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REGENERATION OF THE LATERAL LINE NERVE OF AMBLYSTOMA FROM DIFFERENT NERVE FIBER SOURCES [1]

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ONE FIGURE

Past experiments on the volume of peripheral nerve fiber regeneration in amphibians have demonstrated that when the size of a nerve fiber source and the mass of its terminal tissues are varied independently, the fiber count in the regenerated nerve portion matches the latter rather than the former (Weiss, '37; Weiss and Walker, '34; Weiss and Litwiller, '37a, '37b; Litwiller, '38a, '38b). But the mechanism by which the periphery exerts this control over the density of its reinnervation is still obscure. Where fibers regenerate into a peripheral nerve stump, its complement of degenerated tubes (Buengner's cords) might be assumed to constitute the limiting factor. A peripheral stump after regeneration actually contains nearly its normal quota of fibers (Greenman, '13; Weiss, '37; Davenport, Chor and Dolkart, '37), just what might be expected if most tubes became reinvaded each by a single fiber. However, all tubes are not invaded; some tubes, in turn, receive more than one fiber, and some fibers grow in between tubes (Cajal, '28). Consequently, restoration of numerical order would appear to be more a matter of secondary adjustments than of limited penetration (Holmes and Young, '42). More facts are needed before the

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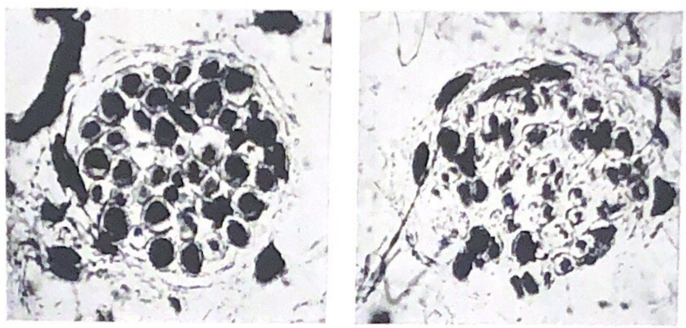
issue can be decided. Realizing that further analysis would be facilitated by the choice of a simpler, smaller and better accessible nerve than the limb nerves hitherto used, we have turned to the trunk lateral line nerve. Since many characteristics qualify this nerve to become a standard object in regeneration studies, we present the results of a preliminary survey of its regenerative capacities under various experimental conditions.

Large larvae of Amblystoma tigrinum, measuring from 3 to 6 cm. between fore and hind limb insertions, served for the experiments. The middle trunk line nerve, which runs anteroposteriorly in a canal under the flank skin, was used. For simple regeneration experiments, the nerve was transected about half-way between the fore and hind limb levels; in some cases, the peripheral stump was left in place, in others, it was pulled out. For regeneration from foreign fiber sources, the nerve was likewise transected in mid-trunk, but the proximal stump was removed and in its place a new fiber source was inserted facing the open end of the peripheral stump. The foreign innervation was provided by (1) the proximal stump of another lateral line nerve of either the same or the opposite side; (2) the deviated proximal stump of the hypoglossal nerve; (3) a transplanted spinal ganglion; (4) a transplanted fragment of spinal cord. Foreign nerves were hauled into the new site by means of a sewing needle through the eye of which they were threaded.

Some animals were sacrificed after 1 to 2 months, others only after 9 to 10 months. The nerves were studied in cross sections after paraffin embedding or in total mounts after dissection; all were impregnated with silver according to Bodian. Fiber counts in total mounts proved consistently too low because of fiber overlap; they require an upward correction of at least 20%.

Normal constitution of lateral line nerve. In animals of the size used, the nerve contains an average of fifty fibers (thirty-four counts in ten animals), falling in three size classes: small, medium, and large, in a numerical ratio of 1 : 2 : 1 (fig. 1). The fiber number does not appreciably decline antero-posteriorly, which means that the many sense organs innervated by the nerve are supplied by collaterals of common fibers, a fact to be taken into account in the physiological interpretation of lateral line function. Preliminary observations on animals of different ages indicate that the majority of these fibers date back to the embryonic phase. Post-embryonically, the neurons increase but little in number though considerably in size (cf.



A B

Fig. 1 Transected and regenerated lateral line nerve, 1 month after operation. A, proximal stump B, distal (regenerated) portion. The large dark bodies in the regenerated nerve portion are nuclei. The young axons appear as small black dots, often several to a single old tube. × 500.

Hursh, '39). On the whole, therefore, this nerve is sufficiently constant in its composition to serve as standard material for experimentation.

Transection with extirpation of peripheral stump. Fifty-three specimens subjected to this operation gave uniform results, namely, abortive regeneration. The outgrowing proximal fiber stumps never reached beyond the immediate vicinity of the cut end; they form small neuromas, the branches of which scatter in near-by muscle and connective tissue, without ever repleting the lateral line canal. The result could perhaps be ascribed to the lack of an adequate number of peripheral sheath cells to support the growth of the nerve. It is noteworthy that the sensory lateral line fibers make what appears microscopically as intimate terminal contact with muscle fibers.

