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A selective recovery of function has been found to follow centripetal regeneration of the optic nerve after its complete transection in amphibians. The ingrowing optic axons make their way into the visual centers of the brain and there re-establish functional reflex associations in a systematic, predetermined manner such that the differential spatial values of the various retinal points are restored in their original form (4–9). The regulatory growth factors responsible for this orderly formation of differential reflex relations in the brain centers are presumably similar in nature to those by which the inherent patterns of intricate synaptic inter-relationships are formed originally and prefunctionally in ontogenetic development. Little is known of the actual governing mechanisms, however, and further evidence is needed regarding the systematic development of synaptic associations both in nerve regeneration and in ontogeny.

The present experiments were undertaken to find out if the 8th cranial nerve root after complete transection in the adult is capable of regenerating axon connections to its proper nuclei in the medulla and, if so, whether the diverse functional properties of the various vestibular fibers, correlated with their central reflex relations, are restored at random or with systematic selectivity as in the case of the optic system. The 8th nerve happens to be well suited for testing selectivity in recovery following regeneration because it contains fibers to a number of separate end organs, the differential reflex properties of several of which are readily determined by simple functional tests. In the frog the end organs supplied by the 8th nerve include the cristae of each of the 3 semicircular canals and the maculae of the utriculus and the sacculus, the lagena, the pars neglecta, and the pars basilaris (1). There is thus enough functional variety among the 8th nerve fibers to render negligible the possibility that the regenerating axons could by chance alone establish suitable central connections in sufficient numbers to restore proper function. Any consistent restoration of normal discrete reflex associations for the different end organs, therefore, must clearly indicate discriminatory, selective factors in the recovery process.

METHODS. In 20 tree frogs (Hyla squirella) bilateral section of the 7th and 8th cranial nerve roots was performed intracranially through an incision in the roof of the pharynx. The roots of the 7th nerve were divided along with those of the 8th because the two enter the medulla together. This did not detract from but rather enhanced the significance of the experiments as far as positive results were concerned, because the presence of the extra regenerating fibers lessened further any opportunities for fortuitous re-establishment of correct central connections. With fine jewelers' forceps the nerve roots were pinched off and pulled and teased apart until all fascicles of the 7th and 8th nerve root complex had been severed, after which the frayed nerve ends were roughly approximated. Eight control cases also were prepared in which the 7th and 8th nerve roots were widely excised and the auditory capsules broken to prevent any recovery. General operative technic and after care of the animals were the same as in previous experiments (4–7).

Tests to detect loss and return of the various reflex functions mediated by the 8th nerve were made at 3 to 5 day intervals after operation. Observations were made separately of the effects of angular acceleration or deceleration of the animals in both directions in the 3 planes determined by the 3 primary axes of the body. Also noted were the effects of linear acceleration in different directions, righting reactions from an inverted position both in and out of water, assumption of postural attitudes on different inclines, leaping, climbing, swimming, other general activities and arousal responses to auditory stimuli. At the end of $3\frac{1}{2}$ months the animals' heads were fixed in Bouin's solution, sectioned at 10 mu in different planes and prepared for microscopic examination by the Bodian activated protargol method.

Results. *Immediate effects of nerve section.* The immediate effects of operation in both experimental and control groups were similar and involved the usual profound loss of equilibration consistent with numerous descriptions in the literature. The following brief account is sufficient for the present purposes. Righting reactions, particularly those performed in water, were severely impaired. The animals were unable to swim without somersaulting or rolling and their inco-ordinated struggles terminated as often in the upside down position as right side up. Leaps through the air also were badly directed and resulted in somersaulting and rolling in mid air with the animals commonly landing on their backs or sides. Instead of clinging fast in normal manner to vertical objects, they frequently fell off when climbing or alighting upon them. The head often assumed and was allowed to remain in various odd postures. All reflexes to angular acceleration in all directions in all three primary planes were abolished except for the optokinetic response which, being slower and weaker and present when there is no acceleration, is readily distinguished from the vestibular reactions. The residual visual adjustments disappeared after transection of the optic nerves in 3 cases so tested. Exaggerated abrupt movements of the head and body along with forced circling reactions were also exhibited but had subsided after a few days and hence were probably irritative rather than deficiency phenomena. There was no indication that the 8th nerve root had escaped complete transection in any of the animals.

