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A Study of Nissl Bodies in the
Autonomic Ganglion Cells of Rodents

Zoology

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A STUDY OF NISSL BODIES IN THE AUTONOMIC
GANGLION CELLS OF RODENTS

BY

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THESIS

Submitted in Partial Fulfillment of the Requirements for the

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June 4, 1913

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Jesse Le Roy Conel

ENTITLED A Study of Nissl Bodies in the
Autonomic Ganglion Cells of Rodents

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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I. INTRODUCTION

1. Purpose of Investigation:- The results of investigators have suggested upon morphological and physiological grounds the possibility that both sensory and motor cells are present in the autonomic (sympathetic) ganglia of vertebrates. The following study was undertaken for the purpose of testing this question by an examination of the Nissl bodies in the autonomic cells of a group of mammals. The group chosen was that of the rodents, the greater part of the material used being obtained from the rabbit. So far as can be learned, no one has ever attempted to obtain light on the problem in hand by means of a study of the Nissl bodies. Yet it is well known that sensory and motor cells in the cerebro-spinal nervous system can be distinguished by means of their Nissl bodies, the latter presenting a striking difference in appearance in the two kinds of cells.

In fact, so little has been done in the autonomic system with the Nissl method that while working with this primary question in mind several others arose. Therefore, in making my investigations I have kept before me the following questions:

(1). Are both sensory and motor cells present in the trunk autonomic ganglia of the rabbit?

(2). If so, how do they compare with reference to Nissl bodies with typical sensory cells in the dorsal root ganglia and with typical motor cells in the ventral

horn of the spinal cord?

(3). How do cells in the cranial autonomic ganglia of the rabbit compare with cells in the trunk autonomic ganglia with respect to Nissl bodies?

(4). How do cells in the trunk autonomic ganglia of the rabbit compare with cells in the same region in other rodents with reference to Nissl bodies and the number of their nuclei?

I am indebted for much assistance to Dr. F. W. Carpenter, under whose guidance the work was done in the Zoological Laboratory of the University of Illinois.

2. Historical Review:- A review of the literature upon the autonomic nervous system reveals the fact that very little has been done within the field outlined by the above questions.

Dogiel (1896) described two types of cells in the autonomic ganglia of fishes, amphibians, reptiles, and mammals, viz., (a) cells with short, thick, much branched dendrites, and (b) cells with long, slender dendrites with few branches. Type a cells he thought were motor, and type b sensory.

Michailow (1908) found in autonomic ganglia five types of cells which differed from each other as to the nature of the endings of their dendrites. In the same year in another paper (1908a) he described six types of sensory nerve endings in autonomic ganglia, some of which he believed belonged to sensory cells in the ganglia.

Müller (1909) observed two distinct kinds of cells in

the autonomic ganglia. Some cells had long, far-reaching, antler-like dendrites which gave them a star-fish appearance. These processes extended beyond the cell capsules. Other cells had a wreath of very small dendrites, which were bent hook-like, and which did not extend beyond the cell capsule, i.e., they were intracapsular, only the axiscylinder projecting beyond the capsule.

The results of physiological experiments in the way of reflexes in the autonomic ganglia would suggest that both sensory and motor cells are present. Langley and Anderson (1894) state that two cases of such reflexes have been established, one in the submaxillary ganglion of the dog (Claude Bernard), and another in the inferior mesenteric ganglion of the cat (Sokownin). Langley and Anderson themselves seem to have established a peripheral reflex in the inferior mesenteric ganglion.

Finally, the manner in which the autonomic trunks develop in mammals offers a suggestion that both sensory and motor cells may be found in the vertebral ganglia and their derivatives, the prevertebral ganglia. Kuntz (1910), in a very interesting work with pig embryos, observed that cells which later form the autonomic trunk, its ganglia and derivatives, migrate both from the dorsal part of the neural tube and the dorsal root ganglion and from the ventral horn of the neural tube. He states, however, that the presence of sensory neurons in the autonomic trunks and prevertebral plexuses has not been demonstrated, and that Froriep, like Langley, Kölliker,

and P. Schultz, denies the existence of sympathetic sensory neurons entirely. As to this point he concludes as follows: "Inasmuch as nervous elements which have the capacity to develop into neurons migrate peripherally along both the sensory and the motor nerve-roots, we are driven to the conclusion that the sympathetic excitatory elements migrate from the neural tube along the fibers of the motor nerve roots, while the sympathetic sensory neurons, wherever such neurons exist, arise from cells which wander out from the cerebro-spinal ganglia. This interpretation makes the sympathetic neurons entirely homologous with the efferent and the afferent components of the other functional divisions of the peripheral nervous system."

