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INVESTIGATIONS ON THE INTERGANGLIONIC AND PERIPHERAL NEURONAL PATHWAYS IN THE CENTRAL NERVOUS SYSTEM OF LYMNAEA STAGNALIS L.

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The simple structure of the central nervous system of *Lymnaea stagnalis* L., the large size and not too great number of its cells facilitate the investigations of morphologists and electrophysiologists on complex neural connections and central localization of physiological functions.

In a previous work (SALÁNKI and GUBICZA, 1969) we have determined the direct axonal connections between different ganglia and localization of neurons innervating the anterior and posterior adductors by the demonstration of retrograde regeneration following transection of various nerve branches in *Anodonta cygnea* L. The same method was employed to identify in *Lymnaea stagnalis* L. the central neurons involved in the direct innervation of the heart following transection of the branch of the intestinal nerve running to the heart (GUBICZA and S.-RÓZSA, 1969).

The purpose of the present work was to identify the neurons changed in consequence of transection of major nerve branches, and relying upon these findings, to describe the direct axonal connections, between different ganglia and organs.

Material and method

For the investigations medium-sized adult specimens of *Lymnaea stagnalis* L. were used. The snails were anaesthetized in a solution of 0.05—0.08% of nembuthal until complete relaxation was achieved. Then the central nervous system was exposed and the following commissures connectives and main nerve branches were cut:

1) cerebro-cerebral commissure
2) parieto-abdominal connective (left)
3) cerebro-buccal connective (left)
4) cerebro-pedal connective (left)
5) cerebro-pedal connective (right)
6) nervus labialis inferior (left)
7) nervus pallialis (left)
8) nervus pallialis (right)

Transection of each nerve branch was performed in 4—6 specimens. After the operative procedure the animals were placed in aquaria containing oxygenized circulating Balaton water for 24—48 hours. Only specimens that survived this post-operative period were used for histological preparations. After the elapse of the period of 24—48 hours following operation the central nervous system was dissected out, fixed in Carnoy solution for 45 minutes, embedded in paraffin and serially sectioned (at 7—8 μ). Embedding was always preceded by careful orientation of the ganglia. Sectioning was made in a horizontal plane parallel with the horizontal posture of the animal. Staining of the sections was made with a mixture of malachite green (E. Gurr, No 315) and pyronine Y (GT Gurr, England) according to the method of Baker and Williams (1965)

The sections were examined under the light microscope. In all the ganglia examined the neurons showing signs of regeneration were measured according to size and classified in three groups:

large cells (120 μ and larger)
medium-sized cells (from 50 to 120 μ)
small cells (under 50 μ)

Then the number of regenerating cells and their localization within the ganglion were determined. Localization of nerve cells was facilitated by dividing the sections of each ganglion into three equal zones:

dorsal third	of central nervous system	(zone I)
medial third	,, ,, ,, ,,	(zone II)
ventral third	,, ,, ,, ,,	(zone III)

On the basis of this division regenerating nerve cells exhibiting granular pyroninophilia could be localized in three zones of depth. The numbers of pyroninophilic cells given in the figures represent the mean values of 4—6 animals, with a limit of error of 10%. For control examinations 4 animals were used in which the central nervous system was only exposed without transecting any of the nerve branches. In addition 4 intact specimens were also processed. In the present work the results of experiments performed on 40 animals with nerve transection and 8 controls are reported.

Results

Transection of the cerebro-cerebral commissure (ccc)

After cutting the cerebro-cerebral commissure, in all the ganglia, except the buccal pair, cells showing signs of regeneration were present in varying numbers *(Fig. 1)*. About 40% of pyroninophilic cells appearing in the central nervous system after injury of the ccc were localized in the cerebral ganglia. In all, 153 neurons were found to send direct fibers to the ccc. Of these cells 59 were located in zone I, 55 in zone II and 39 in zone III. The approximate zonal localization of the cells is shown in *Fig. 1* and their numerical and size distribution in *Table 1.* The number of pyroninophilic cells appearing after injury of the ccc was nearly the same in the specimens examined.

No regularity was observed in the distribution of cells within the ganglion. There was, however, a difference in localization between the neurons of the left and right ganglia, except the parietal ganglia in which a giant cell was always present in the ventral third of each ganglion, localized symmetrically *(Fig. 1,* III). After transection of the ccc the left ganglia were found to contain fewer pyroninophilic cells than the right ones. The difference was particularly great between the right and left parietal ganglion where the former contained 24 nerve cells, whereas the latter only 13 *(Fig. 1)*.

