ANNAL. BIOL. TIHANY	40	55–71	HUNGARIA 1973

THE ROLE OF IONIC ENVIRONMENT IN THE POTENTIAL GENERATION OF THE GIANT NEURONES OF *LYMNAEA STAGNALIS* L.

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Received: 8th January, 1973

In our earlier papers (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971) a number of identifiable neurones were described, which exhibited a marked variation in activity, but the type of activity was always characteristic of a given neurone. Then the question arose, what delicate mechanisms are responsible for the differences in the generation of the pacemaker activity of individual neurones? In examining this question it has to be considered that the pacemaker activity of giant neurones is generated by a different mechanism (WAZIRI et al., 1965) and in a different part of the membrane (TAUC, 1962) than the activity evoked either by natural or artificial stimulation. On the other hand, there are some differences in the special ionic conductances underlying the pacemaker generation of the spike in the nervous system of various species (KOSTYUK, 1968), furthermore the individual neurones of the same animal can also have different properties in this respect. As the maintenance of the membrane potential as well as the generation of the action potential can be explained on the basis of the concentration gradients and conductances for Na^+, Ca^{++}, K^+ and Cl^- ions (HODGKIN and HUXLEY, 1952), it might be supposed that different ionic processes underlie the variety of the spontaneous generation of activity. In close relation to this the possibility of electrogenic ion-currents has to be considered, too (THOMAS, 1972).

Concerning the CNS of *Lymnaea stagnalis* only a few contradictory data have been reported on this problem (MAGURA et al., 1971; JERELOVA et al., 1972; SATELLE and LANE, 1972). In the present paper experiments were performed in order to study the effect of Na^+-, Ca^{++}-, K^+- and Cl^--free solutions on some identified neurones of *Lymnaea stagnalis*. In the first place we wished to elucidate whether the ionic requirements for the potential generation of different neurones are different, and to obtain convincing data referring to some questions like the problem of ions involved in the generation of membrane and action potentials and the problem of the ionic dependence of spontaneous and synaptically driven activity.

Material and methods

Examinations were conducted on the abdominal and right parietal ganglia of isolated CNS of *Lymnaea stagnalis* using the identifiable giant neurones demonstrated in *Fig. 1.* All the experiments were performed at room

temperature. The examined neurones are located on the dorsal surface of ganglia, whose most important electrophysiological parameters have already been described (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971).

Membrane and action potentials were recorded by glass microelectrodes filled with 2.5 M KCl. The current for polarizing the membrane was transmitted through the same electrode. The membrane potential and its changes were measured after the compensation of tip potential of the electrode using a digital voltmeter. For recording we used an amplifier with FET input (KISS et al., 1972).

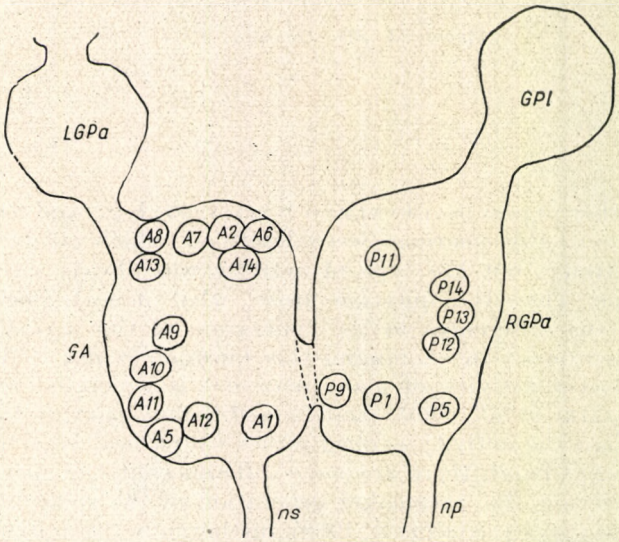

Fig. 1. Localization of the identified neurones. GPl — ganglion pleurale; LGPa — left ganglion parietale; RGPa — right ganglion parietale; GA — ganglion abdominale; ns — nervus splanchnicus; np — nervus pallialis

The isolated ganglion ring, from which the thick connective tissue was removed was placed in a chamber containing 3 ml physiological saline. The composition of this saline was: NaCl 46.2 mM; KCl 4 mM; $CaCl_2$ 5.6 mM (JULLIEN and RIPPLINGER, 1948).

Test solutions with no content of a given ion were made as follows:

NaCl was replaced in 3 different ways: by osmotically equivalent quantity of cholin-Cl, Tris-Cl or saccharose alternatively. No significant differences could be found between these solutions so they are to be described collectively.

$CaCl_2$ was replaced by osmotically equivalent quantity of saccharose.

In the K^+-free solution equivalent concentration of NaCl was used instead of KCl.

For making a Cl^--free solution various combinations of the following compounds were used: Na-, K-, and Ca-propionate, Na- and K-acetate, Na- and K-sulphate. As to replace $CaCl_2$, Ca-propionate was used in every case the effect of Ca-propionate was separately tested under normal conditions at normal Cl^--concentration.

After recording the control activity in physiological solution the whole volume of this solution was exchanged for a given ion-free one. Following

this the chamber was continuously perfused with the latter solution at least for 5 min.

In some of the K^+-free solution experiments the microelectrodes were filled with 3 M NaCl instead of KCl.

For examining the excitability of the soma depolarizing current or chemical stimulation was applied to the membrane. For the chemical stimulation ACh was added to the bath at a similar concentration and in similar way to that described in one of our earlier papers (KISS and SALÁNKI, 1971).

During recording the bioelectric signals were fixed on a magnetic tape and the desired portions were photographed later by means of an EMG oscilloscope and MF 1—1 photorecorder.

Results

On the basis of the reaction to the removal of Na^+ the neurones can be classified into the following categories: The spontaneous activity of the cells belonging to the first category is abolished within 2—3 min. in Na-free solution It is most frequently preceded by a continuous reduction of the spike amplitude and simultaneously the firing rate decreases, too *(Figs 2* and *3)*. This type of reaction was shown by A1, A5 and P5 neurones, whose activity seemed to be under compound synaptic control.

The neurones included in another category cease their activity within 6—8 min. *(Fig. 4)*, e.g. the P12 pacemaker and the A11 driven neurone belong into this group. In addition two pacemaker neurones were found, which continued to generate action potential more than 10 min. (A10 and P1 cell). From the latter cell sometimes pacemaker activity can be registered even 30—40 min. after the removal of Na^+. The neurones included in the latter two categories are less sensitive to the absence of Na^+, their action potential is characterized by only a slight reduction of amplitude, furthermore after a transitional decline it frequently tends to be normalized *(Fig. 5)*.