II. MATERIAL AND METHODS

Autonomic ganglia from the rabbit, rat, mouse, thirteen-lined spermophile, prairie dog, guinea pig, muskrat, and porcupine were examined. For all comparative studies ganglia from different regions in the same animal were used. All of the animals were healthy, normal adults which had enjoyed a long period of rest before they were killed.

1. Fixation:- Material was dissected from the animals immediately after they had been killed by means of chloroform. Three different methods were used for fixing:

- a. 80 parts of 100% alcohol and 20 parts of glacial acetic acid - - - - - 15 minutes
- 90 parts of 100% alcohol and 10 parts of glacial acetic acid - - - - - 15 minutes
- 100% alcohol - - - - - 12 to 18 hours
- Xylol - - - - - 1 hour

- b. 40% formalin - - - - - 48 hours
 Wash in water
 40%, 70%, 95% alcohol- - - - - 1 min. each
 100% alcohol - - - - - 1 hour
 Xylol - - - - - 1 hour
- c. Corrosive sublimate (saturated aqueous solution)
 leave in until tissue becomes opaque.
 Wash several hours in water containing a small
 amount of tincture of iodine.
 40%, 70%, 95% alcohol- - - - - 1 min, each
 100% alcohol - - - - - 1 hour
 Xylol and melted paraffin (equal parts) - 1 hour.

2. Imbedding:- All material was imbedded in paraffin. I found that paraffin melting at 50--53^o was the most satisfactory grade to use. The ganglia were transferred directly from xylol or xylol and paraffin to melted paraffin, and left in the latter for one hour. They were then imbedded in the usual way.

3. Sectioning:- The material was cut in sections varying in thickness from 2 to 10 micra. For the purposes of this investigation sections 10 micra thick were found most satisfactory.

4. Staining:- Several modifications of the Nissl method of staining were tried:

- a. A methylene blue stain was made as follows:

Methylene blue - - - - - 3.75 g.
 Venetian soap - - - - - 1.75 g.
 Distilled water - - - - - 1 liter

This was allowed to stand for three or four months before it was used.

The sections were fastened to slides by means of albumen fixative, and brot down in the usual way to

water thru xylol and the alcohols. Some of the above staining fluid was then heated to steaming in a flask, and poured upon the sections on the slides. The stain was allowed to act for from 4 to 6 minutes, and was then poured off, and the slides flooded with anilin alcohol (9 parts of 95% alcohol, and 1 part of anilin oil). This differentiation fluid was permitted to remain on the slides until the sections had assumed a pale blue color. The anilin alcohol was then drained off, and the slides were put into 95% alcohol for 1 minute, and then transferred to 100% for the same length of time. The sections were finally cleared in xylol, and mounted in Canada balsam.

A counterstain of eosin or erythrosin (Aqueous solution) was sometimes introduced just after the differentiation had taken place.

b. Sections on slides were brot down thru xylol and the alcohols to water. A cold, saturated, aqueous solution of thionin was then poured upon the slides and allowed to remain for from three to five minutes. The stain was then poured off, and the sections were taken thru the alcohols rather rapidly, about 1/2 minute in each grade being sufficient. The alcohol acts as a differentiating agent, and care must be taken not to leave the sections in it too long, else the stain will be entirely removed.

Xylol, clove oil, or cajeput oil were used for

clearing agents. Mounts were made in Canada balsam.

A counterstain of eosin or erythrosin was occasionally used just after the thionin solution had been poured off.

c. This method was the same in all respects as method b, excepting that a cold, saturated, aqueous solution of toluidin was used instead of thionin.

d. Sections affixed to slides were brot down in the usual manner to water. A cold, saturated, aqueous solution of basic fuchsin was left on them for from 3 to 5 minutes. This was poured off, and the slides were taken up thru the alcohols. The latter act as differentiating agents. The sections were cleared in xylol, and mounted in Canada balsam.

e. & f. These methods were identical with method d, excepting for the fact that either neutral red or magenta was used in place of basic fuchsin.

Methods a, b, and c stain the Nissl bodies blue. I found that thionin brot out the Nissl bodies more clearly than the other two dyes. Material fixed in the three different reagents (acetic-alcohol, formalin, and corrosive sublimate) was used with each stain, and my results showed no perceptible difference due to fixation. Counterstains assisted in setting off the Nissl bodies by furnishing a colored background for a contrast.

Methods d, e, and f stain the Nissl bodies red. I did not find these stains at all satisfactory. They do not differen-

tiate the bodies distinctly.

