The Location of the Ganglion Cells of the Sensory Component of the Splanchnic Nerve in the Albino Rat

by

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C O N T E N T S

I N T R O D U C T I O N

Kölliker('96), apparently, was the first even to venture an opinion on the origin of the visceral afferent fibers. He believed that such fibers of the sympathetic nerves originate from cells of the spinal ganglia. Dogiel ('96), on the contrary, thought that they must have their cells of origin within the ganglia or plexuses of the sympathetic system. This latter opinion was upheld also by Onuf and Collins ('98). Langley ('05), however, cut a spinal nerve in a dog just peripherally of the dorsal root ganglion, and found that all the fibers in the white ramus communicans degenerated. He then cut the splanchnic nerve, and found that there were no degenerating fibers in any of the white rami communicantes of the thoracic region. Hence, he reached the conclusion that none of the afferent fibers of the splanchnic nerve have their trophic center in the ganglia of the solar plexus. Rossi ('22)

has recently seen fibers passing from cells of the spinal ganglia into the white rami communicantes.

None of these studies were on the visceral afferent cells. Biedl ('95) cut the splanchnic nerve in dogs, and studied the resulting degeneration in the spinal cord and spinal ganglia. The purpose of his researches, however, was not so much to establish the localization of the splamchnic nerves in the spinal cord, as it was to study the histological character of the cell changes occurring after the section of these nerves.

Warrington and Griffith ('04) studied the cell changes in the spinal ganglia after the extirpation of the stellate ganglion in cats. They classified the normal cells of the spinal ganglia in four groups: clear cells, obscure cells, coarsely granular cells, and smallest clear cells. The clear cells vary in size, and have small chromophile granules scattered almost equally throughout the cyto-

plasm. The obscure cells are of medium size, are dark, and have very fine closely packed chromophile elements. The coarsely granular cells are clear, while their chromophile elements are rather large and few in number. The smallest clear cells resemble the clear cells, but are much smaller. Since this same simple classification may be applied to the spinal ganglion cells of the albino rat, I shall use it in this paper.

Warrington and Griffith ('04) removed the spinal ganglia of the upper thoracic nerves after extirpation of the stellate ganglion, and observed the number and types of degenerated cells in the spinal ganglia. Since the ganglia were removed, there could be no localization of the cells. They found that the number of visceral afferent cells in these ganglia was, at the highest estimate, not more than two per cent of the total number of cells. The degenerated cells were nearly all of the obscure type. The cells which degenerated as

a result of this operation were located in the first three thoracic ganglia only.

I wish to thank Professors G. E. Coghill and H. C. Tracy for their valuable criticism and encouragement.

METHODS AND PROCEDURE

No experimental investigation of the segmental localization of the visceral afferent cells of the splanchnic nerve has been recorded. From clinical observations on patients with lesions of the intestines, Ross ('87) noticed that the pain was referred to the region innervated by the tenth thoracic nerve. Head ('93), as a result of a more thorough clinical study, suggested that the referred pain from the intestines was localized almost entirely in the ninth and tenth thoracic segments. These observations imply that the sensory cells of the splanchnic nerve lie in these segments.

Since no experimental work on localization of the sensory cells of the splanchnic nerve seems to have been done, I undertook this problem from an anatomical standpoint. The adult albino rat was selected for my investigation. As a preliminary to the microscopic study, I made several

dissections to determine the exact gross anatomical relations of the left splanchnic nerve. This nerve arises from the sympathetic trunk between the tenth and eleventh thoracic sympathetic ganglia, as diagrammatically shown in Figure 1, and lies laterally of the aorta. The fibers composing this nerve are not all grouped together, but lie in five or six separate fasciculi. There is no lesser splanchnic nerve in the albino rat.

Having determined the exact gross anatomical location of this nerve, I performed the operation of sectioning it in the live rat. Ether was used as an anesthetic, the anesthesia being induced by placing the animal in a closed glass jar and dropping in a piece of cotton saturated with ether. Subsequent anesthesia was maintained by the drop method, with the use of a small cone of filter paper.

An incision through the skin was made on the back of the animal, just beneath the

lowest rib of the left side. The muscle fibers of the lateral body wall were forced apart by blunt dissection, making an opening about three-fourths of an inch in length laterally of the left kidney.

Through this opening I was able, by using a blunt needle, to dissect the fibers of the left splanchnic nerve loose from the body wall just below the diaphragm. With a small curved hook, I then gathered these fibers into a single bundle. Since a small artery was usually included in this group, two fine silk ligatures were passed around the whole bundle, and securely tied. I then cut the nerve between these two ligatures, and sewed up the incision. The operation was performed under nearly aseptic conditions, and no infection took place in any of the rats.

This operation was performed successfully on nine rats. During the first two or three days following the operation, the animals were inactive and weak. Following this preliminary period of

depression, which was probably due to the shock of the operation, the rats rapidly returned to normal, and apparently remained so indefinitely.

In answer to a possible criticism that control experiments were not performed in which the same operation was performed on other animals with the exception of severing the splanchnic nerve, it should be emphasized that the possibility of cutting other nerve fibers than the splanchnic was obviated by the careful operative procedure. The cutaneous nerves enter the skin near the median dorsal line, so they were not cut. After the skin incision was pulled apart, the segmental nerves lying in the muscles of the lateral abdominal wall could be clearly seen, and hence easily avoided in pulling the muscles apart. Due to this careful operation, it is not probable that any somatic nerves were injured.

Further evidence which indicates that there was no need for a control in my experiment is by Warrington and Griffith ('04). They ran

a control in their experiments upon the stellate ganglion, exposing the ganglion in one animal, but not touching it. They were unable to find a single degenerated cell in the spinal ganglia of the segments which would be involved. For this reason, and because of the careful operative technique, I believe that my results may justifiably be considered as due to cutting the splanchnic nerve.

