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ON THE NUMBER OF NERVE CELLS IN THE GANGLION CERVICALE SUPERIUS AND OF NERVE FIBERS IN THE CEPHALIC END OF THE TRUNCUS SYMPATHICUS IN THE CAT AND ON THE NUMERICAL RELATIONS OF PREGANGLIONIC AND POSTGANGLIONIC NEURONES

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It is well known that the preganglionic fibers of the white rami divide and terminate in connection with a number of sympathetic ganglion cells. But no attempt has as yet been made to secure data with regard to the number of nerve cells which may be activated by one preganglionic nerve fiber.

The drawing made by Huber ('99) of the preganglionic fibers in the frog shows one fiber with seven branches, four of which end in pericellular baskets. This would indicate that in the frog one preganglionic fiber might be associated with at least seven postganglionic neurones.

Langley ('03) has given us data regarding the number of ganglia which may receive nerve fibers from a given white ramus and the number which may receive branches from a given preganglionic fiber.

> It must be noted that in the sympathetic system the preganglionic fibres of any given spinal nerve have a more extensive connection with the peripheral ganglia than any single fibre in it has. As an example I may quote the probable arrangement of the pilomotor fibres of the first lumbar nerve. The nerve sends fibres to five ganglia, the separate fibres usually send branches to three ganglia only.

Gaskell ('86) has called attention in a forceful manner to the great increase in the number of fibers leaving the sympathetic ganglia by way of the gray rami and other branches of distribu-

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tion over those which enter the ganglia by way of the white rami and truncus sympathicus.

It is generally acknowledged, since the publication of Bidder and Volkmann's paper, that an increase of nerve fibres takes place at the various ganglia. The nature of such increase is easily seen by the mere inspection of the nerves which are in connection with such ganglia as the superior cervical; the number of non-medullated fibres which pass out of it to proceed peripherally along the internal and external carotid nerves and along the peripheral grey rami communicantes of the upper cervical and lower cranial nerves is immensely greater than all the fibres both medullated and non-medullated which pass to it from the central nervous system along the cervical splanchnic (cervical sympathetic) nerve. So too the masses of non-medullated fibres which leave the semi-lunar ganglia to be distributed to the stomach, liver, intestines, etc., are very much greater than all the fibres contained in the rami afferentes of these ganglia. It is only necessary to picture to one self the number of fine medullated nerves contained in the various nerve roots, in comparison with the number of non-medullated fibres which pass out of the various ganglia of the body, to see what a great increase of nerve fibres must have taken place in the course of the nerves between the central nervous system and the periphery. Doubtless such increase is partly to be accounted for by the direct division of non-medullated nerve fibres. Such division however takes place chiefly in connection with the passage of the nerve through a ganglion.

It is obvious from all this that the impulses carried by one preganglionic fiber must be passed on to several postganglionic neurones. But no observations are furnished which would enable us to estimate the number. It would add precision to our conception of the interrelation of these neurones if a fairly definite numerical ratio could be assigned.

The superior cervical ganglion offers a favorable field for the investigation of this question. As we have seen in the preceding paper, there is no reason to suppose that fibers enter it except those which ascend in the cervical trunk. Aside from a small peripheral bundle consisting chiefly of unmyelinated fibers, the truncus just caudal to the ganglion consists of ascending myelinated fibers. These vary in size from 1.5μ to 4.5μ, i.e., are typical preganglionic fibers. In some specimens there are also a few fibers as large as 6μ or 7μ which might be interpreted as being sensory. But in the nerve counted, the largest fiber measured 5μ and only eight other fibers approached this in size. In this specimen the peripheral bundle of unmyelinated fibers formed a separate fascicle entirely outside of the perineurium of the trunk. The few myelinated fibers which this fascicle contained were descending from the ganglion and were not enumerated. In the absence of large myelinated fibers which might possibly be interpreted as being sensory, we believe that all of the fibers in the cephalic end of the trunk proper are ascending preganglionic fibers (p. 317). So far as we can determine these fibers are not mixed with any unmyelinated axons.

From these considerations it is evident that an enumeration of the myelinated fibers in the sympathetic trunk just below the superior cervical ganglion should give the number of preganglionic fibers entering the ganglion. We have also ascertained the number of cells in that ganglion and the ratio between these cells and the preganglionic fibers.