Immediately after the application of the Na^+-free solution a transitional hyperpolarization of the membrane of 8—10 mV can be registered and during this period the spontaneous activity is often completely blocked, but it returns again in 4—5 min. *(Fig. 6)*. However the final disappearance of activity does not yet occur in this period, on the contrary, sometimes it is preceded by a depolarization of the membrane.

After the spontaneous activity ceased in the Na^+-free solution all the examined neurones can be activated by depolarization indicating that the excitability of the soma membrane is maintained *(Fig. 7)*. However, after prolonged treatment with the Na^+-free solution also the electric excitability of membrane disappears. The time required for this is only slightly variable ranging between 18 and 20 min. except P1 cell, which can be activated by depolarization for undefined time within the period of the investigation.

The question raised, whether a neurone of such condition is able to react to electric stimulation only. As the majority of the identified neurones can be excited by ACh, this substance seemed to be suitable for trying to activate the neurones became silent in the absence of Na^+. Some of the cells — for example A10 — are depolarized and generate a short train of spikes following

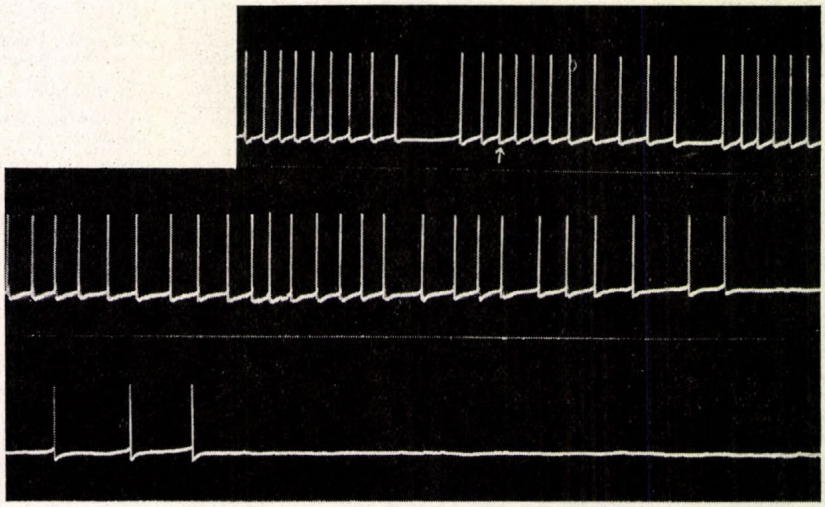

Fig. 2. Course of the abolition of spontaneous activity in the Na-free solution on P5 neurone. Arrow marks the exchange of physiological solution

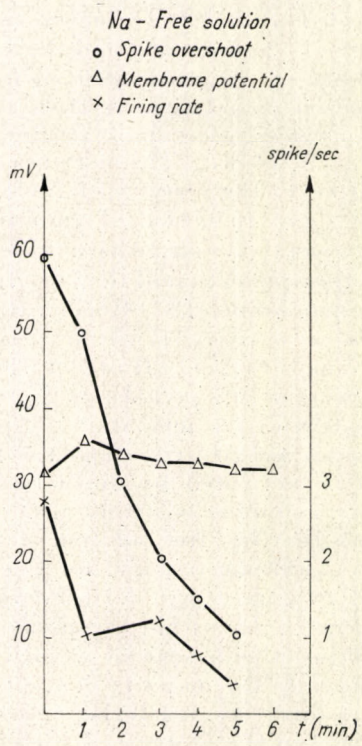

Fig. 3. Changes in the parameters of spontaneous activity of A1 cell as a function of time

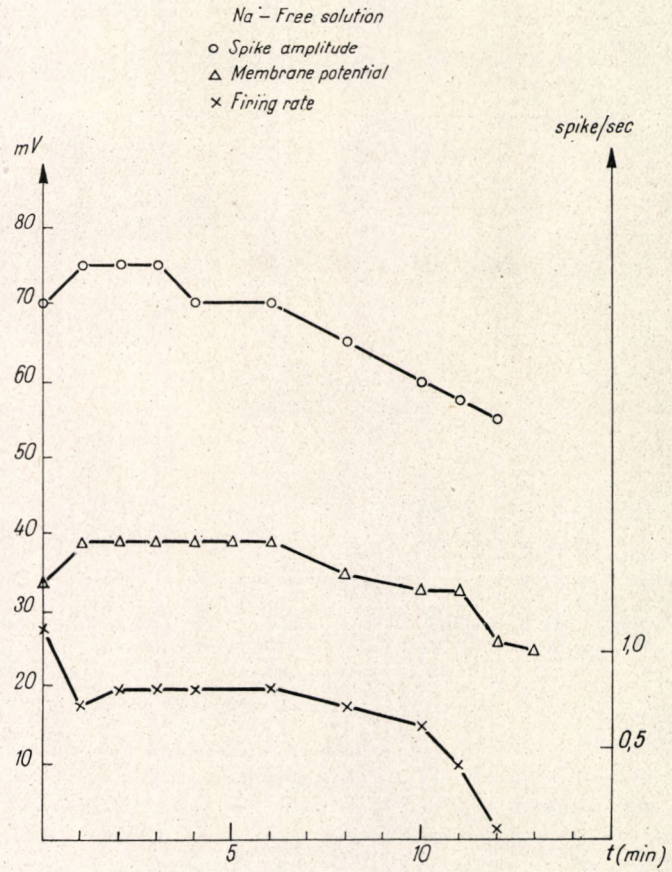

Fig. 4. Changes in the parameters of spontaneous activity of A10 cell as a function of time

the application of ACh even in Na^+-free solution *(Fig. 8)*. But the other neurones cannot be activated by ACh.

On the cell A10 mentioned above a special effect of Na^+-free solution was occasionally found. Its firing rate — diverging from normal — increased in the early period. It can be attributed to an early abolition of ILD-s determining the general character of the activity when the firing rate of rhythmic pacemaker activity does not yet significantly declined *(Fig. 9)*.