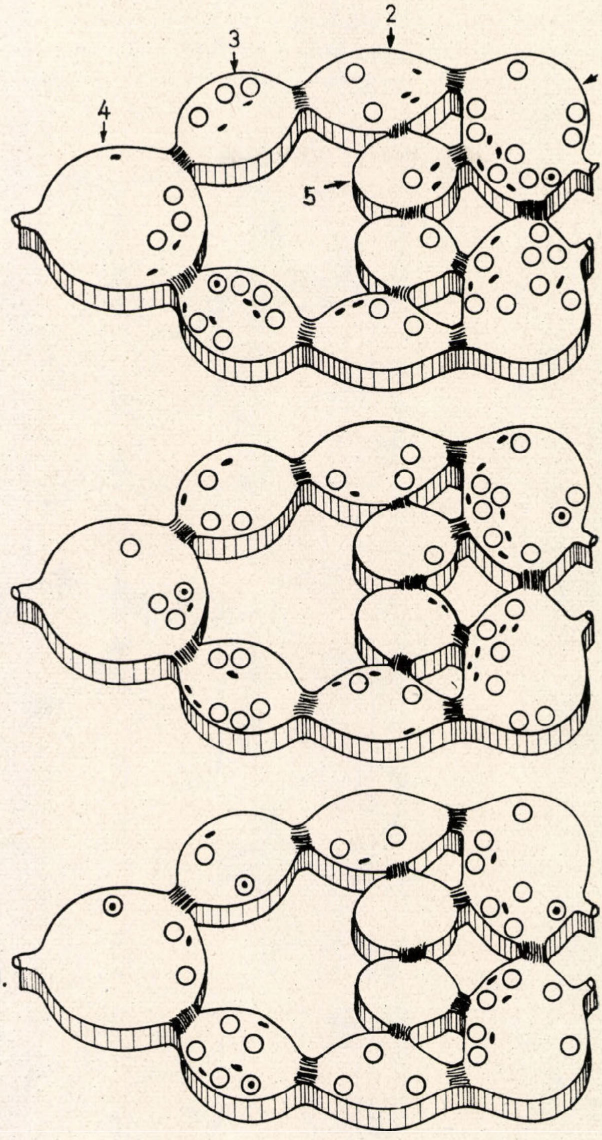
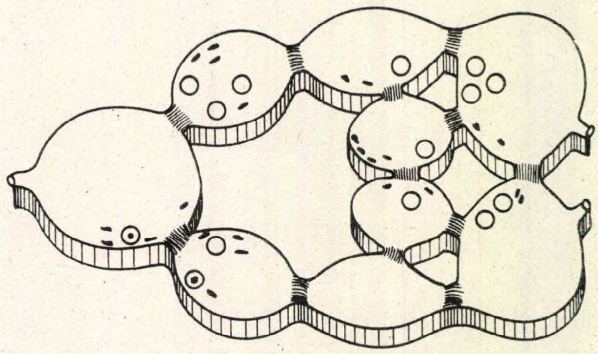


Fig. 1. Distribution of pyroninophilic nerve cells in the central nervous system of *Lymnaea stagnalis* L., after transsection of the cerebro-cerebral commissure
I = dorsal third, II = median third, III = ventral third of the central nervous system. Ganglia: 1. cerebral, 2. pleural, 3. parietal, 4. abdominal, 5. pedal. The left ganglia are marked on the *Figure*.
⊙ = large neurons (over 120 μ in diameter), ○ = medium-sized neurons (from 50 to 120μ)
– = small neurons (under 50 μ)
Legend to Figures refers to all the *Figures* (1—9)

By cutting the cerebro-cerebral commissure evidence was obtained that the cerebral ganglia receive nerve fibers from the contralateral pedal, pleural and parietal ganglia, as well as from the single abdominal ganglion. Thus, direct fibers are emitted from the left pedal, pleural and parietal ganglia and from the single abdominal ganglion to the right cerebral ganglion. On the other hand, the left cerebral ganglion is in direct axonal connection with the corresponding right ganglia and the single abdominal ganglion, at least in

I.



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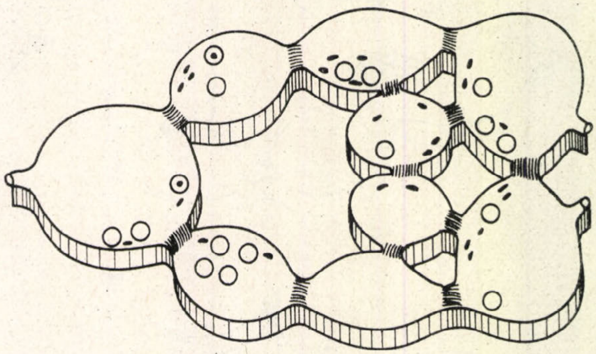

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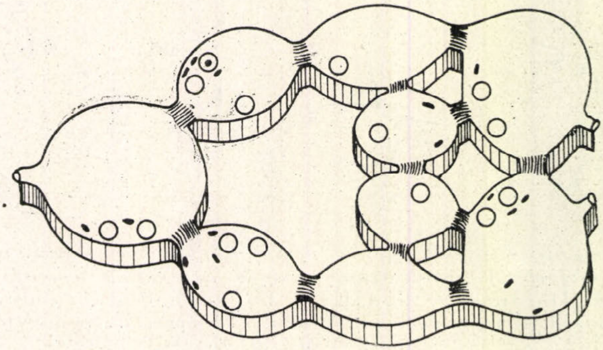

Fig. 2. Localization of pyroninophilic cells in the central nervous system following transection of the left cerebro-pedal connective

ascending direction. Existence of descending pathways from the cerebral ganglion to other ganglia was not demonstrable by cutting the ccc but only by transection of other connectives.

