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Myotypic Respecification of Regenerated Nerve-fibres in Cichlid Fishes[1]

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From the California Institute of Technology

INTRODUCTION

IN man and other mammals normal motor co-ordination is not restored, as a rule, after regeneration of a severed peripheral nerve-trunk (Sperry, 1945). The random misdirection of regenerating fibres into foreign muscles tends to prevent normal dissociated action within the re-innervated musculature. In contrast, larval amphibians have been found to show excellent recovery of motor function in the form of 'homologous or myotypic response' (Weiss, 1936, 1941) following the cutting and regeneration of limb-nerves, limb transplantation, and the cross-connecting of limb nerves to foreign muscles. Similarly, good restoration of muscle co-ordination has been observed in the pectoral fin of adult teleost fishes (Sperry, 1950, 1956).

It has been suggested (Sperry, 1941, 1951) that such recovery is most easily explained in terms of a central readjustment of synaptic connexions to suit the altered pattern of peripheral innervation. Morphological or other direct evidence for such synaptic changes, however, has not been found. For further investigation of the problem fishes appear to offer promising possibilities in that the technical difficulties of staining the synaptic terminals in fishes appear to be somewhat less formidable than in the amphibians.

The following study was undertaken to ascertain whether the type of motor recovery observed in the pectoral fin centres of adult teleosts might also occur in a cranial nucleus where the conditions for histological demonstration of synaptic end-feet are much more favourable. The experiment involves regeneration following surgical section and cross-union of branches of the mandibular ramus of cranial nerve V in the cichlid fish, *Astronotus ocellatus* (Aggassiz), with a study of the extent of subsequent functional recovery. The results demonstrate motor readjustment in the trigeminal system similar to that reported previously for the innervation of limb and fin musculature in amphibians and fishes respectively.

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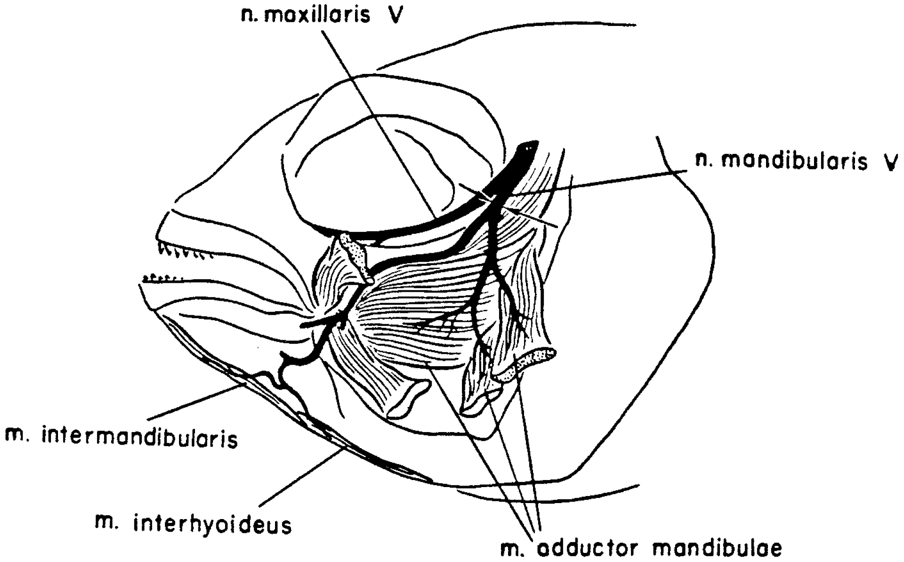
MATERIALS AND METHODS

Mandibular muscles and their innervation

The closing and opening of the mouth in *Astronotus* is governed by two sets of antagonistic muscles which act to elevate and depress the mandible respectively. The closing of the jaw is effected by a set of muscles, the adductor, or levator, mandibulae, which in this species has three subdivisions (Text-fig. 1). The opening of the jaw is controlled by the intermandibularis and the interhyoideus muscles. The posterior portion of the intermandibularis is fused with the interhyoideus, thus forming a common sheet of muscle that pulls the mandible downward. The latter muscles will be referred to here as the 'depressors' and the former as the 'levators'.



