Huygens Institute - Royal Netherlands Academy of Arts and Sciences (KNAW)

Citation:

J. Boeke, Nerve-regeneration after the joining of a motor nerve to a receptive nerve, in: KNAW, Proceedings, 15 II, 1912-1913, Amsterdam, 1913, pp. 1281-1289

This PDF was made on 24 September 2010, from the 'Digital Library' of the Dutch History of Science Web Center (www.dwc.knaw.nl) > 'Digital Library > Proceedings of the Royal Netherlands Academy of Arts and Sciences (KNAW), http://www.digitallibrary.nl'

mica was crossed by a radiation in a direction perpendicular to the cleavage plane. The photogram so obtained was much weaker, although the time of exposition was taken equally long, and although the intensity of the primary radiation was the same. This may be explained by observing that in the reflection the cleavage plane rich in molecules gives a spot, which does not appear with the transmitted radiation. But the other images are to be taken with respect to corresponding planes. The explication therefore must run otherwise. In both cases a cylindrical pencil with cross-section of about 1 mm. strikes the plate. Consequently the part struck by radiation of the plane richest in molecules, the reflection taking place under an angle α near 90°, is a good deal greater, namely in the proportion $\frac{1}{\cos \alpha}$, the number of working layers being the same. In the most unfavourable case of the vector of radiation lying in the plane of incidence, the working vector of radiation, if $\alpha = 90 - \beta$ where β is a small angle, is $-S \sin 2\beta$. The intensity of the image reflected thus will be proportional to $\frac{I^2 \sin^2 2\beta (o\omega)^2}{\sin^2 \beta}$ (where o is the diameter of the pencil, ω the number of particles pro unit of surface). For the case of the vector of radiation lying in the plane of incidence, $\sin 2\beta$ in the numerator is to be substituted by the unity; then the intensity will be great. As the incident pencil is not polarised, we have to expect a stronger effect with the reflection than with the light being directly transmitted.

5. The reflection on rock-salt (perpendicular to a cubical axis) again gave a set of spots very clearly observable, situated on conical sections through the central spot. The spots were lying close together on the plate; as may be supposed they are partly to be assigned to different not wholly parallel layers in the crystal.

Anatomy. — "*Nerve-regeneration after the joining of a motor nerve to a receptive nerve.*" By Prof. J. BOEKE.

(Communicated in the meeting of February 22, 1913).

After the primary discoveries of FONTANA, MONRO, CRUIKSHANK, at the end of the 18th century, no phenomenon of life has been more closely studied than the process of nerve-regeneration. Attention was drawn to the primary degeneration of the peripheral portion of a cut nerve deprived of its trophic centre, the ganglion cells (WALLER), and the manner after which a new nervous union was established

by the growing out of the fibers of the central end into the old path of the peripheral nerve-portion became better and better known. It was seen how the new nerve-fibers growing out from the cut-end may extend to the organs normally supplied by the nerve in question, form new end-organs and how thus even a functional regeneration may take place. It was seen how regenerating nerve-fibers may even grow into a nerve-path belonging to another (cut) nerve, and how motor fibers from the cut-end of the nervus accessorius for example may grow into the peripheral degenerated portion of a cut facialis nerve and thus in the end provide with motor nerve-endings the atrophying muscle-fibers of the mimic muscles.

This phenomenon leads naturally up to the question, whether it would be possible, after a nerve containing motor and receptive fibers has been severed in its course, that motor nerve-fibers from the cut-end grow into degenerated receptive fibers of the peripheral portion of the nerve, and vice-versa.

This question, which was studied for the first time by BIDDER in 1849 and more closely by PHILIPEAUX and VULPIAN in 1863 and 1873, and by different authors in the course of the years, has been answered almost universally in a negative sense. Even LANGLEY and ANDERSON, who studied the question as late as 1904, denied the functional and trophic regenerative union of motor and receptive fibers, and BETHE, who studied the question for (as far as I could gather) the last time in 1907 [1]), gives as the results of his investigations the following statement: "dass auch unter den für die Vereiniging günstigeren Bedingungen (nach Durchschneidung der motorischen Wurzeln) eine functionelle oder auch nur trophische Verwachsung zwischen rezeptorischen und motorischen Fasern nicht eintritt." (l. c. page 481).

