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SHORT PROPRIOSPINAL NEURONS AND INTRINSIC CONNECTIONS OF THE SPINAL GRAY MATTER

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I. INTRODUCTION

Recent advances in neurophysiology have renewed interest for the neuronal patterns within the gray matter of the spinal cord and for shorter intersegmental pathways. Excellent papers of *Lloyd* (1941, 42), *Bernhard* and *Rexed* (1945) and several other authors have given a fairly good insight into the functional organization of lower spinal mechanisms, and methods of precise manipulation of electrodes have supplied histologically exact data on the activity in different nuclei under various circumstances.

Concerning the anatomy of propriospinal connections very little is known and almost no advance can be recorded since the classical investigations of *Cajal* (1909), *Herrick* and *Coghill* (1915) and *Bok* (1928). The so called "bouton degeneration method" furnished important results concerning termination of descending spinal pathways and of dorsal root fibers (*Rasdolsky* 1923, *Hoff* 1932, 35, etc., *Schimert* 1938, 39, *Szentágothai-Schimert* 1941, *Szentágothai* 1948, *Szentágothai*—*Kiss* 1949), which accord with many of recent neurophysiological results.

No attempt was made as far as we know to introduce the bouton degeneration method in the investigation of the propriospinal connections. In this paper we try — based on experiments made with the aid of a simple stereotaxic method, — to solve some problems of the shorter intraspinal pathways.

II. METHODS

Our experiments were performed on dogs and cats, in which small electrolytic lesions had been placed into various points of the spinal gray matter in the upper part of the lumbar enlargement (L_4—L_6). Subsequent collateral and bouton degeneration was investigated after the operation in the level of the lesion as well as above and below.

1. Apparatus (fig. 1.)

In order to place lesions of proper localization and extension on various points in the cross section of the spinal gray matter, we designed a very simple stereotaxic apparatus, which can be fixed by brackets on the vertebral spinous processes. The electrode carrier is to be

Fig. 1. Spinal stereotaxic apparatus during operation, 2/3 of natural size. 1. transverse guide, 2. brackets for fastening apparatus on spinous process, 3. screw for clamping spinous process into brackets, 4. vertical guide, 5. pinion for racking vertical guide on transverse guide, 6. millimeter scale and vernier for adjusting horizontal movements, 7. needle carrier, 8. screw for vertical movement of needle carrier, 9. millimetre scale and vernier for adjusting vertical movements, 10. needle electrode (thick part), 11. clamped brackets for fixing neighbouring spinous process on operating tab. frame. *Fig. 3.* Small electrolytic lesion in the intermediate region. *Fig. 4.* Degenerated unmyelinated collateral entering the gray matter. Characteristic for axonal degeneration (early stage) is vacuolar staining of beaded fiber. *Fig. 5.* Degenerated end-bulb. Its afferent fiber is also beaded and vacuolized. *Fig. 6.* Degenerated end-bulb in later stage of degeneration, when afferent fiber is broken into fragments. 1. degenerated end-bulb, 2. fragment of afferent fiber. Other fragments are lying in another level and therefore not to be seen on photograph. Magnifiication of figs. 4—6 about 1500 times.