Fifteen days time was allowed for the degeneration of the cells. Nicholson ('23) found that in the albino rat fifteen days for degeneration permit the maximum effects of chromatolysis to be observed with methylene blue, toluidin blue, and similar stains. This time was also used successfully by Warrington and Griffith ('04).

Three of these rats in which the left splanchnic nerve had been cut were killed with chloroform at the end of the time mentioned. I then removed the thoracic region of the spinal cord, with the spinal ganglia attached by the

nerve roots. This was done by first cutting the ribs and muscles on each side close to the spinal column. The block of tissue, consisting of the spinal column of the thoracic region with the enclosed spinal cord, was then hardened for two hours in the fixing fluid. After removing the bodies of the vertebrae by cutting with scissors in a frontal plane, I exposed the spinal cord on the ventral side. The laminae of the vertebrae were next divided, exposing the dorsal side of the spinal cord. By careful dissection, I was able to free the spinal ganglia from the remaining particles of bone, muscle, and connective tissue. By this procedure, I was able to keep the spinal ganglia attached to the spinal cord, and oriented in their normal position.

This thoracic region of the spinal cord, with the attached ganglia, was placed for twelve hours in Van Gehuchten's fluid, prepared according to the following formula: absolute alcohol, 60 c.c.; chloroform, 30 c.c.; glacial acetic acid, 10 c.c. The tissue was dehydrated in four

changes of absolute alcohol for six hours, and cleared in toluol for two hours. After an hour's infiltration in paraffin, the tissue was embedded in fresh paraffin of a melting point of 56° C.

I cut and mounted serial sections of the spinal cord from the fifth thoracic to the eleventh thoracic segments, inclusive. These sections were ten micra thick.

The staining technique was taken largely from Hardesty ('02), but since some variations were made, it will be given in full. The slides were placed in toluol for twenty minutes; absolute alcohol, five minutes; 95 per cent alcohol, three minutes; one per cent erythrosine in 70 per cent alcohol, two minutes. The slides were then washed in fifty per cent alcohol until the sections were light red. They were placed in distilled water for two minutes; one per cent aqueous toluidin blue, forty minutes; 0.1 per cent potassium alum, twenty seconds. The sections were decolorized to a faint blue by dipping the

slides in 70 per cent alcohol. Then the sections were rapidly dehydrated by passing through 95 per cent and absolute alcohols, and they were cleared in toluol for twenty minutes. The sections were mounted in damar which had been neutralized with sodium bicarbonate.

O B S E R V A T I O N S

Of the three spinal cords that were sectioned and stained as described above, one was taken for a detailed study. I carefully examined the spinal ganglia of both sides, from the fifth to the eleventh thoracic. The sections were examined serially, using the 4 mm. objective and the 10x ocular. The oil immersion objective was used in the study of doubtful cells.

I found no cells having the slightest indication of chromatolysis in any of the ganglia of the right side, and so considered it to be normal.

All of the degenerated cells found were in the ganglia of the left side. The prevailing type of degeneration consisted in a massing of the Nissl granules to one side of the cell, or around the cell membrane (Figure 2, b). The nucleus was usually eccentric, with the nuclear

wall broken down in places where the Nissl granules were clumped along the nuclear membrane. The nucleolus was enlarged, and a few of the degenerated cells contained branching vacuoles (Figure 2, c). Almost all of the degenerated cells belong to the obscure type, thus agreeing with the observations of Warrington and Griffith ('04).

For the purpose of locating the cells in the sections of the spinal ganglia, a division into nine regions was made, as shown in Figure 3: dorso-lateral, dorsal, dorso-medial, lateral, central, medial, ventro-lateral, ventral, ventro-medial. These divisions are arbitrary and are purely directional, there being no actual division of the ganglia into the regions illustrated.

Figure 4 is a chart of the spinal ganglia that were studied. This shows the number of degenerated cells in each arbitrary region of each ganglion.

In the left spinal ganglia of the fifth

and sixth thoracic nerves, I found no degenerated cells.

In the left spinal ganglion of the seventh thoracic nerve, I found four degenerated cells. These were located as follows: one in the dorsal region; one in the central region; one in the dorso-medial region; and one in the medial region.

In the left spinal ganglion of the eighth thoracic nerve, I found seven degenerated cells. These were located as follows: two in the central region; one inbthe ventral region; three in the dorso-medial region; and one in the ventro-medial region.

In the left spinal ganglion of the ninth thoracic nerve, I found seven degenerated cells. These were located as follows: one in the dorsal region; one in the central region; two in the ventral region; two in the dorso-medial region; and one in the ventro-medial region.

In the left spinal ganglion of the tenth thoracic nerve, I found fourteen degenerated

cells. These were located as follows: one in the dorso-lateral region; one in the lateral region; one in the dorsal region; three in the central region; four in the dorso-medial region; three in the medial region; and one in the ventro-medial region.

In the left spinal ganglion of the eleventh thoracic nerve, I found no degenerated cells.

D I S C U S S I O N

1. The segmental location of the sensory cells of the splanchnic nerve.

The segmental localization of the afferent cells of the splanchnic nerve is apparent from the description given above, and from the charts. The cells are present, beginning with the seventh ganglion, in an increasing number in each ganglion to the tenth, where a maximum is reached. No cells were found in the eleventh ganglion.

This localization of the sensory cells of the splanchnic nerve agrees in the main with Gaskell's ('86) localization of the motor segments in the dog. Gaskell found that the thoracic nerves whose white rami communicantes contained efferent fibers going to the splanchnic nerve were those from the sixth to the eleventh, inclusive.

