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THE SYNAPTOLOGY OF CLARKE'S COLUMN

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I. Introduction

Functional properties of synapses within the dorsal horn of the spinal cord, especially in Clarke's column, seem to differ considerably from those of motor radicular neurons. The almost complete lack of summation required for transmission, as found in Clarke's column by *Lloyd and McIntyre* [3] suggests that there probably are synapses with dominance of one single afferent path and perhaps a one-to-one relation of primary and secondary neurons, similar to that described by *Therman* [10] in Burdach's nucleus. Nevertheless, there are some features of impulse transmission in Clarke's column, which are not consistent with this explanation. As *Grundfest and Campbell* [2] have shown, after single shocks delivered to dorsal root neurons, some impulses are recorded in Flechsig's tract so late after the early monosynaptic discharges, that they must very probably be conducted over internuncial chains. Essentially the same was noticed by *Lloyd and McIntyre* as late random discharges in Flechsig's tract, but these authors deem it necessary to know more of the fundamental functional properties of synapses within the dorsal horn, before an exact explanation could be attempted.

Difficulties in explanation are mainly due to lack of anatomical information concerning the synapses within Clarke's column. As the method of secondary degeneration of «boutons terminaux» has proved itself extremely useful in the analysis of synapses, especially of the motoneurons (5, 6, 7, 8, 9,), we have attempted an experimental-morphologic analysis of the synaptology of Clarke's column.

II. Methods

Investigations were performed 1. on normal dogs, 2. after extirpation of spinal ganglia L_1-S_3 in different combinations, 3. after minute lesions of different parts of the gray matter, especially in the dorsal horn at the levels L_{2-3}, 4. after lesions of the superficial dorsal part of the lateral white column, 5. after hemisaction of the cord at L_1.

Ad 2. Dorsal root transaction was performed in the segments L_{1-6} without laminectomy, by approach from the intervertebral foramen and extirpation of the spinal ganglia. In lower segments dorsal root section was performed extradurally. In some cases all dorsal roots below L_1 were cut, thus the lower part of the cord being totally deafferented.

Ad 3. Minute electrolytic lesions, of about 0,5 mm in diameter, were exactly set into the gray matter at certain sites with the aid of a simple stereotaxic instrument and by a method specially designed for working on the spinal cord, as described elsewhere by one of us *(J. Sz.* 9.).

The animals were let to survive generally five days after operation, when axonal degeneration was to be studied. In the cases of complete deafferentation of the lower part of the spinal cord, when the aim was to find out what remains intact after operation, the animals were let to survive 2—3 weeks to ascertain that all transected fibres underwent complete secondary degeneration.

All material was fixed in neutral formaldehyd (1 : 4) and frozen sections were stained according to the method of *Reumont—Lhermitte.** For investigation of Clarke's column, because of the complete longitudinal orientation of the fine terminal network between the nerve cells, transversal sections are inconvenient. We therefore made sections from pieces about 2 cm long (from the segments $Th_9—L_3$) most carefully oriented in the sagittal and frontal plains.

III. Results

1. *Observations on intact columns*

It is impossible to get clear information from transversal sections, as generally practiced in routine investigation of the cord. Numerous collaterals originating especially from the posterior white column are entering Clarke's column and are forming rich plexuses, the fibre course of which are mainly longitudinal. Better information on the relations of these terminal plexuses and the nerve cells may therefore be gained from longitudinal sections. — Highly favourable for the investigation of the synaptology of this nucleus is the known fact, that the dendrits of Clarke's neurons are strictly confined to the boundaries of the column, and on the other hand no dendrits of neighbouring cells of other nuclei penetrate into the column. Hence all synapses found in the territory of Clarke's column may safely be considered to belong to the neurons of the column itself. On longitudinal sections (in the sagittal, as well as in the frontal, plane) it appears, that the synaptic termination of afferent fibers on Clarke neurons are very difficult to stain. In many preparations only occasionally are found some «boutons terminaux» of medium size. In well stained preparations, however, a strange type of synapse appears which as far as we know has not been described hitherto (Fig. 1/A). Afferents of somewhat larger diameter than generally seen in terminal fibres, are ramifying in close neighbourhood of the neurons, then the rami thicken and are closely joining the main dendrits of the column neurons. One dendrit is closely followed by several (often 3 to 4) terminal fibres, seemingly originating from the same afferent, for several hundred microns (Figs. 1/A. B/1). They are at last terminating on the dendrit — very often at its origin from the cell — in gigantic endbulbs, often as large as 10 to 15 by 6 to 8 μ, which sometimes are half buried into a notch of the cell surface (Fig. 1. C/2). Sometimes the terminal parts of the afferents are giving off side