III. OBSERVATIONS

1. The question of sensory and motor cells in the trunk autonomic ganglia of the rabbit:- It is well known that in the cerebro-spinal nervous system the Nissl bodies in sensory cells present an appearance, both as to size and arrangement, entirely different from that seen in motor cells. Figures 1 and 2 illustrate typical sensory cells. The former is a cell from a dorsal root ganglion of the rabbit, and the latter from the Gasserian ganglion of the same animal. The Nissl bodies of sensory cells range in size from very fine granules to rather large bodies. They have clean-cut, distinct outlines, and the larger ones are triangular in shape. The fine granules and large bodies are intermingled and scattered evenly thruout the entire area of the cell-body from the nuclear wall to the cell boundry. None occurs in the nucleus. Barker (1901) refers to circular areas in spinal ganglion cells which do not have many Nissl bodies, causing a distinct clear space. These clear areas may be one to three in number and are about the same size as the nucleus, or larger. However, I failed to find any such areas in my preparations.

The condition found in motor cells is quite different. Figures 3 and 4 represent typical motor cells; both are from the ventral horn of the spinal cord of the rabbit. The Nissl bodies in all motor cells are large, coarse masses, somewhat oval in shape, arranged more or less concentrically. The out-

lines are not so clean-cut as those of Nissl bodies in sensory cells. The bodies are fairly uniform in size, i.e., there is no gradation from fine granules to large bodies as is to be found in sensory cells. There may often be seen suggestions of fusion of Nissl bodies.

For the purpose of determining, if possible, whether or not typical sensory and motor cells as described above are present in the trunk autonomic ganglia of the rabbit preparations were made from thoracic, superior mesenteric, and coeliac ganglia. Among the hundreds of cells which they contain there do not seem to be any which can be classified as sensory cells on the basis of their Nissl bodies. On the contrary, all of the cells possess the characteristics, so far as Nissl bodies are concerned, of typical motor cells. In none are there present gradations from fine granules to large, triangular Nissl bodies with clean-cut, distinct outlines; nor can there be seen the circular, clear areas mentioned by Barker as characteristic of dorsal root ganglion cells. Also, the bodies are not distributed evenly thruout the cell. They are all large and coarse, more or less oval in shape, and show suggestions of concentric arrangement in the cell (Figs. 5-10).

2. Comparison of cells in trunk autonomic ganglia of the rabbit with cells in ventral horn and dorsal root ganglia:- Some of the points of similarity and difference shown by the cells from these three sources have already been mentioned. However, the cells of the trunk autonomic ganglia of the rabbit differ

from the cells of both the ventral horn and dorsal root ganglia in two more very important respects, viz., (a) the peripheral arrangement of the Nissl bodies, and (b) the presence of cells with two nuclei. In fact, under the microscope a field of autonomic cells may be distinguished from a field of cells in the dorsal root ganglion or ventral horn of the spinal cord by either or both of these two characteristics.

In cells of both the dorsal root ganglia and the ventral horn the Nissl bodies, as has already been explained, are distributed all over the cell from the wall of the nucleus to the periphery. But in the cells of the trunk autonomic ganglia the Nissl bodies are arranged around the periphery of the cell with only a few scattered in the more central area and around the wall of the nucleus (Figs. 5-10). This arrangement was found to obtain in almost all cells which showed an optical section thru the center in the long axis. Occasionally cells were found in which the peripheral arrangement was not pronounced, and the Nissl bodies were distributed fairly evenly thruout the cell-body. Figures 7 and 8 represent two such cells. Figure 7 is a cell at the periphery of the ganglion. In this region the cells are elongated and are much more heavily stained than the cells in the central part of the ganglion. As they enter the processes of the cell, the Nissl bodies assume a spindle-like form and become rather evenly distributed thruout the width of the process. The literature upon the subject so far examined contains only one record of the observation of the characteristic

peripheral arrangement of the Nissl bodies in autonomic ganglion cells. Eve (1896) mentions this peculiar distribution in a study of the "basophil particles" in the sympathetic ganglia of the rabbit.

The second characteristic by means of which a field of cells in the trunk autonomic ganglia may be distinguished under the microscope from a field in the ventral horn or the dorsal root ganglia is the presence of many cells with two nuclei (Figs. 6-9). Estimated roughly, about one-third of the cells in a given field have two nuclei. No binucleate cells were found in either the ventral horn or dorsal root ganglia. However, Apolant (1896) reports cells with two nuclei as occurring occasionally in the dorsal root ganglia of the rabbit. He also found very rarely cells in the coeliac ganglion with three nuclei. I did not see in any of the trunk ganglia a single cell in which three nuclei could be definitely determined, altho I did see two cells which suggested a trinucleate condition.