TECHNIQUE

The cervical portion of the sympathetic trunk was exposed for its entire length and fixed in osmic acid. During fixation it was held taut by stretching it over a glass cover-slip with fine silk threads tied at either end, the upper enclosing the branches of the internal carotid nerve well above the superior cervical ganglion. All the other branches of the ganglion were cut off close to their origin. The tissue was blocked in paraffine and serial sections prepared, 10μ in thickness, from the superior to the inferior pole of the ganglion, and sections 7μ in thickness were made through the trunk.

The number of fibers in the trunk was determined as follows. A ruled ocular, No. 10, was used, the ruling enclosing an area of 1 sq. cm. subdivided into one hundred forty-four smaller squares. The lines of the ocular were made parallel to the anteroposterior and lateral lines of movement of a mechanical stage, the latter being at right angles to each other. A 7a objective was used. Beginning at the left side of a section, the fibers within the area of the ruled square were counted. Then, using only the anteroposterior movement of the stage, the section was moved the full width of the ruled square, using some well-isolated fiber as a landmark. This was continued until a column of fibers was counted extending anteroposteriorly clear through the section. Then by means of the lateral movement of the stage, the section was moved the full width of the ruled square and a second column of fibers counted, and so on until the field was covered.

The number of cells in the ganglion was determined by counting the nucleoli in every fourth section and multiplying the result by four. The method of using the square ruled ocular and mechanical stage was the same as in counting the fibers. Here especial care had to be taken to avoid overlooking small nucleoli which fell behind the ruled lines as well as those which might be out of focus.

There are several possible sources of error in counting the cells by this method. Since only every fourth section was counted and the result multiplied by four to find the total number of cells, an inaccuracy is introduced, which, however, is made negligible by the large number of sections counted. A second source of error may be found in the fact that some few cells contain two nucleoli and the knife may pass between them and they will then lie in adjacent sections and may each be counted as representing a cell. This possibility would represent an error so small as to be negligible. A third and real source of error is found in the fact that a certain percentage of all nucleoli are cut and the parts come to lie in adjacent sections. Parts of nucleoli would then be counted as whole ones.

Measurements showed that the diameter of the average nucleolus is 2.25μ, and since the sections of the ganglion were 10μ in thickness we must assume that $22\frac{1}{2}$ per cent of all nucleoli were cut at some point in their diameters. If the knife passes through the nucleolus at any point in the middle one-half of its diameter, each of the resulting parts will probably be thick enough to permit of its being seen and counted as if it were an entire nucleolus. If the cut passes through either of the outer one-fourths the major part will be counted but the minor part will be so thin as to be overlooked. We may therefore assume that one-half of the $22\frac{1}{2}$ per cent of cut nucleoli will be so cut as to be seen in two sections and one-half will be so cut as to be recognizable in one section only. For example, if there actually were four hundred nucleoli in a ganglion, 77½ per cent of these, or 310, would lie wholly in the sections and be correctly counted. Of the remaining 90 nucleoli which are cut, 45 will be counted twice, and 45 will be counted once, so that the total number of nucleoli will seem to be 445. This source of error, amounting to approximately 10 per cent of the number obtained by enumeration, was not taken into account in the enumerations made by Ranson ('06) and others on the cells of the spinal ganglia. It seems probable to us that the results of earlier enumerations are therefore somewhat too high.

RESULTS

The total number of fibers in the sympathetic trunk just below the ganglion was 3851. In the 138 sections of the ganglion which were searched for nucleoli 34,334 of them were found. Since this was done in every fourth section the total for all the sections would be approximately 137,336. As already stated, we believe that some nucleoli were cut in such a way as to be recognizable in two succeeding sections, and for this error a correction of 10 per cent must be made. This would give us 123,603 as the number of cells actually present in the ganglion.

In this particular specimen, then, there were 3851 myelinated preganglionic fibers entering the superior cervical ganglion which contained approximately 123,603 cells. The ratio of fibers to cells was approximately 1 to 32.

DISCUSSION

Does this ratio of 1 to 32 represent the proportion of preganglionic to postganglionic neurones? This question raises two others: Are all the neurones in the ganglion postganglionic, i.e., cells with axons which run from the ganglion to the tissue innervated, and to what extent have the preganglionic fibers given off collaterals to postganglionic neurones in ganglia located farther caudalward in the truncus sympathicus? The first question has been discussed in detail on pages 345–354 of this issue.