Simple transection. Of nine cases in this group, two were studied in cross sections (early stages) and seven in whole mounts (terminal stages). In the latter, the peripheral stump was reinnervated to the extent of from eighteen to twenty-eight fibers, with an average of twenty-four. Allowing for undercounting in total mounts, the actual number would probably lie above thirty. Even though this value represents a deficit from the average of fifty fibers of normal nerve, it is still of the same order.

The two cross-sectioned cases illustrate regeneration 1 month after transection. The regenerated axons are immature, consisting of neurofibrillar bundles not yet identifiable as distinct nerve fibers. In one case (fig. 1), some ninety fibrillar units were counted near the cut, and about sixty-five farther distally. They were grouped into forty-three bundles, and this may well be an anticipation of the future fiber total. Eleven tubes were empty in the regenerated portion near the cut, but only three to four farther distally. The second case contained twenty-four fibrillar bundles.

Crossing of different lateral line nerves. Most cases of this series failed because the crossed nerve slipped back. The peripheral stump remained uninnervated, but persisted as such. Of a total of twenty cases, only three can be properly evaluated. In two, the smaller upper nerve (normal count in total mounts: ten to seventeen fibers) was connected with the peripheral middle nerve and regenerated twelve and fourteen fibers, respectively. In the third case, the larger nerve was connected with the peripheral stump of the smaller one and regenerated ten fibers. These values are consistently below those obtained in ordinary regeneration of the middle trunk nerve and could be ascribed, in the first two cases, to the undersized fiber source, and in the third, to the reduced size of the peripheral nerve stump.

Proximal hypoglossal connected with peripheral lateral line nerve. A sample count of a proximal hypoglossal nerve gave over 300 fibers. This large nerve regenerated extensively, but only a very limited number of fibers found admission in the peripheral lateral line nerve. Of ten cases studied in whole mounts, five showed no connection between hypoglossal and lateral line nerve, evidently because the gap between the stumps was too wide. In the remaining five cases, the peripheral lateral line nerve contained 32, 40, 13, 36 and 23 fibers, respectively, which is again within the range observed after mere transection. Cross sections of an early regeneration stage contained ninety fibrillar units, grouped into forty-two bundles, near the base, declining to thirty-five units in twenty bundles farther distally. This also compares favorably with the early transection cases reported above. The motor fibers of the hypoglossal seem to have had no difficulty in regenerating into the purely sensory lateral line nerve.

Spinal ganglion transplants. In nine cases, a spinal ganglion was transplanted in front of the cut end of a distal lateral line nerve stump, the proximal portion having been removed. Five cases were discarded because of staining failure. In one case the ganglion had disappeared and the nerve contained no fibers. The three remaining cases (cross sections) showed the ganglion with numerous nerve cells intact and 22, 28 and 33 fibers, respectively, present in the lateral line nerve reinnervated from the ganglion.

Spinal cord transplants as regeneration source. In contrast to similar grafts to the dorsal fin (Weiss '40, '41), flank grafts rarely survive, presumably for mechanical reasons. Only in two out of nine cases, the graft was evident, and in these it had regenerated an average of twenty (cross section) and fifteen (total mount) fibers into the lateral line nerve.

Discussion. It is clear from the experiments that the number of fibers in the regenerated portion of the lateral line nerve is independent of the number of fibers produced by the proximal stump. The hypoglossal nerve and the spinal ganglion transplants provided a source several times larger than the regular lateral line nerve, without causing an increase in the yield of peripheral fibers. Similarly, a small lateral line nerve stump, when supplied from a larger lateral line nerve, contains fewer fibers than would be regenerated in the latter's own stump. The number of regenerated fibers in the peripheral stump of the lateral line nerve tends to approach the full normal quota; it rarely reaches it, never exceeds it. Evidently, therefore, the size of the peripheral stump limits the volume of fiber regeneration. The lateral line nerve is a singularly favorable object to prove that the nerve itself, rather than the terminal receptor surface, constitutes the limiting factor, for, as was stated above, even in the normal nerve, the fiber number bears no relation to the number of end organs innervated. How the old nerve controls the quantity of regeneration, remains to be determined. The observed grouping during intermediate regeneration stages of fibrillar units into approximately as many bundles as the normal nerve has fibers, is suggestive. However, we do not know how this grouping comes about. While some old nerve tubes contain but a single bundle, others contain several, and still others, although a minority, remain empty.

In contrast to limb nerves, which in urodeles can regenerate to full length even after removal of the peripheral nerve stumps, no nerves in the lateral line canal have ever grown out for appreciable distances unless inside of an old peripheral stump. It is uncertain whether this failure is to be ascribed to inadequate sheath cell outgrowth or to immediate absorption of the fiber branches by the surrounding segmental muscle and connective tissue. Further experiments will have to decide these points, as well as the interesting question of restitution of fiber diameters after regeneration from diverse sources.

Summary. The small and relatively constant fiber complement of the urodele lateral line nerve recommends this nerve as standard object for nerve regeneration studies. The number of fibers regenerated in the peripheral stump stays within the normal range, even if excessive fiber sources (hypoglossal nerve, spinal ganglia) are made available. Thus, the peripheral rather than the central stump determines the volume

of regeneration, provided that the proximal fiber supply is adequate.

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