3. Comparison of cells in the cranial autonomic ganglia of the rabbit with cells in the trunk autonomic ganglia:- For this study cells of the otic, ciliary, spheno-palatine, thoracic, superior mesenteric, and coeliac ganglia from the same rabbit were used.

The Nissl bodies of the cranial ganglion cells are like those of the trunk ganglion cells in respect to size, shape, and peripheral arrangement (Figs. 11, 12, 13). On the basis

of the size and arrangement of the Nissl bodies all of the cells in these three cranial ganglia seem to be motor. There is one point of difference in distribution of the bodies which is quite striking and constant. In the majority of the cranial ganglion cells showing an optical section thru the center in the long axis there is a rather large area immediately surrounding the nucleus which contains very few, if any, Nissl bodies. Such a clear area is not evident in the cells of the trunk ganglia, since in the latter there are quite a number of bodies scattered immediately around the nucleus.

Another decidedly marked dissimilarity between the cells of the cranial ganglia and those of the trunk ganglia is found in the number of nuclei. As previously stated, binucleate cells are numerous in the trunk ganglia. In contrast to this condition, not a single cell possessing two nuclei was found in any of the cranial ganglia. Apolant (1896) reports that he once found a binucleate cell in the otic ganglion of the rabbit.

4. Comparison of cells in the trunk autonomic ganglia of the rabbit with cells in the same region of other rodents:- All of the cells from the trunk autonomic ganglia of the rat, mouse, thirteen-lined spermophile, prairie dog, muskrat, guinea pig, and porcupine show the same general condition of Nissl bodies as is found in the cells of the trunk autonomic ganglia of the rabbit, and upon the basis of the Nissl bodies all of the cells seem to be motor.

In rat, mouse, spermophile, and prairie dog only cells with one nucleus each were found. Apolant examined the autonomic ganglia of the rat, mouse, and squirrel, and states that it was an exception to find a cell with two nuclei, thus leading one to infer that he occasionally found binucleate cells in these animals. He reports that the guinea pig shows the same condition as the rabbit. I found that the trunk autonomic ganglia of guinea pig, muskrat, and porcupine contain many cells which have two nuclei. From the following table, which was compiled by examining fields of cells under the microscope at random from various regions, it will be seen that the rabbit possesses the greatest number of binucleate cells, with the guinea pig, muskrat, and porcupine following in the order in which they are named.

Table I.

Ganglion	Rabbit		Guinea pig		Muskrat		Porcupine	
	Bi-nucl	Uni-nucl	Bi-nucl	Uni-nucl	Bi-nucl	Uni-nucl	Bi-nucl	Uni-nucl
Thoracic								
Field 1	16	40	15	43	4	40	3	37
" 2	15	32	14	40	8	88	3	26
" 3	17	36	15	34	6	32	3	27
" 4	13	42	17	36	9	36	5	32
Sup.Mes.								
Field 1	10	31						
" 2	10	33						
" 3	14	35						
" 4	12	28						
Coeliac								
Field 1	17	33			6	22		
" 2	12	35			5	32		
" 3	13	43			5	40		
" 4	14	34			4	29		
Lumbar								
Field 1					4	40		
" 2					7	37		
" 3					7	25		
" 4					3	36		
Sacral								
Field 1					2	22		
" 2					3	27		
" 3					6	28		
" 4					5	32		

Cells having two nuclei have been reported in both the cerebro-spinal and autonomic nervous systems of other animals, viz., the cat, frog, dog, man. But in none have they been found to be as numerous as in the four animals named in the above table.

Fields in the thoracic ganglion only were studied for all four of the animals. The percentage of binucleate cells in these fields may be expressed as follows:

	Total binucleate cells	Total uninucleate cells	Percent. of binucleate cells
Rabbit	61	150	28.91
Guinea Pig	61	153	28.50
Muskrat	27	196	12.10
Porcupine	14	122	10.29

IV. SUMMARY

1. A study of the cells in the autonomic ganglia of the rabbit by the Nissl method seems to indicate that only motor cells are present in these ganglia.

2. A comparison of cells in the trunk autonomic ganglia with cells in the dorsal root ganglia and the ventral horn of the rabbit reveals the fact that the autonomic cells resemble the cells of the ventral horn (motor) with respect to the Nissl bodies. Many binucleate cells were found in the autonomic ganglia, while no cells with two nuclei were seen in either the dorsal root ganglia or the ventral horn.

