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Some Effects of Electrically
Stimulating Ganglion
Cells.

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SOME EFFECTS OF ELECTRICALLY STIMULATING GANGLION CELLS.¹

C. F. HODGE.

The experiments about to be described were made in the Psycho-physical Laboratory of the Johns Hopkins University during the winter of 1887-8 and fall and winter of 1888. I desire at the outset to make the fullest acknowledgment of my indebtedness to Dr. H. H. Donaldson, under whose guidance the work has been carried on, and to whom much of its success is due.

THEORY AND PURPOSE OF THE INVESTIGATION.

The theory that underlies physiology is that all function is due to chemical changes taking place within the living organism ; and, further, that the functional activity of a specialized tissue depends primarily upon these changes in its individual cells. That these changes may reach a magnitude easily demonstrable with the microscope has been proved, as every one knows, for the cells of certain glands. My present purpose is to ascertain to what extent and by what methods changes due to the functional activity of ganglion cells may be in like manner demonstrated.

¹ Dissertation submitted to the Board of University Studies, Johns Hopkins University, for the degree of Doctor of Philosophy, April, 1889.

A preliminary communication upon this work appeared in the AMERICAN JOURNAL OF PSYCHOLOGY for May, 1888.

HISTORICAL.

The great amount of work devoted to the morphology of the nerve cell is in the main aside from the problem in hand. It need be here referred to simply as it furnishes the structural mechanism necessary for a conception of the physiological working of the ganglion cell. This is furnished by Schultze,¹ to whom is justly ascribed the credit of demonstrating the fibrillar and granular structure of nerve cells. Later methods have, however, demonstrated this more sharply; and for the best of these we are chiefly indebted to Professor Kupffer² and Boveri.³ This method consists, essentially, of hardening a perfectly fresh nerve in osmic acid and subsequently staining with acid fuchsin. Seen in section after this treatment, the medullary sheath appears black, and, what is of most interest here, the axis cylinder shows fine red fibrils in a finely granular matrix. The ganglion cell by this method is found to consist, beside nucleus and nucleolus, of a dense tangle of fibrils, unquestionably the same as those occurring in the axis cylinder, with an irregularly granular material filling the spaces between the fibrils. We are thus given at least the two things necessary for a nerve mechanism: the fibril to conduct, and, in close connection with this, some sort of substance, changes in which may serve to originate or modify the nerve impulse.

Of great significance to the problem in hand is such work as Heidenhain and Langley have done on the

¹ General characters of the structures composing the nervous system. Max Schultze, Stricker's Manual of Histology, p. 116.

² Ueber den Axencylinder markhaltiger Nervenfasern. Prof. C. Kupffer. (Sitzgb. d. math. phys. Klasse d. k. bayr. Akad. d. Wissensch. 1883, II. 3.)

³ Beiträge zur Kenntniss der Nervenfasern. Theodor Boveri, Munich, 1885.

histology of resting and secreting glands. There are evident points of similarity between the active processes of gland and ganglion cells. There seem also to be points of difference which make it difficult at present to homologize the processes of the two. At any rate this is not the place to discuss that phase of the subject.

The pathology of nerve tissue is, of course, closely related to its functional activity. The subject, however, is so large, and so little of it bears directly upon the point in hand, that any general discussion of the pathological literature may be best relegated to a subsequent chapter. Two papers may be briefly referred to as throwing some light upon the results of my own experiments.

In 1878, Angelucci made a study of the histological changes in spinal ganglion cells of four cases of nervous disease, one of chronic, two of acute myelitis, and one case of paralytic insanity. I will only note that in the series of degenerative changes described by him, the nucleus plays an important part. It early loses its rounded outline, becoming "stelliforme," shrinks up and disappears, leaving the cell a lump of pigment and fat.

More nearly physiological is some work of Rosenbach² upon histological changes in the ganglion cell due to hunger. Rosenbach worked upon dogs. His method consisted in depriving the animals of food for different lengths of time. At the expiration of the desired period, or upon the death of the animal, sec-

¹ Osservazioni sulle alterazioni dei gangli intervertebrali in alcune malattie della midolla. Arnaldo Angelucci. Atti della R. Accademia de' Lincei, Serie III^a V^o 2^o. Rome, 1878.

² Das Nervensystem in Hungerzustande. P. Rosenbach. Centralblatt für Nervenheilkunde, 1884.

tions were made of the spinal ganglia and cord, and compared with similar preparations from well-fed dogs. Among other changes, and preceding degeneration or atrophy, he finds that the cells shrink and become vacuolated. Upon the death of the animal the nuclei had disappeared from many of the cells. The nerve fibers, however, appeared normal.

But little has been done upon the purely physiological histology of the ganglion cell.