TEXT-FIG. 1. Sketch of jaw musculature and its innervation in *Astronotus ocellatus*. The two superficial adductor muscles are cut and deflected to expose the deeper adductor muscle. Arrows indicate point of section of mandibularis V nerve in Series I.

Both the levator and depressor muscles are innervated by the *ramus mandibularis trigemini*. The ramus mandibularis receives centrally all of the motor-fibres of V and some sensory fibres and, soon after its emergence from the cranium, gives off a motor-bundle (Text-fig. 1) to the levator muscles of the mandible. The nerve then turns laterally and ventrally into the lower jaw and divides into several branchlets, one of which carries all of the remaining motor-fibres and supplies the depressor muscles.

Surgical procedure

The fish were anesthetized by placing them in a solution of Tricane (MS 222, Sandoz Chemical Co.) mixed in the proportion of 0·05 grammes per litre of conditioned tap-water. The surgery was performed with the fish out of water under the dissection microscope with a magnification of 10 times. The anesthetized fish, wrapped in a wet cloth, was placed in a plasticene mould padded with gauze to hold it in the desired position. Anesthesia was maintained by dropping slowly over the gills a diluted solution of Tricane.

The nerve-sections were performed within the orbit. The conjunctiva was cut on the ventral side to free the eyeball, which then was raised and retracted gently to the dorsal edge of the orbit. This procedure exposed the infraorbital nerve-trunk which runs laterally along the floor of the orbit. The infraorbital trunk in this region consists of three main divisions, the middle one being the mandibularis illustrated in Text-fig. 1. Section of the nerves was done with fine scissors and forceps. The nerve-ends were reunited by simply apposing them and allowing a few moments for coagulation between the severed ends. Later the eyeball was replaced and the fish were put in a beaker of aerated aquarium water until they had recovered, after which they were returned to aquaria, two fishes per 3-gallon tank. The tanks were continuously aerated and the water temperature was thermostatically controlled at approximately 25° C. Penicillin was added to the tanks, 40,000 units per gallon, along with quinine hydrochloride, 1 grain per gallon, on the day of operation to minimize postoperative infection.

After regeneration and subsequent testing of functional recovery, the operated region was examined again under anesthesia, the surgical procedure being similar to that described above.

The fishes used in this experiment measured 4–6 cm. in total length at the time of operation, and they increased approximately 0·5 cm. in length during the period of observation, roughly 4 weeks. These specimens were quite young; the adults reach a length of 20–25 cm.

OBSERVATIONS

Series I. Proximal section and regeneration of mandibular trunk

In ten fish, the left mandibular nerve was completely divided at a point proximal to its bifurcation into levator and depressor branches (level of section is marked by arrows in Text-fig. 1). Before their reapposition the two ends were teased open to favour a random disarrangement of the interneuronal fibre-pattern in regeneration.

Partial paralysis was visible on the left side during the first week following the operation. However, since the two mandibles are fused in front, the whole lower jaw continued to move up and down in a near-normal fashion owing to the action of the muscles on the right side. The fish was able to ingest and masticate its food, including whole snails and small bits of meat, much as usual.

In one specimen of this series the mandibles were separated at the time of operation by an incision at the symphysis extending ventrally and medially through the intermandibularis muscle-plate. In this case the left mandible hung open and immobile except for a slow feeble tremor associated with the action of the opercular and branchiostegal muscles mediated by nerve VII. This residual movement, which was not sufficient to enable the fish to close the mandible, disappeared when the hyoid and the mandible were disconnected from the operculum and the branchiostegal rays.

The right mandible, meanwhile, continued to move up and down normally, and the fish was able to pick up and eat very small pieces of meat. This separation of the mandibles was not carried out in the other specimens at this time, because it had been found that it results, in the course of growth, in an abnormal curvature of the lower jaws that renders post-recovery observations difficult.