When cutting the ccc, in addition to the appearance of typical pyroninophilic cells, considerable changes were noted in the cells of the mediodorsal body attached to the cerebral ganglion. These cells are small, never exceeding 10 μ in diameter. Their nuclei are uncommonly large (7—10 μ) containing several small nucleoli hardly visible in the light microscope. In the control animals the diameter of the nucleoli was usually smaller than 1 μ,

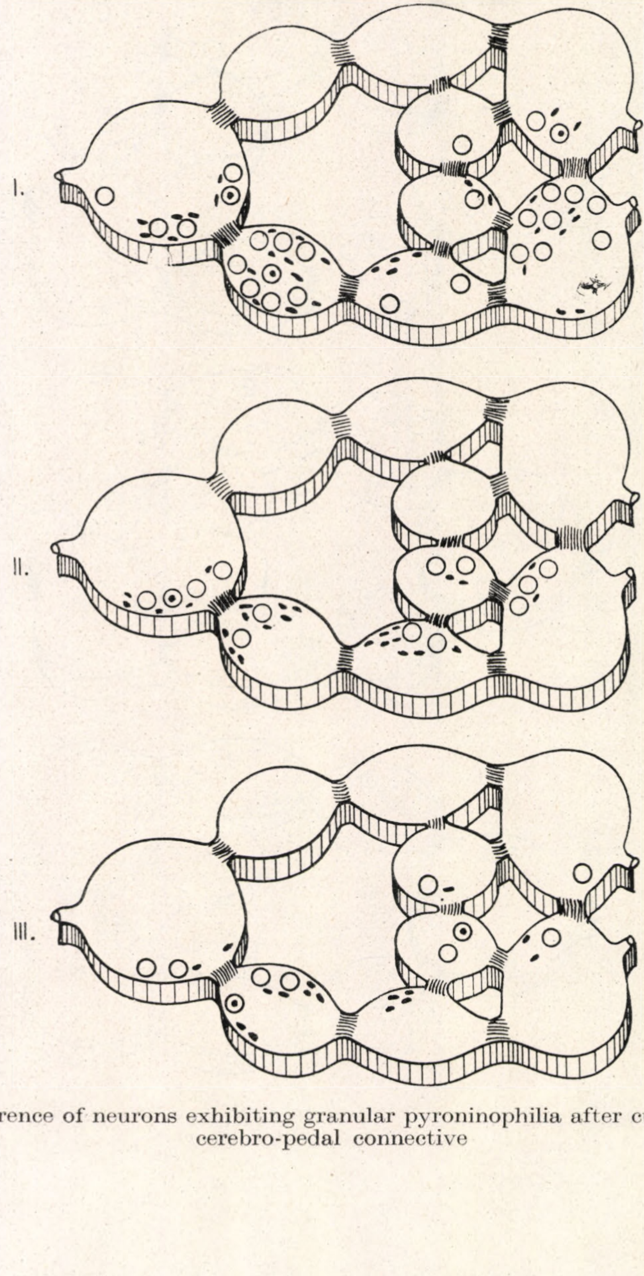

Fig. 3. Occurrence of neurons exhibiting granular pyroninophilia after cutting the right cerebro-pedal connective

whereas following transection of the ccc, the nucleoli in some cells of the mediodorsal body enlarged so much that they entirely filled the nuclei, ranging from 2 to 7 μ in diameter. Even nucleoli 8—9 μ in size were not rare. With pyronine these enlarged nucleoli stained a vivid red. The red colour could be removed by digestion with ribonuclease which is an evidence of the increased RNA content of the nucleoli.

These results have verified that the neurons of the mediodorsal body also send axons to the cerebro-cerebral commissure and injury of the latter brings about a characteristic reorganization of the nucleoli of the involved cells.