Effect of the removal of Ca^{++}

In Ca^{++}-free solution the spontaneous activity generally disappears in 5—10 min. The changes in the membrane potential are not unequivocal, although in most of the cases a transitional depolarization can be observed in the early phase. It is accompanied by a marked increase in the firing rate giving place to a decreased frequency a few minutes before the activity is abolished. There is a continuous marked reduction in the amplitude of the action

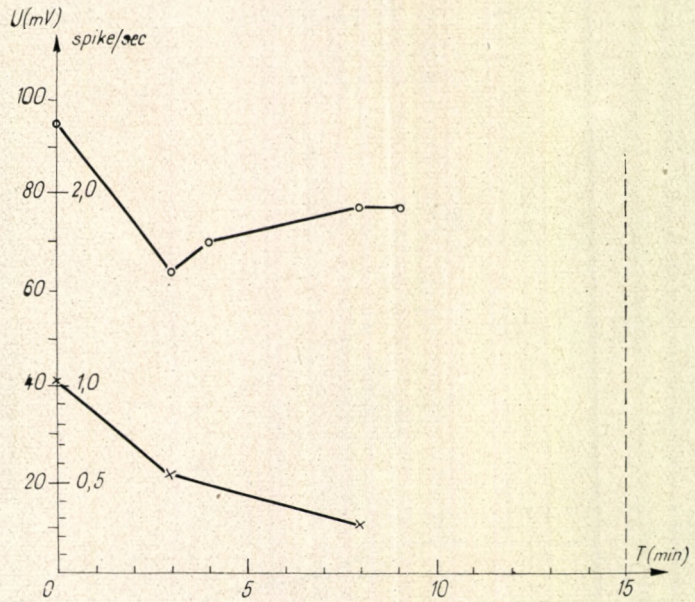

Fig. 5. Alteration of the spontaneous firing rate (— x —) and amplitude of the spike produced by A11 cell in the Na^+-free solution. The vertical broken line marks the time of the cessation of activity

potential *(Fig. 10)*. After the cessation of the spontaneous activity it cannot be restored either spontaneously or by depolarization.

Ca^{++}-free solution is able to activate the silent neurones, although for only a short time, because later there is a damage of the mechanism involved in the generation of the spike *(Fig. 11)*. There was only two neurones (A5 and P12) whose spontaneous activity was maintained in Ca^{++}-free solution for as long as 15—20 min. or even longer *(Fig. 12)*.

On an individual neurone we failed to demonstrate any definite correlation between time courses of the capability for producing spikes in Na^+- and Ca^{++}-free solution.

Effect of the removal of K^+

In K-free solution the membrane potential of the neurones increases by 5—10 mV. It is to be expected because of the increased K^+-concentration gradient between the two sides of the membrane. This hyperpolarization is accompanied by an increase in the amplitude of action potential and a decrease in the frequency of spontaneous activity *(Fig. 13)*. The rate of decrease in the frequency is rather variable, in most of the cases it is below 50%, in some other cases, it can be much greater until the activity is eliminated. Sometimes this elimination occurs in such a way that the spontaneous, continuous activity is broken off by a hyperpolarization of 10—20 mV *(Fig. 14)*.

In a general given identified neurone cannot be characterized by any definite type or degree of the above changes, as there is a great variability

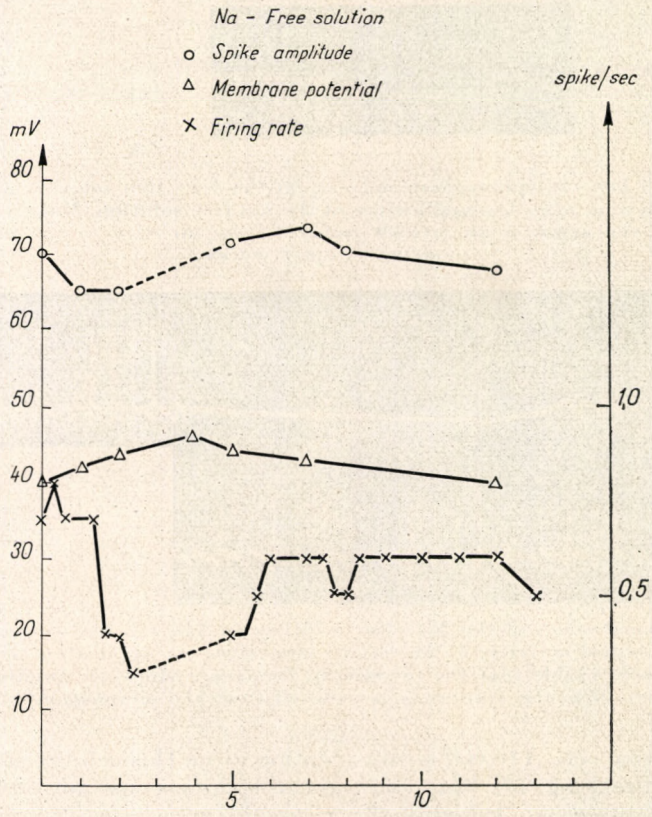

Fig. 6. A10 cell; time relations of the temporary cessation and recovery of the spontaneous activity in the Na^+-free solution. During the interval marked by broken line the neurone is silent. Notice, that this interval coincides with a transitional hyperpolarization of the membrane

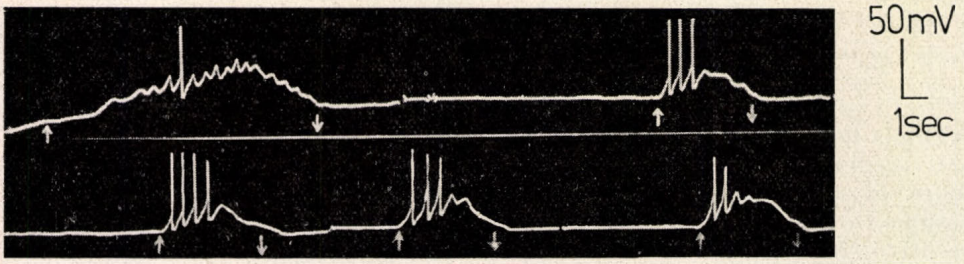

Fig. 7. Activation of P5 neurone by an artificial depolarization in the Na^+-free solution after the complete cessation of the spontaneous activity. Arrows mark the onset and interruption of depolarizing DC in order at 6; 10; 14; 18 and 22 min. from the application of the Na^+-free solution. Notice, that in the 6th min. only local potentials are produced in addition to a single spike, after this there is a continuous recovery of the generation of spike, but later it is blocked again

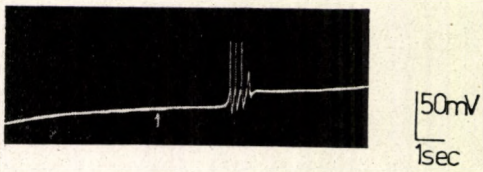

Fig. 8. A10 cell lost the spontaneous activity in the Na^+-free solution is activated by ACh in the 10th min. after the application of the ion-free solution. Note: the shift in DC level preceding the arrow is an artifact resulted from an error in the function of the magnetic tape-recorder