And yet, notwithstanding these statements, the question must be answered in a positive sense.

To study the question, the same course was taken as that followed by PHILIPEAUX and VULPIAN making their classic experiments in 1863 and 1873 (VULPIAN). The nervus lingualis and the nervus hypoglossus of the same side were both cut through. Only I did not join the central end of the lingualis to the peripheral portion of the nervus hypoglossus [2]), as was done by the investigators mentioned above, but followed the example given by BETHE in 1903, and joined the

[1]) PFLUGER'S Arcniv, 116 Bd. 1907.

[2]) In a second note I hope to describe the results of this line of experiments.

central end of the n. hypoglossus to the peripheral portion of the nervus lingualis. The two other nerve-ends were both exstirpated as far as they could be reached.

The entire cycle of experiments was the following:

a. In a number of fullgrown hedge-hogs (14 in all) the right nervus hypoglossus was cut through, and the ends joined together. After a lapse of several days, weeks or months the animals were killed, the bloodvessels were rinsed by means of the fluid of RINGER-LOCKE, and the tissues were preserved by means of an injection of a very slightly alcaline solution of formalin into the aorta; afterwards the nerves and the nerve-endings inside the tongue were stained by the BIELSCHOWSKY-method, and cross-sections or sagittal sections of the tongue examined under the microscope.

The phenomena of regeneration of the motor fibers after the reunion of the severed ends of the n. hypoglossus I will not discuss here. In this connection it only interests us to know, that in preparations made of the tongue of animals killed 5 to 10 days after they were operated upon, all the fibers of the n. hypoglossus of the right half of the tongue were entirely degenerated, the fibers of the nervus lingualis having of course remained entirely intact. In this way I obtained a very accurate insight into the topographical relations, the course and distribution of both nerves throughout the tongue. These relations are very systematic, so that when we only take care to compare analogous cross-sections of different tongues with each other we are able to tell immediately in a given cross-section the places where the nerve-fibers of the n. lingualis and those of the n. hypoglossus (at least the larger rami) are to be found. For a safe and accurate judgment of the results of the following group of experiments (*b*) these preliminary experiments are absolutely necessary.

b. In another series of full-grown hedge-hogs at the right side of the neck the nervus lingualis and the nervus hypoglossus were cut through, great care being taken to make as small a wound as was possible and to injure no other elements. After this the central cut-end of the n. hypoglossus was joined with the peripheral portion of the n. lingualis, the two other ends were exstirpated as far as possible, and the wound closed. After a lapse of some weeks or months the animals were killed, and stained sections through the tongue examined after the manner described above. To prevent ulcerative processes to occur in the lamed and anaesthetic half of the tongue, before the operation all the teeth of the right side of the mouth were stripped of their crowns. After that ulcerative processes in the tongue did not occur any more.

Examination of the place of section of the nerves showed in the first place that in the greater half of the cases, viz. in 11 of the 20 aminals of group *b* which were operated upon, a complete union of the heterogeneous nerves had taken place. The central cut-end of the hypoglossus adhered firmly to the peripheral portion of the lingualis, and after one or two months the peripheral portion of the joined nerve had turned white again, viz. had become myelinised. After a due lapse of time even the place of union of the nerves, the cicatrice itself, was white. I however got the impression, that the process of union of the cut-ends has a somewhat longer duration than after the dissection and joining of homogeneous nerve-portions. The experiments of group *a* showed, that already after the lapse of one month regenerating nerve-fibers were visible in the tongue, and after one and a half month regenerating motor endplates were visible on the muscle-fibers even at the tip of the tongue. In the experiments of group *b* it was only after 2 or 3 months, that I was able to detect the regenerating fibers inside the tongue.