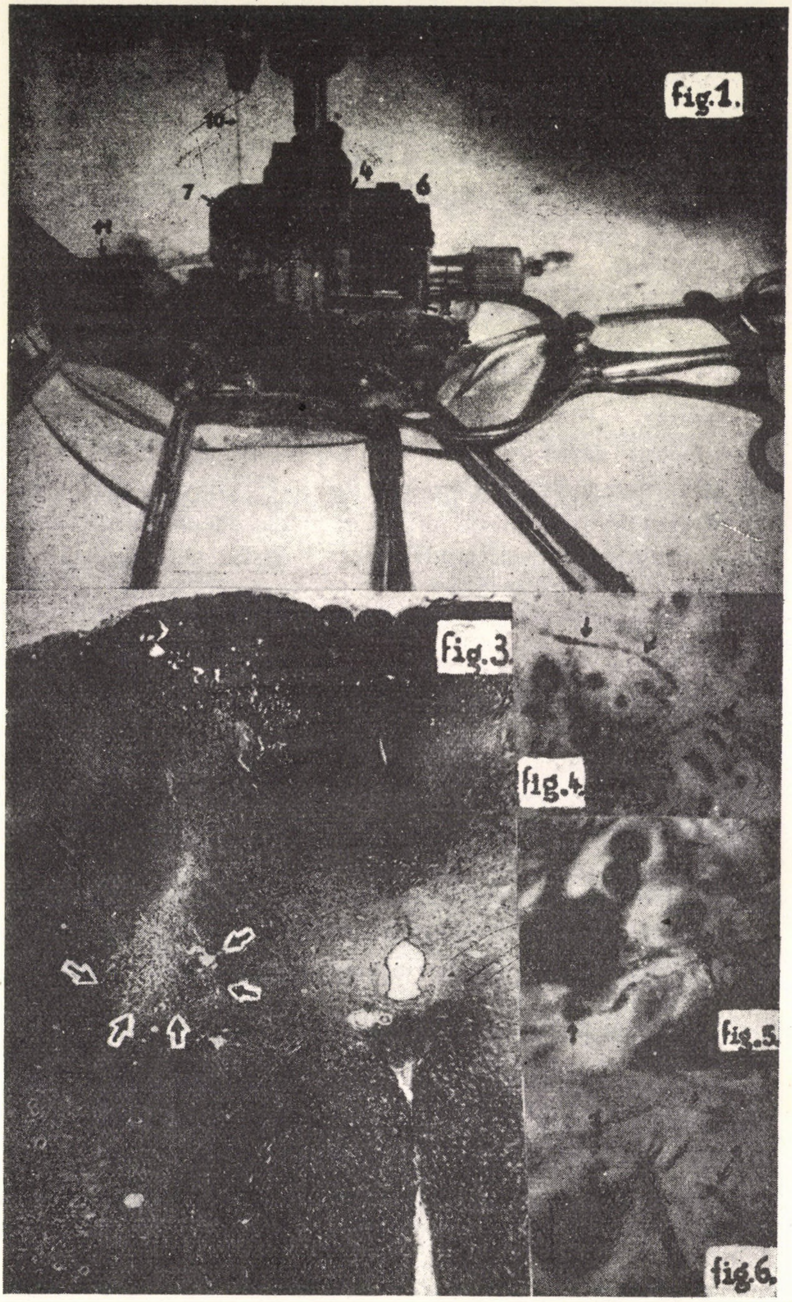

Fig. 1, 3, 4, 5, 6.

moved in transversal direction by rack and pinion, in vertical direction by a screw. The apparatus consists of a tranverse and a vertical guide. The transverse guide (1) is fixed to the basis of the instrument ; two brackets (2) which can be clamped upon a spinous process by a screw (3). On the transverse guide the vertical one (4) can be moved in transversal direction by rack an pinion (5). The exact position of the vertical on the transverse guide is indicated on a millimetre scale (6) by a vernier. On the vertical guide the needle holder (7) can be moved in vertical direction by a screw (8), and its movements can exactly be determined and adjusted on a vertical millimetre scale by a vernier (9). No movements in sagittal (cranio-caudal) direction are necessary while working on the spinal cord, since the level of the lesion can always be changed by clamping the apparatus on the spinous process of another vertebra. In order to place more lesions in slightly different levels e. g. two or three lesions caudad from another the needle electrode holder is provided with three parallel grooves 1 mm apart. Thus if the needle electrode is clamped into the first groove, the lesion lies 1 mm craniad from that produced by the needle fixed into the second groove, and this again 1 mm craniad from a lesion produced by the needle clamped into the third groove.

2. *Coordinate "charts"*

For exact localization of the lesions it is very important to select animals of equal size. One of each group is killed and perfused with a formaldehyde solution of 4 %. After some days the vertebral canal is opened by removing spine and arcus of one vertebra of that segment, in which lesions should be set. The apparatus is then fastened on the spine of the neighbouring vertebra and the place is exactly determined into which the electrode will penetrate if the apparatus is fixed on the same vertebra in a living animal of equal size. From this part of the cord frozen cross sections are cut and an about 30 times magnified camera lucida drawing is prepared of the outlines of the cord and the gray matter. The section should be mounted for drawing only in water, in order to avoid shrinkage. A scale of 0,1 mm gradation is drawn on the diagram in the same magnification (fig. 2.). Separate "charts" are in the same way prepared for every relevant site. The coordinates of any part of the gray or white matter are X = distance from the midsagittal plane, and Y = vertical distance from the dorsal surface of the cord. For instance as indicated in fig. 2. the lateral column of a medium sized cat in Th_2 has an X-coordinate of 1,15 mm. and a Y-coordinate of 1,8 mm.