3. The Nissl bodies in the cells of the trunk autonomic ganglia of the rat, mouse, spermophile, prairie dog, guinea pig, muskrat, and porcupine are in every respect like those in the cells of the same region in the rabbit. The cells all seem to be motor.

4. The cells in the cranial autonomic ganglia of the rabbit differ from those in the trunk autonomic ganglia in that they have, immediately surrounding the nucleus, a clear area which is almost, if not entirely, without Nissl bodies.

5. The cells in the cranial autonomic ganglia of the rabbit have but one nucleus each.

6. As is the case with rabbit, many cells in the trunk autonomic ganglia of the guinea pig, muskrat, and porcupine have two nuclei each.

7. No binucleate cells were found in the trunk autonomic ganglia of the rat, mouse, thirteen-lined spermophile, and prairie dog.

V. BIBLIOGRAPHY

- Apolant, Hugo.
1896. Über die sympathischen Ganglienzellen der Nager.
Arch.f.mikros.Anat.u.Entwickl., Bd. 47, pp. 461-471, 8 figs.
- Barker, Lewellys F.
1901. The Nervous System.
D. Appleton & Co., New York.
- Dogiel, A. S.
1896. Zwei Arten sympathischen Nervenzellen.
Anat. Anz., Bd. 11, Hefte 22, pp. 679-687, 2 figs.
- Eve, F. C.
1896. Sympathetic Nerve Cells and their Basophil Constituent in Prolonged Activity and Repose.
Jour. Physiol., v. 20, pp. 334-353, 1 plate.
- Huber, G. Carl.
1897. Lectures on the Sympathetic Nervous System.
Jour. Comp. Neurol., v. 7, pp. 73-139, pl. 8-11, 14 figs.
- Kuntz, Albert.
1910. The Development of the Sympathetic Nervous System in Mammals.
Jour. Comp. Neurol. and Psych., v. 20, no. 3, pp. 211-258, 18 figs.
- Langley and Anderson.
1894. On Reflex Action from Sympathetic Ganglia.
Jour. Physiol., v. 16, pp. 410-440.
- Michailow, S.
1908. Zur Frage von der feineren Struktur der peripheren sympathischen Ganglien.
Anat. Anz., Bd. 33, Hefte 6/7, pp. 129-134, 4 figs.
- Michailow, S.
1908a. Mikroskopische Struktur der Ganglien des Plexus solaris und anderer Ganglien des Grenzstranges des N. sympathicus.
Anat. Anz., Bd. 33, no. 22/23, pp. 581-590.
- Müller, L. R.
1909. Studien über die Anatomie und Histologie des sympathischen Grenzstranges insbesondere über seine Beziehungen zu dem spinalen Nervensysteme.
Verhandlungen des Kongresses für Innere Medizin, pp. 659-681, pl. 12-22, 19 figs.

VI. EXPLANATION OF PLATES

All figures are of autonomic ganglion cells from the rabbit, stained by the Nissl method.

All drawings were made with the camera lucida at a magnification of 1250 diameters. Plates I and II are photographs of the original drawings, reduced approximately one-half.

Plate I.

- Fig. 1. Sensory cell from a dorsal root ganglion.
- Fig. 2. Sensory cell from the Gasserian ganglion.
- Fig. 3. Motor cells from the ventral horn of the spinal cord, showing evidence of the fusion of Nissl bodies.
- Fig. 4. Typical motor cells from the ventral horn.
- Fig. 5. Typical autonomic ganglion cell from a thoracic ganglion, showing the peripheral arrangement of the Nissl bodies.
- Fig. 6. Cell from a thoracic ganglion showing two nuclei.

Plate II.

Figs. 7 and 8. Cells from a thoracic ganglion which do not show the characteristic peripheral arrangement of the Nissl bodies.

Fig. 9. Cell from the superior mesenteric ganglion showing two nuclei.

Fig. 10. Typical cell from the coeliac ganglion showing the peripheral arrangement of the Nissl bodies.

Fig. 11. Typical cell from the otic ganglion.

Fig. 12. Typical cells from the ciliary ganglion.

Fig. 13. Typical cells from the spheno-palatine ganglion.

Figures 11, 12, and 13 show the peripheral arrangement of the Nissl bodies and the clear area surrounding the nucleus which is characteristic of the cranial autonomic ganglion cells.

PLATE I.

FIG 1

FIG 2

FIG 3

FIG 4

FIG 5

FIG 6

FIG 8

FIG 9

PLATE II.

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

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