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CHANGE OF THE NISSL-SUBSTANCE IN CONNECTION WITH THE PERIODIC ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF FRESH-WATER MUSSEL *ANODONTA CYGNEA* L.

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The periodic activity of the fresh water mussel in connected with the tonic function of the adductors (BARNES 1955) while the control of the active and rest periods lasting both of them for several hours and alternating with a comparatively high grade of regularity is a function of the central nervous system (SALÁNKI 1963). The periodic activity can be readily influenced with various chemical agents as well as with the change of the oxygen supply (KOSHTOYANTS and SALÁNKI 1958, SALÁNKI 1960, 1965), but also these effects bring about the periodical tonus changes of the adductors only through the central nervous system (MINKER and ÁBRAHÁM 1959). Therefore it seems evident that the periodic activity must be in correlation with the change of the activity condition and/or of the amount of enzymes and biologically active substances of the central nervous system.

Histochemical and biochemical examinations conducted on various tissues point to the fact that numerous synthetizing enzymes of the cytoplasm are found in the ergastoplasm (microsomatic fraction) (CASPERSSON 1941, which has been identified with the basophilic component of the cell and with the electron microscopically well identifiable endoplasmic reticulum (CLAUDE 1946, WEISS 1953,. It has been established earlier that the affinity of the submicroscopic substance of the cytoplasm to basic stains is connected with the presence of RNS (BRACHET 1940, LANDSTRÖM, CASPERSSON and WOHLFAHRT 1941, EDSTRÖM and HYDEN 1954). In nerve cells it is the Nissl-substance or tigroid (LENHOSSÉK 1896) which corresponds to the basophilic component (BRACHET 1940, PALAY and PALADE 1953, HESS 1955), the presence of which has been demonstrated also in the central nervous system of Lamellibranchiates (NAGY 1962).

Departing from the above data in the course of our experiments we wanted to examine the connection of the basophilic substance of the cytoplasm with the periodical activity, namely whether in the active and rest phases of the periodic activity it is possible to find a difference in the neurons concerning the amount of the Nissl-substance or not.

Material and method

Examinations were conducted on adult specimens of *Anodonta cygnea* L. The animals were kept separated from each other in aquaria in running Bala-

ton-water and their activity was recorded during several days on mussel-actograph (SALÁNKI and BALLA 1964).

For histological examination of the ganglions we killed animals at the beginning of the two opposite phases of the periodic activity, *i.e.*

a) at the beginning of the active period,

b) at the beginning of the period of rest *(Fig. 1)*.

The cerebral, visceral and pedal pairs of ganglia were prepared out which took at most 2—3 minutes.

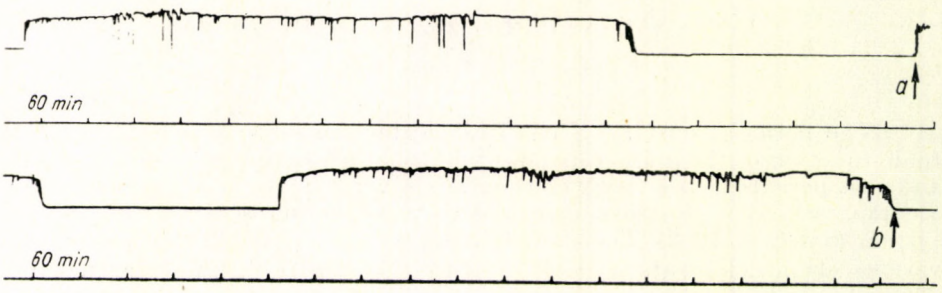

Fig. 1. Detail of two actograms: *a)* beginning of the active period; *b)* beginning of the rest period

1. ábra. Két aktogramm részlete: *a)* aktív periódus kezdete; *b)* nyugalmi periódus kezdete.

Histological methods: The ganglia prepared out were fixed in SUSAI, than carried out subsequently through alcohol, methyl benzoate and benzo they were embedded in to paraffin. 5 μ series sections were made and stained with gallocyanin (EINARSON 1932), with tionin (SPIELMEYER 1930), and kresylviolet (KISZELY—BARKA 1958). (The original Nissl-staining is impracticable owing to the dimensions of the ganglions.)

Statistical evaluation of the staining of the Nissl-substance in the cells was carried out by counting the cells from each ganglion in the microphotogram of a whole section (1600 to 2500 cells per ganglion) and ranging them in three groups. We considered as empty those cells in which no Nissl-substance could be seen at all and as full those, the whole cytoplasm of which was stained, while as transitory those in the cytoplasm of which both stained and empty portions occurred.

