, at least a connecting strand of the dural sheath was left intact. The eyeball was rotated by first severing all attachments to surrounding structures except the blood vessels and the optic nerve. This was done through two incisions, one in the roof of the mouth, the other around the outside of the cornea. The globe was then grasped by the stumps of the ocular muscles and rotated anterodorsally on its optic axis through 180 degrees. Some of the blood vessels were inevitably broken in the course of rotation, but a sufficient number remained to maintain circulation through the iris. The degree of rotation was estimated by distinct landmarks in the iris and pupil which varied in the different species. After it had been adjusted, the eyeball was allowed to set and heal in its new position after which the nerve of the rotated eye was sectioned as described. The appearance of the eye about 6 weeks after its rotation in four representative cases is illustrated in Fig. 1.

Vision in one eye is not easily tested in the presence of vision on the opposite side in anurans due to binocular overlap of the visual fields. Hence the contralateral optic nerve of the experimental group was sectioned either 3 days before rotating the eye (6 cases) or about 25 days after (13 cases). Thereafter the contralateral nerve was sectioned once or twice again after intervals of about 28 days whenever it became apparent that vision had been recovered in the unrotated eye.

Recovery. Functional regeneration of the optic nerve, though not so consistently successful as in the adult urodele, did occur readily in the great majority of these adult anurans. The first indications of recovery of vision began to appear on the average about 25 days after nerve section. The recovery period varied in different cases from 21 days to about 33 days. In two of the experimental cases the rotated eye became necrotic and was sloughed off. Two other experimental cases failed to recover vision in the rotated eye and two of the control cases recovered vision on only one side. The eye in these latter four instances retained a healthy external appearance, but microscopic examination revealed that regeneration of the optic nerve had been defective. Except for a very fine strand of fibers in one case the regenerating axons had frayed out along aberrant courses and had failed to reach the chiasma. In a fifth experimental case some signs of response to visual stimuli reappeared but the responses were too weak, infrequent, and inconsistent to permit any conclusions. A substantial strand of fibers about $\frac{1}{3}$ the size of the distal nerve stump was found in this case connecting with the chiasma but many small bundles of fibers had misregenerated along nearby structures of the orbit. The remaining 14 experimental cases which showed successful return of vision included 2 *B. terrestris*, 3 *H. cinerea*, 2 *H. crucifer*, 2 *H. squirella*, 3 *R. clamitans*, and 2 *R. papiens*. The eight control cases included 1 *B. terrestris*, 1 *H. cinerea*, 2 *H. crucifer*, 2 *R. clamitans*, and 2 *R. pipiens*.

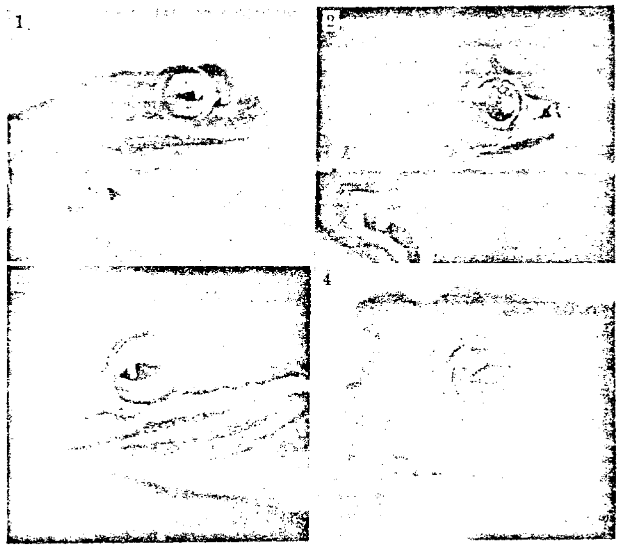

FIG. 1. Appearance of eye after 180 degree rotation in four species of anurans. 1. *H. squirella*. 2. *R. clamitans*. 3. *H. cinerea*. 4. *B. terrestris*.

Tests of vision. The ability of these 22 animals to localize objects in space was tested regularly over a ten-day period beginning about one week after the first signs of recovery of function. A minimum of 8 trials per day was recorded for each animal. A housefly impaled on the end of a thin wire set endwise in a glass rod handle served as the lure. This lure was presented in different sectors of the visual field in random order. It was held with a slight oscillatory motion because the animals apparently strike almost exclusively at moving objects. Care was taken in testing visual localization to eliminate sensory cues other than visual and to avoid misleading reflections of the test object from the glass walls of the containers.

