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RECOVERY OF SIGHT AFTER TRANSPLANTATION OF EYES AND REGENERATION OF RETINA AND OPTIC NERVE*

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GENERAL CONSIDERATIONS

The eye of the newt and eye of the salamander, once noted for magical potencies, have been shown experimentally to possess regenerative capacity far beyond that found elsewhere among the vertebrates. For example, Stone and Farthing (30) succeeded in transplanting the same eye of an adult newt repeatedly to four different animals. In each new host the eye was left in place until visual function was recovered. Counting the original owner, this single eye thus served the vision of five different animals.

In each new host the lens, retina, and optic nerve of the transplanted eyeball underwent degeneration, as regularly happens in adult newt eyes when the blood supply is interrupted. A new lens and retina regenerate from a reserve of undifferentiated cells in surviving portions of the eyeball after vascularization of the transplant has been re-established. The newly regenerated retina then sends out its own new optic nerve fibers that grow centrally to connect with the brain.

It was also demonstrated by Stone (27) that explanted eyes of the adult newt can be stored in the refrigerator for up to seven days and still retain their capacity to regenerate visual function when transplanted to the denuded orbit of a new host. The eyes of the urodele amphibians have also been successfully transplanted in larval stages to different related species. Large eyes can be transplanted to a small-eyed species and vice versa, with the possibility of thereby improving somewhat the vision with which an animal is normally endowed (29). In this connection, however, the in-

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herited central integrative machinery of the host species seems to be a relatively fixed limiting factor.

The urodele eye has also been transplanted to different parts of the body such as the top or back of the head, the neck, or the flank. Under these conditions the cornea regains its clear transparency, the retina regenerates and presumably if the new optic fibers could find their way to the optic lobe of the brain, such ectopic eyes could serve visual perception. It was recently shown that eyes transplanted embryonically to the top center of the head in frog tadpoles established connections with the brain and were functional (12). However, the vision of ectopic eyes, in these lower vertebrates at least, must be expected to be of the sort that would obtain if the eye were transmitting the same information from the site and orientation that is normal for an eye connected with the given brain center (25).

Eye transplantation has not been similarly successful among the fishes nor among the frogs and toads even in larval stages of the latter, and it was thought for a time that the optic nerve in these forms lacked the capacity for functional regeneration (30). We found later, however, that severance of the optic nerve without interrupting the central artery of the retina is followed by excellent regeneration with recovery of vision in these forms also (22). If the blood supply to the retina is cut off in fishes or anuran amphibians, the retina generally regresses and fails to regenerate, although in a few exceptional instances visual recovery has been obtained in transplanted eyes (15). These latter appear to have been cases in which the original retina survived, in part at least, rather than cases of degeneration and regeneration of an entirely new retina.

In recent work with the goldfish involving surgical removal of parts of the retina, incidental observations suggest that large lesions of the retina are rapidly repaired by regeneration (4 and in press) and the same seems to be true for lesions in the optic lobe of the brain where the retinal fibers terminate (14). The tissue repair under these conditions involves more than just regeneration of interrupted fibers. The interrupted fibers do regenerate, but also a new supply of neural cells is multiplied from indifferent reserve or germinative cells in the surround.

In general the entire central nervous system as well as the optic system among the fishes and amphibians has strong potential for functional regeneration and repair. In this respect, the CNS of these forms stands in marked contrast to that of the mammals including man. The possibility of obtaining visual recoveries like the above in vertebrates above the amphibians, particularly in man, by future perfection of surgical procedures or of

biochemical treatment is a prospect that for the present at least cannot be said to look hopeful. Very little evidence is avilable with respect to reptiles and birds, but quite a number of experimental efforts have been directed at obtaining recovery of vision in mammals after eye transplantation and after optic nerve section. In no one of these cases can it be said that recovery of vision has been satisfactorily demonstrated in a mammal as a result of regeneration of the divided optic nerve. A few claims of success in the past cannot be definitely ruled out, nor can they be accepted, either, in the face of a large mass of well-documented counterevidence. In general the divided optic nerve in mammals not only fails to achieve functional reconnection with the brain, but tends to undergo a retrograde degeneration back to and including the ganglion cell bodies in the retina (6). My own efforts to obtain functional regeneration in the mammalian optic nerve were abandoned back in 1941 when I found that even a delicate local crush of the nerve performed under the microscope that left the outer sheath of the nerve and the retinal artery intact—and was carried out in young rats shortly after birth before the eyes had opened—lead only to retrograde changes and subnormal development of the whole eye. In the entire series of 18 such cases there was no functional indication of any vision, and from the anatomical checks it was judged unlikely that any retinal fibers had made a successful crossing of the subdural break in the nerve (26). Nevertheless, demonstrated species differences in regenerative capacity and in capacity to resist retrograde degeneration encountered among the vertebrates suggest that similar crushing in other species and at different stages of growth would be worth trying.