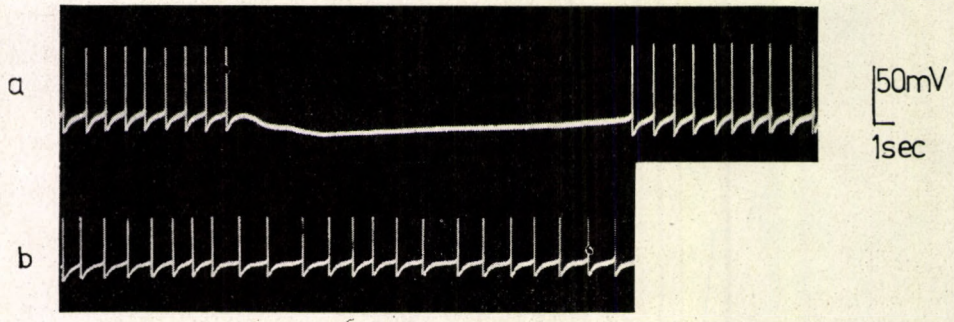

Fig. 9. Transitional effect of the Na^+-free solution on A10 cell realized in an increased firing rate. *a)* control activity; *b)* in the 3rd min. after the application of the Na^+-free solution it is fairly visible that the increase in frequency observed simultaneously with a reduction in the amplitude is resulted from the elimination of ILD

even on the same cell. A10 cell is an exception as on this cell the sudden hyperpolarization breaking off the continuous spontaneous activity can most frequently be observed. Occasionally it results in a permanent cessation of firing, on another occasion the silence appears to be transitional with a time course quite similar to that of ILD *(Fig. 15)*.

A series of the measurements was done by means of microelectrodes filled with NaCl. Under these conditions the K^+-free solution caused much greater hyperpolarization *(Fig. 16)*. At the same time no significant difference was found in the changes observed in the amplitude and frequency of the spikes compared with that measured by means of the KCl-filled microelectrodes.

Effect of the removal of Cl^-

Cl^--free solution results in characteristic changes in the type of activity within a few minutes. The discharge pattern becomes irregular, the continuous activity tends to be replaced by groups of the spikes *(Fig. 17 a, b)*. Furthermore, in some measurements performed in the absence of Cl^- the activity assumed a bursting character *(Fig. 17 c)*, where the duration of the consecutive spikes also showed peculiar changes *(Figs 17 d, e)*. Changes of this nature were produced for example on A10 cell. On this cell a burst-like grouping of spikes had been observed also in the control state *(Fig. 18 a)*, and this character became more pronounced upon the removal of Cl^- *(Fig. 18 b)*. It can frequently be observed that the potential generation during a burst is inactivated for a short time at a depolarized level *(Fig. 19)*.

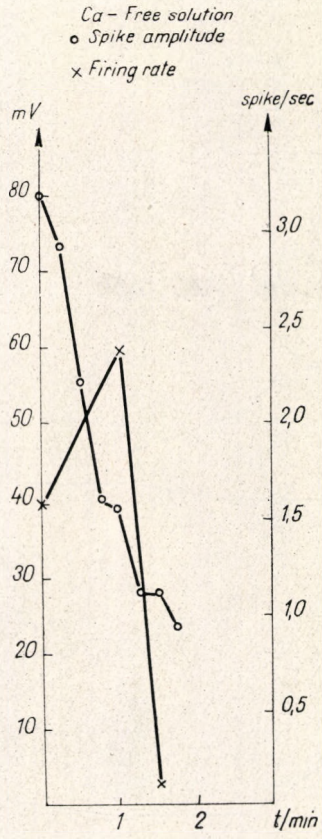

Fig. 10. The most frequently observable influence of the Ca++-free solution on the spontaneous activity: a fast reduction the firing rate shows a biphasic change

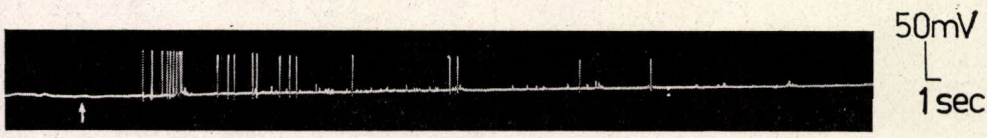

Fig. 11. Activation of a silent neurone by Ca++-free solution. Arrow marks the exchange of physiological solution. Notice, that after a temporary activation the firing rate and the amplitude rapidly decrease, following this the activity disappears at a depolarized level

In the absence of Cl^- all the neurones are characterized by a decrease in the spike amplitude and an increase in the impulse duration.

If ACh was applied to the preparation in a solution containing no Cl^- the burst-like sequence of the potentials was considerably prolonged *(Fig. 18 c)*.

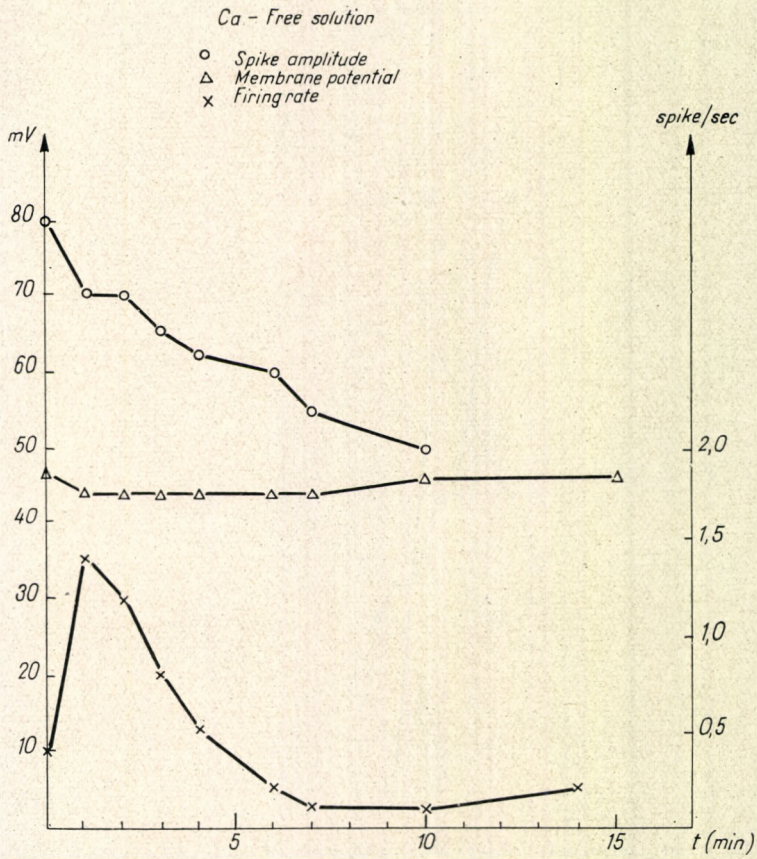

Fig. 12. Changes in the parameters of spontaneous activity in the Ca^{++}-free solution on P12 neurone, which continues to produce spikes more than 15 min. after exchanging the physiological solution