Results with unrotated eye. After recovery the control cases with eyes in normal position had no difficulty in locating and catching flies presented in any sector of the visual field. They struck with normal accuracy at the lure within the 15 cm. range tested. Although attempts to approach the lure from distances as great as 40 cm. were noticed a few times in some of the more aggressive animals, no systematic tests were conducted at these greater distances because of the great variability even among normal animals in tendency to respond. The animals made correctly directed preparatory turning movements of the head and body as the lure was moved about from one part of the visual field to another. That direction of movement was accurately perceived was further indicated by the fact that the more aggressive animals frequently struck and caught the lure as it was moving quite rapidly across the visual field in front of them.

The optokinetic response was also tested, but it proved to be rather variable in these adult anurans even before operation and not a very satisfactory index of visual recovery. Some animals showed quite good reactions while in others the response was barely discernible or lacking. Even the reactions of individual cases varied considerably from one test to another. In general the reactions were about the same in the control cases after recovery as they had been in preoperative tests. In the two animals which recovered on only one side, however, responses toward the seeing side were absent. The optokinetic reactions after recovery were always correctly correlated with the direction of rotation of the visual field. All tests indicated that the recovered vision in the control group was normal in character and not confusedly blurred as might have been expected if functional termination of the regenerating fibers in the visual centers had been random and non-selective.

It should perhaps be pointed out that the term "normal" is used in describing these results merely in a qualitative sense to distinguish normal from randomly confused vision and from reversed vision. It is quite possible that quantitatively the recovered vision was not up to normal standards. Visual acuity and intensity discrimination, for example, might well have been subnormal without noticeably affecting the animals' proficiency within the range covered by these tests. Crude tests of the size of moving objects at which the animals would strike, however, failed to reveal any significant difference between the operated and unoperated animals. Also, the term covers only the particular capacities involved in localizing small objects in space (in the adults) and in making correct optokinetic reactions (in the tadpoles). Form perception, such as it is in amphibians, color discrimination, if present (11), and any other aspect of visual function not involved in these tests may or may not have been normal in character after nerve regeneration. At the same time it must be recognized that the localization of objects in space is very probably in adult anurans the primary and most highly specialized function of vision.

Results with rotated eye. Localization of the lure by the experimental cases in which the eye had been rotated was from the start reversed about the optic axis. When a fly was held in front of the animals within easy jumping distance, they wheeled rapidly to the rear instead of striking forward. Contrariwise when the lure was held in back of them and a little to the side they struck forward into space. When the animals came to rest in such a position that the lure could be presented well below eye level, they tilted the head upward and snapped at the air above. When the lure was held above the head and a little caudad to the eye the animals struck downward in front of them and got a mouthful of mud and moss. When the lure was presented successively in front of the animals they kept shifting around in circles as if the lure had appeared behind them each time instead of in front. This brief description of the reversed reactions applies particularly to the responses of the specimens of *R. clamitans* and *R. pipiens* and also to the tree frogs when they were resting on the bottom of the terraria rather than clinging vertically to the walls. The picture was of course somewhat different in the tree frogs when responding from a vertical position and in the toads, which struck mainly with a rapid flick of the tongue. The essential reversal of the striking reactions, however, was clearly evident in all 14 cases. As in the adult urodele, *Triturus*, and in the tadpoles the results in these adult anurans showed that the character of the recovered vision is determined systematically by intrinsic anatomical relations irrespective of the functional adequacy for the organism.

There was a definite decrease in tendency to display the optokinetic response to movement of the visual field after eye rotation. In 12 of the 14 cases optokinetic reactions could be elicited in some degree in preoperative tests but only four (1 *R. pipiens*, 1 *H. crucifer*, and 2 *H. squirella*) displayed discernible optokinetic reactions after recovery. In 5 of the 14 cases the contralateral nerve had been sectioned 8 days prior to section of the nerve of the rotated eye in order to test in the interval the effect of eye rotation alone with the original nerve connections intact. When tested all five showed nicely reversed striking responses, but only two (both *H. squirella*) showed any optokinetic reactions to rotation of the visual field. In both these cases the responses were reversed and abnormally exaggerated. Both animals also showed spontaneous turning movements of the head and body whenever they were aroused from their characteristic repose. These movements were made toward the blind side and were thus comparable to the spontaneous circus movements displayed by the tadpoles and adult *Triturus* (8) after eye rotation. After nerve regeneration in these 5 cases, the optokinetic responses were the same as before nerve section, *i.e.*, absent in 3 cases and reversed and abnormally exaggerated in the two *H. squirella*. Apparently the failure in the majority of cases to get as good optokinetic reactions in reverse as were made in the correct direction preoperatively was thus correlated with eye rotation and not with optic nerve regeneration. Since the spatial localization of small objects was a decidedly more critical test for the purpose of the present experiments, no further attempts were made through adaptation of the testing apparatus and method to obtain more consistent elicitation of the optokinetic reflex. In the 4 cases which exhibited the optokinetic response after recovery, it was made in reverse.