As already indicated in a preceding paper, damaged neurons in the developed retina and central nervous system of mammals are not replaced. Even if they were, it is questionable whether the fibers of such new cells would succeed in growing to appropriate distant connections under the same conditions where the severed fibers of the mature neurons have been found to fail.

In brief, it is clear that regenerative vigor in the visual system varies greatly among the different vertebrate classes and orders and in different stages of growth. Among mammals regenerative capacity is known to be quite different among different nerve cell types even within the same nervous system. This is apparent not only in the capacity of neurons to regenerate fiber processes, but also in the mere ability of different fiber systems to withstand retrograde degeneration after section. The problem of understanding and perhaps controlling some of the factors that make for functional regeneration is an old one, already the subject of considerable investigation (32) and as yet without promise of a quick solution.

It has been thought that perhaps the main difference behind the excellent central regeneration in lower forms and the lack in mammals may be largely a matter of the kind of tissue obstacles met by the new nerve sprouts in each case. It has seemed more likely to the writer that the more limiting differences probably lie in the intrinsic growth and regenerative potential of the nerve cells themselves. If so, it then becomes a problem in the biochemistry of neuron growth and differentiation. The answer might lie in simply finding and supplying a critical biochemical constituent or several that are present in nerve tissues that regenerate but are lacking in those that do not. It must be admitted, however, that the answer could equally well lie within a complex series of sequential steps in neuronal differentiation involving a number of complicated interlocking and delicately balanced intracellular conditions impossible to control in any practical way within the immediate future.

QUALITY OF VISUAL RECOVERY

We turn now to problems concerning the quality and functional properties of the vision as it is recovered in the lower vertebrates by regeneration. Although we still deal here with the restoration of sight through retinal and optic nerve regeneration, the main concern shifts from practical aspects relating to regenerative capacity as such, to various other things that one can learn from the regeneration phenomena about vision in general and about its neural machinery. The observations carry implications regarding, among other things, requirements for devices that might simulate, bypass, or replace the functions of the eye and optic nerve and also bear on some of the problems of substituting other sensory systems for vision. However, I shall not attempt from this point on to relate the material to any such practical problems; I will consider the evidence with reference purely to its theoretical implications, particularly those concerning the developmental patterning of the fiber pathways and genetic organization of the central connections.

The formation of brain connections by the optic nerve presents a problem in the allocation and assignment of proper functional associations among the half-million or so optic fibers typically involved. For normal vision the spatial or directional 'local sign' properties of each fiber must be arranged systematically with topographic correspondence to the locus of origin in the retinal field. For normal color and brightness discrimination the red, green, blue, yellow, and luminosity units must evoke their differential central effects in a systematic pattern that accords with their relations to the various photoreceptor units. Similarly, the 'on' fibers and the 'on-off' and 'off' fibers must each be 'plugged in' centrally in its own appropriate manner. If additional fiber types are present like the "convexity edge detectors," etc., described by Maturana et al. (16, 17) for the frog, these too must attain each its special functional associations in the brain centers. Presumably many of the specialized fiber types have spatial or local sign properties in addition to their color or 'on-off,' etc., specifications. All of these different physiological properties must then be woven appropriately into the total systematic pattern of functional associations.

In spite of the above complexities, it is nevertheless generally true that whenever there is good regeneration of the optic nerve, anatomically, the result is an excellent restoration of all aspects of visual function. The directionality factors are restored in their original form. Color discriminations trained prior to section of the nerve are reinstated in the same form by regeneration without retraining (1, and in press). Incidentally, these color discrimination habits, based on the fish's jumping at the correct one of two colored targets hung above the water, exhibit good interocular transfer. They survive combined ablation of the forebrain plus the cerebellum, and if the directly-trained tectum is ablated after training, the discrimination habit is retained presumably through a second memory system developed in the opposite tectum. Visual acuity is difficult to measure after regeneration, but as far as we can tell it appears to be recovered to approximately its normal level under optimal conditions.

Since section and regeneration of the optic nerve involves inevitably a rather chaotic scrambling of its thousands of fibers within the nerve scar, the question arises as to why the recovered vision should be orderly, accurate, and clear instead of being a blurred confusion. With respect to directionality factors, it was found that the same orderly recovery of retinal local sign occurred consistently in the face of experimental conditions like eye inversion and rotation and nerve cross-unions that ruled out functional readaptation as the organizing factor (21-26).