3. *Operative procedure*

Laminectomy is made exactly at the place from which the coordinates have already been determined. The arcus of only one vertebra is removed. The operation is made under deep ether anaesthesia. The spinous process of the neighbouring vertebra is carefully cleaned and prepared for the apparatus. One or two other neighbouring processi are clamped (11) to frames, which are fastened on the operating table in order to fix this part of the vertebral column as much as possible. The apparatus is then fixed on the prepared spine in a way that the electrode should lie exactly perpendicular to the dorsal surface of the cord. A small transversal slit is made in the dura. The tip of the electrode — (for lesions we are using unipolar platinium electrodes with glass insulation of 0,15 mm total caliber ; the uninsulated tip of the electrode must be prepared according to the wanted shape and size of the lesion) — is exactly placed upon the dorsal median sulcus of the cord (fig. 2. I.) and the position of the vertical guide on the transversal scale (6) is exactly determined. Then the vertical guide is moved from the midsagittal plane into the direction to the site corresponding to the X-coordinate. The tip of the lesion is by vertical movement again exactly placed on the dorsal surface of the cord (fig. 2. II.). Now the position of the electrode carrier on the vertical guide is exactly determined by the vernier on the vertical scale. Then the needle-electrode is lowered into the cord by vertical movement of the electrode carrier until the depth corresponding to the Y-coordinate is reached. Since the spinal cord is often somewhat deformed by the penetrating needle and a little shifted in ventral direction, the cord should be brought back into its original position by pushing of the slit edges of the dura. Now the electrode has reached its final position (fig. 2. III.).

Electrolytic lesions (anodal) are produced by constant current of 2 MA. The relation between time of action of the current and size of the lesion must be determined by experience. Foci of 0,5 mm diameter are produced in about 20 secs. Intensity of the current must be increased gradually and the animal must be kept in very deep anaesthesia in order to avoid stronger movements.

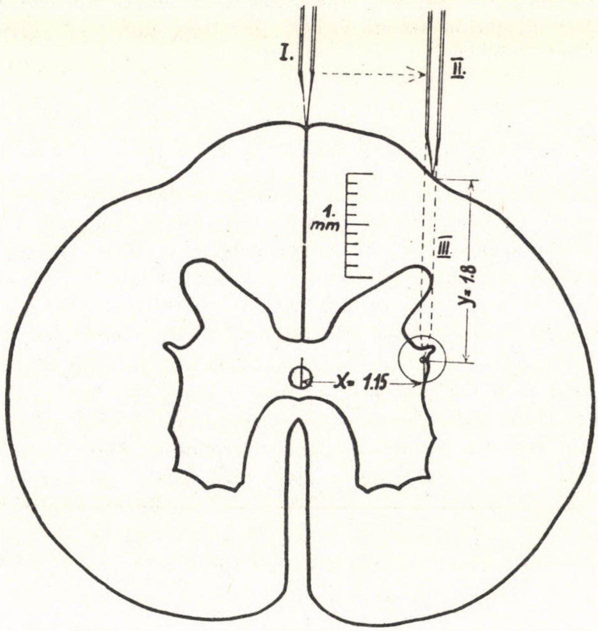

Fig. 2.

Coordinate chart. Outlines of spinal cord cross section, medium sized cat, Th_2. Scale of 0,1 mm gradation in same magnification. Coordinates of the lateral column indicated. I. first position of the needle electrode on the dorsal median sulcus, II. second position of the needle in the distance of X-coordinate with tip on the surface of the cord. III. Needle electrode in final position.

4. Investigation of material

Operated animals were kept alive for 5 days. In cases of pure gray matter lesions they should show no neurological symptoms at all. The spinal cord is fixed in neutral formaldehyde solution of 10%. Cross, — and for studying intrinsic connections of the posterior horn parasagittal longitudinal frozen sections are stained with the Bielschowsky-Gross method. Signs of axonal and terminal degeneration (fig. 4, 5 and 6) were very carefully investigated and maped.