In 1869, Svierczewski¹ described changes in the living cells of the frog's sympathetic ganglia, changes in part probably due to functional activity of the cell. He kept the cells alive in aqueous humor or lymph under the microscope, and subjecting them to different conditions, observed the effects. It is significant to note that his attention centered upon the nucleus and its contents. The nucleoli were seen to wander about in the nucleus, sometimes in the most lively fashion, for as long as twenty-four hours. On exposing the cells to carbon dioxide, a finely granular precipitate suddenly formed within the nucleus, which redissolved on treatment with oxygen or hydrogen ("paraglobulin-reaction"). This process was accompanied, under certain conditions, by a marked shrinking of the nucleus, its rounded form being altered to an angular or "zick-zack" outline, the nucleolus being at the same time lost to view.

Somewhat similar observations were made by Freud² upon the living ganglion cells of *Astacus*. He describes shreds and angular-shaped particles which change form and position in the nucleus.

¹ Zur Physiologie des Kerns und Kernkörperchens der Nervenzellen des Sympatheticus. Svierczewski. *Centralblatt für die medicinischen Wissenschaften*, 1869, p. 641.

² Ueber den Bau der Nervenfasern und Nervenzellen beim Flusskrebs. *Wiener Sitzgb.* 1882, p. 1.

Kühne,¹ in the course of his other studies, notes a fact which bears directly upon the present subject. This is a disarrangement and a shrinking together of the axis-cylinder fibrils of the nerves in the nictitating membrane of a frog, due to ten minutes unipolar stimulation of the nerve root within the skull. Also vacuoles make their appearance among the fibrils.

The only paper devoted to the exact problem under consideration has come out within the last month. The author states the exact question: "Is the activity of the central nervous system accompanied by changes recognizable with the microscope?"² He proceeds to answer the question under the idea that staining reveals much finer differences than changes of form. This determines his method, which consists in choosing two frogs of the same weight and sex, the one to be experimented with, the other for control. He then proceeds to stimulate by induction shocks the eighth nerve of one for one hour, keeping the control frog as quiet as possible during the same time. The spinal cords of both are hardened in corrosive sublimate solution and alcohol, and sections made through both cords opposite the origin of the eighth nerve. The sections are stained on the slide with haematoxylin, nigrosin, eosin, and safranin, the Gaule combination, in the order named. In *some* cases, the author states, sections of each cord are treated on the same slide. It is significant that here too the interest centers about the nuclei. These, by a difference of staining, fall into two categories, the red and the blue ;

¹ Neue Untersuchungen über motorische Nervenendigung. Kühne. Zeitschrift für Biologie, 1887, p. 56, Table D, Fig. 64.

² Wird der thätige Zustand des Centralnervensystems von microscopisch wahrzunehmenden Veränderungen begleitet? Bohdan Korybutt-Daszkiewicz. Archiv für mik. Anat. 1889, p. 51.

and a greater proportion of the nuclei stain red in the cord of the stimulated frog. A count of all the red and all the blue nuclei in a large number of sections shows that 3.31 to 3.66 times more nuclei stain red in the stimulated than in the unstimulated frog. The results are derived from four frogs, two stimulated and two control.

We gather from this brief résumé that nerve tissue of the frog, crawfish, dog and man has been examined with special reference to the observation of changes occurring in it. The main points noted up to date are, first, changes in the appearance of the living nucleus; second, vacuolation and shrinkage of the cell protoplasm and also of the axis cylinder; and third, that the nuclei in the spinal cord of a stimulated and unstimulated frog stain somewhat differently.

METHOD OF INVESTIGATION.

The value of results, especially in this branch of histology, depends so much upon the soundness of the method employed, that a somewhat detailed description of some features of my method must be given. I have used in all cases the spinal root ganglia. My scheme of procedure has been throughout to stimulate electrically a nerve going to one or more of these ganglia on one side of the animal, leaving the corresponding parts on the other side at rest. To avoid possible confusion, the right side was invariably used for stimulation, the left for control. At first a double control was used, consisting of the corresponding ganglia from a similar animal of the same size and sex. This practice was soon abandoned, however, for it was found that the ganglion cells of two frogs that could not be distinguished externally might differ

very widely in staining and general appearance. The stimulated nerve was not divided, so that the contractions of the muscle supplied by it could be used to indicate the healthy condition of the nerve. If the nerve was conducting impulses peripherally to its muscle, it was taken for granted that it was conducting impulses in like manner centrally to its ganglion.

In general, as a means of stimulation, the ordinary combination was used, of Du Bois-Reymond coil, platinum electrodes, and bichromate or copper sulphate cell; and the strength of stimulus was determined within physiological limits by touching the electrodes to the tongue before beginning to stimulate. Special apparatus to regulate the strength of primary current and number of stimuli per second may best be described in connection with the purpose of special experiments. Intervals of rest were generally allowed. This was at first managed by having a key in the primary circuit and making and breaking the circuit by hand. Later, this part was relegated to clockwork, which spaced the intervals with more precision and removed the chief feature of irksomeness from the operation.