Discussion

It has been described by a number of investigators that the activity of some giant neurones is maintained in Na^+-free solution for quite a long time. In these cases it was supposed that the rising phase of the spike is produced by a Ca^{++} current (Gerasimov et al., 1965; Oomura et al., 1961; Jerelova et al., 1972). In some of the cases the "calcium-spike" hypothesis was reconsidered and either a contribution of both Na^+ and Ca^{++} to the generation of action potential was suggested, (Meves, 1968; Geduldig and Junge, 1968; Chamberlain and Kerkut, 1969), or a passive retention or active depot of Na^+ was conceived, which might be able to assure the Na^+-requirement for the spike generation even if the extracellular space is apparently free from Na^+. (Krasts and Veprintzev, 1972; Moreton, 1972). In our examinations the spontaneous activity of most neurones ceased in the Na^+-free solution within several minutes suggesting that Na^+ appears to be the main carrier of the current involved in the generation of action

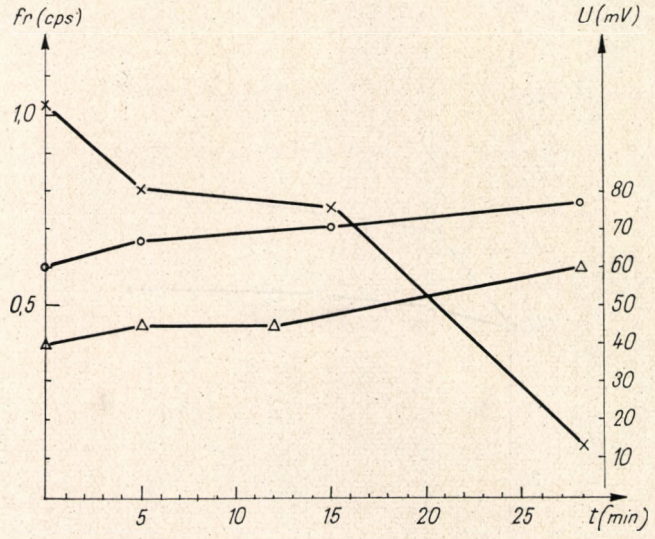

Fig. 13. Influence of the K^+-free solution on the parameters of spontaneous activity of Pl neurone

Fig. 14. Sudden hyperpolarization under the influence of the K^+-free solution in the 6th min. after exchanging the physiological solution on Pl neurone

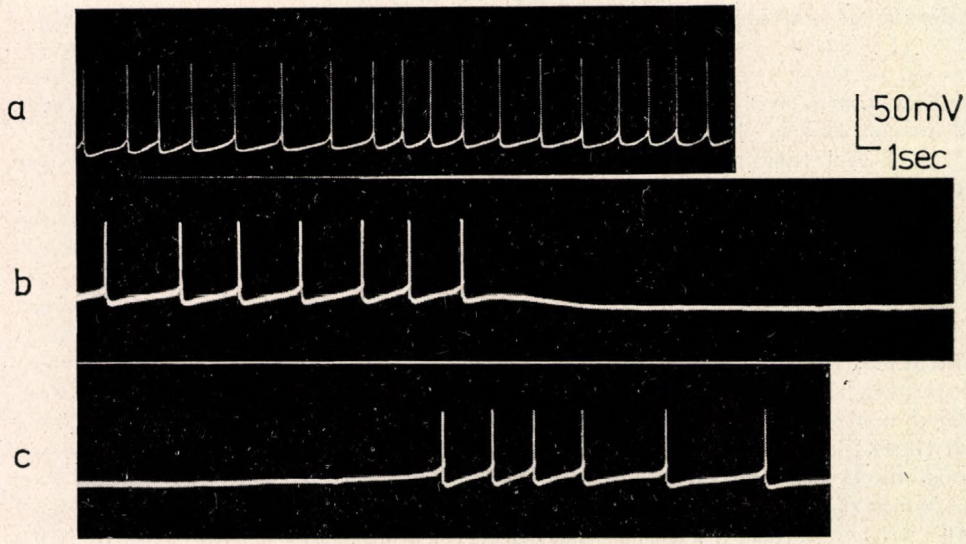

Fig. 15. Effect of the K^+-free solution on A10 cell. *a)* control activity which is not interrupted by ILD-s; *b)* and *c)* in the 4th min. after the application of the K^+-free solution, the continuous spontaneous activity is broken off by a hyperpolarizing phase similar to ILD

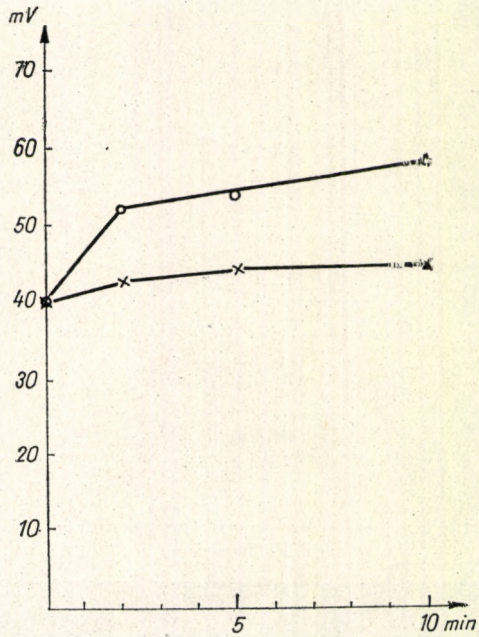

Fig. 16. Development of the hyperpolarizing effect of the K^+-free solution in the case of recording with a KCl-filled (—x—) and a NaCl-filled (—o—) electrode. Both curves show an average of values obtained on the examined neurones

potential. Generally, considering especially the case, when the spike generation in the Na^+-free solution is abolished after a considerable time only, some considerations contradict the presumption of a "calcium-spike":

1. If Ca^{++} were the main carrier of current, one would expect the removal of Ca^{++} to block the generation of the spike most rapidly on those cells whose activity is less sensitive to the removal of Na^+, e.g. P1 neurone. However, this is not supported by the experimental results.