A possible explanation of this limited arrangement of the sensory cells may be found in the development of the parts innervated by the nerve. In general, the splanchnic nerve supplies the small intestine . Since the intestine develops by the elongation of a tube which originally is but a few segments in length, its innervation would quite naturally be derived from only a few segments of the nervous system.

On the other hand, it is possible that this segmental localization develops by the amalgamation of certain functional actions into particular regions. For example, in the case of the cranial nerves, the general somatic sensory fibers are almost entirely localized in the fifth nerve and the ramus auricularis of the tenth. The cranial visceral sensory fibers are confined essentially to three nerves: the seventh, ninth, and tenth. Hence, it may be that there is a similar massing of the visceral sensory cells of the spinal region into a few segmental nerves.

Further evidence for this last view is given by the work of Warrington and Griffith ('04). In their experiments, extirpation of the stellate ganglion in a dog cut practically all of the visceral sensory fibers in the region above that which connects with the splanchnic nerve. A search of the spinal ganglia for degenerated cells following the above-mentioned lesion resulted in a localization of the visceral afferent cells whose axones pass through the stellate ganglion, to the first, second, and third thoracic segments.

Since my work shows that the splanchnic afferent cells are restricted to the seventh, eighth, ninth, and tenth thoracic segments (Figure 5), it would seem that the visceral afferent cells might be localized into three or four segmental regions, with no over-lapping, and probably gaps between. These regions, as already discovered, would be:

First, the cranial localization

Second, the upper thoracic or stellate group

Third, the lower thoracic or splanchnic group

As yet, no work has been recorded on the regions caudal of the splanchnic.

Some clinical writers (Head ('93); Onuf and Collins ('98)) have suggested that if these three or four segmental regions of visceral afferent cells did over-lap, somatic pain at a particular point might not always signify a lesion in the same visceral field. My own work, in connection with that of Warrington and Griffith, indicates that such a possibility is not the case.

2. The longitudinal grouping of the sensory cells of the splanchnic nerve.

In undertaking this study, as I mentioned previously, I divided the spinal ganglia into nine arbitrary regions for the purpose of definitely locating the sensory cells of the splanchnic nerve. At the conclusion of my study, I found that there is some real longitudinal localization of the cells into regions. That this is true, at least in a general way, is shown by Figure 4. This is a syudy of each separate ganglion, showing the number of degenerated cells in each arbitrary region of each ganglion. There is considerable similarity of these charts for all of the ganglia involved.

The lateral and dorso-lateral regions appear only in the tenth spinal ganglion, and hence are not characteristic of all the ganglia. The ventral region is represented only in the eighth and ninth ganglia, while the medial

region is represented only in the seventh and tenth, so these are also irregularities.

On the other hand, the dorsal region of cells is equally represented in the seventh, ninth, and tenth ganglia. The central and dorso-medial regions are prominently represented in all the regions which contain cells of the splanchnic nerve, and the ventro-medial region is represented in the eighth, ninth, and tenth ganglia (Figure 6).

The single cell found in the lateral region was near the arbitrary boundary line between the lateral and central regions. Also, the single cell found in the dorso-lateral region was close to the dorsal region. The possibility of slight differences in the relative position of the ganglia due to manipulation during removal of the tissue might account for some of these minor irregularities.

In summing up this arrangement, it would seem that the predominantly characteristic

regions of the ganglia in which these visceral cells are found are the central and the dorso-medial, with the dorsal and ventro-medial regions also characteristic but not so large (Figure 7). As a result of this localization, a region can be described in which all the afferent cells of the splanchnic nerve are found. It consists of the six medial arbitrary regions, and overlaps slightly into the dorso-lateral and lateral arbitrary regions.

My work indicates that all the visceral afferent cells of the splanchnic nerve lie in this region of the spinal ganglia. There remain, however, several questions which my work has not answered. Are all the visceral afferent cells of these ganglia in this region? Are all the cells in this region visceral?

It is probable that a number of visceral afferent cells which lie in these ganglia would be unaffected by cutting the splanchnic nerve. Their axones might pass peripherally, or in the sympathetic trunk to other segments. The

location of these cells, if they exist, can be determined by section of the rami communicantes of the segments involved, and subsequent search for the degenerated cells in the ganglia.

On the other hand, some negative evidence as to the location of the visceral cells might be produced. By cutting the spinal nerves of these segments distal to the point where the white ramus communicans is given off, all the somatic sensory cells would be expected to show degenerative changes. This operation has been performed ((Warrington and Griffith ('04))) for the purpose of studying cell changes, but no study of cell distribution is recorded.

Finally, can the cells of this region be considered as forming a true visceral longitudinal grouping, or column?

My own work is the first recorded suggestion that such a visceral sensory cell column may be present. Additional research, along the lines indicated above, will be

necessary before such a sensory column can be definitely and exactly demonstrated. At least, it would seem from my results that the visceral afferent cells tend to lie more toward the medial and dorsal parts of the spinal ganglia.

The fact that these cells tend to lie toward the medial part of the ganglia may have a developmental significance. The structures which the splanchnic nerve innervates were embryonically all in the median plane of the body. The location of the sensory cells in the medial part of the ganglia might be considered as a resultbof the phenomenon of neurobiotaxis as conceived by Kappers ('20). It is more probable, however, that it is due merely to mechanical pulling processes, which tend to draw these cells toward the median plane during the course of development.

3. The function of the sensory cells of the splanchnic nerve.

While an anatomical investigation such as mine cannot determine the function of the cells in question, it at least brings the matter to mind. As described above, nearly all the cells which degenerated after section of the splanchnic nerve belonged to the class of obscure cells. Ranson ('12) noted that the axones of the "small cells", which is his name for the obscure cells, were non-medullated. Ranson ('13) announced that these non-medullated fibers of the dorsal roots could be traced clearly into Lissauer's tract. He decided at that time that these fibers had little or nothing to do with proprioceptive impulses. He later ('14) suggested that these cells probably carry pain and temperature sensations, and are involved in vaso-motor reflexes.