These results were confirmed in all points by the microscopic examination. The regenerating nerve-fibers of the hypoglossus had grown through the cicatrice, had reached the peripheral portion of the lingualis and had grown into it just as in the regenerative union of homogeneous nerve-ends. Sections through the place of union tend to show the same intertwisting of the neurofibrillar bundles, the regenerating axons, in the cicatrice, the slow forward movement, and at the end the same picture of the regenerating axons penetrating into the channel of the degenerated peripheral portion, in casu the n. lingualis. Nearly all the regenerating fibers of the hypoglossus penetrate into the peripheral nerve-end, in casu the n. lingualis. A few fibers only pass alongside and are seen growing out into the surrounding tissue, the perineural connective tissue.

The examination of the microscopic sections gave me however the same impression as the macroscopic inspection, viz. that the process of regeneration, especially of the penetrating of the regenerating axons into the peripheral nerve-end (lingualis) has a somewhat longer duration and slower movement than in the union of homogeneous nerve-ends. The intertwisting of the axons is more dense, and a greater number of the so-called spirals of PERRONCITO are formed. As however the nervus hypoglossus possesses a far greater number of nerve-fibers than the nervus lingualis, finally the peripheral nerve-end (lingualis) becomes entirely filled-up with the regenerating axons of the hypoglossus nerve.

The examination of the cross-sections through the tongue gives

corresponding results. When we examine such a cross-section in a successful experiment (and only those are considered), we find all the sections of the branches of the n. lingualis filled with regenerating nerve-fibers, whilst those of the n. hypoglossus are entirely (or nearly so) devoid of them, showing only the so-called bands of BÜNGNER of the degenerated nerve-tubes.

This is — and that gives us the answer to the question mentioned above, why no *physiological* regeneration is to be found — not only the case with the larger branches, but also the smaller and smallest branches present the same aspect. When the larger branches of the hypoglossus are devoid of regenerating axons, no trace of these is to be found even in the smallest branches of the hypoglossus, whilst even the smallest branches of the lingualis are full of regenerating axons, and a dense plexus of regenerated nerve-fibers is present in the mucous membrane of the tongue, in the connective tissue of the submucosa, but not a single motor nerve-plate is to be found on any of the muscle-fibers, in sharp contrast to what we find after the regeneration of the nerve-fibers of the hypoglossus into the peripheral end of the hypoglossus itself (group *a*), where we find everywhere the regenerating end-plates on the muscle-fibers.

When regenerating nerve-fibers have penetrated into the old channel of a peripheral degenerated nerve, it clearly is impossible for them to get out of it and they are compelled to travel it to the end. Nowhere is this rule demonstrated so clearly as it is done here. The branches of the lingualis nerve wind their way towards the final station, the mucous membrane, between the bundles of muscle-fibers, and often seem to come into close contact with them, as is clearly shown by the examination of the sections in the experiments of group *a*. And yet not a single nerve-fiber of the regenerating hypoglossus nerve leaves the channel of the lingualis in group *b* to form an endorgan on the muscle fibers as it is to be seen everywhere in the experiments of group *a*[1]).

Now the question might be asked, whether these regenerating nerve fibres growing into the peripheral end of the nervus lingualis are in reality hypoglossus fibres, and whether it is not more probable that the ingrowing fibres are after all lingualis fibres, which grew out from the central end of the lingualis and have found their way into the old nerve channel. To exclude this source of errors, in a number of animals, in which 3 and 4 months ago the central end of the n. hypoglossus had been joined to the peripheral end of the

[1]) J. BOEKE, Ueber De- und Regeneration motorischer Endplatten, etc in Verhandl. der Anat. Gesellsch. Versamml. in München. April 1912. S. 152

n. lingualis (group *b*), the cicatrice was opened again, and after it had been ascertained, that the nerve-ends had grown together and that the peripheral portion was myelinised already, the central cut-end of the nervus lingualis was prepared again, and cut out with a part of the surrounding connective tissue as far as it was possible to reach it, the connective tissue being exstirpated because it might be possible that some nerve fibres from the central end of the lingualis had grown into the connective tissue and from there had reached the point of joining of the two nerve-ends. Ten days were allowed to the eventually cut nerve fibres to degenerate, and after that time the animals were killed and prepared after the manner described above. Ten days may be supposed to be entirely sufficient for the degeneration of all the nerve fibres eventually supplied by the central portion of the lingualis nerve.