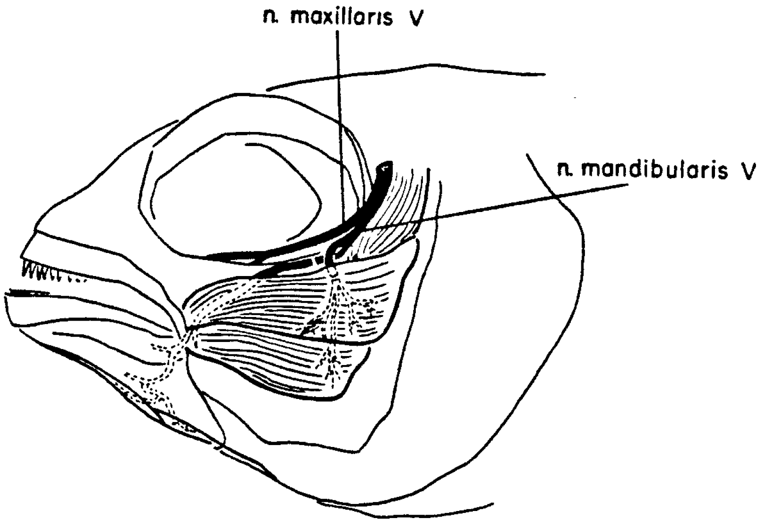
The first signs of recovery in the specimen with divided mandibles appeared on about the 7th day after operation. By the 10th day there were definite movements on the operated side, and by the 16th day the mandibular movement had been almost completely recovered. The recovered movement when first detected on the paralysed side was timed synchronously with that of the normal right side. The abnormal growth of the mandibles during the subsequent recovery period made further observations difficult. In the other fish of the series also the paralysis on the left side was no longer visible by the 16th day.

On the 21st day after operation the right mandibular nerve was cut in four specimens of the series in a position corresponding to that of the original section on the left side. In all four cases the movement of the lower jaw, activated now through the regenerated left nerve, was near normal except for a slight paralysis on the right side produced by the nerve-section on that side. The fish were able to pick up and chew food without difficulty. Two days later the regenerated left mandibular nerve in these four cases was cut at the site of the scar. This left the lower jaw hanging open and almost immobile except for the residual movement referred to above. The fish were unable to close the mouth or to feed. The results were the same in all four specimens.

In the remaining five specimens the two mandibles were separated by a medial incision at the symphysis on the 24th day after operation, in order that the independent movement of the two halves of the jaw might be compared for timing and co-ordination. In all cases the two mandibles were found to open and close with perfect synchrony, indicating normal timing in the re-innervated muscles. On the following day when the right mandibular nerve was cut, the right mandible hung open but the left continued to move normally. Two days later the regenerated left mandibular nerve was cut at the site of the scar in two of the latter five specimens, resulting in paralysis of the lower jaw except for the slight residual movement noted previously.

Series II. Cross-union of levator and depressor nerves

In the second series of operations the left mandibular nerve was sectioned distal to its bifurcation; that is, the levator and depressor branches were cut separately. The four nerve-stumps were then cross-united so that the levator fibres would grow back into the depressor muscles and the depressor fibres into the levator muscles as indicated in Text-fig. 2. Fourteen fishes were operated, eleven of which survived for the testing of functional recovery.



TEXT-FIG. 2. Sketch showing cross-union of levator and depressor branches of mandibularis V nerve.

The visible effects of the operation were the same as in Series I. In two of the specimens the mandibles were separated at the time of the operation to facilitate detection of the first signs of recovery. On about the 8th day after operation, slight movement was observed in the paralysed left mandible of these two cases. By the 14th day recovery was pronounced, though the movement of the two separated mandibles was restricted because the mandibles had grown to overlap anteriorly.

Checks for recovery were carried out as in Series I, beginning on the 23rd to 29th days after operation. In four cases the right (unoperated) mandibular nerve was cut. Three of the four showed partial paralysis of the right mandible as noted in Series I, but without serious impairment of the movement of the jaw which now was activated by the regenerated crossed nerves. The fourth specimen had already shown a lack of complete recovery in that the slight paralysis observed after the original operation had not been corrected. The downward movement in the left mandible was extremely weak and this fish was hardly able to ingest usual-sized pieces of food, snails, or bits of meat. Unlike the three normally recovered specimens, it had to make repeated attempts to pick a piece of food from the bottom of the tank. After the right mandibular nerve was sectioned in this specimen, the jaw remained in a nearly locked position. An examination of the regenerated left mandibular nerve revealed that the levator branch had grown back towards its own stump, along with the crossed depressor, apparently leaving the distal stump of the depressor branch without any motor connexion. The operated region of the three normally recovered specimens was then examined and in all these cases the cross-union of levator and depressor branches was complete and the nerves had grown into the foreign muscles.