Lennander ('02) was of the opinion that visceral pain was mediated through the parietal

peritoneum, but this was later disproven by Neumann ('10). Hertz ('11) showed by experiment that all viscera were insensitive to tactile stimuli. He was of the opinion that heat and cold could be appreciated only in the esophagus and anal canal, and that tension was the only cause of true visceral pain. Ranson ('21) sums up the present knowledge of this subject as follows:

> "We now know that afferent impulses of visceral origin may appear in consciousness as painful sensations, and that at least in the stomach and large intestine there is also a crude form of temperature sensibility. The majority of the afferent impulses from the viscera do not rise into consciousness at all, but expend themselves in the production of reflexes."

In other words, physiological experimentation has shown that the visceral impulses result in pain and temperature sensations, and reflexes

which are mostly vaso-motor.

The cells in the spinal ganglia whose fibers run in the splanchnic nerve belong to the type described by Ranson as taking part in pain and temperature sensations, and in vaso-motor reflexes. This corroborates on an anatomical basis the physiological results stated in the preceding paragraph, and at the same time increases the knowledge of the anatomical paths involved in physiological visceral reactions.

C O N C L U S I O N S

1. After section of the splanchnic nerve in the albino rat, cells in chromatolysis occur in the seventh, eighth, ninth, and tenth thoracic spinal ganglia.

2. These cells are located chiefly in the proximal and medial portions of the ganglia.

3. The sensory component of the splanchnic nerve in the albino rat involves the seventh, eighth, ninth, and tenth thoracic spinal ganglia.

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Figure 1.

Diagram showing the relations of the left splanchnic nerve to the sympathetic trunk and the thoracic nerves in the albino rat. The numerals indicate the serial numbers of the spinal ganglia.