At the end of the desired length of time the stimulated ganglion, with its mate of the opposite side, was as quickly as possible excised and the process of fixing and hardening begun. The method from this point on is directed toward having the two ganglia pass through identical treatment. *In no single instance, no matter how many controls were used, were they separated from the time they left the animal to the time when, placed side by side upon the same slide, they were ready for study.* Not only were they carried through the same reagents, but, *in every case*, through the

same reagents in the same bottles or dishes, from the first fixing fluid to the solid paraffin. And from this point the two are cut at the same stroke of the microtome knife, fastened to the slide together, stained together, and appear side by side in the same field of the microscope.

All this was made easy by a simple device, which may be of use to others. The ganglia were usually left attached to their segment of the spinal cord until ready to pass into strong alcohol. They are then trimmed for cutting, and arranged in the same position relative to each other upon one end of a small strip of mica: 1x3 cm. is a convenient size. As they lie upon the slip, a drop of the thin white of an egg is placed over them. This is allowed to dry somewhat and the whole carefully laid in the alcohol, where the egg speedily coagulates, holding the ganglia firmly to each other and to the mica slip. The rule of always placing the stimulated ganglion nearest the end of the slip aids in simplifying matters. Any desired record may be scratched upon the other end of the slip. Not only one, but several pairs can be fastened to the same slip, arranged in a row so that they all may be cut at the same time. For example, it was my practice to stimulate the right brachial and sciatic plexuses of a frog. This places at our disposal five pairs of ganglia. These may be hardened by five different methods, and all be arranged as described above on a single slip of mica. They are all cut together, fastened to the slide together (invariably by the alcohol fixing method where differences of staining or granulation are to be studied), and all stained together. Many slides are obtainable from one such set of ganglia, and each slide may be stained in a different way. This

device has given me, incidentally, a permutation of hardening and staining combinations which might well form the basis of a separate study.

In this way not only may a dozen specimens be manipulated as easily as one, but they are held in the desired positions relative to each other and, of special importance, they are cut together. However perfect the microtome, sections do not come from it of absolutely uniform thickness; and where minute, or even gross, differences of staining are to be studied, this is of prime importance.

Apropos of Korybutt-Daszkiewicz's work, I have sections, no thicker than his and obtained by essentially the same method, which show a most striking differentiation into red and blue nuclei. It requires but little focusing, however, to demonstrate that the red nuclei occupy the superficial, and the blue the deeper, layers of the section. A slight difference in the thickness of the section might thus change the proportions of the two quite materially. The thinner the section, according to the above, the larger would be the proportion of red nuclei. That this may be an explanation for Korybutt-Daszkiewicz's result is indicated by the fact that in equal areas of section he finds nearly 400 (4127 to 3759) nuclei less in the stimulated than in the control cords. This would suggest that the sections of the stimulated cords are thinner than those of the control; and from these he gets his preponderance of red nuclei.

The essential feature, then, of my method is that it compares *corresponding ganglia of the same animal* which have been subjected to *identical treatment* in passing from the animal to the slide; *the only point of difference being that the one has had its nerve stim-*

ulated for a longer or shorter time, while the other has not. Methods of hardening and staining do not concern us so long as the two ganglia go through every step of the processes together.

For the encouragement of others I may say that I have tried almost every method practicable and impracticable, in the hope of finding a striking reaction. Some such were found, but up to date they have all proved inconstant. Trzebinski¹ has made a special study of the influence of hardening reagents upon the ganglion cells of the spinal cord. He finds corrosive sublimate one of the best reagents, and states that it does not produce vacuolation of the cell. This method followed by Gaule's quadruple staining has furnished my best preparations for the study of granulation and staining. Trzebinski did not, it would seem, experiment with osmic acid. This, with fuchsin, safranin, or all four of the Gaule stains, has given a most beautiful preservation of the form of the nucleus and the minute structure of the cell protoplasm.

Two widely different animals, the frog and cat, were purposely selected to furnish the material for investigation. And the results which I will now consider are derived from fifteen experiments upon frogs and ten or eleven experiments upon cats. *All the experiments* will be referred to either singly or in groups.

RESULTS.

Frog No. 1 was given three drops of 1 per cent curare solution, and the sciatic nerve of the right side was stimulated continuously for thirty minutes. The

¹Einiges über die Einwirkung der Härtungsmethoden auf die Beschaffenheit der Ganglienzellen im Rückenmark der Hunde und Kaninchen. Trzebinski. Virchow's Archiv, Vol. 107.

three pairs of sciatic ganglia were excised and, with those of a control frog, hardened in corrosive sublimate. The ninth pairs were stained *in toto* in soda carmine; and for some unaccountable reason scarcely any nucleoli could be found in sections of the stimulated ganglion, while they appeared as usual in the ganglion of the other side and control ganglia. A count of the two gave the following:

		Nuclei.	Nucleoli.
		122	92
Six sections of each contained	{ Resting,	177	28
	{ Stimulated,		

The seventh and eighth pairs stained in other ways (Kleinenberg's haematoxylin and by Weigert's method) gave no such result; and in fact the phenomenon could not be made to reappear in any subsequent experiment. Other than this, no results could be made out.

Three frogs were next taken, each with a control; each was given the same amount of curare, and the right sciatic nerves of the three were stimulated continuously one, two, and three hours respectively. From the nine stimulated ganglia thus obtained no effect of activity could be discerned.