The evidence is against the existence of any purely intraganglionic or commissural neurones; and there is no evidence of the existence of any sensory neurones in this ganglion. No doubt the high ratio of fibers to cells will appear to some as an evidence of intraganglionic commissural neurones. But a careful reading of the paragraph from Gaskell quoted on page 360 should do away with any feeling that the ratio of preganglionic to postganglionic neurones here given is unreasonably high.

To one who is familiar with the intricate feltwork produced by the fine branches of the preganglionic fibers which we have described under the name of intercellular plexus (p. 337) it does not seem unreasonable that one preganglionic fiber should form direct synaptic connections with thirty-two postganglionic neurones. However, we do not wish to urge this point and must admit that, although no satisfactory evidence of their existence has ever been presented and although we have very strong evidence against their presence in certain ganglia, it is nevertheless possible that there may be some intraganglionic commissural neurones in sympathetic ganglia. If there were any in the superior cervical ganglion the ratio here stated would be by that much reduced. With regard to the second question we cannot say with certainty to what extent the fibers ascending in the cervical sympathetic trunk may have given off collaterals in the middle cervical and stellate ganglia. We possess, however, information which makes it possible to form an intelligent opinion on the question.

The middle cervical ganglion is small and inconstant. The stellate ganglion, on the other hand, is large and contains many cells. All the preganglionic fibers running to the superior cervical ganglion must pass by or through it. To what extent do they give off collaterals to its cells? Some information on this subject may be gained by a study of the results obtained by Langley ('92) from stimulating the upper thoracic nerves of the cat within the spinal canal. So far as the fibers running to the superior cervical ganglion are concerned, he has shown that those for the dilation of the pupil arise from the first three thoracic nerves, those for the nictitating membrane, submaxillary salivary gland, and blood-vessels of the head from the first five, those for the hairs of the face and neck from the first seven. The fibers to the middle cervical and stellate ganglia for the acceleration of the heart arise from the second to the fifth thoracic nerves, inclusive. The origin of the fibers terminating in the stellate ganglion has been determined as follows: pilomotor fibers from the fourth to the ninth thoracic nerve, secretory and vasomotor fibers as determined by reactions of the fore-foot from the fourth to the ninth thoracic nerves.

Since there is no cardiac branch from the superior cervical ganglion in the cat it is unlikely that the cardiac accelerator fibers from the second to the fifth thoracic nerves send any branches beyond the middle cervical ganglion. With the exception of the accelerator fibers, those from the first three cervical nerves appear to run exclusively to the superior cervical ganglion. So far as we can tell, then, there are no fibers running from the first three cervical nerves which give off collaterals in the stellate or the middle cervical ganglion and pass on to end in the superior cervical ganglion.

But the fourth and fifth thoracic nerves send many fibers to both the superior cervical and stellate ganglia while the sixth and seventh send a few to the superior cervical ganglion. To what extent single fibers from these nerves may be connected with cells in both of these ganglia is uncertain. But there are certain points worth considering in this connection. Most of the functions controlled through the superior cervical ganglion are highly specialized, such as dilation of the pupil, movement of the nictitating membrane, salivation and lacrimation; and it is not probable that preganglionic fibers controlling these functions give off collaterals in a ganglion of quite different functions like the stellate. On the other hand, it is quite possible that vasomotor preganglionic fibers from the fourth and fifth, and pilomotor fibers from the fourth to the ninth thoracic nerve send branches to both the stellate and superior cervical ganglia.

The inference to be drawn from this discussion is that while a majority of the fibers ascending to the superior cervical ganglion in the truncus sympathicus pass by the other ganglia in their path without giving off collaterals, a certain unknown percentage of them may possibly give off such collaterals. Since in our enumeration the fibers were counted just below the superior cervical ganglion the possibility that collaterals had been given off from some of these fibers at a lower level makes it not unlikely that the ratio of postganglionic to preganglionic neurones as determined in this paper is somewhat too low.

SUMMARY

Careful enumerations show that the superior cervical ganglion in the cat contains some 123,603 nerve cells and that the truncus sympathicus near the ganglion contains 3851 ascending preganglionic myelinated fibers. The ratio between these fibers and the cells in the ganglion is 1 to 32. We believe that this ratio may be taken as expressing the approximate numerical relations between preganglionic and postganglionic elements for this ganglion.

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