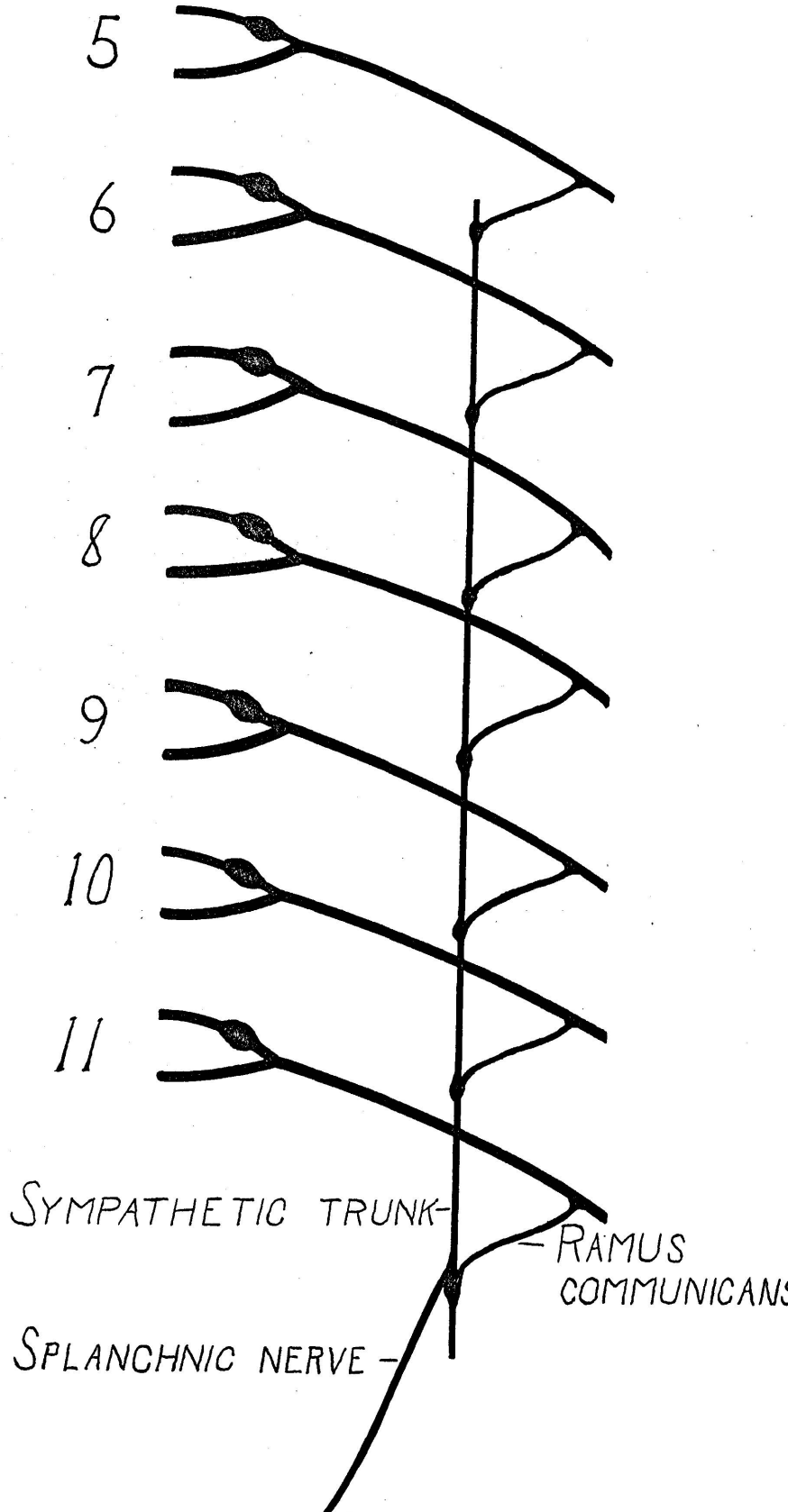

5
6
7
8
9
10
11
SYMPATHETIC TRUNK
RAMUS COMMUNICANS
SPLANCHNIC NERVE

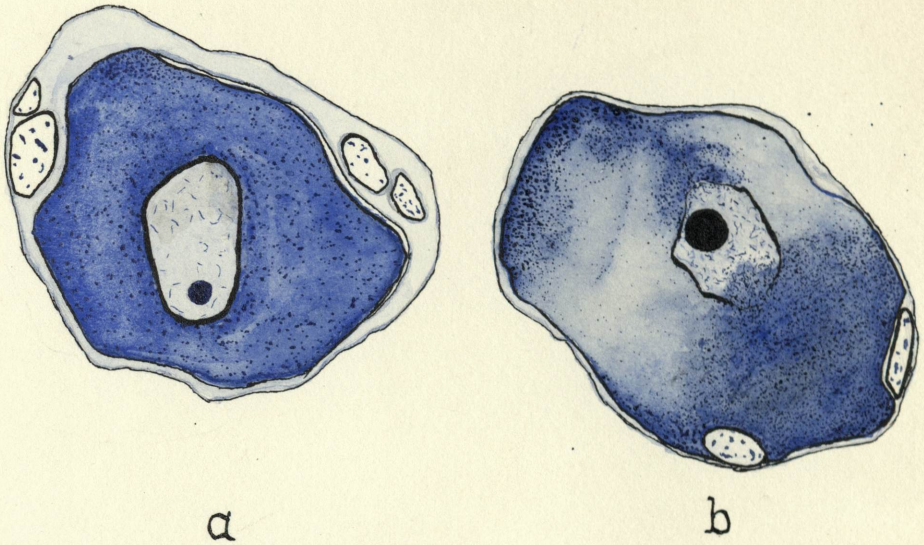
FIGURE 1

Figure 2.

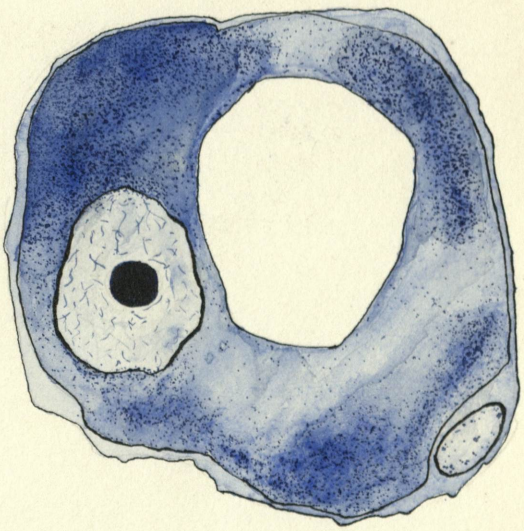
a. Normal spinal ganglion cell of the obscure type.

b. Usual type of degenerated spinal ganglion cell.

c. Degenerated spinal ganglion cell, showing vacuole.



a b



c

FIGURE 2

Figure 3 .

Sketch of cross-section of spinal cord with ganglia, showing the arbitrary divisions of the ganglia.

DL - Dorso-lateral

D - Dorsal

DM - Dorso-medial

L - Lateral

C - Central

M - Medial

VL - Ventro-lateral

V - Ventral

VM - Ventro-medial

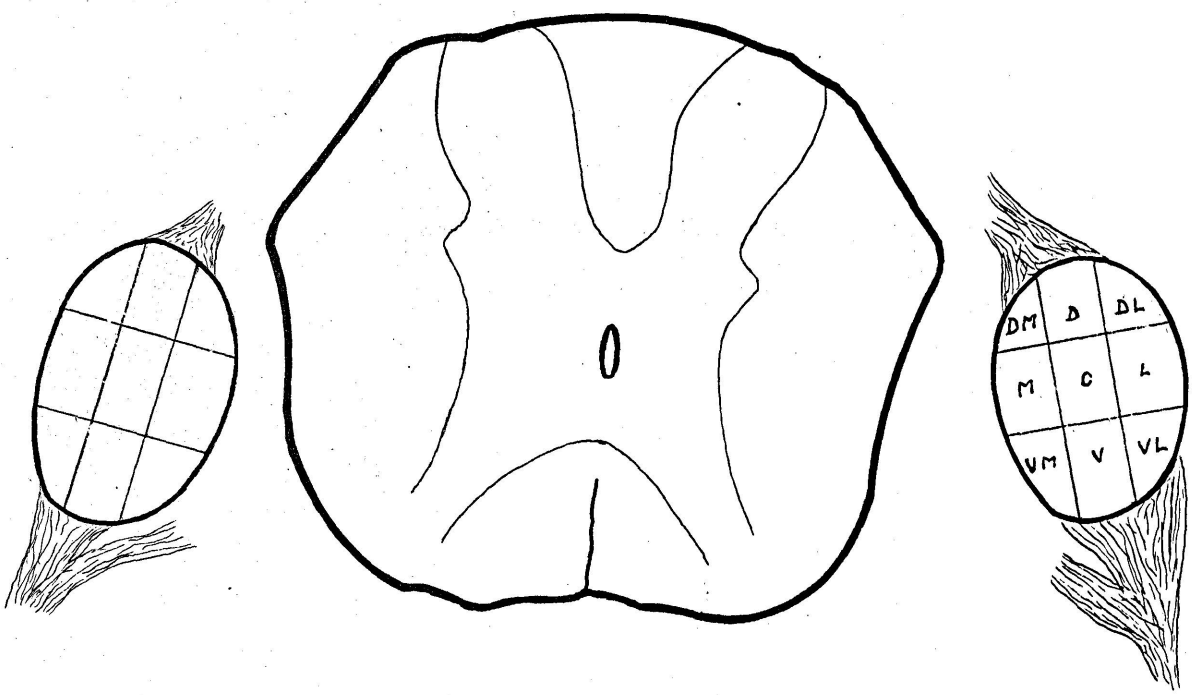


FIGURE 3

Figure 4.