The orderliness of the visual recovery in the above-mentioned experiments could not be accounted for in terms of any of the earlier concepts regarding nerve fiber growth and the selective patterning of fiber connections in the central nervous system, such as neurobiotaxis, disuse atrophy, mechanical contact guidance, stimulogenous fibrillation, electrodynamic patterning, reflex conditioning, trial-and-error learning, and the like. The findings did conform with and support the neuroaffinity or 'chemoaffinity' hypothesis of synaptic formation that I proposed in 1938 to explain 'homologous response' (19) and applied soon after to the developmental patterning of synaptic connections in general (21). The hypothesis holds that the formation and maintenance of synaptic junctions throughout most or all of the nervous system is selective and orderly from the start, being regulated by differential biochemical affinities among the neuronal elements involved.

Applied to the visual system it means that the thousands of individual fibers in the optic nerve differ chemically among themselves according to the locus of origin in the retinal field and also according to the particular color, 'on-off,' and other specific physiological functions of the fiber units. It means also that the receiving neurons of the midbrain optic tectum are similarly endowed with 'identification tags' in the form of matching differential specificities. The regenerating fibers re-entering the brain centers are assumed to show a strong or exclusive preference for synapsis among the neurons they contact, by-passing the great majority of neurons they encounter to connect only with the appropriate matching neurons of the tectum and other visual centers. In the case of the tectal connections, "attraction of opposites" is suggested for the underlying physiochemical forces in that the fibers from the anterior quadrant of the retina project to the posterior quadrant of the tectum, and those from dorsal retina go to the ventral tectum, etc.

The requisite chemical 'tagging' of retinal and tectal neurons and of their fiber extensions is presumed to arise through orderly processes of embryonic differentiation and induction (24). This would involve at the minimum a front-back gradient of differentiation of the retinal field followed shortly by the appearance of an up-down gradient of different quality. Radial and surface-depth gradients are also to be inferred (the former thought to be especially important in mammals). Later in development more localized differentiation of the three retinal layers, and of the several cell types within each layer, is visibly evident.

Similar differentiation of axial gradients, layers, and cell types must take place centrally as well as within the lateral geniculate body and the optic lobe to provide countermatching chemical specificities. The output fibers of the tectobulbar and tectospinal tracts are inferred from similar regeneration experiments to possess their own biochemical tags by which they in turn are enabled to form proper bulbar and spinal 'downstream' associations again on a chemoaffinity basis (23).

It has been proposed that the inherent integrative patterning of the entire central nervous system, including the complex circuity of instinctive behaviors as well as simpler sensory and motor integration patterns, is assembled in development on a similar basis. The 'neuroaffinity' hypothesis fits well with related known principles of embryology and genetics to furnish a feasible and credible basis for the organized growth and inheritance of innate behavior patterns.

In general it makes an attractive theory that would explain a great deal about the inherited organization of vision, but is it correct? The whole idea of nerve fibers growing to specific connections on a selective chemical basis runs counter to a large mass of evidence behind the widely accepted doctrine established in the nineteen twenties and thirties that the growth and termination of nerve fibers is indiscriminate. Chemotropic along with galvanotropic factors appeared to have been ruled out by most of the evidence in favor of mechanical contact guidance.

The strongest support for chemical selectivity in synapsis has come so far from the studies on optic nerve regeneration. To a large extent the entire theory of selective synapsis via chemoaffinities can be said to hang on the answer to the single question: Is the optic fiber reconnection in the brain really selective and specific as inferred?

Selectivity of reconnection is not directly proven by the orderliness of the recovered vision which is also explainable hypothetically in terms of physiological coding or resonance phenomena. Nor is it proven by the orderly topography of maps obtained by making tectal lesions (22) or electrical recordings (7, 8) following regeneration since both of these might conceivably reflect the postsynaptic organization of the tectum rather than that of the regenerated optic nerve. Certain difficulties in the specific reconnection hypothesis have been raised by Gaze and Jacobson (10) who report previously unknown ipsilateral projections of the retina to the tectum that overlap but are not in register with the main contralateral projections. They also find indications of an early nonspecific phase of tectal re-innervation that is retracted and differentiated only later after visual function is established. Further, the above scheme of chemical gradients proposed for the fish and amphibians obviously cannot be applied similarly to mammals where there is partial crossing in the chiasma.

Nevertheless, the evidence for specific reconnection has been greatly strengthened just recently in histological studies being carried out currently in fishes, mostly eichlids and the common goldfish (4 and in press; 2). Particularly pertinent are the findings on regrowth of the severed optic nerve

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