An experiment was then made to test the influence of curare upon the working of the central portion of the reflex arc, and the indications seemed quite strong that, although curare does not entirely suspend nervous action in the cord, it does reduce the activity very materially. Its further use was for this reason abandoned.¹

Frog No. 6 was used to demonstrate *post mortem* changes in the ganglion cells, and does not concern us now.

¹The proof that curare influenced the effect of stimulation is not conclusive, since continuous stimulation was also given up at the same time. Further experiment is needed on this point.

Frog No. 7 was made reflex, and the right brachial and sciatic plexuses were stimulated, with two minutes of rest alternating with two minutes of stimulation, for two and a half hours. The stimuli were regulated so as to be as strong as possible without causing reflex contractions of the muscles of the other side. At the end of this time but slight muscular contractions could be obtained from the arm or leg of the right side, and no reflex contractions whatever from stimulating the skin of this side, while stimulating the skin of the left side gave normal reflex contractions in that side.

Sections of the ganglia from the two sides show marked differences. Perhaps the most pronounced of these is a difference, noted independently by a number of observers, viz. that the nuclei appear shrunken in the stimulated ganglia. This led to a series of measurements, the results of which are given in the following table. The nuclei were measured, long and short diameters, in sets of one hundred, fifty stimulated and fifty unstimulated being taken from as nearly corresponding sections as possible of the two ganglia. A definite rule precluded any willful selection of the cells to be measured, the rule being that only nuclei containing nucleoli should be measured, and that all such should be taken in the order of their occurrence in the section. The measurements were made to the nearest μ under a magnifying power of Leitz, Oc. 3, Obj. 7 (≈ 600 diameters).

TABLE I.¹

Frog No. 7. Made reflex, stimulated 2½ hours, in-

¹The tables have been recomputed, and so differ slightly from those in the preliminary communication (*Am. Jour. Psychol.*, Vol. I, p. 479 ff.). The difference is due to using the square root of the average surface of the nuclei, instead of the arithmetical mean, for the mean diameter.

tervals of rest and stimulation being two minutes. Three sets of 100 nuclei each.

Ganglia hardened in corrosive sublimate. 8th pair. 9th pair. 2d pair.	NUCLEI IN μ .		CELLS IN μ .	
	Mean Diameters.		Mean Diameters.	
	Resting	13.57	Set 1.	39.69
	Stimulated . .	12.23		35.00
	Diff. 1.34			
	Resting	13.94	Set 2.	
	Stimulated . .	12.56		
	Diff. 1.38			
	Resting	14.48	Set 3.	
	Stimulated . .	13.26		
	Diff. 1.22			

Sets 1, 2 and 3, volume shrinkage, 24 per cent.

The volume shrinkage per cent is computed from the mean diameters, treating the nuclei as spheres.

Besides shrinkage of nuclei, other changes are plainly visible. The protoplasm of the stimulated cells is much more vacuolated than that of the resting, and the staining, by Gaule's quadruple method, is less intense. Instead of the coarsely and densely granular constitution of the resting cell, we find the protoplasm of the stimulated cell finely granular and vacuolated. Owing in part to this absence of granules, the nuclei are more distinct in the stimulated cells. In part this is also due to a deeper staining of the nucleus itself, the open reticular appearance of the nucleus giving place to an evenly dense stain. There can be no doubt that in my preparations the stimulated nuclei stain much bluer than the resting, except in cases where pathological conditions were present.

The five experiments succeeding this were made with the purpose of getting the greatest amount of change possible; and under the supposition that this

might be obtained, for the frog at least, in shorter time if the nutrition of the cells were prevented, the frogs were thoroughly bled or the capsules of the ganglia torn off. None of these experiments gave definite results. Sections of both ganglia stained by the above method appear redder than normal. This is presumably due to a clogging of the cells with decomposition products which would normally be carried away in the circulation. Stimulated and resting alike show vacuolation, probably the same as that found by Rosenbach in starving dogs. The nuclei in both appear shrunken, but do not show any marked difference in size.

Results of but a single experiment of this class need be given.

TABLE II.

Frog No. 8. Bled. Stimulated for 7 hours, five minutes of stimulation alternating with five minutes of rest. One set of 100 nuclei.

MEAN DIAMETERS IN μ .

Ganglia of 8th pair, hardened in corrosive sublimate and stained by the Gaule method.	{	Resting	12.36	Volume shrinkage, 8%.
		Stimulated	12.01	

One experiment, in which the ganglia were suspended in normal salt solution while being stimulated, gave more definite results.

TABLE III.

Frog No. 14. Sciatic ganglia of right side suspended in salt solution and stimulated $3\frac{1}{2}$ hours, five stimuli per second, one minute of stimulation alternating with one minute of rest. The ganglia of left side kept during this time in blood of same frog. Two sets of 100 nuclei each.

MEAN DIAMETERS IN μ .