In five other fish of Series II the two mandibles were separated at the symphysis so that the function of each could be observed independently. In four of these specimens the right and left mandibles moved up and down with perfect co-ordination and timing. The movement on the left (regenerated) side was normal except that the mandible did not close quite as tightly as did the right mandible. Sectioning of the right (unoperated) mandibular nerve in these four specimens produced paralysis of the right mandible, but the fish continued to use the left mandible in approximately normal manner. On separation of the mandibles in the fifth specimen, it was found that the movement of the left mandible was impaired. When the right mandibular nerve was cut, the right mandible hung open and the left mandible was locked in an almost closed position. Examination of the regenerated nerve revealed that in this exception the levator branch had grown back towards its own stump, instead of into the depressor stump as intended.

The regenerated nerve in the four completely recovered specimens was then examined by dissection under anesthesia. In one case the levator and depressor branches were found to have slipped and grown back into their own respective muscles. In the other three specimens the cross-union of levator and depressor branches was complete. Careful examination was made to ensure that no stray fibres connected the levator and depressor branches to their own respective muscles. A further electrical check was performed in which the connexion between the levator branch and the depressor stump was severed, leaving the other cross-connexion (depressor branch into levator stump) intact. The mandibular nerve was then lifted from the surrounding tissue by means of a curved needle, and electric stimulation from an induction coil was applied at a point above the scar. The result was a pronounced contraction in the levator muscles, indicating that functional connexion had been established between the depressor nerve components and the levator muscles. Sectioning the depressor branch at the site of the scar eliminated all response in the levator muscles.

The two specimens in which the mandibles had been separated at the time of operation were not tested further because of the lopsided regrowth of the mandibles.

DISCUSSION

The recovery of normal vigorous movement of the mandible observed in Series I could have been effected only through re-innervation of the paralysed mandibular muscles and activation of these muscles in their proper timing. This means either (*a*) that the regenerating motor-fibres must have re-established their functional connexions selectively with their original muscles, or (*b*) that those fibres that connected with foreign muscles must have changed their central timing to suit the new terminations. The latter alternative is strongly favoured by the occurrence of similar recovery in the second series in which the nerve-fibres were definitely cross-connected by surgical means to antagonist muscles. In view of the rapidity of recovery in Series II and the results of the combined physiological and anatomical checks, it is extremely improbable that the mandibular muscles could have become re-innervated by their original motoneurons.

The possibility that the central readjustment in timing could have been effected on a reflex or other purely functional basis such as learning is contraindicated by the fact that such readjustment has not been observed to occur in other vertebrates including man. Nor was there any visible evidence of re-learning during the recovery of mandibular movement. Accordingly it is inferred that the trigeminal motoneurons automatically adjusted their central timing to suit whatever trigeminal muscles their regenerating axons happened to connect with in the periphery. This is the type of motor re-adaptation observed originally by Weiss (1936, 1941) in the limb innervation in larval urodeles and later in newly metamorphosed toads, and found more recently in the limb innervation of adult *Triturus* (Sperry, unpublished) and in the fin innervation of adult fishes (Sperry, 1950, 1956). Investigation has failed to reveal this kind of readjustment in the limb innervation of adult frogs, in the oculomotor system of the adult swell-fish, and of mid-larval and adult amphibians, and in the motor system generally of young and adult mammals (Sperry, 1945, 1947, 1956, and unpublished data).