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*In *Romeis:* Mikroskopische Technik. Leibnitz, München 1948. 15th edition. § 796/a.

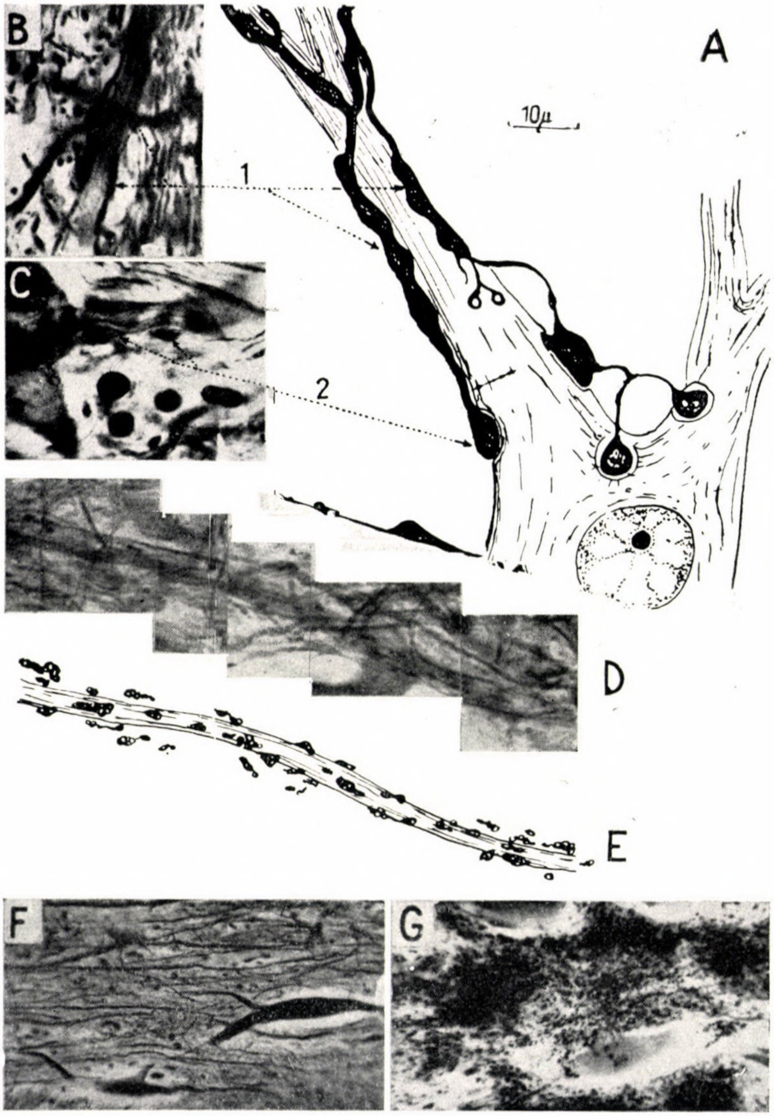

Fig. 1. A) Semidiagrammatic drawing of a «giant synapse» in Clarke's column. B) Photomicrograph of large parallel contacts with the main dendrits (1). C) Photomicrograph of large endbulbs (2), lying in depression of the cell surface. — D) 5 days after transection of the dorsal root L_5. Main dendrit of Clarke's column neuron (in the level L_2), surrounded by numerous degenerated fragments. The picture is assembled from five photomicrographs of different optic levels. E) Same as D., explanatory sketch. — F) Longitudinal section of normal column in L_3 with lower power. G) The same, 15 days after complete deafferentation below L_2. Almost complete degeneration of the intercellular fibre plexus