Graph showing the regional distribution of the cells in each individual spinal ganglion. The thoracic segments are numbered; the regions are shown by the letters.

DL - Dorso-lateral

D - Dorsal

DM - Dorso-medial

L - Lateral

C - Central

M - Medial

VL - Ventro-lateral

V - Ventral

VM - Ventro-medial

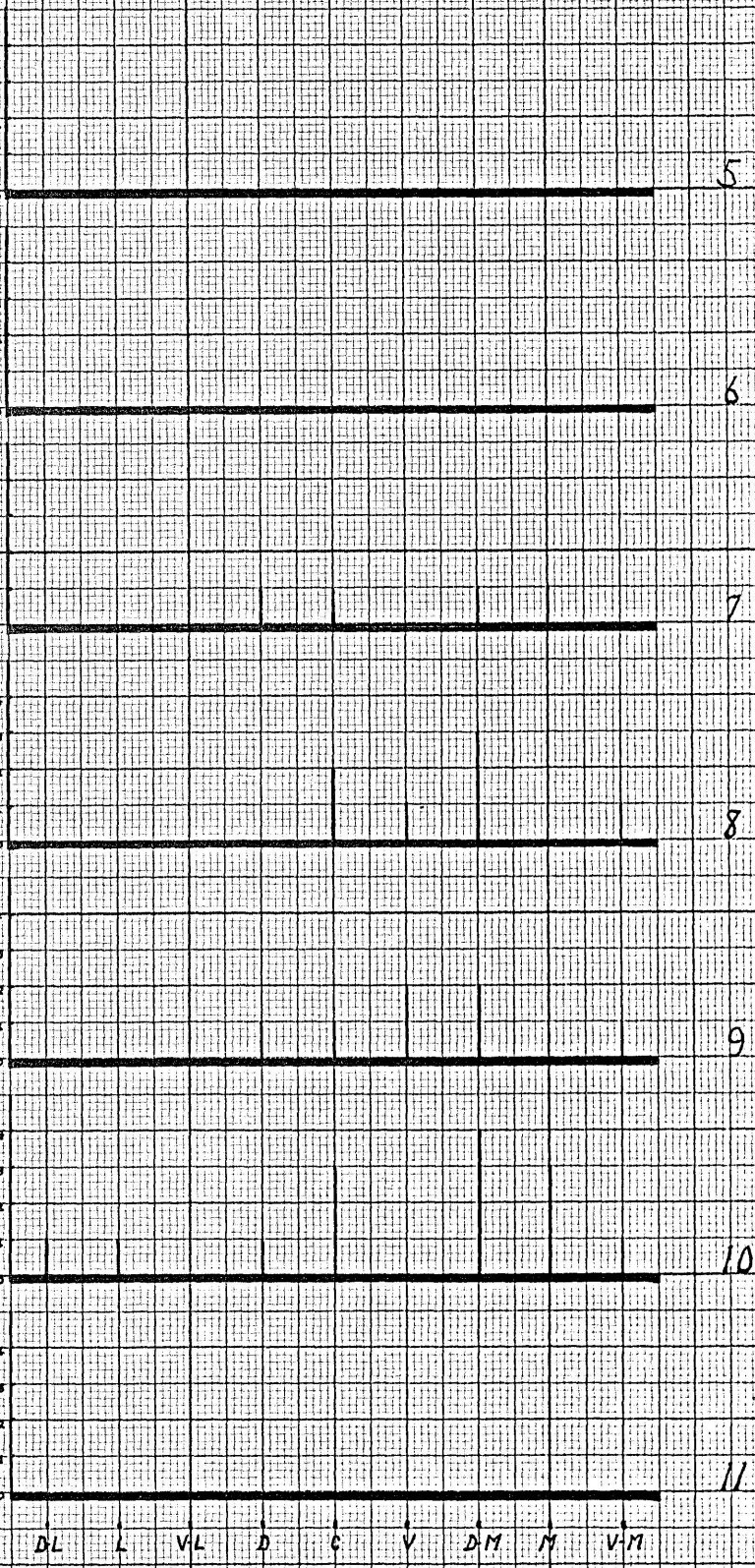


FIGURE 4

Figure 5 .

Diagram of the segmental localization of the visceral afferent cells of the splanchnic nerve.