9th ganglia; corrodio sublimato and Gaule's solution.	{	Resting14.70	Set 1. Measured by myself <i>previous to</i> Mr. W.'s measurement of set 2.
		Stimulated . .13.10	
		Diff. 1.60	
	{	Resting14.57	Set 2. Measured by Mr. W. <i>without</i> <i>knowledge of my results</i> , and having but one of the ganglia in the field at the same time, and <i>not knowing which</i> <i>had been stimulated and which not.</i>
		Stimulated . .12.14	
		Diff. 2.43	

Sets 1 and 2, volume shrinkage, 36 per cent.

It was thought that greater changes might be obtained at a higher temperature; accordingly one experiment was made to test this, and though not altogether successful, the results may be given.

TABLE IV.

Frog No. 15. Cerebrum removed and wound allowed to heal before experiment. Stimulated $5\frac{1}{2}$ hours at a temperature of 35° C.; intervals of rest and stimulation being one minute. Three sets of 100 nuclei each.

MEAN DIAMETERS IN μ .

Ganglia of 3d pair. Hardened in Pierre nchl. Gaule's soln.	{	Resting16.53	Set 1. Measured by myself <i>previous to</i> measurement of set 2.
		Stimulated . .15.66	
		Diff. .87	
	{	Resting17.40	Set 2. Measured by Mr. L. without knowledge of my own meas- urement, and <i>not knowing which of the</i> <i>ganglia had been stimulated.</i>
		Stimulated . .15.84	
		Diff. 1.56	
9th pair. Fluorine. Gaule's soln.	{	Resting20.90	Set 3.
		Stimulated . .19.13	
		Diff. 1.77	

Sets 1, 2 and 3, volume shrinkage, 12.5 per cent.

It is to be noted that both Mr. W.'s and Mr. L.'s measurements make the difference greater than my own. Staining and structure of protoplasm not well defined; due probably to the fact that the frog died

toward end of experiment. At its close the muscles were beginning to pass into *rigor mortis*.

This closes the series of experiments upon frogs. It is desirable, for a more general application of the results above detailed, to ascertain whether they hold good for some other animal. Experience has shown that the most marked results are to be expected from experiments in which the condition of the animal is most nearly normal. I think I am justified in distrusting the influence of curare; from the following experiment, chloroform also would seem to be a disturbing factor. A cat was killed with chloroform, and several of the spinal ganglia were examined. Many of the cells were found to show considerable vacuolation, strikingly similar to that produced by stimulation. The point needs further investigation, but it is not altogether improbable that a substance which produces such marked physiological effects may also give rise to histological changes in nerve centers. However this may be, it was determined not to run any risk of complicating matters by the use of anaesthetics. A mode of producing insensibility without the use of drugs was accordingly resorted to.

The method¹ adopted consists in trephining the skull at about the point of greatest convexity and a centimeter to one side of the middle line. A small slit is made in the dura, and through this a blunt glass rod is thrust directly to the floor of the skull, and worked carefully across along the floor to the opposite side. The crura are thus severed at their entrance to the cerebrum; and if successful, complete anaesthesia, with normal

¹The method was obtained from a paper entitled "On the Renal Circulation during Fever" (Walter Mendelson, Am. J. M. Sc., Phila. 1883), where the method is credited to Ludwig.

pulse and respiration, should result. The operation is performed while the animal is under slight anaesthesia from ether. The right brachial plexus is then laid bare in the axilla, and, with great care as regards injuring the nerves or blood-vessels going to them, freed from fat and disentangled from the subclavian artery and vein, so that these may not be included between the electrodes. By including the whole plexus at this point, four ganglia are stimulated. My own practice has been to slip a two-tined platinum electrode over the plexus from behind, in such a way that the two tines of the electrode touch opposite sides of the nerves and make it necessary for the stimulus to pass obliquely through them. The greater number of the fibers, however, from the 6th and 7th cervical escape stimulation, and possibly, too, the nerves from the 1st dorsal and 8th cervical coming first between the electrodes, tends somewhat to short circuit the current, thus depriving the other nerves of an equal share of the stimulation. At any rate, the 6th and 7th cervical have failed to show the effect of stimulation to the extent that the 8th cervical and 1st dorsal do. Hence for the clearest results it is best to include in the circuit only the medius and spiralis nerves and the branches lying behind these, and then use only the 8th cervical and 1st thoracic ganglia. A double control was employed at first, consisting of thoracic ganglia from the same animal, a pair of these being carried through with each pair of test ganglia. This was soon found to be entirely unnecessary, since the cells of these control ganglia invariably resembled those of the resting ganglion.

TABLE V.

Cat No. 1. Stimulated for 7 hours; intervals, one minute.