branches which are also ending in end feet on the same dendrit. When compared with the synapses on spinal motoneurons, these «boutons terminaux» are really gigantic. But another difference is also highly significant. The small afferent fibres terminating in end-feet, especially on motoneurons, are reaching the cells by a course of nearly at a right angle to the surface, while the terminals in Clarke's column are establishing long and very close parallel contacts with the dendrits of the postsynaptic neurons. Along these parallel contacts there occur thickenings on which the contact seems to be especially close. It is very probable that not only the terminal knobs, but also these parallel contacts may be considered as true synaptic articulation surfaces. Similar, though much smaller and less characteristic, parallel contact synapses are seen also on internuncial neurons in the posterion horn and the intermediate zone. These synapses on Clarke neurons may justly be called «giant synapses» when the difference of the interneuronal articulation surface covered by one synaptic terminal is taken into consideration, especially when compared with the synapses of motor radicular neurons. They may be better compared with the well-known basket type synapses of Held in the anterior ventral cochlear nucleus. — Beside these «giant synapses» ordinary «boutons terminaux» are found mostly on the cell somata, which are terminations of thin fibres running in transversal direction. — A third type of afferent fibres are very thin fibres of longitudinal direction with characteristic coils lying 20 to 50 μ apart. Their exact relation or termination on the column neurons was not detected.

2. *Extirpation of spinal ganglia*

After extirpation of single spinal ganglia between the segments L_2 and S_2 signs of secondary degeneration are encountered in frontal or sagittal sections of Clarke's column irrespective of the site of the removed ganglion, beginning with the caudal end of the column (between L_3 and L_4). Only when the ganglion L_3 or L_2 had been removed is the degeneration found to start about one segment upwards from the entrance level of the degenerated dorsal root. Extremely few descending afferents to Clarke's column have been found. In cranial direction degeneration was traced clearly to the level Th_9, irrespective of the severed segment. The number of degenerated fibres decreases sharply in the lower thoracic segments, but very probably some fibres could be detected also at higher levels, though this was not investigated. — Some of the «giant synapses» are clealy degenerated. The fragments of degenerated terminals are strangely distributed. Some of the main dendrits are completely enclosed by degenerated fragments (Figs. 1/D, E), while synapses on other dendrits are completely free of degenerated afferents.

After extirpation of two or three neighbouring spinal ganglia signs of degeneration are more apparent, but essentially the same as after removal of a single ganglion. The site of dorsal root afferents during their ascendent course and their synapses shows a certain regularity. Afferents originating from lower segments are always situated medially from those originating from more cranial levels and afferents belonging to the sacral segments are establishing contact with medial Clarke neurons, those from upper lumbar segments with those situated in the lateral part of the column. — Afferents originating from the same segment are entering Clarke's column —, as clearly visible on sagittal sections, on which degenerated entering collateral groups can easily be followed —, beginning with the level of the caudal end of Clarke's column until the lower thoracic segments.

After *complete deafferentation* of the lower cord by unilateral extirpation of the spinal ganglia L_1—L_7 and transection of the lower dorsal roots (S_{1-3} and Cocc.), at first sight all afferents seem to be degenerated within 15—20 days (Fig. 1/G). More close examination reveals, however, that two types of afferents are left intact and may therefore be considered to originate somewhere in the cord. The smaller type of «boutons terminaux», which are the endings of finer transversal fibres and do not have preterminal parallel contacts with the dendrits, are preserved in fair numbers. The very thin coiled fibres hardly noticeable in normal preparations, since the coarser dorsal root afferents are completely dominating the picture, are completely unchanged. As a matter of fact, at first we did not notice them in normal preparations. Our attention was called to these «coiled fibres» only in the preparations of deafferented cords. — No signs of degeneration were ever found after deafferentation in the Clarke's column of the contralateral side. Thus we may conclude safely, that no crossed dorsal root afferents reach the Clarke neurons.