One of these experiments, which looked entirely successful, was studied as accurately as possible, and gave the following results: from the central portion of the lingualis nerve not a single nerve fibre entered the peripheral lingualis, nor had any other nerve (a small muscle nerve for example) regenerated into the peripheral lingualis, except the nervus hypoglossus. From the central cut-end of the hypoglossus, which was in full process of regeneration, a large number of regenerating nerve fibres had grown out and had all penetrated into the peripheral end of the nervus lingualis. Only a very few fibres had grown into the perineural connective tissue around the lingualis nerve. Inside the tongue all the lingualis branches were full of regenerating fibres, the hypoglossus branches were entirely devoid of them.

The regenerating fibres, which here could have no other source than the hypoglossus, had followed the course of the lingualis nerve down to the smallest branches of the nerve plexus in the mucous membrane of the tongue. Of so-called autogenic regeneration (A. Bethe) no trace was found (only full-grown animals were used for experiments).

The fibres of the hypoglossus nerve, having arrived at the end of the terminal branches of the lingualis, begin to form nerve-endings of different patterns. It is here not the right place to describe elaborately the differences in form and in extension of the nerve-endings. I hope to do that in extenso elsewhere. Here I will only mention two or three points.

It is certainly an interesting fact that the hypoglossus fibres after having penetrated into and arrived at the end of the lingualis tract, begin to form terminal branchings and different end-bulbs. But not only that they form nerve-endings in the connective tissue, but

they even penetrate into the epithelium. In most cases the terminal fibrillae do not penetrate far into the epithelium, but remain

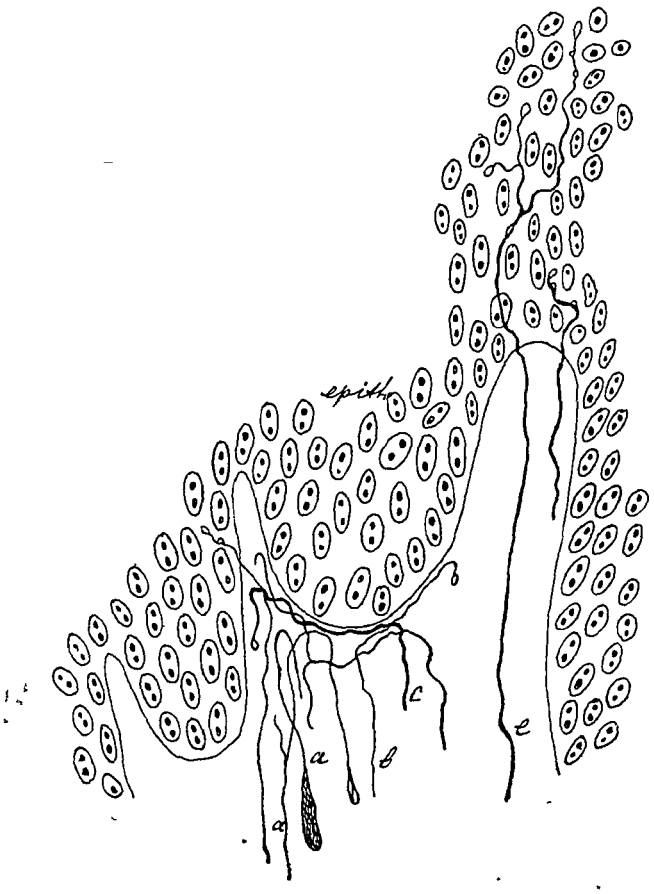

Fig. 1. Nerve endings of hypoglossus fibres in the epithelium and the connective tissue of the mucous membrane of the tongue.

a, *b*, *c*. Ascending fibres, not penetrating into the epithelium, but turning round and descending again towards the connective tissue.

e = fibres penetrating into the epithelium

in the basal layers, where they form small endnets around different epithelial cells, but sometimes they penetrate into the upper layers of the epithelium (fig. 1 *e*).