	NUCLEI (four sets 100 each).		CELLS.	
	Mean Diameters in μ .	Shrinkage, in Volume.	Mean Diameters in μ .	
1st thoracic.	Resting		59.06	
	Hardened in osmic acid.		57.19	
	Stimulated	35%		
	Diff. 2.22			
8th cervical.	Resting			
	Hardened in corrosive sublimate.			
	Stimulated	51% (Selected). (T.)†		All over 50 μ .
	Diff. 3.21			
7th cervical.	Resting		57.50	
	Hardened in Flemming's fluid.		56.25	
	Stimulated	20%		
	Diff. 1.19			
6th cervical.	Resting		44.21	
	Hardened in picric acid.		44.74	
	Stimulated	14.6%		
	Diff. .75			

Stimulation severe, frequently varied and regulated so as to produce the greatest amount possible of muscular contraction in the right fore-leg without giving rise to reflex contractions in other parts of the animal. Muscular contractions in right fore-leg toward close of experiment feeble but constant. Within five minutes after the animal was bled the muscles of this leg had passed into *rigor mortis*, the muscles of other limbs being normal and irritable. Pulse and respiration remained normal the whole time.

Besides shrinkage of the nuclei, other important changes occur. For the first thoracic pair, hardened in osmic acid, the nuclei are plump and round in the resting ganglia and stain lighter than the protoplasm. In the stimulated ganglion they are irregular in outline and stain much darker than the rest of the cell. Especially marked in this case also is the extreme vacuolation of protoplasm in the stimulated cells, and this does not occur in the ganglion of the other side or

† See explanation page 395, note.

in the two thoracic ganglia used as control. It was noticed independently by three observers that the nuclei of the cell capsule were shrunken in the stimulated ganglion. (See Figs. 3 and 4 of plate.) The eighth cervical ganglia, hardened in corrosive sublimate, show the characteristic appearance of the nuclei, with slight difference in the staining of the protoplasm.

TABLE VI.

Cat No. 2. Stimulated 1 hour 40 minutes ; intervals, one minute.

	100 NUCLEI.		100 CELLS.	
	Mean Diameters in μ .		Shrinkage in Volume.	Mean Diameters in μ .
Ganglia of 1st thoracic. Osmic acid.	Resting	14.91	25.6%	14.94
	Stimulated . .	13.51		14.45
	Diff. 1.40			

Examination of the sections shows changes similar to those described for cat No. 1, but less in degree.

Though no attempt was made to render the stimulation equal for cats No. 1 and 2, it is hinted by the results of the two experiments that some sort of a quantitative relation exists between amount of stimulation and amount of change in the cells. Such a relation should obtain if we are dealing with cause and effect. To test this point with theoretical precision is, of course, impossible, for we must know, in order to do this, not only the strength of stimulus used, but also that the same amount of stimulation is distributed to the same number of cells ; and, further, that the ganglion cells of one animal are exactly as irritable as those of another animal. In the following series, therefore, we assume that the irritability of cats is in general the same, and that the same nerves in different cats connect approximately with the same number of ganglion cells. To render these factors as

nearly alike as possible, half-grown kittens were used throughout. The amount of stimulation was regulated by using the same apparatus for all the experiments, and by placing a rheocord, resistance-box and galvanometer in the primary circuit derived from two one-liter copper sulphate cells. By manipulation of the resistance-box and rheocord, the galvanometer needle was brought to a given position and held at this point during each experiment of the series. The results require little more than tabulation.

TABLE VII.

SERIES WITH EQUAL STIMULATION.

Intervals of 15 seconds of stimulation alternate with 45 seconds rest. Ganglia of 1st thoracic pair, hardened in same fluid.	Cat No. 7 (operated upon and left without stimulation for 2½ hrs.)	Length of Stimulation.	No. of Nuclei Measured.	Mean Diameters of Nuclei in μ .	Shrinkage In Area of Section of Nucleus.	Shrinkage In Volume of Nucleus.
	0 hrs.	100	14.20	- 4.7%	* - 6.9%	
			14.54			
	Cat No. 5	1 "	100	14.70	+ 14.1%	+ 22%
			13.51			
	Cat No. 6	2½ "	200 (T.)†	11.86	+ 13.8%	+ 21%
			10.95			
	Cat No. 8	5 "	100	15.97	+ 17.1%	+ 24.3%
			14.38			
	Cat No. 11	10 "	100	16.19	+ 31%	+ 43.9%
		‡ S. 100	13.35			
		§ S. 100 (T.)				
		100 (T.)				

* The minus sign indicates that the nuclei of the right ganglion are larger in this case. In the only other set measured from a normal pair, the nuclei were also slightly larger in the ganglion of the right side.

† Sets marked T. are measured by a third person, with whom every precaution was taken to obtain a purely mechanical and unprejudiced measurement.

‡ Sets marked S. are those in which only nuclei in cells of over 50 μ diameter were measured.

TWO EXPERIMENTS WITH STRONGER STIMULATION.