3. *Small lesions within the dorsal horn*

Abundant signs of degeneration are present above the level of the lesion, whenever Clarke's column itself is affected. Descending degeneration does not proceed further than the next segment and affects only small fibres, the exact termination of which could not be detected. In ascending direction the degeneration extends to about 2 cm, the synaptic contacts of degenerating fibres with the neurons are similar, though finer, than after extirpation of spinal ganglia. From this we may infer that some dorsal root afferents, after entering Clarke's column, — or rather some of their branches — do not terminate at the level of their entrance into the column, but are ascending for a certain distance, which does not exceed 2 cm, and terminate on Clarke neurons. — In cases when Clarke's columm was not involved into the lesion, i. e. when the lateral and dorsal part of the dorsal horn are severed, there are also signs of secondary degeneration, which can be followed for about 2 segments in cranial and for 1 segment in caudal direction. The degeneration in these cases spares the coarser dorsal root afferents, but affects some of the «coiled fibres». Unfortunately it was not possible to determine the exact termination of these. From these results we conclude that the so-called «coiled fibres» are originating from internuncial neurons of the posterior horn.

4. *Lesions of the lateral funiculus*

When the superficial laye · of the dorsal part of the lateral white column is transected or, better, if parts 2 mm long and ½ mm deep are excised, signs of secondary degeneration are found in the ipsilateral column of Clarke. They are beginning at the level of the lesion and are extending about ½ to 1 cm upwards. The longitudinal fibres of the column are intact in these cases, but small fibres of a transversal course and their terminal knobs on Clarke neuron somata are occasionally degenerated. The solitary «boutons terminaux», mentioned already several times, must therefore be considered as recurrent collaterals of dorsal spino-cerebellar fibres. They are following the rule of *Cajal*, according to which initial collaterals are generally returning to, and terminating on, the same sort of cells from which their fibres come forth.

IV. Discussion

On the basis of these results we may try to sketch the synaptic connections of Clarke's column, as seen on our diagram (Fig. 2). The diagram represents a frontal section of the right side of the lower cord. The dorsal root fibres ascending in the posterior white column are entering Clarke's column, starting from the level of its caudal end, and are establishing «giant synapses», which are partly long and coarse parallel contacts and/or terminate with very large end knobs. Some of the branches of dorsal root afferents ascend further 1—2 segments and are establishing smaller synapses with upper Clarke neurons. Dorsal root afferents arising from the same segment are entering Clarke's column not only at its caudal end, but in every segment below the lower thoracic segments. Whether or not the dorsal root afferents entering the column from the dorsal funiculus at different levels may be branches of the same afferent as indicated in the diagram, or are strictly the terminal parts of differents within the same root, cannot be decided by degeneration experiments. The finding, however, of speed drops of muscular afferent volleys *(Lloyd and McIntyre)* ascending in the posterior white column, especially between L_4 and L_3, indicates that dorsal root afferents are branching before one of the branches is entering Clarke's column. The other branch, which ascends further in the posterior white column, very probably branches again at the level of L_1, where again a speed drop is experienced. A third speed drop is found in the lower thoracic segments, which probably