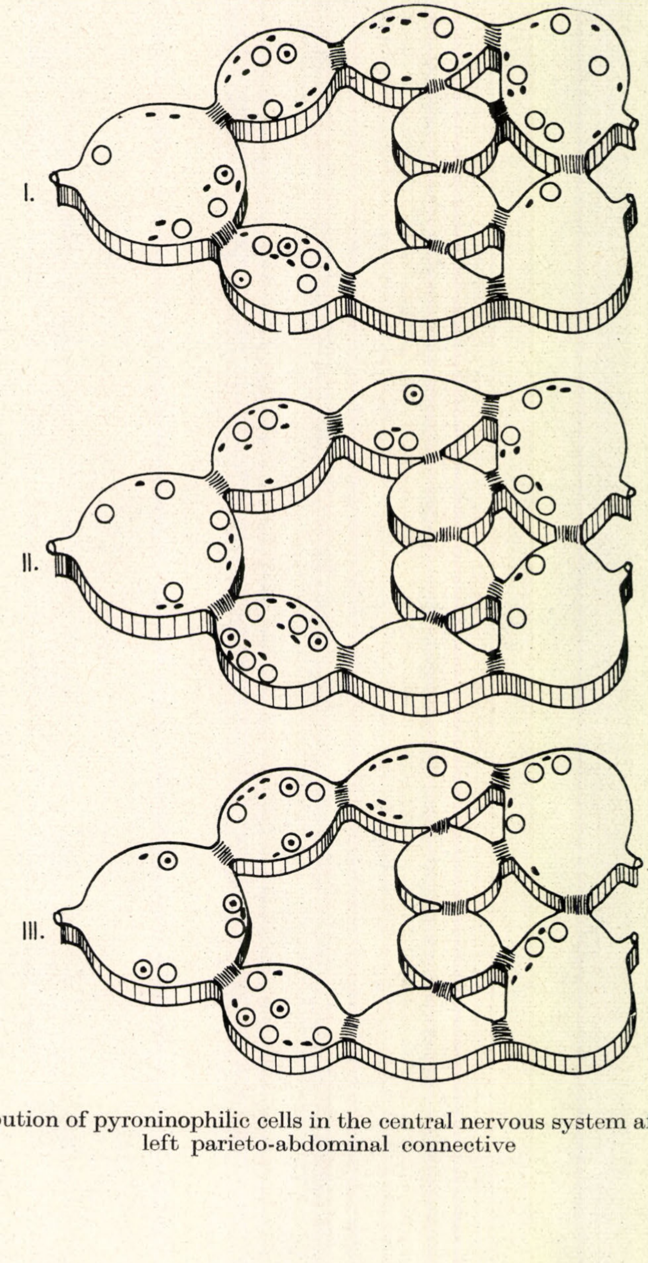

Fig. 4. Distribution of pyroninophilic cells in the central nervous system after cutting the left parieto-abdominal connective

Transection of the cerebro-pedal connectives (cpc)

By cutting the left cerebro-pedal connective cells exhibiting granular pyroninophilia were found in all the ganglia examined except the right pleural ganglion *(Fig. 2)*. In all, 112 pyroninophilic cells were present localized as follows: 38 in zone I, 42 in zone II, and 32 in zone III. When cutting the right cpc, no pyroninophilic cells were found in the left pleural and parietal ganglia in any of the specimens *(Fig. 3)*. Following transection of the right cpc 125 pyroninophilic nerve cells were counted in the central nervous system. Of these neurons 60 were found in the dorsal, 39 in the median and 26 in the ventral zone.

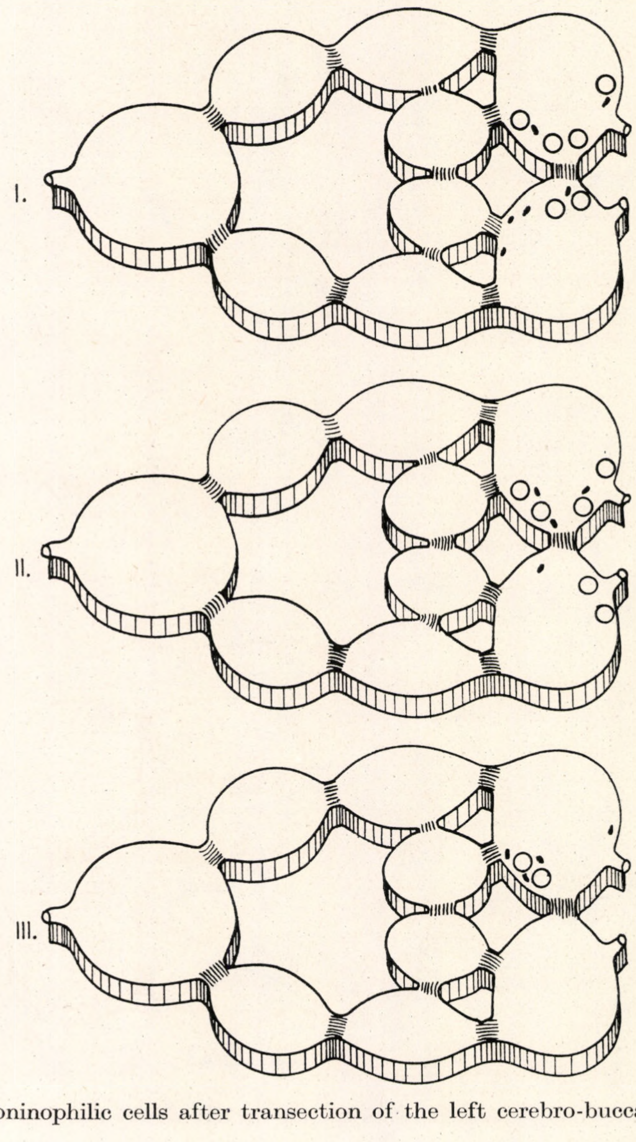

Fig. 5. Pyroninophilic cells after transection of the left cerebro-buccal connective

After cutting the cerebro-pedal connectives a total of 237 neurons exhibiting pyroninophilia were found in the central nervous system but only about 10—14% of these cells were localized in the pedal ganglia. Distribution of pyroninophilic cells according to size is shown in *Table 1.*

Localization of the involved cells within the ganglia was found to be identical in the various specimens. Injury of the right cpc was followed by the appearance of the greater number of pyroninophilic neurons in the right cerebral and right parietal ganglia *(Fig. 3).*