1st thoracic ganglia. Osmic acid.	Time.	No. of Nuclei Measured.	Mean Diam.	Area Shrinkage.	Volume of Shrinkage.
{	<i>Cat No. 9</i> (45 seconds rest to 15 seconds work).....	2 hrs. 100	12.39 10.45	31%	40.9%
	<i>Cat No. 10</i> (intervals 15 seconds rest to 15 seconds work).2 hrs.	100 (T.)	13.82 12.04	23.9%	32.7%

In the above series the stimulus used was very slight, too slight for the most definite results. Still no special study of the sections is necessary to detect the gradation expressed in the table, staining as above described. The use of the one reagent throughout the series has emphasized the fact that osmic acid is not so strongly reduced by the cells of the stimulated ganglia. This sometimes results in so marked a difference in staining that sections of the two ganglia may be easily distinguished by the naked eye. The vacuolation, so striking in the stimulated cells of No. 1, is only slightly present in these experiments, presumably because the stimuli were not strong enough.

Many devices were resorted to to eliminate the personal equation and obtain mechanical measurements. Three persons unacquainted with the methods of the investigation have kindly consented to assist in the work of measuring. Even here the differences are too marked to make an absolutely neutral state of mind possible, since each of the three, before completing the measurement of a single set, had a very definite notion that the nuclei in the two ganglia were different. In my own measurements I was wont from

the first to throw all thought of the work as completely as possible off my mind, to think about something else, to have a story in which I was interested read aloud, or something of the kind to divert my attention. In general also all the measurements were made before the results were footed up, so that the way they were tending could have no unconscious influence upon the measurements.

The objection has been raised that the changes above described, especially shrinkage of the nucleus, may be pathological. It is true they resemble changes hitherto described as pathological; but up to the present no attempt has been made to distinguish changes due to fatigue from those caused by disease, and on *a priori* grounds we should expect the former to precede and shade into the latter. The fact that the change becomes steadily greater as the stimulation is prolonged, would further indicate that it is due to active processes of the living cell. It would be interesting to know whether stimulated cells will return to the normal condition if given a sufficiently long period of rest. But whether they do this, or whether they die and give place to new cells, is a point for future investigation, and not the question in hand. In either case we are safe in assuming that the changes are such as occur in the normal working of the ganglion cell.

CONCLUSION.

A method has been attained by which changes due to functional activity can be as easily and certainly demonstrated in a ganglion as in a gland. The chief of these changes for the spinal ganglion cells of the frog and cat are :

As a result of electrical stimulation :

1. For the nucleus: 1. Marked decrease in size.

2. Change from a smooth and rounded to a jagged, irregular outline. 3. Loss of open reticular appearance with darker stain.

B. For the cell protoplasm: 1. Slight shrinkage in size. 2. Lessened power to stain or to reduce osmic acid. 3. Vacuolation.

C. For the cell capsule: Decrease in size of the nuclei.

INCIDENTAL OBSERVATIONS.

A number of suggestive observations, made in the course of the investigation and not belonging properly to the body of the paper, may be mentioned here.

A strange differentiation of some sort between the large and small cells of the spinal ganglia is brought to light by stimulation. The large cells show the effects of work; the small cells, very little or not at all. The fact is too marked to pass by unnoticed. Considering all the cells large which have one diameter 50 μ or over, and those small which have not, a count gives the following result:

Cat No. 11. First Thoracic Ganglia.

	In 100 large cells nuclei:		In 100 small cells nuclei:	
	Shrunk.	Not shrunk.	Shrunk.	Not shrunk.
Resting,	5	95	0	100
Stimulated,	94	6	8	92

A few fibers going to a ganglion of course escape stimulation by our method. This accounts very well for the few large cells which do not appear worked in the stimulated ganglion. It cannot account for this condition in the multitude of small cells which comprise over half the cells in a ganglion. No explanation will be attempted until further experiment is made. As might be expected, a few cells in the resting ganglion appear worked.

In close relation to our work is the question of the

minute structure of a spinal ganglion. What is the path of a nerve impulse through the ganglion? The supposition has been, since Ranvier's work, that a fiber enters a spinal ganglion, unites with one of its cells by a "T" process, and passes out in the opposite direction. This supposition finds support in the way a nerve degenerates when separated from the ganglion, and in the fact that the same number of fibers enter a ganglion as leave it.¹ It is also supported by Birge's² work, in which he finds a single ganglion cell in the anterior horn of the frog's spinal cord for each nerve fiber in the anterior root. If this relation holds for the cells and fibers of the spinal ganglion, we should evidently find a cell in the ganglion for each fiber in the posterior root. Expecting to demonstrate this, Dr. Nelson,³ working under the direction of Birge in the University of Wisconsin, counted the fibers in the posterior root and the cells in the corresponding ganglion. The work was done on the frog, and in all about ten ganglia were counted. Nelson found, allowing 2-4 per cent for error in counting, *two* ganglion cells for every fiber in the root. This counting has been repeated for two ganglia by myself, and for one more by still another observer. Our figures are as follows:

	No. fibers in root.	No. of cells in ganglion.
Seventh ganglion, right side,	1128	2767
Eighth " left "	1811	5416
Seventh " left "	1340 (T.'s count) 1364 (my count) 4456 (T.)	

¹ Ueber den Bau der Spinalganglien. M. Hall. Wiener Acad. Sitzungsber. LXXII, 3, p. 31-37.

