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THE EFFECT OF THE CHANGE OF ELECTRODE DIMENSIONS AND DISTANCE ON THE ACTION POTENTIAL INDUCED BY ELECTRIC EXCITATION OF THE UNMYELINATED NERVE *ANODONTA CYGNEA* L.

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The cerebrovisceral connective (CVC) of the fresh water mussel *(Anodonta cygnea* L.) is paired nerve consisting of $0.1-2\ \mu$ diameter unmyelinated elementary fibres (LÁBOS, ZS.-NAGY, BENKŐ, SALÁNKI 1963).

The shape of the action potential induced by electric excitation is very manyfold (ZHUKOV, 1946, KAHN and KUZNETZOV 1938, SALÁNKI, LÁBOS 1964).

In the following, using square wave electric excitation and RC amplification of 0.75 sec time constant we are going to systematically examine the effect of some conditions of stimulation earthing and recording — in the first place of distance between the electrodes and electrode dimensions — on the shape of the action potential, the size, number and polarity of its components.

The question is topical because some contradictions can be observed examining the electrophysiological properties of the nerve which may be summarized as follows:

1. Acting with various pharmacons on the CVC one component of the action potential induced by electric excitation in every case remained unchanged although some components beyond doubt changed (SALÁNKI, LÁBOS 1964).

2. In the determination of velocity of conduction a difference arises between literary data (ZHUKOV 1946) and our own values (LÁBOS and SALÁNKI 1964).

3. Number and size of the components related to each other showed deviations which can not be explained by differences of the stimulus-parameters.

4. The artefact is in all cases substantially differentiated. This points to the fact that the action potential led with RC amplification is itself distorted, showing more components than are in reality.

The above facts and a communication of GASSER (1960) prompted us to subject the effect of the methodical assumptions to a careful examination. GASSER changed the distance of the leading electrodes in the course of common bipolar lead and observed at a certain length the phenomenon of the so-called "lead separation effect" which appears as a positive wave in the action potential, does not reflect a new component and is regarded by GASSER as a biophysical property of the normal nerve fibre which is connected with the longitudinal polarisation. GASSER, applying a proper technique (delayed integration) obtained that the fibre spectrum of the saphenus nerve of cat became considerally more homogeneous and a more direct connection with the histological fibre spectrum could be demonstrated. Thus it appeared that waves which were considered as important do not reflect any fibre group.

A similar problem exists concerning the CVC of the fresh water mussel. The electron microscopic fibre spectrum is considerably more homogeneous than the action potential, even in the case when in the theoretical reconstruction of the action potential a certain connection between fibre diameter, fibre cross section, velocity of conduction and amplitude is assumed (LÁBOS, ZS.-NAGY, BENKŐ, SALÁNKI 1963).

Results of the technique applied by A. GASSER (1960) are reminding us as pointed out by GASSER himself that the biophysical properties of the nerve and the mechanism of the spreading of waves are not sufficiently known. Probably beside the geometrical data of the fibres other factors not directly connected with these are involved in the formation of the velocity of conduction.

In the following some controversial issues are raised in the case of a nerve in which the methodical conditions on account of the great longitudinal resistance of the nerve are stressed. The high longitudinal resistance is a consequence of the low values of the elementary fibre diameter (LÁBOS and coworkers 1962). The ohmic resistance of 1 cm of the frog's ischiadicus is 40—60 kΩ while the resistance of 1 cm of CVC is 15—20 times greater. This conditions for the observation of the lead — separation — effect are 20 times more favourable.

Method

The freshly excised nerve was kept in MARCZINSKY's (1959) physiological solution until the lead off. The composition of the solution is 1.23 g $CaCl_2$, 5.12 g $MgCl_2 6H_2O$, 0.04 g $MgSO_4 \cdot 7H_2O$, 0.76 g KCl, 28.3 g NaCl, 0.08 g $NaBr.2H_2O$, 0.35 g $NaHCO_3$ ad 1000 ml aqua dest. This basic solution must be further diluted with distilled water in the 4 : 96 ratio.

In the course of leading and excitation the nerve was placed in paraffin oil.

The leading and stimulating electrodes were 0.15 mm diameter Ag wires while the earth plate was Ag-plate.

The stimulator used has cathode output and its parameters can be changed independently of each other. Single square wave impulses were employed. There was generally no isolator between preparation and nerve but in some cases high frequency isolator was used.