a - Spinal ganglion

b - Ventral root

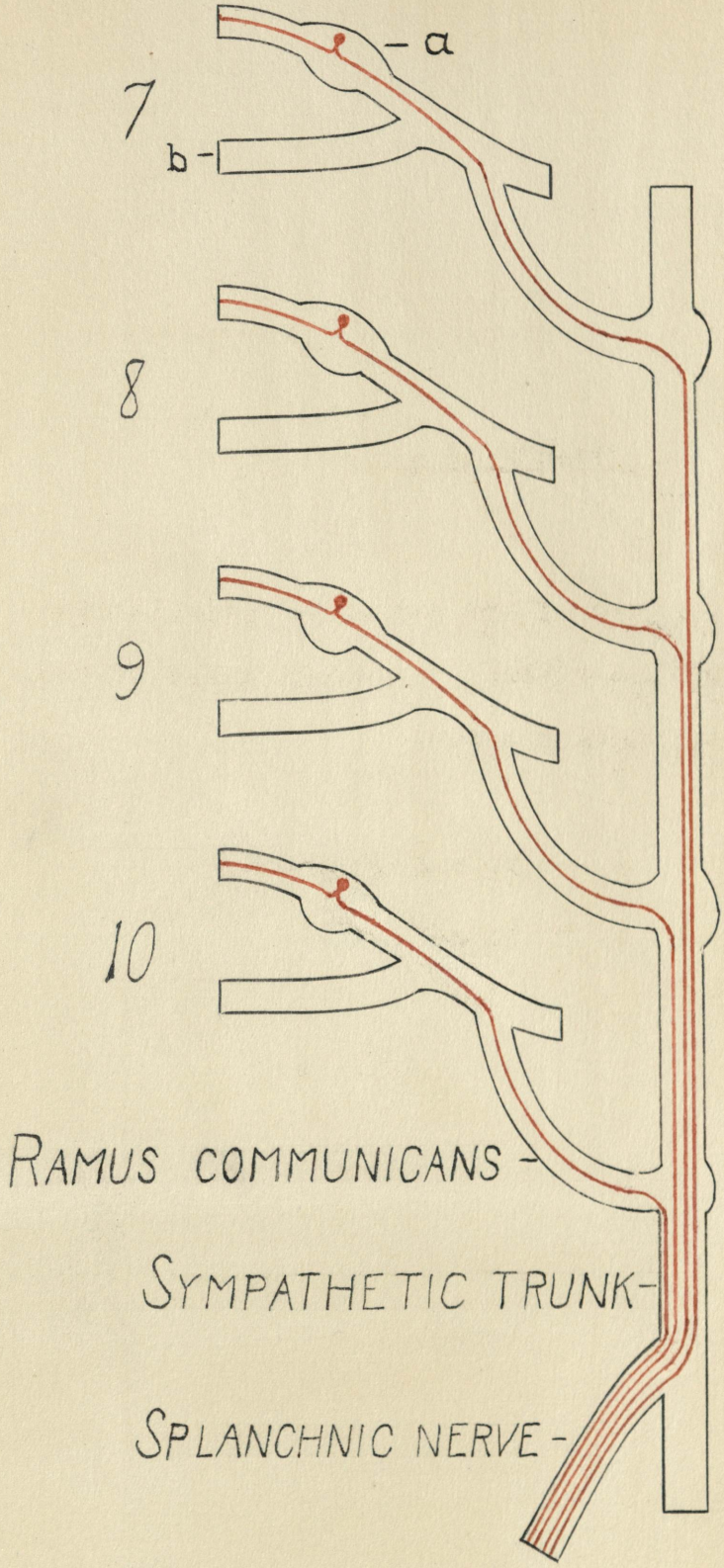

a
7
b
8
9
10
RAMUS COMMUNICANS
SYMPATHETIC TRUNK
SPLANCHNIC NERVE

FIGURE 5

Figure 6 .

Graphs showing the segmental distribution of the cells in the arbitrary regions of the spinal ganglia.