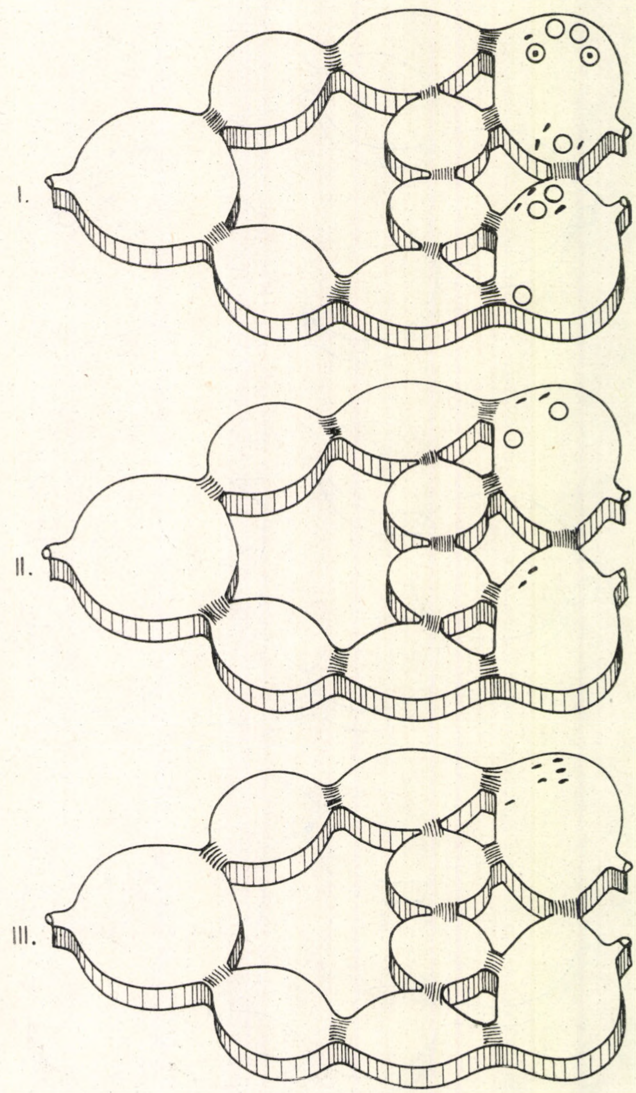

Fig. 6. Pyroninophilic cells after cutting the left nervus labialis inferior

Transection of the parieto-abdominal connective

Injury of the left parieto-abdominal connective resulted in the appearance of 148 pyroninophilic neurons in the central nervous system of the *Lymnaea stagnalis* L. In this case nearly the same number of neurons showing signs of regeneration were found in all three zones. The pedal pair of ganglia and the right pleural ganglion do not send direct fibers to the parieto-abdominal connective *(Fig. 4)*. In the right parietal ganglion the number of cells exhibiting pyroninophilia was strikingly high. Distribution of pyroninophilic cells in the right and left ganglia was assymetrical. By transection of the parieto-abdominal connective it was proved that direct axonal con-