It seems however that the epithelium offers a certain resistance against the ingrowing fibrillae, that makes it difficult for them to penetrate into the epithelial membrane. In the normal half of the tongue at all points of the epithelium the neurofibrillae may be seen

to penetrate far into the epithelium, sometimes as far as the superficial layers of cells. In the other half of the tongue, where the fibres of the hypoglossus nerve are regenerating along the nerve paths of the lingualis, one sees often strikingly how the nervous fibrillae grow right up against the basal side of the epithelium, but then do not penetrate it, but turn round and descend again, ending inside the connective tissue with an endknob or endnet, or run for a shorter or longer distance along the basal side of the epithelium as if seeking entrance, and then turn round and end between the elements of the connective tissue as described above (fig. 1 *a*, *b*, *c*).

In the second place it is an interesting fact, that the terminal branches of the hypoglossus nerve fibres often show a striking resemblance to the endplates formed on the muscle fibres during regeneration after simple cutting of the hypoglossus nerve (*a*-group of experiments). An example is given in the figs. 2 and 3. In fig. 2 is drawn a set of terminal branches formed by a hypoglossus nerve fibre against the basal membrane of the epithelium, in fig. 3 is drawn a regenerated motor end-plate on a muscle fibre of the tongue after

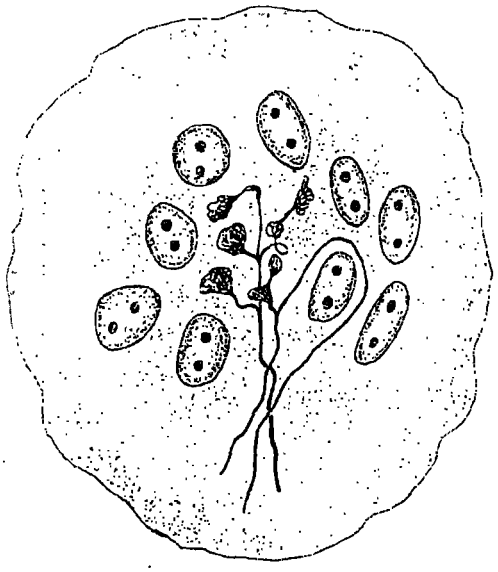

Fig. 2. Terminal branches of a hypoglossus nervefibre in the connective tissue of the mucous membrane of the tongue (group *b*).

Fig. 3. Regenerated motor endplate on a muscle fibre of the tongue (hedgehog group *a*).

the cutting of the hypoglossus nerve. It is certainly interesting, that even in such atypic surroundings the hypoglossus nerve fibres try to build up their proper typical endformations.

In the third place the following point may be mentioned. In the course of the branches of the lingualis nerve are distributed groups of ganglion cells of sympathetic nature, probably belonging to the chorda-tympani part of the lingualis nerve. The fibres of the lingualis (chorda tympani?) form a beautifully impregnated network with meshes and interwoven fibrillae on the surface of these cells. After the cutting of the lingualis nerve this network of fibrillae disappears entirely, the cells themselves undergoing apparently no alteration. The fibers of the hypoglossus nerve appear to be unable to regenerate this network of neurofibrillae, at least in all my preparations, even there where the nerve plexus in the mucosa and the submucosa was very well regenerated, and all the branches of the lingualis nerve were full of regenerating fibres, no trace of the above mentioned network could be found.

To conclude, it appears from these facts that fusion of heterogenic nerve-ends is not only possible, but may lead to distinct regenerative processes which do not differ much from these following on the fusion of homogenic nerve-ends. A functional (physiological) regeneration however does not take place, because the regenerating fibres are not able to reach their proper destination, and no contact with the muscle fibres is acquired.

And yet a certain amount of *functional* regeneration may be obtained after all. Firstly some fibres of the hypoglossus nerve will grow out, not into the neural tubes of the lingualis, but in the connective tissue of the perineural sheath. These fibres after a time will reach their destination, the tongue, and these fibres will have no difficulty in coming into contact with the adjoining muscle fibres and will form new motor end-plates on them. Secondly here and there in the preparations a fibre was found, which in forming terminal branches in the connective tissue of the mucous membrane of the tongue, had come in contact with the end of a muscle fibre, and was seen to run alongside it for a distance (towards the centre of the tongue) and then to form a small end plate on the surface of the muscle fibre. This last mode of functional regeneration I met with however only in a few cases.

Leiden, 18 February 1813.