III. RESULTS

1. Lesions of the dorsal column

It is rather difficult to get isolated dorsal column lesions, without injury of the white matter. We could select only from a large number of experiments, a few in which the lesion was strictly confined to the gray matter of the dorsal horn.

At the level of the lesion a large number of degenerated axons and boutons-terminaux was found in the gray matter of the contralateral side. On the ipsilateral side naturally too, but in close neighbourhood of the lesion and because of the injury of posterior funiculus collaterals it is useless to study the degeneration since no conclusion can be drawn from any findings. The majority of axons running to the other side is crossing through the posterior commissure, while only an insignificant number do so through the ventral commissure (!). The crossing fibers are terminating chiefly in the nucleus proprius corn. posterioris and in the intermediate region. Only few degenerated axons terminate in the ventral column, none in the gelatinous substance of Rolando. Some degenerated axons are entering the opposite lateral funiculus.

Below the level (caudad) of the lesion extensive degeneration is to be seen as far as for 3—4 segments in the ipsilateral ventral column especially around motor radicular cells. More caudad signs of degeneration disappear. In the posterior column and the intermediate region no degeneration is noticed more than two segments below the lesion. There are few and very short descending intrinsic pathways of the posterior column. Through the intermediate region degenerated collaterals of the ipsilateral lateral funiculus (fig. 4) can be followed through the posterior commissure, which are terminating chiefly on the contralateral nucleus cornu-commissuralis posterior and in the contralateral intermediate region.

Above the level of (craniad to) the lesion degeneration on the ipsilateral side is confined to the posterior horn. On longitudinal parasagittal sections the degeneration of the ascending intrinsic posterior column pathway is easily to be followed as far as 3 segments (about 2 cm on smaller dogs) craniad from the lesion. The termination of these fibers on posterior horn neurons can also be seen very well. No similar ascending system is to be seen in Lissauers tract and no endings of ascending posterior horn neurons in the gelatinous substance of Rolando were detected. On the contralateral side as far as for 2 or three segments degeneration is to be seen in lateral parts of the ventral column.

2. Lesions of the intermediate region

At the level of the lesion there are numerous signs of axonal and terminal degeneration in the gray matter of the contralateral side. Degenerated boutons-terminaux are to be seen in the medial part of the posterior horn, in the intermediate zone as well as in the motor column. Degenerated fibers are crossing through both commissures.

At levels below the lesion degeneration of end-feet is abundant in the ipsilateral ventral column even as far as five segments caudad from the lesion. In the dorsal column no signs of degeneration are found with the exception of the dorsal cornu-commissural nucleus, which is highly developed in the lower sacral segments. These nuclei of both sides are receiving from the level of L_5 and L_6 numerous afferent fibers, which are descending through the lateral funiculus. Degenerated terminals are also found on the contralateral side around motor horn cells until about 3—4 segments caudad from the lesion. Degenerated terminals are also found on smaller internuntial neurons of the ventral horn.

At levels above the lesion there are even in the next segment almost no signs of degeneration, neither in the ventral nor in the dorsal column of the ipsilateral side. Very few degenerated collaterals are entering the intermediate region one segment craniad. On the contralateral side there are clear signs of terminal degeneration in the ventrolateral part of the ventral horn, which gradually disappear after the third segment.

3. *Lesions of the ventral column*

At the level of the lesion no degeneration is recorded in the gray matter of the contralateral side, in case when the foci are situated in lateral parts of the column. When the focus is situated more mediad, signs of degeneration are frequently seen in the ventral column of the contralateral side.

Below the lesion there is abundant degeneration both in the ventral horn and the intermediate region of the ipsilateral side. Degeneration on the contralateral side is seen only when the lesion lies mediad in the ventral column. Signs of degeneration are tracable in caudal direction as far as three or four segments both to motor radicular and internuncial cells.

Above the lesion only very few signs of degeneration are found. As far as one or at the highest two segments above the lesion few degenerated boutons terminaux are to be seen in the ipsilateral ventral column. In case when the lesion is situated mediad as to involve the ventral cornu-commissural nucleus ascending degeneration is tracable to motor cells of the contralateral ventral column. Degenerated boutons-terminaux are most frequently found on radicular cells lying in the dorsoventral part of the ventral horn. This degeneration extends for about two segments.