Results

The Nissl-substance can be demonstrated in the nerve cells of *Anodonta cygnea* with each of the methods employed. The best results were obtained, however, with the kresyl-violet method. The Nissl-substance is stained the least intensively with gallocyanin, even when the period of staining was increased to double of the originally described. Therefore the statistical evaluation was carried out on preparations obtained with kresyl-violet. It should be noted that the general picture of the sections stained with thionin

and gallocyanin is perfectly similar to the preparations stained with kresyl-violet.

Considering the staining of the Nissl-substance significant differences were found between the ganglions of animals killed at the beginning of the active and rest period.

a) At the beginning of active period: The overwhelming majority of the cells in all three pairs of ganglia is almost completely full with Nissl-substance. Entirely empty cells hardly, only sporadically occur. Also the number of cells only partly containing Nissl-substance is low *(Fig. 2)*. It is to be noted that in the pedal ganglion the proportion of the empty cells is somewhat higher *(Table 1)*. In some cells the Nissl-substance is conspicuously granulated.

b) At the beginning of the period of rest. In all three pairs of ganglia the cells with empty, not stained cytoplasm preponderate *(Fig. 3)*. The distribution of the cells according to Nissl-substance shows the opposite picture as against the beginning of the active period, although the numerical difference is not so large *(Table 1)*. At the same time in some cells the strongly granulated Nissl-substance readily staining with kresyl violet remains *(Fig. 4)*.

Table 1

Distribution of neurons according to Nissl-substance contents

Ganglion	Beginning of the activ period				Beginning of the rest period			
	cell number	full %	transit. %	empty %	cell number	full %	transit. %	empty %
cerebral	604	83.12	10.59	6.29	659	11.38	23.06	65.56
visceral	2540	86.82	9.17	4.01	1115	6.22	13.88	79.90
pedal	1410	72.41	15.88	11.71	1376	6.51	18.22	75.27

Discussion

It is well known that the basophilic component of the cytoplasm, exactly as the electron-microscopically examinable endoplasmic reticulum which is considered as adequate with it, shows varied forms even in the same kind of cells, which is generally explained with the different functional condition (Palade and Porter 1954). The change of cytoplasmatic elements resulting in basophily can be brought about also with an external impact, under experimental conditions (Shabadash and Zelinka 1961, Aghajanian 1963). Nagy (1962) regards the differences in Nissl-substance among the ganglions of different individuals of lamellibranchiates as a phenomenon connected with senescence.

Our examinations demonstrate that the differences of Nissl-substance in the neurons of the fresh water mussel are in close connection with the physiological condition of the animal. Selecting adequately the moment of the killing of the animal we can arbitrarily demonstrate much or little Nissl-substance in the ganglions. This points also to the fact that the change of the Nissl-substance can be hardly connected with the age of the mussels.

It is remarkable that, depending on the actual frequency of the periodical activity, the basophily of the cytoplasm is able to almost completely dis-

appear comparatively rapidly, within a few hours and then again to appear in a short time. This agrees with the observation of SELMAN and JURAND (1964) who after an ultrasound treatment of short duration demonstrated rapid destruction then restitution in the endoplasmic reticulum of *Triturus* and with the results of KONECKI and KOZUBSKA (1961) who described the diurnal changes of the Nissl-substance in the motoric cells of the mouse's spinal marrow. It is possible, however, that the point in question is not about the disappearance and appearance of the Nissl-substance but only about such change of the structure of the substance responsible for basophily which influences the intensity of staining.

On the basis of examinations which identified the Nissl-substance of the neuron with ribonucleoprotein and attributed to it a significant enzymatic role (HYDEN 1960) it may be assumed that the change in the staining of the Nissl-substance is not only accompanying the periodic activity closely connected with the vital function of *Anodonta*, but it is in relation with the specific metabolic processes involved in the central control of the periodic activity. In this respect our earlier observation seems to be remarkable, according to which serotonin which presumably has an important function in the nervous system of molluscs is localised for the most part in the endoplasmic reticulum of the neurons of the mussel (ZS.-NAGY et al. 1965).

Summary

It has been established that in the cerebral, visceral and pedal ganglia of the fresh water mussel (*Anodonta cygnea* L.) the demonstrability of the Nissl-substance shows a change connected with the periodic activity of the animal; at the beginning of activity the overwhelming majority of cells exhibit a massive basophily, while at the beginning of the period of rest neurons containing Nissl-substance can be found comparatively rarely.

It is assumed that the change of the basophily of the cell connected with the periods activity and rest is not an accompanying phenomenon but it is in direct connection with the central control of the periodic activity.