Effect of experience. After the reversed nature of the recovered vision had been ascertained, the animals were thereafter fed and tested only irregularly and no special attempt was made to determine the anurans' ability to correct reversed visual reactions by the learning process over an extended period of time. The following observations are therefore only suggestive, not conclusive.

All 14 of the adult cases with rotated eyes were kept at least 30 days after recovery of vision and four were kept longer than 70 days. Eight of the animals, never especially voracious feeders in the laboratory, showed no reliable change in their tendency to respond in reverse. Four cases, two of which had at first been quite aggressive in their misguided efforts to catch the lure, gradually became less responsive and finally refused to strike at the lure at all. After reaching this state, however, they also refused to eat even when flies were brought into direct contact with the nose, the regular method by which the animals with reversed vision were fed. It was thus not entirely clear in these four cases that the gradual decrease in frequency and vigor of reversed reactions was indicative of learning. Two other cases sacrificed at 78 and 83 days after recovery remained, on the other hand, particularly active and aggressive to the end in their misdirected attempts to catch the lure whenever it came in sight.

During the post-recovery period living flies placed periodically in the terraria with the animals with reversed vision remained uncaught except for the few that lighted or walked directly upon the animals, whereas they were immediately snatched up when placed in terraria with the animals with unrotated eyes which had recovered normal vision. The reversed optokinetic reactions and the spontaneous circus movements observed in the two *H. squirella* were still present at 40 days and 44 days after recovery when the animals were sacrificed. Thus in summary, there was a suggestion that the adult anuran may in some cases learn to inhibit the useless reversed reactions, but no indication in any case of a positive correction.

RETINAL PROJECTION ON THE OPTIC LOBE

In trying to determine how the central associations are formed in regeneration it becomes important to know whether fibers from any given retinal region have to make their way to a special localized area of the optic lobe in order to establish correct functional relations or whether it makes no difference in which region of the optic lobe the ingrowing fibers happen to terminate. If there exists an orderly projection of the retina upon the optic lobe, localized lesions therein should produce scotomas or blind spots in the visual field. Such blind spots should be detectable by noting the sectors of the visual field in which a lure can be held without eliciting any response, provided, of course, the animals respond readily when the lure is shifted into other sectors.

The anurans are exceptionally suitable for this type of test. Their tendency to remain in a set position for long intervals without any movement of head or eyes makes it easy to bring the test object into such position as to stimulate only the particular retinal area desired. The fact that responses to the lure are made primarily with the head and body without any appreciable independent exploratory movement of the eyeball also makes for clear-cut results. Walls' (11) statement, however, that "no amphibian is known to perform any eye movements other than retraction and elevation" is hardly accurate. Rotatory and turning movements of the eye in the orbit are pronounced and striking in the tadpole. Such movements are also common, though of less amplitude, in adult frogs and toads. These movements, however, seem to be associated primarily with vestibular reflexes, and if any initial exploratory movements of the eyeball occur independently of head and body movements, they are certainly so slight as to be negligible factors as far as the following experiments are concerned.

Operations. Tests were run first on 15 animals (1 *B. terrestris*, 5 *H. cinerea*, 7 *R. clamitans*, and 2 *R. pipiens*) with normal optic nerves. Lesions involving one half to two thirds of the entire lobe were made bilaterally in the anterior, dorsal and posterior portions of the lobe, each type of lesion being made in five animals. There was no attempt to produce lesions in the ventral portion, mainly because of the difficulty of avoiding injury to afferent and efferent fibers running to and from the other parts of the lobe. After the skin had been cut and reflected the cranium was broken away in small pieces and the outer meninges were cut or torn off until the dorsal aspect of the lobes was completely exposed. The lesions were begun with fine-pointed jewelers' forceps and completed by the suction method using a drawn-out point of glass tubing about 0.3 mm. inside diameter. Animals were selected which were hungry and approached and struck readily at the lure when it was presented from any direction above, below, behind, or in front of them.