IV. EVALUATION OF THE RESULTS

In evaluating these results it should be kept in mind that in order to avoid destructions of the white matter, lesions should be very small and since they are of sphaeroid shape, they involve only a very small part, about one tenth of the column in one segment. Degeneration therefore gives no true picture of the connections arising from one segment. We therefore must be

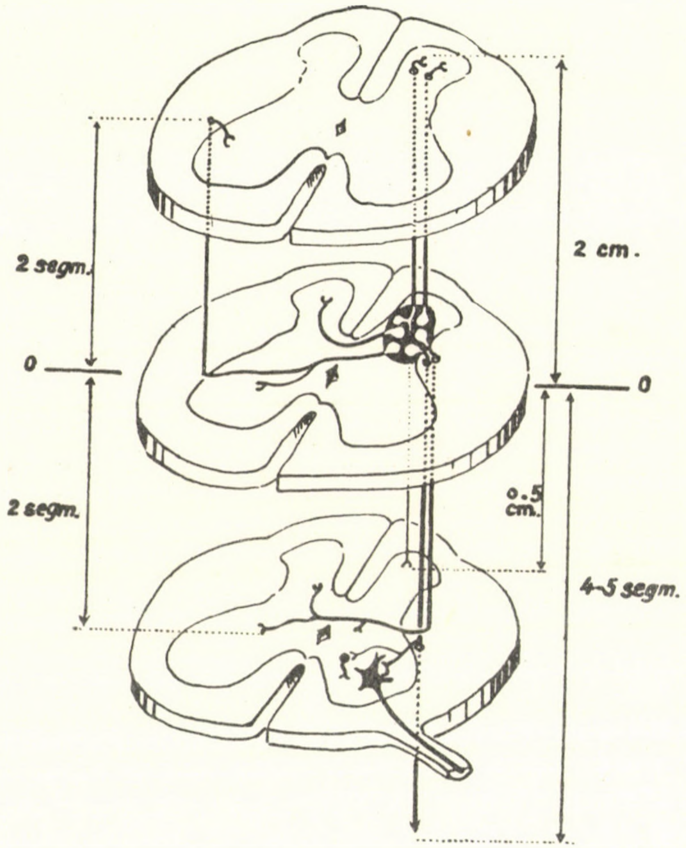

Fig. 7.
Short propriospinal and intrinsic gray matter pathways originating from the posterior horn. Arrows on side of the figure indicate the maximal distance bridged by ascending and descending systems as determined by anatomic investigation. Further explanation in the text.

aware of the fact that our results are indicating only the most important and abundant connections. Also the limits which had been found for asecnding and descending degeneration may be in reality somewhat wider than indicated. The distances given on the sides of figs. 7, 8 and 9 refer to the average distances for which clear signs of degeneration were tracable in our experiments. Fibers running through the foci of destruction must also be taken into account (fig. 8).

In general we have to stress that signs of degeneration are always most abundant in close neighbourhood of the lesion and are steadily decreasing with growing distance from the level of the lesion. This indicates that short propriospinal connections cover distances less than one segment as well as five segments.

The most important short propriospinal connections are diagramatically shown for different parts of the gray matter in figs. 7, 8 and 9.

Short connections arising form the posterior column are indicated in fig. 7. They can be divided into intrinsic gray matter pathways, and into connections running over the white matter. Intrinsic gray matter connections are established in the level of the lesion with the contralateral posterior horn and with upper and lower levels of the ipsilateral posterior horn. Descending posterior column pathways are very short (0,5 cm.) and few, whereas ascending connections are abundant and of considerable length (2 cm.). They are building up the known intrinsic longitudinal fasciculi of the posterior column. Authors often are speaking of ascending chain-like pathways within the posterior column, but they were localized generally into the gelatinous substance of Rolando and Lissauers tract. From results obtained by this method no far reaching conclusions can be drawn concerning connections of the gelatinous substance, nevertheless the existence of any chainlike ascending pathway appears from our results not to be very probable. The ascending connections, as shown by our experiments, may play an important part in chain-like ascending mechanisms. The main connections of the posterior horn are leading to the ventral column. On the ipsilateral side they are descending, on the contralateral ascending. The descending connections are probably nothing else than relay neurons connecting long descending pathways of extraspinal origin (pyramidal, tectospinal etc.), which are ending in large number on posterior horn neurons, with motor radicular cells. Other descending pathways are crossing before termination through the posterior commissure, which seems to contain in considerable number preterminal crossing collaterals of descending systems.