Extent of recovery. About $1\frac{1}{2}$ months after operation, the animals were all blinded by section of the optic nerves. This made it possible to estimate more easily and accurately the final degrees of recovery by quieting the animals and by eliminating all visual equilibratory actions that might otherwise be confused with vestibular function.

In the control group 2 animals died shortly after operation but the other 6 survived in fair condition. These controls showed no recovery of the principal test reactions. There seemed to be some slight improvement in their posture and ability to move about and remain in an upright position and to cling to the substrate, but even this may have been in part a result of the subsidence of irritative phenomena. The amount of recovery achieved in the various test performances by the controls over the $3\frac{1}{2}$ month observation period was practically negligible, in any case, and for purposes of comparison with the experimental group, the state exhibited by the controls is designated as one of zero recovery.

On the 21st day after operation 3 of the experimental group showed signs of recovery of vestibular responses to horizontal angular acceleration. By the 26th day these and 4 additional cases were displaying unmistakable responses of the vestibular type on the horizontal turntable. Thereafter, recovery continued to make noticeable progress in the experimental group as a whole over a period of about 3 weeks. Five of the 20 experimental cases attained complete recovery by this time in that they came to exhibit responses closely approaching normal in all of the different tests. Two cases, on the other hand, never showed any recovery at all and another one died during the second week after operation. The remaining 12 of the experimental group achieved various intermediate degrees of recovery ranging from nearly complete recovery in most tests to almost no recovery.

Selective character of recovery. In those animals which showed complete recovery, the reflex reactions elicited by different patterns of labyrinthine stimulation were not randomly confused, disorderly, or massive in nature as if the fibers from the different end organs had made central connections in a fortuitous manner. On the contrary, discrete reactions of normal quality were evoked consistently by different types of stimulation. Horizontal angular acceleration to the right caused compensatory horizontal turning of the head to the left, while conversely, deceleration caused an opposite movement to the right. Corresponding responses in the opposite directions were produced by horizontal spin to the left. Tipping the animals on their longitudinal axis to the right or to the left caused in each case typical compensatory tilting movement of the head in the proper direction to tend to keep the head on a horizontal plane. Tipping the animals head-downward on the transverse body axis resulted in a counteracting elevation of the head just as in the normal animal and, vice versa, tilting the body head-upward caused an active depression of the head. Thus these various types of labyrinthine stimulation elicited discretely each its own particular appropriate response.

Righting from an inverted position was again performed with proficiency indistinguishable from normal. The animals recovered their ability to swim in a straight line correct side up, to make long leaps through the air without landing upside down, and to alight and climb on vertical walls without falling off. Normal postures were regularly assumed and in all general activities behavior of apparently normal quality was displayed.

Some attempt was made to test functional recovery of the auditory as well as vestibular fibers of the 8th nerve in these animals. The method used was to see if the animals could be aroused from their characteristic daytime repose to a state of alertness by means of auditory stimuli. It was found that tearing, rustling, crushing, or scratching of paper (noises with high overtones) would elicit arousal reactions with reliable consistency in 3 of these 5 cases showing complete recovery. The arousal response consisted of elevation of the eyeballs, withdrawal of the nictitating membranes, dilatation of the pupils, increase of respiratory rate, and sometimes raising of the head or even movement of the whole body. The lack of consistency in the responses of the other 2 of the 5 experimental cases tested does not necessarily indicate that the hearing of these 2 was abnormally poor, for there was comparable individual variation in tendency to respond among a group of 7 normal animals. Although the positive responses seemed convincing and were not made by any of the control cases, this evidence must be accepted with caution because of the impossibility of ruling out completely other avenues of stimulation such as convection currents in the air, sympathetic vibration of the substrate, or other uncontrolled factors. These arousal reactions were abolished by severance of the 8th nerve root in 5 cases so tested (2 experimental, 3 normal), but this might have been a secondary result of the severe effects of 8th nerve section.