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A NISSL-ANYAG MENNYISÉGÉNEK VÁLTOZÁSA A PERIODIKUS AKTIVITÁSSAL ÖSSZEFÜGGÉSBEN *ANODONTA CYGNEA* L. KÖZPONTI IDEGRENDSZERÉBEN

Összefoglalás

Salánki János, Zs.-Nagy Imre és H. Vas Éva

Megállapítást nyert, hogy tavi kagyló *(Anodonta cygnea* L.) cerebrális, viscerális és pedális ganglionjaiban a Nissl-anyag kimutathatósága az állat periodikus aktivitásával összefüggő változást mutat: aktivitás kezdetén a sejtek döntő többsége masszív bazofiliát mutat, nyugalmi állapot kezdetén viszont csak viszonylag ritkán találhatók Nissl-anyagot tartalmazó idegsejtek.

Feltételezzük, hogy a sejt bazofiliájának az aktivitási állapottal összefüggő változása nem kísérőjelenség, hanem direkt összefüggésben van a periodikus aktivitás központi szabályozásával.

ИЗМЕНЕНИЕ ВЕЩЕСТВА НИССЛЯ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ БЕЗЗУБКИ В СВЯЗИ С ПЕРИОДИЧЕСКОЙ АКТИВНОСТЬЮ

Я. Шаланки, И. Ж.-Надь, Ева Г.-Ваш

Было установлено, что выявляемость вещества Ниссля в центральном, высцеральном и педальном ганглиях беззубки зависит от периодической активности животного; в начале активности большинство клеток обладает интенсивной базофилией, а в начале состояния покоя относительно редко встречаются нервные клетки, содержащие вещество Ниссля.

Предполагается, что изменение базофилии клеток, которое идет параллелльно ритмической активности, не является сопутствующим явлением, а находится в прямой взаимовсязи с центральной регуляцией периодической активности.

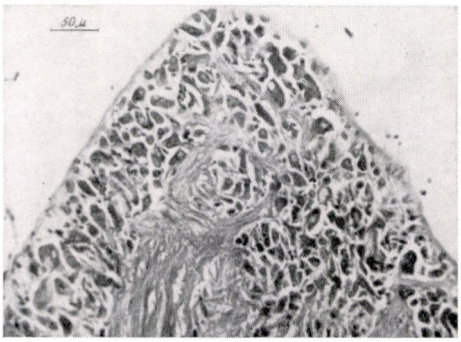

Fig. 2. Detail of cerebral ganglion at the beginning of the active state, with neurons full of Nissl substance. Susa, Kresyl violet, 252×

2. ábra. Cerebrális ganglion részlete, aktív állapot kezdetén, a Nissl-anyaggal tele levő idegsejtekkel. Susa, kresylviolet, 252× nagyítás

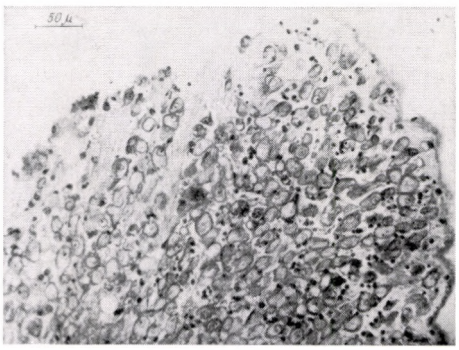

Fig. 3. Detail of cerebral ganglion at the beginning of the rest period with neurons of no stained cytoplasm. Susa, kresyl violet, 252×

3. ábra. Cerebrális ganglion részlete, nyugalmi állapot kezdetén, a nem festődött citoplazmájú idegsejtekkel. Susa, kresylviolett, 252× nagyítás

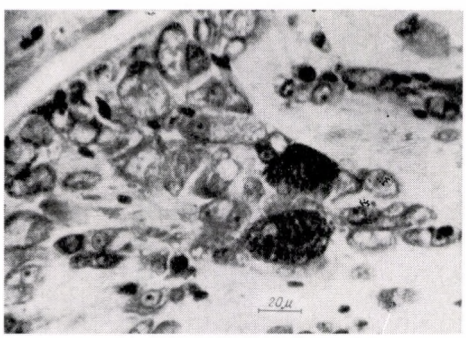

Fig. 4. Detail of cerebral ganglion at the beginning of the rest period. Cells containing conspicuously granulated Nissl substance. Susa, kresyl violet, 554×

4. ábra. Cerebrális ganglion részlete a nyugalmi állapot kezdetén. Feltűnően szemcsézett Nissl-anyagot tartalmazó sejtek. Susa, kresylviolett 554× nagyítás