Short propriospinal systems originating from the intermediate region are mainly descending on the ipsilateral, both descending and ascending on the contralateral side. The descending systems on both sides establish direct connections with motor radicular cells, but many fibers terminate also on smaller internuntial cells of the ventral column. Very probably the ascending system of the contralateral side is no short propriospinal system, but represents only collaterals given by the *spino-thalamic* tract to the motor radicular cells. It perhaps may seem strange that collaterals should be given off by spinothalamic fibers only in the first three segments of their ascent, but the same can be seen very clearly in the posterior funiculus, where ascending posterior root fibers are giving off collaterals only in the first few segments after their entrance

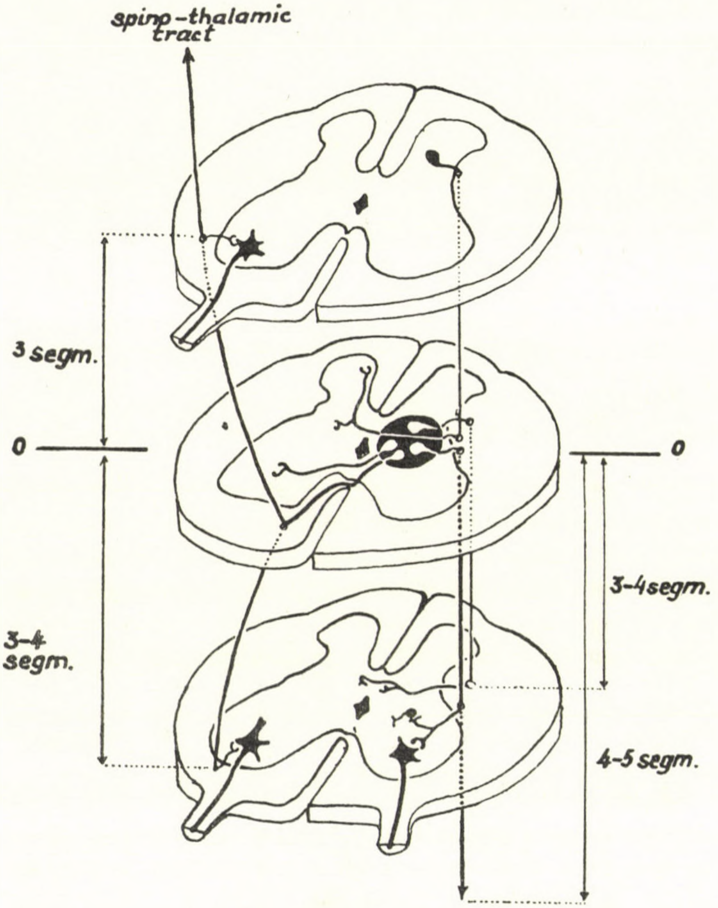

Fig. 8.

Short propriospinal pathways originating in the intermediate zone. Further explanation as fig. 7.

into the cord. Descending short neurons are terminating also in the nuclei of the porsterior commissure, which are highly developed in lower sacral segments (fig. 8).

Short propriospinal neurons, which originate from the ventral column are both ascending and descending on both sides. Ipsilateral systems arise from lateral parts of the column, contralateral ones originate from medial parts, especially from the ventral cornu-commissural nucleus. Endings are not only contributed to motor radicular cells, but always also to smaller internuncial cells of the ventral horn and the intermediate region. The distance covered by these short propriospinal neurons or connections is largest in descending direction on the ipsilateral side, and shortest on the same side in ascending direction. Neither in ascending nor in descending direction any fibers were tracable from the ventral column towards the posterior horn.