In the cases showing intermediate degrees of recovery, as well, the recovered labyrinthine reflexes were of normal character. Functional restoration was defective in these cases only in the lack or weakness of responses, and not in the mode of response; i.e., the defects appeared to be basically quantitative, not qualitive. For example, an animal might show fair or good responses in the correct direction to angular acceleration in the horizontal plane, while at the same time reactions to tilting in the vertical plane would be absent or extremely weak. In 5 cases recovery was definitely asymmetrical with respect to the two sides so that the animals tended to lean or turn predominantly to one side. But the various test responses superimposed on these postural biases were made in the correct direction. Only one exception to the foregoing statements was noted. This was a case in which for a period of over a week during recovery, rapid lateral tilting caused the animal to lean farther off balance rather than to compensate in the normal manner. These reversed responses, however, are apparently characteristic of frogs in which only the utriculus remains functional (11). They cannot, therefore, be considered a sign of atypical central reflex associations and more probably signify that recovery of function of the end organs other than the utriculus was tardy and deficient. In summary the only defects in the recovered functions were such as to indicate that they arose not from abnormal functional associations but rather from absence of function.

Judging by the number of different kinds of sensory end organs supplied by the 7th and 8th nerves, at least 11 distinct classes of afferent fibers had regenerated from the point of transection with ample opportunity among them for a completely chaotic interspersion into abnormal pathways and with very little chance for particular fiber types to be directed by mechanical factors alone to their proper central terminations. And yet in recovery there was no reliable sign of any abnormal central associations while it was quite clear on the other hand that in all cases showing recovery different classes of vestibular fibers had managed consistently to re-establish their own proper type of reflex associations. The conclusion is evident that restoration of reflex relationships in the medulla had not been fortuitous but had been systematically regulated in a prearranged order.

Further checks and controls. That the observed recovery of vestibular reflexes was a product of 8th nerve regeneration and not ascribable to compensatory action of other sensory systems was clear from the following: *a*, the striking difference between those cases which showed complete recovery and those, including the controls, which showed no recovery at all; *b*, lack of deterioration of the principal test reflexes following optic nerve section; and finally, *c*, abolishment of all the recovered reactions by resection of the regenerated 7th and 8th nerve roots. The conclusion that functional abnormalities where they occurred were due to a lack of recovery and not to a positive recovery of atypical central associations was supported further by the histology. The severed nerve roots were found to have undergone a dense regeneration in those cases in which recovery of function was good while in those cases in which functional recovery had been lacking or poor, nerve regeneration proved to be absent or very slight. In many cases there was a severe reduction in the number of cells in the 8th nerve ganglion and it is very possible that lack of complete recovery was caused in large part by direct or secondary damage to the nerve ganglion itself which lies close to the point of transection. An additional but less important factor appeared to be the escape and misdirection of regenerating fibers outside the dural sheath of the medulla. In the region of transection there was considerable intermixing and rearrangement of fibers and fiber bundles.

Attempts to determine the regulatory factors. The experimental results thus far show that the 8th nerve root is capable of regenerating centripetally to re-establish functional associations within the central nervous system and that the resultant restoration of vestibular reflexes is achieved in an orderly systematic manner. They raise the additional problem, however, of whether the orderliness of functional recovery is attained directly by growth mechanisms alone or whether it involves functional readjustment processes of some type such as learning. The following observations and few additional experiments conducted mostly on late larval stages are concerned with this further problem.

The following indicated that the learning process is not involved in regulating recovery. *a.* The recovered reflexes were found to survive decerebration plus optic nerve section in both the adult (*H. squirella*, 4 cases) and in the tadpole (*R. grylio*, 5 cases). *b.* There was no indication of a practice period in recovery. The first time that a response could be elicited by angular acceleration, for example, it was made in the correct direction. *c.* The vestibular system is highly reflex in nature and relatively unsubject to reintegration by learning. Even in mammals no direct connections with the cortex or a cerebral cortical center have been found (3). Finally, *d*, what is known of the learning capacity of amphibians in comparable situations indicates that it is far too limited to achieve readjustments of this sort (8).