2. Activation of the silent neurones by exposure to the Ca^{++}-free solution is hardly compatible with a "Ca-spike" hypothesis.

3. It was previously shown, that the inward current which had disappeared in Na^+-free solution did not reappear by raising the Ca^{++} concentration.

The question arose, what causes the different sensitivity of different identified neurones to the removal of Na^+? In our earlier papers (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971) it was described that the membrane potential, rhythmic activity and chemical sensitivity are specific to an identified neurone, thus it is conceivable that the ionic mechanisms underlying all these phenomena may also be characteristic of a given cell. Nevertheless, most likely a retention of Na^+ near the membrane has to be considered, too — similarly to other objects. MORETON (1972) attributed the retention of Na^+ to the function of an active pump, which requires some time to turn into an active state under Na^+-free conditions. This suggestion is based on the observation that after an early transitional decrease in the amplitude the spike can be restored. In that cases, when the spontaneous activity of the

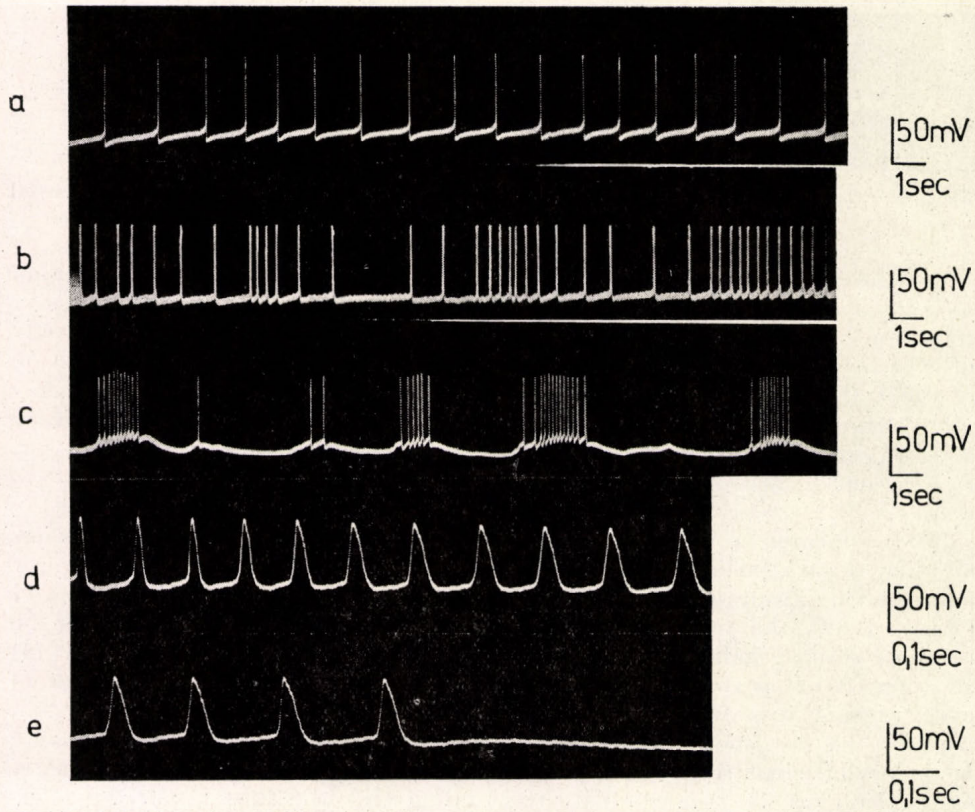

Fig. 17. Effect of the Cl^--free solution. *a)* control activity of A11 neurone; *b)* activity of the same neurone in the 3rd min. following the application of Cl^--free solution. *c)* in the 16th min. the activity assumes a bursting character *d)* and *e)* (continuously) changes in the shape of the action potentials during a burst

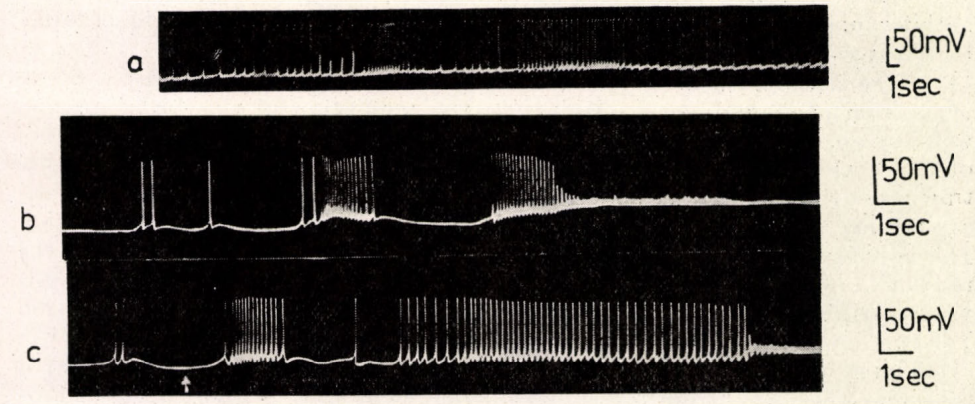

Fig. 18. Effect of the Cl^--free solution on A10 neurone. *a)* control activity demonstrating well the burst-like grouping of the spikes; *b)* in the Cl^--free solution the bursting activity becomes more pronounced; *c)* in the Cl^--free solution ACh applied at the arrow prolongs the time course of the burst

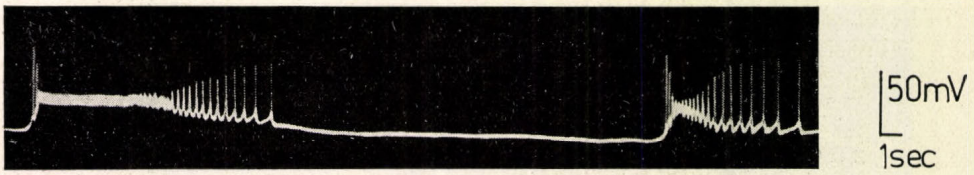

Fig. 19. Effect of the Cl^--free solution on A10 cell. It is fairly visible that the potential generation is inactivated within a burst at a depolarized level

cells examined in the present experiments ceased within 10 min., we found no sign of this phenomenon thus, in this case one can consider a passive retention of Na^+ at the most, variations of which depending on the anatomy of cells may account for the variability of the time required for the abolition of the activity of different identified neurones. However, on the cells firing more than 6—8 min. in absence of Na^+ one can frequently observe a recovery after an early decrease in the spike amplitude and in the firing rate consequently if an active depot exists, it requires at least 6—8 min. to turn into an active state.