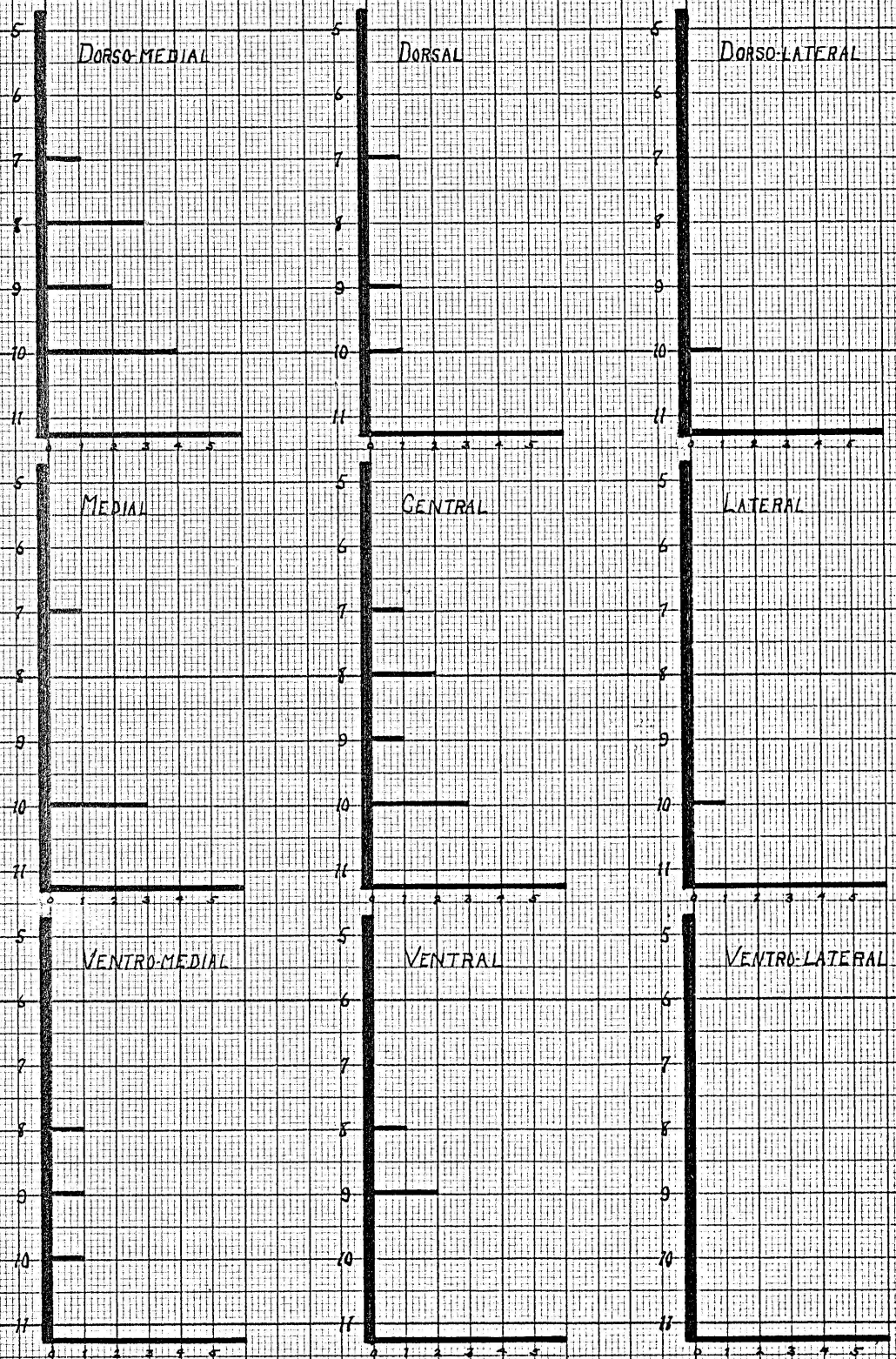

DORSO-MEDIAL
DORSAL
DORSO-LATERAL
MEDIAL
CENTRAL
LATERAL
VENTRO-MEDIAL
VENTRAL
VENTRO-LATERAL

FIGURE 6

Figure 7 .

Graph showing the total number of visceral afferent cells of the splanchnic nerve in the arbitrary regions of the spinal ganglia.

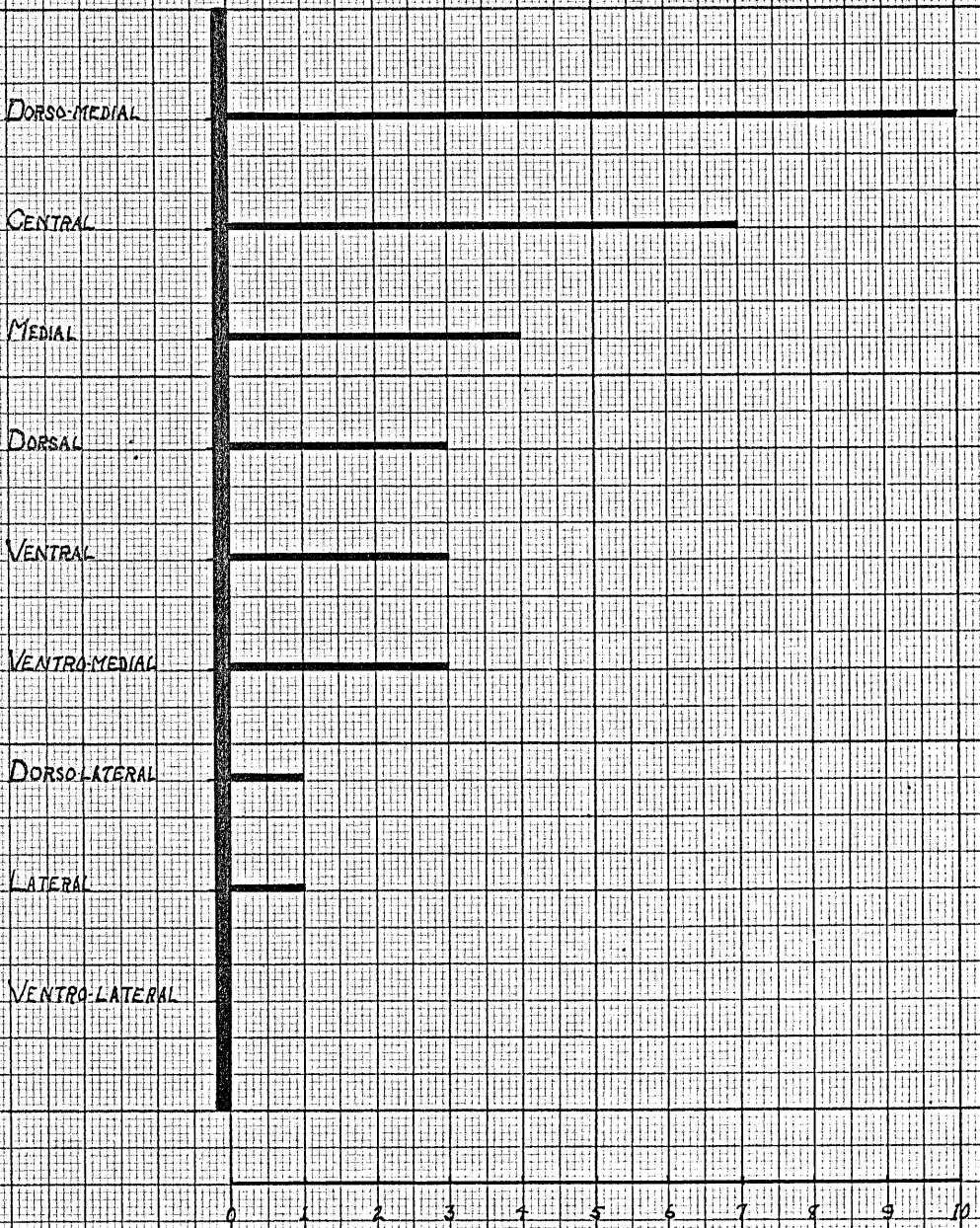

DORSO-MEDIAL
CENTRAL
MEDIAL
DORSAL
VENTRAL
VENTRO-MEDIAL
DORSO-LATERAL
LATERAL
VENTRO-LATERAL
0
1
2
3
4
5
6
7
8
9
10

FIGURE 7