The inference that no type of functional regulation whatever is involved in the recovery is suggested by the following: Those vestibular reflex movements of the eyeball which on the occurrence of imposed head movements tend to maintain a steady view of the visual field are pronounced in the tadpole and readily demonstrated by tilting the animals in water either in an open dish or in a close-fitting test tube. It was found that these reflexes were restored in their proper form by regeneration of the 8th nerve root in the tadpole (*R. grylio*, 4 cases; *R. clamitans*, 2 cases) even though the animals had been blinded by previous excision of the optic nerve. Blinding presumably eliminated the functional value of these eyeball responses and yet in spite of this the vestibular eye movements were systematically re-established in their regular pattern. This evidence against functional adaptation, though suggestive, is not crucial because the eye movements may conceivably be part of a larger motor adjustment, other components of which retained a regulative functional value.

An attempt was made to set up a more critical test of the rôle of functional regulation by reversing the orientation of the vestibular sense organs. Recovery of normal responses under such conditions would indicate functional readaptation whereas recovery of responses in reverse would indicate that the re-establishment of reflex relations is predetermined by growth factors regardless of functional adaptation. Homoplastic transplantation of the left labyrinth into the site of the excised right labyrinth with reversal of the antero-posterior axis was carried out in a range of late larval stages of *R. grylio* and *R. clamitans* (16 cases), but none of these animals showed any signs of functional recovery although kept as long as 3 months after operation. Even in premotile embryonic stages such transplants or even direct reimplantations have previously been found to fail functionally, although apparently good nerve connections with the medulla are formed (10). Possibly a developmental stage or condition will eventually be found in which the inverted labyrinth will establish functional central nervous connections and thus provide a conclusive answer to the above question.

SUMMARY AND CONCLUSIONS

Fibers of the 8th nerve root after its complete transection in 17 adult and in 11 larval anuran amphibians succeeded in regenerating across the nerve gap, into the central nerve stump and into the medulla where they re-established functional reflex connections. Furthermore, despite inexact apposition of the nerve ends and distortion of the intraneural fiber pattern in the region of severance, the different fiber types of the vestibular root succeeded in regaining their original innate modes of function in an orderly selective manner. The normal character of the recovered reflexes indicated that those nerve fibers connected peripherally to the crista of the horizontal semi-circular canal formed their own type of central reflex relations distinctly different from those formed by neighboring fibers innervating other end organs of the labyrinth. Similarly the fibers of the anterior and posterior canals systematically re-established in each case their own distinct and appropriate type of central associations. As far as could be determined from the tests made, the fibers of all the various end organs of the labyrinth succeeded in recovering selectively their original proper type of central reflex relations.

Just how this systematic recovery of specific functional associations was accomplished remains unknown. That the precision of recovery can be attributed simply to indifferent mechanical guidance of the regenerating fibers is excluded by the manner in which the nerves were broken and apposed as well as by the microscopic appearance of the nerve scar. General knowledge of nerve regeneration (13) moreover indicates that such an interpretation is quite out of the question and may be disregarded. That the ingrowing fibers terminated in a random manner in the centers and these random anatomical associations were later readjusted to function adaptively by a "conditioning" or learning process, may also be excluded for reasons already enumerated above.

Two possible alternative interpretations remain open. First, function may conceivably have influenced the formation of synaptic terminals in some such manner as presumed in the theory of neurobiotaxis (2). It has often been supposed, particularly with regard to the development of reflex patterns in ontogeny, that the adequacy of functional effects may somehow have an organizing influence on the growth of synaptic associations. Some evidence counterindicating any functional regulation in the present cases is cited above but it is admittedly incomplete. Secondly, it is possible that the systematic re-establishment of central associations was predetermined essentially by non-functional factors such as physico-chemical axon specificities and selective contact affinities between the different axon types and neurons of the vestibular centers. This latter interpretation is strongly favored by other available evidence regarding both the establishment of basic integration patterns in ontogeny (12) and the re-establishment of central synaptic associations in nerve regeneration (5–8). The required constitutional specification of the vestibular nerve fibers could conceivably arise embryologically through induction effects of end organ differentiation in the labyrinth.

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