It appeared to be characteristic of all the neurones that the spontaneous activity ceased earlier than the excitability of the soma. As on the giant neurones the spontaneous activity is generated in a distant part of the axon (TAUC, 1962), the present observation has from a new side confirmed the results obtained indirectly by voltage clamp technic (MAGURA et al., 1971) showing that it is the soma which reacts later to the removal of Na^+. However, present investigations do not allow to formulate a definite decision, whether this fact shows an actual difference in the ionic mechanisms, or there is a more efficient retention of Na^+ near to the soma membrane. The latter assumption can be supported by the finding that the time required for the abolition of the excitability of the soma does not considerably vary from neurone to neurone, it is 18—20 min. after removing the external Na^+. On the basis of the latter assumption it means the time required for a total running out of Na^+-depot.

The difference in the Na^+ requirement for spontaneous and evoked activity observed in our experiments is in contrast with the data obtained by CARPENTER and GUNN (1970).

The ionic permeability of the membrane is an important factor of the generation of spontaneous activity. In the regulation of this Ca^{++} plays a well-known role. After the elimination of the membrane-stabilizing action of Ca^{++} the alteration of Na^+- and K^+-permeability can account for the transitional depolarization, increased firing rate and finally a complete disappearance of the excitability of the membrane in the Ca^+-free solution. The individual identified neurones show differentiated behaviour also in this respect, however, the time required for the abolition of spontaneous activity varies within a more limited interval compared with the Na^+-free solution. For the explanation of the observed deviation of data concerning Ca^{++} it is even more obvious to suppose a certain degree of retention, because after removing the Ca^{++} from the solution some membrane-bound Ca^{++} remains.

Recently a number of authors have proved the role of an electrogenic Na^+-pump in addition to the unequal distribution of the ions in the maintenance of the membrane potential of giant neurones (KERKUT and THOMAS,

1965; CARPENTER and ALVING, 1968; KOSTYUK et al., 1972; CHRISTOFFERSEN, 1972). On the basis of our results there is a reason to suppose a contribution of electrogenic Na^+-pump to the membrane potential also of the examined neurones of *Lymnaea*. This is indicated by the fact that in the K^+-free solution there is only a slight increase in the resting membrane potential although the $[K]_o - [K]_i$ concentration gradient has increased considerably. It can be explained if the resting potential measured under physiological conditions is not a pure K^+-potential but it is resulted also from the simultaneous function of an electrogenic Na^+-pump. The latter can be blocked by the removal of external K^+, thus in the K^+-free solution the increase in the resting potential cannot reach the theoretically expected value. On *Aplysia* neurones CARPENTER (1970) obtained a pronounced depolarization under K^+-free conditions at 25° C and explained this phenomenon in a similar way.

The above explanation is confirmed by our examinations with NaCl-filled electrodes. The microelectrodes with tip-diameter of about 1 μ had been kept in the cell for 5—10 min. before the K^+-free solution was applied. During this time some spontaneous outflow of Na^+ probably occurred, which might have been of extremely small amount though, nevertheless, sufficient to stimulate the Na^+-pump. The fact that on increasing the intracellular concentration of Na^+ this stimulating effect can be obtained has been described by several authors (KERKUT and THOMAS, 1965; THOMAS, 1969; CHRISTOFFERSEN, 1972).

One possible explanation of the transitional hyperpolarization observed in the Na^+-free solution is a high resting Na^+-permeability of the membrane. In consequence of the removal of Na^+ the voltage opposed to K^+-potential resulted from the Na^+-gradient is eliminated, thus the membrane potential can much better approximate the value to be expected on the basis of the concentration gradient of K^+.

The reason of the burst-like activity developed in the Cl^--free solution may be approximated by the following assumption: In the Cl^--free solution HODGKIN and HOROWITZ (1959) demonstrated an about 20 mV transitional depolarization of the membrane, which lasted for 15—20 min. This coexisted with an increased outflow of K^+. The decrease in resting potential may temporary activate the potential generation. It might be supposed, that the K^+-conductance of the membrane is also damaged resulting in a delayed repolarization phase and an inactivation of generation of the spike at a depolarized level as the increased Na^+-influx during a burst cannot be balanced by the outward K^+-current. Besides, after a certain time a hyperpolarizing phase is resulted by stimulating the activity of the electrogenic Na^+-pump.

Concerning the aberrant behaviour of some neurones obviously it has to be considered that the majority of the examined neurones has synaptic inputs. On the cell A10 and on several other cells sometimes it can fairly be observed that the bursts produced in the Cl^--free solution are preceded by EPSP-s. The possibility cannot be disregarded that on the cells having excitatory synaptic input the effect of the absence of Cl^- may be realized through the postsynaptic membrane, too.

The reactions given to depolarization and ACh in different ion-free solutions generally did not differ from the control. However, in the Na^+-free solution there were some cells which could be activated only by depolarization but were uneffected by ACh. In these cases the mediator effect probably is

realized by changing the Na^+-permeability. CHIARANDINI et al. (1967) suggested the existence of such a mechanism on a cell of CILDA type. In our earlier experiments (KISS and SALÁNKI, 1971) performed on CNS of Lymnaea one of the neurones — marked A10 — was identified as a cell of CILDA type. However, the absence of Na^+ has no influence on the reaction to ACh on this neurone, consequently, the above suggestion cannot be generalized.

Summary

On changing the ionic environment of CNS of *Lymnaea stagnalis* it has been established that

1. On removing the Na^+ the activity of several neurones ceased within a short time, while that of the other neurones ceased later. Even such a neuron was found, which continued to generate action potentials for as long as 30 min. in the absence of Na^+.

After the activity stopped the cells could be activated by depolarization, while only a part of the neurones was affected by ACh under such conditions.

2. In Ca^{++}-free solution the spontaneous activity of the cells was abolished within a relatively short time, but the activity of several cells was maintained for 15—20 min.

3. In the absence of K^+ a relatively small hyperpolarization was accompanied by a decrease in the firing rate.

4. In the absence of Cl^- the rhythm of activity of the cells became irregular, sometimes it assumed a bursting character.

The obtained data can be accounted for partly by different changes in the ionic concentration gradients and in the permeability of the membrane, partly by an influence on the electrogenic Na^+-pump.

REFERENCES

CARPENTER, D. O. (1970): Membrane potential produced directly by the Na^+ pump in *Aplysia* neurons. — *Comp. Biochem. Physiol.* **35,** 371—385.

CARPENTER, D. O., ALVING, B. O. (1968): A contribution of an electrogenic Na^+ pump to membrane potential in *Aplysia* neurons. — *J. Gen. Physiol.* **52,** 1—19.