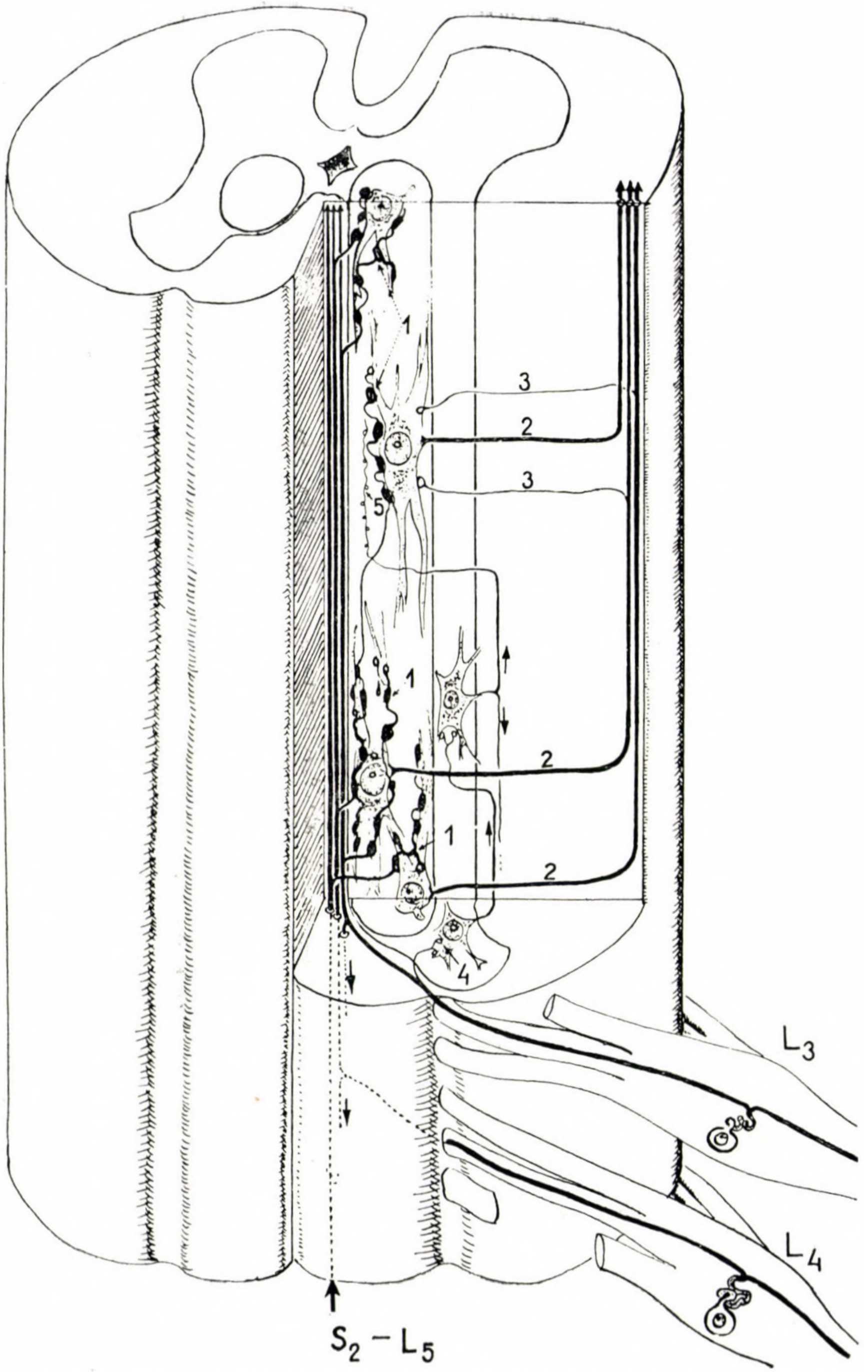

Fig. 2. Stereodiagram of the cord at the level of L_{1-4}. Collaterals from dorsal root afferents (originating from the segments S_2-L_3) are entering Clarke's column from its caudal end (L_{3-4}) until lower thoracic segments. They are establishing «giant synapses» (1) with column neurons. Clarke neuron neurits (2) which ascend in the dorsal spino-cerebellar tract are giving recurrent collaterals (3) to the somata of upper Clarke neurons. Dorsal root collaterals are also terminating on dorsal horn internuncials (4), which, forming ascendent chains, are terminating in Clarke's column as «coiled» fibres (5)

indicates a third branching of the fibres. Anatomic and functional findings are thus in fair accordance.