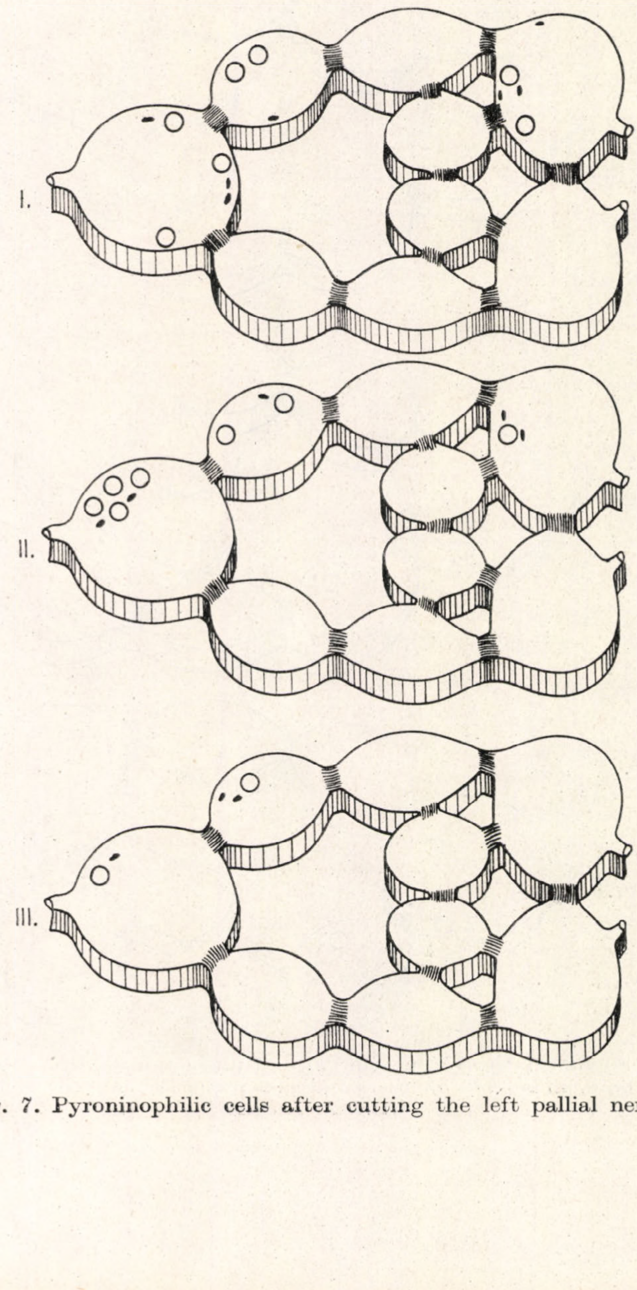

Fig. 7. **Pyroninophilic cells after cutting the left pallial nerve**

nections passing through the abdominal ganglion exist between the right and left parietal ganglia. Direct fibers are sent also from the abdominal ganglion through the left parieto-abdominal connective to the left pleural ganglion and both cerebral ganglia *(Fig. 4)*.

Transection of the left cerebro-buccal connective

After cutting this connective regenerating cells were present only in the cerebral ganglia *(Fig. 5)*. The number of these pyroninophilic cells was

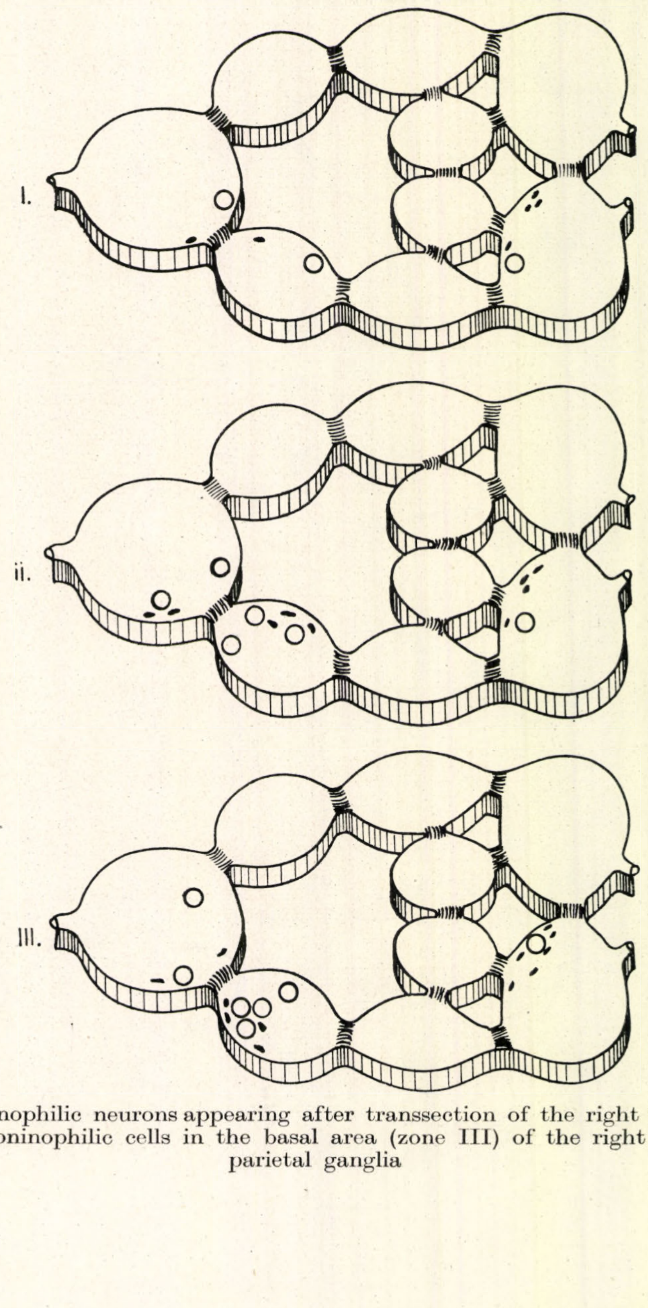

Fig. 8. Pyroninophilic neurons appearing after transsection of the right pallial nerve. Groups of pyroninophilic cells in the basal area (zone III) of the right cerebral and parietal ganglia

28: 12 in the dorsal, 10 in the median and 6 in the ventral area of the ganglion. No pyroninophilic cells were found in the buccal ganglion in any of the specimens examined following transection of any nerve branch.

Transection of the inferior labial nerve

By injury of the left inferior labial nerve similar results were obtained as by cutting the cerebro-buccal connective. Pyroninophilic neurons (in all, 27 cells) were found only in the cerebral ganglia. Distribution of these cells is shown in *Fig. 6*. Transsection of the nerves leaving the central nervous system was always marked by relatively few pyroninophilic cells.

Transection of the left and right pallial nerves

When cutting the left pallial nerve pyroninophilic cells appeared in the left cerebral and parietal ganglia and in the single abdominal ganglion *(Fig. 7)*. A total of 31 neurons became pyroninophilic, of which 14 were localized in

Fig. 9. Grouping of pyroninophilic cells in the third zone of the right cerebral ganglion after injury of the left pallial nerve

the dorsal, 12 in the median and 5 in the ventral zone. In the abdominal ganglion the pyroninophilic cells were grouped around the site of origin of the intestinal nerve.