The RC amplifier used was of 0.75 sec time constant; its input was symmetric. The values of the grid leak resistance: 2×3.3 MΩ.

Results and discussion

1. *Stimulation and lead off with 3 electrodes*

Since two electrodes are needed for each excitation and lead, one of the three electrodes is a common leading and stimulating electrode. Such type of leading-excitation is the local lead *(Fig. 1/A)*. One of the electrodes of the cathode output stimulator (500 or 2000 Ω cathode resistance) is grounded and thus the common electrode will be earthed since otherwise it could not carry out the absolutely necessary "anti-interference filter" function because it does not fall between the loci of stimulation and lead.

Consequently in this case the lead is anyway asymmetric.

CVC is a nerve of high resistance. The resistance of its 1 cm stretch is 0.7—1 MΩ depending on the diameter of the nerve. In the following the influence of the situation related to each other of the stimulating earth and leading electrode on the properties of the action potential led was examined. Presumably it is the consequence of the high resistance of the nerve fibre that slight electrode dislocations are accompanied by considerable changes on the action potential.

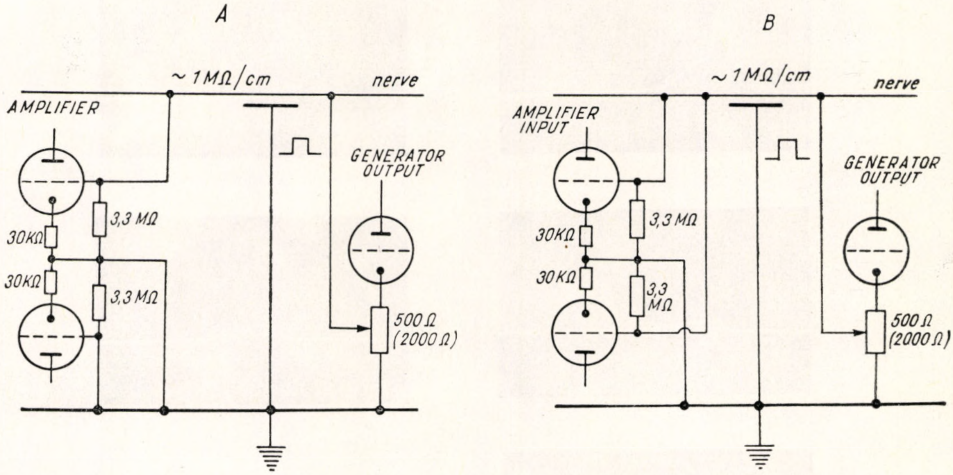


Fig. 1. A) Excitation and leading off with 3 electrodes. Connection between nerve, output and amplifier input
B) Excitation and leading off with 4 electrodes

1. ábra. A) Ingerlés és elvezetés 3 elektródával. Az ideg, az ingerlőkimenet az erősítőbemenet kapcsolata. B) Ingerlés és elvezetés 4 elektródával

a) The change of the $\overline{SG_1}$ distance

Increase of the $\overline{SG_1}$ distance is accompanied by the same type of changes of the action potential as the reduction of nerve tension or period with a constant $\overline{SG_1}$ distance *(Figs. 2, 3)*. The effect of the increase of the $\overline{SG_1}$ distance can be compensated *e.g.* with the increase of the voltage.

One of the causes of the phenomenon is that 1 mm electrode motion means the putting in the stimulating circuit of 70—100 kΩ resistance which leads to a reduction of the current. On the other hand, beside the decrease of the effective excitation, the geometrical conditions of the origin of the impulse are different. The importance of the latter factor is less conspicuous in this experiment. The exact role of the capacitive factor can not be appreciated but beyond doubt the equivalent circuit of the passive elements of the system from the view point of signal transmission is an RC circuit of resistance output, since the artefact is highly differentiated even in the case of a stimulation signal of a few msec, which can not be distorted so far by the 0.75 sec time constant of the RC amplifier. This means that for the stimulation signal the nerve behaves as a system of small time constant, while in the development of the action potential the longitudinal resistance of 1 MΩ/cm is effective which speaks in favour of a high time constant of the system. It may be explained by thin electrolyte-layer on the surface of the nerve.