From the results of our experiments it emerges clearly that though being the main afferent source, dorsal root afferents are not the only presynaptic system of Clarke's column. As indicated in the diagram (Fig. 2/5) the so-called coiled fibres, which remain intact after complete deafferentation, must originate from dorsal horn internuncial neurons. Since dorsal horn internuncials are always in rich synaptic connections with dorsal root fibres, and very probably are forming long ascendent intragriseal (or mainly intragriseal) neuron chains (9.), we may infer that afferent impulses originating in primary sensory neurons are reaching Clarke's column not only directly but also relayed by internuncial chains. — Another afferent source for the column are very probably recurrent collaterals from Flechsig's tract. They are terminating on the cell somata as isolated «boutons terminaux». No evidence was, however, found of their terminating in the close neighbourhood of the axon hillock.

It is germane to inquire how far our findings concerning the synaptology of Clarke's column are in accord with recent results on the transmission of impulses. (i) The inference of *Lloyd and McIntyre* on the relay established by lower extremity dorsal root afferents (independent from their segmental origin) with Clarke's column neurons from its caudal end, on the lower edge of L_3, to lower thoracic segments, is substantiated. The assumption of branching of primary afferents before one of the branches is entering Clarke's column, is also in fair accordance with anatomic findings. (ii) Anatomical investigation reveals the causes of the striking difference between the modes of synaptic transmission from the same large muscle afferents to motoneurons and those of Clarke's column. As clearly pointed out by *Lloyd* and *McIntyre*, in Clarke's column practically no summation is required for transmission, whereas considerable summation is necessary in the motor nuclei. The differences between synapses of motoneurons and Clarke's column neurons are very characteristic. In motoneurons several hundreds of end feet are terminating, from which only a few come from the same source and these are also scattered on the surface of the neuron. No two «boutons terminaux» originating from the same system are found to lie in close neighbourhood of another. This was described by one of us on the basis of degeneration studies *(J Sz.* 5., see espcially Figs. 3. & 4) in the same year when *Lorente de Nó* (4) came to the conclusion that synaptic transmission in motor nuclei requires spatial summation from different channels converging to a local area of the surface of the neuron. — The synapses of dorsal root primary afferents in Clarke's column are strikingly different. They are establishing enormous (both long and coarse) parallel contacts especially with the main dendrits, terminating with extraordinary large end bulbs, sometimes deeply buried into a depression of the dendrit surface. Another important difference is the fact emerging from direct observation but especially from degeneration experiments, that the several parallel contacts around the same dendrit belong very probably to the same primary afferent, or at least to afferents originating from the same ganglion. — The main difference between the two types of synapses is as follows. *(a) The synaptic articulation surface between a single presynaptic afferent and postsynaptic cell surface is several times larger* *in Clarke's column than on motor neurons. (b) A large circumscript area of the postsynaptic cell surface (a large part of one, or more, main dendrits) is on Clarke neurons in synaptic articulation with the same presynaptic neuron, a fact never seen in motor neuron synapses.* Thus the inference of *Lloyd and McIntyre* on «an extremely powerful synaptic articulation of primary afferents in Clarke's column» is in complete accordance with our anatomical findings. On the other hand, these findings are offering strong anatomic support for the correctness of modern concepts on synaptic transmission mechanisms (especially spatial summation) elaborated on a physiological basis, and make it possible to draw further conclusions based on anatomic data in cases when physiological analysis gives nsufficient information.

(iii) The assumption that there may be a one-to-one synaptic relation between primary muscle afferents and Clarke neurons would, however, be incorrect. We can often see that after extirpation of a single spinal ganglion one or even more dendrits of the same Clarke neurons are completely buried into degenerated fragments, but other dendrits and another part of the cell surface is covered by normal dorsal root afferent synapses. This shows clearly that there is a certain convergence of primary afferents belonging even to different segments also in Clarke's column. But apparently no summation of impulses carried by these converging presynaptic elements is required for transmission.