When tested within an hour after operation the five animals with the anterior part of the lobe intact made no response when the lure was shown in the back part of the visual field but struck vigorously and accurately when it was shown in front. The five animals in which the ventral part of the lobe remained intact made no response when the lure was presented anywhere in the visual field above them but turned or struck forward readily when it was presented below eye level. When the lure was presented behind the five cases in which the posterior part of the lobe was intact, they turned quickly so as to face the lure in preparation to strike just as do normal animals but, when they had thus turned and the lure was directly in front of them, they made no further response until it was again moved into the back part of the visual field. By repeating the performance these animals could be made to turn around in circles without ever striking at the lure although they came into a good striking position with each turn. These 15 cases were again tested on several occasions during the following three days with similar results.

That the retina normally is projected upon the optic lobe in an orderly manner with the retinal axes reversed in the tectum is clearly indicated by these effects of tectal lesions. The results confirm the conclusions regarding tectal termination of optic fibers in anurans reached by Ströer (10) on the basis of anatomical studies. Just how precise, that is, how close to a point-to-point correlation, the retinal projection is in these anurans cannot, of course, be deduced from the above results. The observations can be taken to indicate only grossly the existence of an orderly projection of the retinal field upon the optic lobe.

RETINAL PROJECTION AFTER NERVE REGENERATION

Since the redistribution of nerve fibers in regeneration has elsewhere been shown to be indiscriminate both in mammals (4) and in amphibians (14) the question arose as to whether the systematic projection of the optic fibers on the tectum might not be drastically disarranged after regeneration, necessitating a rather complete reorganization of secondary synaptic associations in the optic lobe. Accordingly, tectal lesions similar to the preceding were made in a group of animals which had recovered vision following complete severance and regeneration of the optic nerve.

Operations. Eight cases (1 *B. terrestris*, 4 *H. cinerea*, 1 *H. squirella*, 1 *R. clamitans*, and 1 *R. pipiens*) were selected which had recovered normal vision after optic nerve regeneration and were quite aggressive in their attempts to approach and snap at the lure when it was presented in any part of the visual field. Anterior lesions were made in 4 cases, posterior in 2 cases, and medial lesions in the remaining 2. Anterior lesions were stressed because the behavioral check is more strikingly conclusive. Under normal conditions an animal partially disinclined to strike at the lure will often not shift the head and body in order to get at the lure to the rear but will strike forward where the lure is easily accessible. Thus animals that would turn around to get at the lure—as they did after anterior lesions in the above cases—would certainly strike at the lure directly in front of them if it could be seen. Three of the eight cases were selected from the control group and the remaining five, all unilateral, were chosen from a group of extra cases prepared especially for the purpose.

The types of scotoma produced in these cases with regenerated optic nerves conformed consistently with those which had resulted in the foregoing group of normal animals. Posterior lesions abolished responses to the lure held behind, dorsal lesions to the lure held above, and anterior lesions to the lure held in front of the animals. Two additional cases (*B. terrestris*, *H. squirella*) from the experimental group which had recovered reversed vision were also tested. A lesion in the anterior part of the optic lobe in one of them abolished responses to the lure presented behind the animal. When the lure was held in front, this animal responded by turning around to the rear in characteristic reversed manner. In the other case a medial tectal lesion abolished responses to the lure when it was held below eye level but did not eliminate the reversed reactions to the lure when it was presented well above eye level. From the nature of the scotomas produced by tectal lesions in these ten cases with regenerated optic nerves it may be concluded that the ingrowing optic fibers reestablish functional associations in the same topographical areas of the optic lobe in which they originally terminated and that no major reorganization of secondary synaptic relations is involved in the recovery of function.

ANATOMICAL CHECKS

All cases in which recovery of vision was absent or deficient, 2 tadpoles and 3 adults which recovered normal vision, and 3 tadpoles and 7 adults which recovered reversed vision were prepared for microscopic examination by the Bodian (1) method. The optic nerve was sectioned longitudinally in different planes at 10μ. The findings in those cases in which visual recovery was unsuccessful have already been mentioned.

The point at which the optic nerve had been cut was recognizable, particularly in the adult animals, by an enlargement of the nerve trunk through which individual fibers and small fiber bundles took intertwined and tortuous routes. From the entangled appearance of the neuromatous scar region it was apparent that there had not been an orderly fiber outgrowth in bridging the gap between the distal and proximal nerve stumps. The systematic restoration of proper central linkages in spite of chaotic outgrowth across the nerve gap seems unaccountable except on the assumption that fibers from different retinal regions have specific inherent properties which influence selectively the formation of central associations.