Transection of the right pallial nerve resulted in the appearance of pyroninophilic cells in the right cerebral and parietal ganglia, as well as in the abdominal ganglion. In all, 43 neurons exhibited signs of regeneration. Their distribution within the ganglion is shown in *Fig. 8.*

Fig. 9 shows the grouping of small pyroninophilic cells in the third zone of the right cerebral ganglion after cutting the right pallial nerve. The basal part of the parietal ganglion also contains groups of pyroninophilic cells *(Fig. 8).*

Following transection of the inferior labial nerve and of the right and left pallial nerves, as well as of the cerebro-buccal connective the number of cells involved even all together, is smaller than that found after cutting the central commissures *(Table 1).*

TABLE 1

Distribution according to number and size of cells exhibiting pyroninophilia following transection of different commissures, connectives and nerve branches emerging from the central nervous system of Lymnaea stagnalis L.

	Over 120 μ	50—120 μ	Under 50 μ	Total
cerebro-cerebral commissure (ccc)	8	87	58	153
left cerebro-pedal connective (cpc)	5	41	66	112
right cerebro-pedal connective (cpc)	7	43	75	125
parieto-abdominal connective (left)	14	50	84	148
nervus labialis inferior (left)	2	8	17	27
nervus pallialis (left)	—	16	15	31
nervus pallialis (right)	—	16	27	43

Discussion

On the basis of our experimental results it was stated that following transection of different nerves pyroninophilic neurons in the central nervous system of *Lymnaea stagnalis* L. are rather scattered within the ganglia. Groups of cells containing a granular pyroninophilic substance were found only after cutting the right and left pallial nerves.

After cutting different interganglionic connections granular pyroninophilic neurons showing signs of regeneration were found in almost all the ganglia. Following transection of the cerebro-cerabral commissure great numbers of pyroninophilic cells were present in all the ganglia of the central nervous system. This finding supports the assumption that the pair of cerebral ganglia plays an integrative role within the central nervous system. In a previous work (GUBICZA and S.-RÓZSA, 1969) it has been reported that following injury of the intestinal nerve, namely, by cutting the branch innervating the heart, the greatest numbers of pyroninophilic cells were located in the cerebral ganglion. A further confirmation of the integrative role of the latter ganglion seems to be provided by the fact that pyroninophilic cells were always present in this ganglion after transection of any nerve or connective. This finding can be taken as an evidence that the cerebral ganglion is the centre of the ascending and descending pathways.

By cutting the parieto-abdominal connective all the ganglia, except the right pleural ganglion and the pedal pair of ganglia, contained pyroninophilic nerve cells which proves that the parietal ganglion receives fibers from all the ganglia situated higher up and that the paths coming from the cerebral and pleural ganglia pass uninterruptedly through this connective in their way to the periphery.

After cutting the cerebro-pedal connectives it was surprising to find a relatively small number of pyroninophilic cells in the pedal ganglion, to which numerous direct fibers arrive from other ganglia. Presumably, the majority of the neurons of the pedal ganglia send direct fibres to the periphery, while the rest transmits information to the cerebral ganglion or receives information from it *(Figs 3 and 4)*.

No cells exhibiting pyroninophilia were found in the buccal ganglion after cutting any of the nerve branches which finding seems to support the assumption that the buccal ganglia send direct fibers only to the periphery (oral organs). According to our data the pair of buccal ganglia is not in close functional connection with the central nervous system.

Another question to be answered is why the right parietal ganglion contains more pyroninophilic cells than the left following transection of the cerebro-cerebral commissure and left parieto-abdominal connective. As shown in *Fig. 4*, when cutting the right cpc the right parietal ganglion numerous nerve cells exhibiting pyroninophilia, whereas in the left ganglion no such cells were present. On the other hand, when the left cpc was cut pyroninophilic cells were present not only in the left parietal ganglion but also in the right one, where their number was even somewhat higher. From the difference in the number of pyroninophilic cells between the right and left parietal ganglia following cutting of other nerve branches as well, it was concluded that the right parietal ganglion has a different functional role. As it is known from earlier anatomical descriptions of the central nervous system of the *Lymnaea stagnalis* L. there is a disparity between the nerve branches emitted by the two parietal ganglia and their areas of innervation are also different. Moreover, the right parietal ganglion contains about three times more neurons than the left (Gubicza, 1970).

In a previous work (Gubicza and S.-Rózsa, 1969) it has been reported that after cutting the intestinal nerve (originating from the abdominal ganglion) at its branch innervating the heart, pyroninophilic cells appeared in the pedal ganglia alike. After transsection of the parieto-abdominal connective, however, no such cells were present in these ganglia in any of the cases, which seems to prove that the nerve fibers running from the pedal ganglion to the heart pass through the right ganglia.

Enlargement of nucleoli exhibiting pyroninophilia in certain cells of the mediodorsal body attached to the cerebral ganglion is undoubtedly connected with the transection of the cerebral commissure as this was never encountered in control animals or when other nerve branches were cut. This observation seems to confirm the earlier assumption that the small cells of the mediodorsal and medio-lateral bodies are in direct connection with the neurons of the cerebral ganglion (BOER, 1965; LEVER, 1958). Enlargement of nucleoli following neuronal injury has been described on various experimental animals (VOGT and VOGT, 1946; COHEN and JACKLET, 1965, SALÁNKI and GUBICZA, 1967).