Following the early proposal of Wiersma (1931), it has been accepted that the exceptionally good motor re-adaptation where it has been found in fish and amphibians must involve a transmission of some kind of specifying influence from the muscle to the regenerated motor axons. Exactly how the specification imposed upon the nerve axon by the muscle subsequently affects the incidence of central discharge remains to be determined. The possibility of checking experimentally the suggestion that the central timing is changed through a readjustment in the synaptic end-feet on the affected motor neurons is rendered somewhat more feasible by the present finding that such motor readjustment can occur in a cranial motor-nerve nucleus in postembryonic stages.

SUMMARY

1. The mandibular portion of cranial nerve V of the teleost fish, *Astronotus ocellatus*, was totally severed and allowed to regenerate into its distal nerve-stump in a series of ten cases.

2. In a second series of fourteen cases, the two nerve branches supplying the elevator and depressor muscles of the mandible were sectioned separately and the nerve of the depressor muscles was crossed into the levator muscles and vice versa.

3. In both series, return of function in the re-innervated muscles was first

detected about 8 days after nerve-section, and full recovery was achieved by the 18th to 21st day save for two exceptions in Series II in which the surgical cross-union proved faulty.

4. The recovered mandibular action was properly timed and co-ordinated from the beginning with the mandibular muscles of the opposite side.

5. It is inferred (*a*) that the regenerating motor-fibres establish functional connexions with foreign mandibular muscles, (*b*) that the muscles induce a local muscle specificity in the regenerated motor axons, and (*c*) that this 'myotypic' specificity determines in some way not yet analysed the central timing of motoneuron discharge in the trigeminal motor nucleus.

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attention will be directed to the influences of Universities (Salerno, Bologna, Padua); Academies (Royal Society, dei Lincei, del Cimento); the short focus microscope (Leewenhoeck); the cluster of kindred sciences. Furthermore, the transmission of anatomical terminology from the Greek, through the Syriac and Arabic to the Latin and the further point that the terms made their way through the fields of the fanciful, the dogma, the ancillary and the precise phases — these too will show that anatomy struggled to attain a rank of dignity in the hierarchy of knowledge. Perhaps there is some justification in speaking of an anatomia ''sanctus'' (Pre-Greek); an anatomia ''doctor'' (Greco-Roman-Arabic); an anatomia ''animata'' (Renaissance); and an anatomia ''scientia'' (Modern).

153. *High-order integrative functions in surgically isolated somatic cortex in cat.*[1] R. W. SPERRY, Division of Biology, California Institute of Technology.

It had been shown that tactile discriminations for hardness, roughness, and shape learned with use of one forepaw in a pedal-pressing apparatus, transfer at high level to the untrained forepaw in unoperated cats whereas such transfer fails in cats with sectioned corpus callosum. In the present experiment most of the neocortex was removed from the right hemisphere in 4 callosum-sectioned cats leaving intact an island of anterior cortex in and around somatic areas I and II. This produced no more than a transient impairment of preoperatively trained tactile discriminations, nor did it markedly retard the learning of new discriminations all performed with the affected (left) forepaw. Subsequent surgical damage centered in the isolated cortical remnant of one case abolished all further discrimination with the left paw. Additional cortical lesions placed some months later in the contralateral (left) somatic cortex of the remaining 3 animals impaired severely the discriminative performance with the right paw to the extent of eliminating it entirely when the lesion approached in size the intact cortical island on the right. However, these later lesions did not impair the discriminative performance with the left paw. The results indicate that the cortical reintegration directly involved in learning, retention, and recall of tactile discrimination can be effected within the limits of the isolated sector of cortex.

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161. *Effects of epinephrine on carbohydrate metabolism in underfed and ad libitum fed rats.*[1] B. N. SPIRTOS and N. S. HALMI, Department of Anatomy, State University of Iowa.

Young adult male rats of the Sprague-Dawley strain were used to study the influence of epinephrine on carbohydrate metabolism in under-fed rats and *ad libitum* fed controls. The experimental animals were fed 10 gm of powdered Rockland chow daily (i.e., approximately one half of the amount of food consumed by rats on a non-restricted diet) for 4-5 weeks. Following a 24 hour fast, the underfed rats showed less rise in liver glycogen and blood sugar levels in response to the injection of epinephrine than did *ad libitum* fed-fasted rats. Gastrocnemius glycogen levels were found to be higher in underfed-fasted