Because of the small size and great number of fibers in the optic nerve of these anurans—Breusch and Arey (2) estimate 29,000 fibers in *R. pipiens*—the histological picture was too complicated to make possible any inferences regarding the question of whether or not the regenerating fibers attain an orderly segregation in the proximal stump or in the central tracts en route to the optic lobe. The results of tectal lesions do not settle the question because it is possible that the ingrowing fibers reach the optic lobe with a random distribution but only those succeed in establishing functional synaptic connections which happen by chance to enter their proper area.

Postmortem examination of the tectal lesions revealed that their general location was as intended, but there was considerable variation in border outline. The nature of the lesions and also of the behavorial tests did not permit any detailed quantitative comparisons of the precision of retinal projection in normal and regenerated nerves.

Comment

The foregoing data on recovery of vision after optic nerve regeneration in six different species of anuran amphibians support the previous conclusions based on visual recovery in the urodele *Triturus* (8). These conclusions and their logical basis are, briefly, as follows: Since stimulation of different retinal areas evokes different responses, each retinal locus must possess functional connections with the brain centers differing from those of all other areas. After optic nerve regeneration these differential relations between retina and visual centers are systematically restored in their original form, as shown by tests of optokinetic responses and visual localization of small objects. This orderly restoration of central reflex relations occurs regardless of the orientation of the retina, despite a maladaptive effect for the organism, and must therefore be regulated by growth factors independently of functional adaptation. As this would otherwise be impossible the ingrowing optic fibers must possess specific properties of some sort by which they are differentially distinguished in the centers according to their respective retinal origins. The previous discussion of these points with some of their implications (8) is in large part applicable to the present data and need not be repeated.

The orderly topographical arrangement of functional relations found in the optic lobe after optic nerve regeneration is difficult to explain without assuming that the secondary neurons of the optic tectum are also biochemically dissimilar, possessing differential affinities for fibers arising from different retinal quadrants. The results thus lend further support to the supposition that neurons of the central nervous system are specified biochemically in much greater degree than is evident from their morphological variations. A degree of central neuron specification is indicated which approaches the degree of innate functional differentiation and which presumably plays an important role in the ontogenetic determination and differentiation of inherent integrating patterns.

Where the functional properties within central nuclei have an orderly distribution corresponding with anatomical dimensions as in the anuran optic lobe, it is conceivable that a basic embryonic specification arises through central self-differentiation of the nuclear mass itself. Under such conditions the conjecture that specification of the tectal neurons may be induced via the more early differentiating motor and adjustor systems becomes unnecessary, although later acquisition of afferent and efferent relations may well result in further individuation superimposed upon the initial nuclear field.

The conclusion that function is not the organizing factor in the reestablishment of systematic reflex relations in these experiments in no way contradicts the possibility that function, as a generalized, non-specific factor, may be of importance for the normal healthy maintenance and development of nerve structures. That neuron discharge acts by itself in any specific manner, in optic nerve regeneration to regulate the formation of proper rather than improper or indiscriminate reflex associations, however, seems untenable. An inherent physico-chemical differentiation of the optic fibers must be inferred.

That such neuron differentiation is extremely important in the establishment of proper linkages between centers and peripheral end organs, in amphibians at least, becomes increasingly evident. Whether these linkages are determined primarily on a physiological basis involving specific modes of excitatory discharge and selective detection as envisaged in the resonance principle (13) or depend upon specific structural associations the formation of which is regulated through the influence of peripheral differentiation on central synaptic growth, as more recently proposed (6, 8), remains uncertain. The excitatory characteristics of mononeural connections between receptor and effector organs (12) and the fact that separate muscles which function asynchronously can be excited independently in their normal action phase by branches of a single motor axon (13, 14) seem more satisfactorily explained by the resonance principle. Until these latter phenomena have been more thoroughly studied, however, it seems advisable to stress at present the alternative possibility of interpreting effects of neuron specification on a more orthodox connectionist basis.

Summary

1. In larval and adult anurans of six different species regeneration of the optic nerve resulted in a return of visual perception which was well organized, not an intermingled confusion. Distinct and consistent responses to position and direction of movement of objects in the visual field were recovered.

2. The orientation of visuomotor responses after recovery, however, was dependent upon the orientation of the retina. It was normal in animals

whose retinas had been left in normal position but reversed about the optic axis in animals whose retinas had been rotated through 180 degrees prior to nerve section.

3. The location of scotomas produced by localized lesions in the optic tectum after optic nerve regeneration indicated that optic fibers from different retinal loci had reestablished functional connections in the same areas of the optic lobe to which they had originally projected.

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