(iv) Since Clarke neurons are also connected with dorsal horn internuncials, internuncial activity must also have some access to spino-cerebellar neurons, as inferred by *Grundfest* and *Campbell*. As, unfortunately, the exact nature of the contact between our «coiled fibres» and Clarke neurons could not be clarified, and also virtually nothing is known on transmission in the so called «nest-and plexus-like synapses», to which these synapses probably belong, nothing can be inferred from these anatomic findings at present.

(v) The recurrent collaterals, originating very probably from initial parts of spino-cerebellar fibres, are terminating as ordinary end-bulbs on the cell somata. Thus, though these synapses are not in close neighbourhood of the axon hillock, they are *axosomatic* and are lying «downstreem», in comparison to the mainly *axodendritic* primary afferent synapses. Since these latter are clearly of excitatory nature, the anatomic situation of the recurrent collateral synapses meets requirements of the hypothesis of *Retzlaff* and *Gesell* (1). The correctness of this hypothesis could perhaps be tested on Clarke's column.

Summary

The synaptology of Clarke's column was studied on dogs by means of the *Reumont—Lhermitte* silver technique on longitudinal sections of the spinal cord. Afferent pathways of Clarke's column, especially dorsal roots, were transected in order to determine the exact site of their termination on Clarke neurons by tracing the subsequent secondary degeneration of the collaterals and their synaptic endings.

The dorsal root afferents (originating from L_2—S_3) are terminating, independently from their segmental origin in the lower part of the column (Th_9—L_{3-4}), with a hitherto unknown

type of synapse. Characteristic features of these «giant synapses» are long parallel contacts between the preterminal parts of the afferent fibre and Clarke neuron dendrits, and the final termination of the afferents in large «boutons terminaux» half buried into depressions of the cell surface. The synaptic articulation surface between dorsal root afferents and Clarke neurons is considerably larger than in any other type of spinal cord synapse. This may account for the almost complete lack of summation in synaptic transmission, as found by *Lloyd* and *McIntyre* in the column. No one-to-one relation between pre- and postsynaptic neurons was, however, found.

Some other details concerning connections between different nerve elements in Clarke's column are presented and their physiological significance is discussed. The results of this experimental- morphologic analysis are in fair accordance with a number of facts recently discovered by physiological investigation.

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СИНАПТОЛОГИЯ КЛАРКОВОЙ КОЛОННЫ

Я. СЕНТАГОТАИ и А. АЛЬБЕРТ

Авторы изучали синаптолоию Кларковой колонны над продольными разрезами сагитального и фронтального направлений спинного мозга собак при помощи метода серебрения по *Рэмонта* и *Лермитта*, с целью определения места синапсов, авторы пересечением разных афферентных путей, ведущих к дорсальному ядру спинного мозга и наблюдением перерождения, принадлежащих к пересеченным системам синапсов, определили точное место и способ окончания разных афферентных систем.

Основную систему Кларковой колонны образуют задние корешковые волоконца, снабжающие задние конечности. Эти волоконца, после их входа в спинной мозг, восходят сперва в заднем пучке, а затем, начиная с нижней границы столба (L 3—4) вплоть до нижних сечений грудной клетки, непрерывно входят в Кларкову колонну. Задние корешковые волоконца оканчиваются, независимо от их происхождения из разных сечений, в нижней части всей Кларковой колонны, с той лишь разницей, что происходящие из более каудальных сечений волокна вступают в контакт с более медиальными клетками, а происходящие из более краниальных сечений с более латериальными клетками.

Задние корешковые волоконца оканчиваются в клетках Кларковой колонны в »гигантских синапсах», отклоняющихся от всех известных до сих пор спиномозговых синапсов; характерным для этих »гигантских синапсов« являются их длинный параллельный контакт с дендритами нейронов Кларка и их большие, проникающие в поверхность клетки, концевые утолщения. Этот своеобразный синапс с большой поверхностью объясняет известную из новейшей нейрофизиологической литературы передачу возбуждений без суммаций синапсов Кларковой колонны.

Авторы выявили на основании своих исследований многочисленные другие анатомические подробности и указывают на предполагаемое физиологическое значение последних.

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