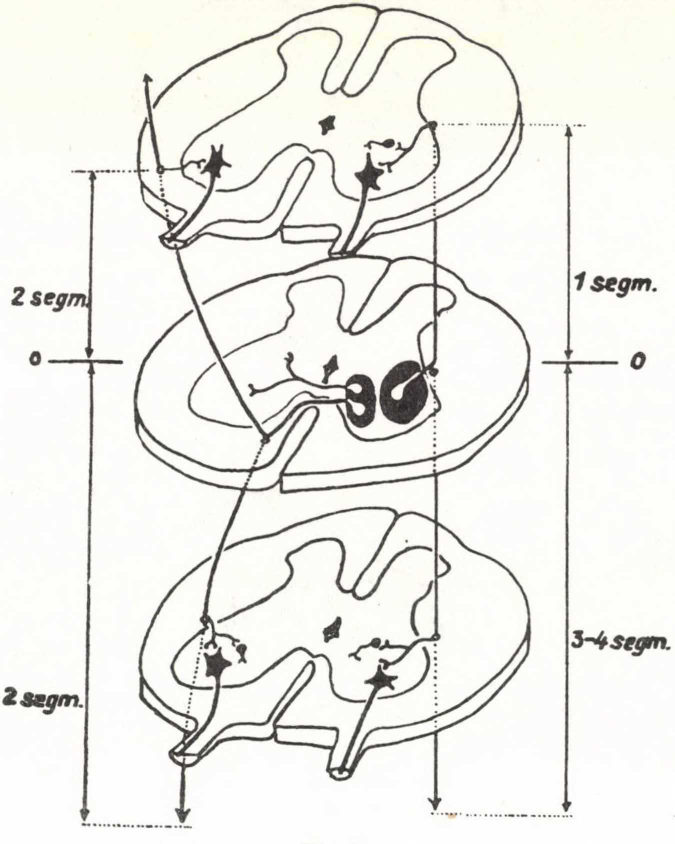

Fig. 9.

Short propriospinal pathways originating from the ventral horn. Further explanation as fig. 7.

V. DISCUSSION

The results of these investigations are giving the first anatomic data on connections established within the spinal gray matter by short proprio-spinal neurons. They are in complete accordance with many data got from recent neurophysiological investigations.

In a remarkable paper *Lloyd* (1941) has shown that activity initiated from the medulla (reticulo- and vestibulospinal systems) and the upper cord which traverses the length of the neuraxis, enters the short propriospinal nuclei at all levels to travel a short distance as relayed impulses along the short propriospinal fibers. *Lloyd* has also determined by response of the first sacral ventral root to ventrolateral column shocks delivered at various distances (craniad) of the stimulating electrodes the maximal distance at which sufficient of the propriospinal neurons are excited directly to effect the discharge of motoneurons with only one synaptic delay. *Lloyd* thus determined the main maximal length of descending short propriospinal fibers to be about 3,5 cm-s in cats. This is in complete accordance with our results, in finding that after lesions of the ventral horn and the intermediate region in the segments below the lesion an abundant degeneration of end feet around motor root cells is to be seen on the ipsilateral side on cats as far as 3 cm. More caudad signs of degeneration rapidly diminish in the ventral horn. Our results indicate that most of the propriospinal neurons are shorter, and the distance of 3,5 cm may really be considered as the maximal length of short propriospinal neurons.

Bernhard and *Rexed* (1945) have localized the premotor neurons of the peroneal nerve into the lateral part of the intermediate region of L_4—S_1, which is also in fair accord with our results. The authors believe the medial part of the zona intermedia to contain premotor neurons, which would activate the motoneurons of the trunc muscles, situated in the medial part of the ventral column. Our results are rather pointing to the fact that propriospinal neurons of the medial part of the gray matter are connected with the motor horn of the contralateral side.

Concerning crossed connections it is tracable from these results that the ventral cornu-commissural nucleus and, as mentioned above, other internuncial neurons lying in the medial part of the zona interedia may play the most important role in crossed mechanisms. The same was inferred also by *Lloyd* (1942) from the fact that during transmission of reflex effect from the forelimb to the hindlimb the most powerful activity was encountered in the nucleus cornu-commissuralis ventralis.