No regularity was demonstrable in the distribution according to size of neurons forming the different nerve paths. The greatest number of pyroninophilic cells was found among the smallest cells and the fewest pyroninophilic cells were found among large cells (over 120 μ in diameter) but this may be in relation with the general cell number (GUBICZA, 1970).

Our results seem to indicate that the central representation of the different nerves is diffuse, several ganglia being involved in it.

Summary

By cutting the different commissures, connectives and nerve branches in the central nervous system of the *Lymnaea stagnalis* L. it was concluded that

1) Neurons containing a granular substance staining intensively with PMaG appearing 1—2 days following nerve transections were scattered in the various ganglia. Grouping of pyroninophilic cells was noted only after transection of the left and right pallial nerves.

2) After cutting different nerve branches, there was a disparity in the number and distribution of pyroninophilic cells between the right and left ganglia.

3) Granular pyroninophilia was found to appear in small, medium-sized and large cells alike. Most pyroninophilic cells were found among the small cells.

4) The number of pyroninophilic cells was higher after transection of the connectives and commissures of the central nervous system than after cutting peripheral nerves.

5) After transection of different nerve branches the right parietal ganglion differed from the left showing a similar pattern to that of the abdominal ganglion.

6) Following transection of the cerebro-cerebral commissure neurons with enlarged nucleoli containing RNA particles were found among the cells of the mediodorsal body. These cells stained intensively with pyronine.

7) The cerebral ganglia can be regarded as the integrative centre of the ascending and descending nerve paths in *Lymnaea stagnalis* L.

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GANGLIONKÖZTI ÉS PERIFÉRIÁS NEURONPÁLYÁK VIZSGÁLATA LYMNAEA STAGNALIS L. KÖZPONTI IDEGRENDSZERÉBEN

Gubicza András és *S.-Rózsa Katalin*

Összefoglalás

A *Lymnaea stagnalis* L. központi idegrendszerének különböző comissuráit, connektivumait és idegágait átvágva, az alábbi megállapításokat tették:

1. Az idegátmetszések után 1-2 nappal jelentkező PMaG-vel jól festődött szemcsés anyagot tartalmazó idegsejtek a különböző dúcokban szórtan fordulnak elő. Csak a n. pallialis sinister és dexter átvágása után volt tapasztalható a pyroninofil sejtek csoportos előfordulása.

2. A különböző idegágak átmetszése után, a jobboldali és a baloldali dúcpárokban, eltérő számban és egymástól eltérő helyen fordultak elő pyroninofil sejtek.

3. Kis, közepes és nagyméretű idegsejt egyaránt található pyroninofil sejtek között. Legtöbb a kis méretű idegsejt.

4. A központi idegrendszer connektivumait és commissuráját átvágva több idegsejt vált pyroninofillá, mint a perifériás idegágak átvágása esetén.

5. A jobboldalon elhelyezkedő ggl. parietale a különböző idegágak átvágása esetén eltért a baloldali ggl. parietálétól és az abdominalis dúchoz hasonló képet mutatott.

6. A cerebro-cerebrális comissura átvágása esetén a medio-dorzális test sejtjei között pyroninnal jól festődött nagy nucleolusú RNS tartalmú sejtek fordultak elő.

7. A cerebralis ganglionok a *Lymnaea* integratív központjának tekinthetők, mivel mind a felszálló, mind a leszálló pályák végső, illetve kiindulási helyének felelnek meg.

ИССЛЕДОВАНИЕ МЕЖГАНГЛИОЗНЫХ И ПЕРИФЕРИЧЕСКИХ ПУТЕЙ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ БОЛЬШОГО ПРУДОВИКА

А. Губица и *К. Ш.-Рожа*

На основе опытов с перерезкой различных комиссур, коннективов и нервов в центральной нервной системе большого прудовика авторы приходят к следующим выводам:

1. Через 1—2 дня после перерезки нервов нейроны, которые хорошо окрашиваются РМаГ обнаруживаются в различных ганглиях. Группировка пиронинофильных клеток появилась только после перерезки левого и правого паллиальных нервов.

2. После перерезки различных нервов, в левых и правых ганглиях пиронинофильные клетки появляются в различных местах и не в одинаковых числах.

3. Мелкие, средние и крупные нейроны в равной степени встречаются среди пиронинофильных клеток. Больше всего нейронов малого размера.

4. После перерезки комиссур и коннективов центральной нервной системы больше нейронов становится пиронинофильными, чем после перерезки периферических нервов.

5. После перерезки различных нервов, правый париетальный ганглий по распределению пиронинофильных клеток отличался от левово париетального ганглия и проявлял сходство с абдоминальным ганглием.

6. В случае перерезки церебро-церебральной комиссуры среди нейронов медиодорзального тела обнаруживаются клетки, с большим ядрышком содержащим РНК, хорошо окрашиваемые пиронином.

7. Церебральные ганглии у большого прудовика являются интегративным центром, так как здесь оканчиваются восходящие пути и отсюда начинаются нисходящие пути.