Thus the analysis of the results obtained by the change of $\overline{SG_1}$ distance reveals that the reduction of amplitude observed at the increase of electrode distances can not be absolutely regarded as a mark of the decremen-

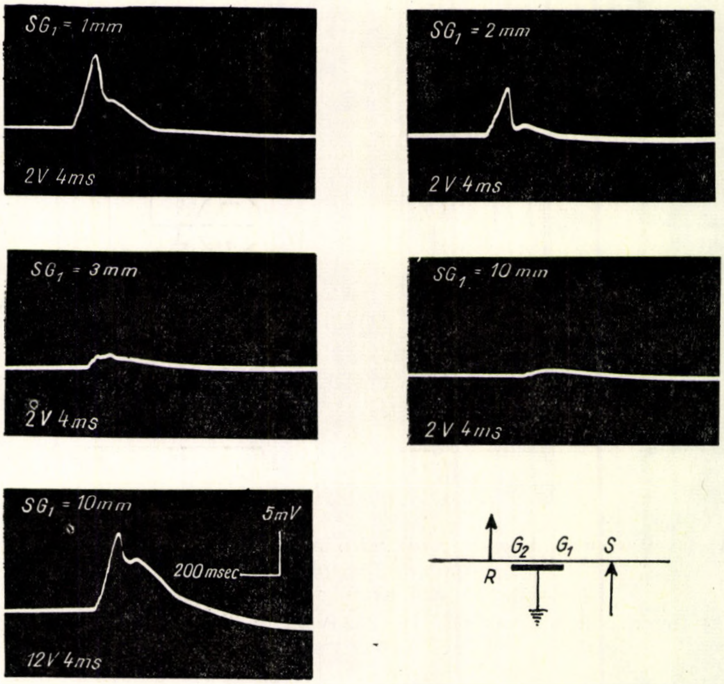


Fig. 2. The external of the excitation circuit is increasing (the $\overline{SG_1}$ stretch increases, the electrode S moves off). The value of the potential led off diminishes. On the last Figure it can be seen that with the increase of the voltage the effect can be over-compensated

2. ábra. Az ingeráramkör külső ellenállása növekszik ($\overline{SG_1}$ szakasz nő, az S elektród távolodik). Az elvezetett potenciál nagysága csökken. Az utolsó ábrán látható, hogy az ingerfeszültség növelésével a hatás túlkompenzálható

tal impulse led of the nerve even if one of the electrodes is stimulating while the other leading (or earth). This phenomenon is called pseudo-decrement. Stimulation with constant current is necessary in the course of further investigations.

The dependence of the amplitude on $\overline{SG_1}$ is linear on an interval only *(Fig. 3)*.

b) Effect of change in length of the earth electrode

Application of an earth electrode of great extension makes the locus where the excitation arises uncertain, since in a cathode output stimulator the negative pole is the earth electrode which generally used to be a plate of a few mm length. The locus where the impulse originates is the negative pole which here — as evidenced by the experiments discussed below — must be longer than 5 mm. This, in the case when the other electrodes are near the earth electrode, with a lead either asymmetrical or symmetrical *(i.e.* when there is no common electrode between the excitation circuit and the lead) may cause a very considerable error in the determination of velocity of lead. From

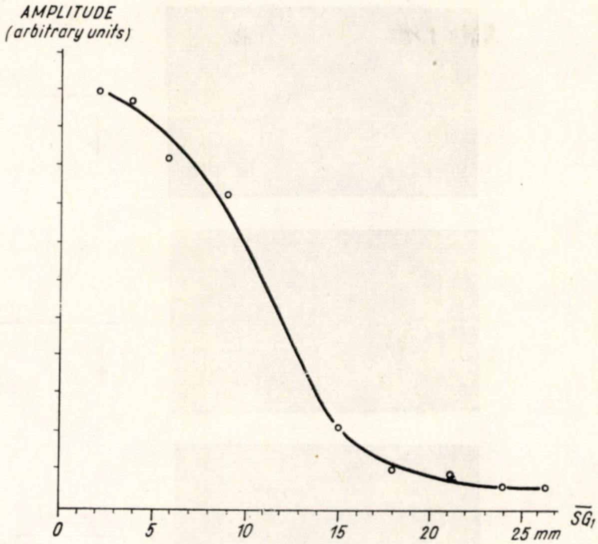


Fig. 3. Abscissa: distance $\overline{SG}_1$. Ordinate: the value of the highest component. Other conditions are unchanged

3. ábra. Abszcissza: $\overline{SG}_1$ távolság. Ordináta: a legmagasabb komponens nagysága. Egyéb körülmények változatlanok

here it originates that apparently the velocity of conduction depends also on the pathway of impulse.