CARPENTER, D. O., GUNN, R. (1970): The dependence of pacemaker discharge of *Aplysia* neurons upon Na^+ and Ca^{++}. — *J. Cell. Physiol.* **75,** 121—128.

CHAMBERLAIN, S. G., KERKUT, G. A. (1969): Voltage clamp analysis of the sodium and calcium inward currents in snail neurones. — *Comp. Biochem. Physiol.* **28,** 787—801.

CHIARANDINI, D. J., STEFANI, E., GERSCHENFELD, H. M. (1967): Ionic mechanism of cholinergic excitation in molluscan neurons. — *Science* **156,** 1597—1599.

CHRISTOFFERSEN, G. R. J. (1972): Steady state contribution of the Na^+- K^+- pump to the membrane potential in identified neurons of *Helix aspersa*. — *Acta Physiol. Scand.* **86,** 498—514.

GEDULDIG, D., JUNGE, J. (1968): Sodium and calcium components of action potential in the *Aplysia* giant neurone. — *J. Physiol. (London)* **199,** 347—365.

GERASIMOV, V. D., KOSTYUK, P. G., MAISKI, V. A. (1965): Excitability of giant nerve cells of various pulmonate molluscs in sodium-free solutions. — *Fed. Proc.* **24,** T676.

HODGKIN, A. L., HOROWITZ, P. (1959): The influence of K^+ and Cl^- ions on the membrane potential of single muscle fibres. — *J. Physiol.* **148,** 127—160.

HODGKIN, A. L., HUXLEY, A. F. (1952): Currents carried by Na^+ and K^+ ions through the membrane of the giant axon of *Loligo*. — *J. Physiol.* **116,** 449—472.

JERELOVA, O. M., KRASTS, I. V., VEPRINTZEV, B. N. (1972): The effect of Na^+, Ca^{++} and Mg^{++} on the amplitude of the action potential from giant neurons of *Lymnaea stagnalis*. — *Comp. Biochem. Physiol.* **40,** 281—293.

JULLIEN, A., RIPPLINGER, J. (1948): Sur l'automatisme du isolé du coeur de Lymnée. — *C. R. Acad. Sci. (Paris)* **226,** 1396.
KERKUT, G. A., THOMAS, R. C. (1965): An electrogenic sodium pump in snail nerve cells. — *Comp. Biochem. Physiol.* **14,** 167—183.
KISS, I., SALÁNKI, J. (1971): The heterogenic chemical sensitivity of the central neurones of *Lymnaea stagnalis.* — *Annal. Biol. Tihany* **38,** 39—52.
KISS, I., SALÁNKI, J., VÉRÓ, M. (1972): Dependence of reaction to ACh on the membrane potential of neurones of *Lymnaea stagnalis.* — *Annal. Biol. Tihany* **39,** 21—27.
KOSTYUK, P. G. (1968): Ionic background of activity in giant neurons of Molluscs. — *Neurobiology of Invertebrates* (Ed.: J. SALÁNKI). *Akadémiai Kiadó, Budapest and Plenum Press, New York* pp. 145—167.
KOSTYUK, P. G., KRYSHTAL, O. A., PIDOPLICHKO, V. I. (1972): Potential dependent membrane current during the active transport of ions in snail neurones. — *J. Physiol.* **226,** 373—392.
KRASTS, I. V., VEPRINTZEV, B. N. (1972): The giant neurons of *Tritonia:* Its electric properties and the ionic dependence of the action potential. — *Comp. Biochem. Physiol.* **41,** 289—296.
MAGURA, I. S., KISS, I., KRYSHTAL, O. A. (1971): Current-voltage relations of the giant neurone soma membrane of *Lymnaea stagnalis.* — *Acta physiol. Acad. Sci. hung.* **40,** 221—228.
MEVES, H. (1968): The ionic requirements for the production of action potentials in *Helix pomatia* neurons. — *Pflügers Arch.* **304,** 214—241.
MORETON, R. B. (1972): Electrophysiology and ionic movements in the central nervous system of the snail, *Helix aspersa.* — *J. Exp. Biol.* **57,** 513—541.
OOMURA, Y., OZAKI, S., MAENO, T. (1961): Electrical activity of a giant nerve cell under abnormal conditions. — *Nature (Lond.)* **191,** 1265—1267.
SALÁNKI, J., KISS, I. (1969): Identified cells in the central nervous system of *Lymnaea stagnalis* L. — *Annal. Biol. Tihany* **36,** 63—75.
SATTELLE, D. B., LANE, N. J. (1972): cit. in: Moreton, R. B. (1972): Electrophysiology and ionic movements in the central nervous system of the snail, *Helix aspersa.* — *J. Exp. Biol.* **57,** 513—541.
TAUC, L. (1962): Site of origin and propagation of spike in the giant neuron of *Aplysia.* — *J. Gen. Physiol.* **45,** 1077—1097.
THOMAS, R. C. (1969): Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. — *J. Physiol.* **201,** 496—514.
THOMAS, R. C. (1972): Electrogenic sodium pump in nerve and muscle cells. — *Physiol. Rev.* **52,** 563—595.
WAZIRI, R., FRAZIER, W., KANDEL, E. R. (1965): Analysis of pacemaker activity in an identifiable burst generating neuron in *Aplysia.* — *Physiologist* **8,** 300—310.

AZ IONMILIŐ SZEREPE *LYMNAEA STAGNALIS* L. ÓRIÁS NEURONJAINAK POTENCIÁL GENERÁLÁSÁBAN

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Összefoglalás

Az ionmiliő változtatása során megállapítást nyert:

1. Na^+ megvonás esetén egyes neuronok aktivitása hamarabb, másoké később szűnik meg. Találtak olyan idegsejtet is, amely Na^+-hiányban 30 perc múlva is generál akciós potenciált.

A sejtek leállás után is aktiválhatók voltak depolarizációval, míg ACh-ra ilyenkor csak a neuronok egy része válaszolt.

2. Ca^{++}-mentes oldatban a sejtek spontán aktivitása viszonylag rövid időn belül megszűnt, de néhány sejt ilyenkor is 15—20 percen át aktív maradt.

3. K^+-hiány esetén viszonylag kismértékű hiperpolarizáció mellett az aktivitás frekvenciája csökkent.

4. Cl^--hiányban a sejtek aktivitási ritmusa szabálytalanná vált, egyes esetekben burst-ölő jelleget vett fel.

A kapott eredmények részben az ion koncentrációgradiensek és a membrán permeabilitás megváltozásával, részben az elektrogén Na^+-pumpa befolyásolásával magyarázhatók.