Since working on the lumbar enlargement we can make no statements on the termination of longer propriospinal neurons. From other experiments — which will be described in a following paper — it appears that descending spinal neurons longer 4 cm are terminating rather on internuncial neurons of the intermediate region and the central part of the ventral horn. From this we perhaps may draw the conclusion that longer propriospinal tracts have more synapses with internuncials and relatively few with motoneurons. Further investigations in this respect are necessary.

It is interesting to mention that the posterior horn does not receive descending propriospinal neuron terminations. The same is stressed by *Lloyd* (1942) since during transmission of reflexes from the forelimb to the hindlimb the posterior horn remains inactive. Intrinsic descending connections of the posterior horn do not exceed the distance of one segment at the highest. The well developed posterior commissure nuclei of the lower sacral segments make an exception, in getting many synapses from descending propriospinal systems. Anatomic investigation thus fully supports the view of *Lloyd*, according to which there exists an important contrast between the spinal mechanisms as activated by the descending spinal reflex system and by the pyramidal system. The predominant connection of the pyramidal fibers with dorsal and intermediate spinal internuncial neurons was first shown by *Rasdolsky* (1923), thoroughly studied by *Hoff* (1932, 35) and later confirmed by many other authors. According to *Szentágothai* (1942), no pyramidal fibers at all terminate at motoneurons as well as no other fiber of the descending midbrain systems (e. g. tectospinal tract). In contrast to these systems the short propriospinal neurons are terminating predominantly on radicular motor cells, whereas the longer propriospinal systems are ending on both kinds of neurons but predominantly on internuncials.

Concerning short ascending systems no direct neurophysiological data are available at present, which could be confronted with our anatomical results. Ascending connections from the intermediate zone and the ventral column are predominantly crossed and fewer than the descending ones. Intrinsic ascending connections of the posterior horn seem to involve a considerable number of the dorsal horn neurons. They bridge distances of 2 cm and perhaps more and may be the links of the often mentioned chain-like ascending posterior horn pathway. Since intimate connections between the posterior horn of both sides are present, crossing (perhaps multiple crossing) of these ascending posterior horn pathways is possible. The gelatinous substance of Rolando does not take part in this ascending system.

Retrograde connections from the anterior horn towards the posterior seem not to exist, or are at least very rare. This is not consistent with the view concerning existence of the so called "reverberating or closed circuits" as far as connections of the posterior horn with the anterior are concerned. Reciprocal connections between the ventral horn and the intermediate zone are frequent.

The results here described may be considered only the first step on a long way. Different kinds of anatomic investigations with the aid of the bouton degeneration method — which has proved itself extremely useful in such questions — are necessary on different levels of the spinal cord with this stereo- taxic method and other methods

too in order to clarify the intricate connections of spinal neurons amongs themselves and with extraspinal pathways.

VI. Summary

The short intersegmental and intrinsic gray matter pathways of the lumbar enlargement have been studied with the aid of a stereotaxic method specially designed for the spinal cord With a "spinal" stereotaxic instrument small electrolytic lesions can be placed into different parts of the spinal gray matter.

Subsequent boutons-terminaux degeneration was studied after lesions of the posterior horn, the intermediate region and the ventral horn with the aid of the Bielschowsky-Gross method on frozen sections.

Anatomically established facts on the pattern of short propriospinal pathways have been found to be consistent with a number of recent physiological findings on spinal reflex mechanisms.

(Краткие межсегментные пути и невронанные системы в сером веществе спинногомозга).

Сентаготаи Я.

Выводы

Мы исследовали краткие междусегментальные пути спинного мозга и невронанные системы серого вещества с помощью, специально выработанного для этой цели, нового стереотактического метода.

Специальных »спинномозговым« стереотактическим аппаратом мы вызывали желкие электролитические повреждения на разных местах серого вещества.

Повреждая задьний рог, промежуточную зону и передний рог, мы исследовали вторичную дегенерацию синапсов с помощью импрегнационного метода Билшовский-Гросс. Рисунки 7-ого, 8-ого и 9-ого схематически покажут краткие невронанные системы, выходящие из этих мест серого вещества. Рядом с рисунками имеются данные относительно приблизительной длинны этих связей.

Определенные анатомическим методом, краткие пути, выходящие из разных ядер серого вещества спинного мозга, полно соответсьуют данным о местных рефлекторных механизмах спинного мозга, полученным современными электрофизиологическими методами.

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