² Die Zahl der Nervenfasern und der motorischen Ganglienzellen im Rückenmark des Frosches. E. A. Birge. Archiv für Anat. und Phys. 1882, Physiol. Abth. p. 435.

³ The above is inserted by the courtesy of Dr. Nelson. His notes of the work were destroyed in the burning of Science Hall, Madison, Wisconsin.

⁴ The most careful count of all, done under magnifying power of Leitz, Obj. 7, Oc. 3 (= 600 diameters) for fibers, and Obj. 7, Oc. 1 (= 325 diameters for cells).

The method consisted in counting all the nucleoli *in every section of a complete series through the ganglion*, and all the fibers in a cross section of the root between the ganglion and the cord, generally close to the ganglion. The tissue is hardened in osmic acid. A nucleolus may be pushed to one side or dragged out of a cell by the edge of the knife, but is never cut in two. So we run no risk on that score of counting a cell twice; and double nucleoli are so rare that they may be left out of the account.¹ My rule throughout was to count everything that could be construed to be a fiber in the root, and nothing but what was most certainly a cell in the ganglion, thus throwing all the doubtful cases upon the same side.

With the above figures approximately correct, either apolar cells in the ganglia must be very numerous, or the relations of fibers to cells must be more complex than formerly supposed. A most careful teasing of spinal ganglia of a frog, using a fine jet of water instead of needles, I think demonstrates the following points:

1. Apolar cells do not occur in the spinal ganglia of the frog in any considerable numbers, none having been observed.
2. Typical bipolar cells do occur. Three have been noted up to date.
3. The axis cylinder of the cell process is often seen to divide and enter the cell as a spiral and straight fiber.
4. At the angles of the "T" the axis cylinder of the cell process may be observed to divide and pass

¹In counting the last ganglion, T. kept careful account of all double nucleoli. In the 4456 cells 38 were found. In all cases they were found in the small cells, lying among the fibers or close to the nerve fiber axis of the ganglion.

both ways in the nerve fiber, of which it *seldom* forms the whole of the axis cylinder.

5. Two cases of double $\rightleftharpoons$ have been found.

6. Two cells, in a number of cases, have been found to unite their processes, not necessarily as a cell junction, but to aid in making the axis cylinder of the same nerve fiber.

No rigor has been spared in this teasing from fear of breaking specimens. The coverslip has been tapped and each specimen rolled over and over while under the microscope until every point in the above description has been clearly demonstrated. A special investigation of these points is under way.

In the course of examining so many cells, an appearance of the nucleus has been noticed which may throw some light upon that most vexed problem, the minute structure of the ganglion cell itself. The marked effect upon the nucleus of stimulation would indicate an intimate relation between the nerve fiber and the nucleus. In general, the jagged points of a worked nucleus give the impression that it is connected at these points with the fibrillar reticulum of the cell protoplasm. At times, and not so very rarely, something more definite makes its appearance. A stream of fibrils is plainly seen to pass from one side of the nucleus to mingle with the fibrils of the cell. These fibrils arise from the nuclear membrane, and in no case have I been able to trace them to an origin within the nucleus.

March 15, 1889.

(For explanation of plate see page 27).

EXPLANATION OF PLATE.

Fig. 1.—*Cat No. 11.* Left 1st thoracic ganglion, resting. A portion of single field. Nuclei full and round, protoplasm darkly stained.

Fig. 2.—*Cat No. 11.* Right 1st thoracic ganglion worked, not severely, 10 hours. Cells *a* and *b* are adjoining cells, taken from a slightly more central portion of the section, and show an appearance of the nucleus, shrunk away from the cell protoplasm, quite characteristic for central portions of the worked ganglion, but not of the resting.

Fig. 3.—*Cat No. 1.* Cell from left 1st thoracic ganglion, *resting*. Adjoining cells outlined in part.

Fig. 4.—*Cat No. 1.* Cell from right 1st thoracic ganglion, *worked severely 7 hours*. Nucleus much shrunk and protoplasm vacuolated. 1 and 1' two larger vacuoles.

Note.—All the above figures were drawn under a magnification of Leitz Obj. 7, Oc. 3 (=600 diameters), and the cells, nuclei and nuclei of capsule were outlined by the aid of a Zeiss camera lucida after Abbe (the form with the longer arm).

Figs. 1 and 2, staining with osmic acid.

Figs. 3 and 4, staining with osmic acid followed by Gaule's quadruple stain.

Vita.

Cyrus Fremont Dodge

was born near Ansonia, Conn., Oct. 10,
1859. The first fifteen years of his
life were spent on the farm. During
the summer he attended
district school in winter.

In Sept. 4, 1874, his parents re-
moved to Ripon, Wis. In the fall
of 1875, he attended a village
academy; and after attending the pre-
paratory and college departments he
received the degree B. A. from Ripon
College, June 27, 1880.

The three years succeeding
graduation were spent at Ripon, Wis.,
in the study of literature.

Early in his college course, the
purpose was formed to devote his life
to the study of biology. He was in-
fluenced by the lectures of Dr. J. B. S. Patten