Therefore we must endevour to reduce the size of the earth electrode. Keeping the distance $\overline{SR}$ and $\overline{SG}_1$ at a constant value, when reducing the size of the earth electrode the following phenomena can be observed *(Fig. 4)* :

1. the amplitude of the potential led off increases,
2. the period of the potential led off decreases,
3. the distribution is of a lesser degree, no components can be separated,
4. the differentiated artefact and the slow potential at an earth length value "separate" from each other (at 5—7 mm) or flow together respectively.

The essence of the phenomenon is, consequently, "synchronization" with the reduction of earth length and "desynchronization" with its increase. The "separation" between the action potential and the artefact points to the character of non-continuous of the function describing the connection between some parameters of the induced potential and the earth length.

The stimulation effect, the current density are decreasing under the large electrode, this is why the potential led off is smaller at a great earth length.

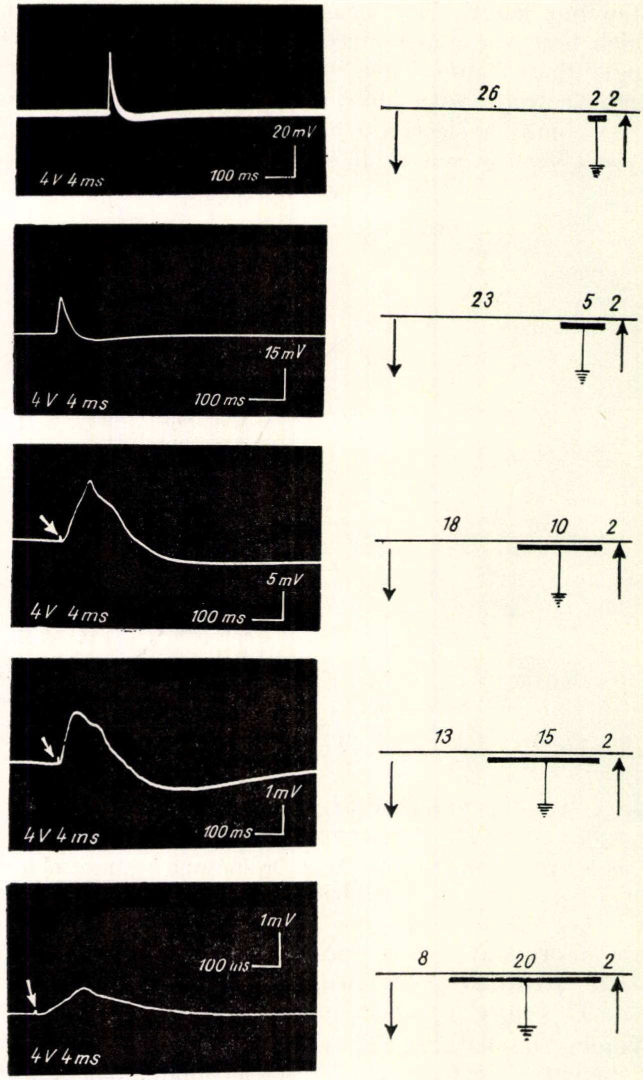


Fig. 4. Increasing the length of the earth electrode. The arrow leading to comes from the warm point of the out put of the excitor, while the arrow leading off from the free grid of the amplifier. Numerals in mm signify the data for length

4. ábra. A földelektród hosszának növelése. Az odavezető nyíl az ingerlő kimenetének meleg pontjától az elvezető nyíl az erősítő szabad rácsától jön. A számok mm-ben a hosszúság-adatokat jelentik

The disintegration into components may be the consequence of increased differentiation which is equivalent to the reduction of the time constant of the system.

Under an earth electrode length of about 5 mm for the greatest part the behaviour of an amplifier is reflected which has received a too great input signal. In such cases the action potential and the artefact can not be distinguished that is the "irruption of stimulus" covers the impulse. It is probable that the slow signal remaining after the application of the blocking pharmacons is the artefact caused by the irruption of stimulus which on account of the

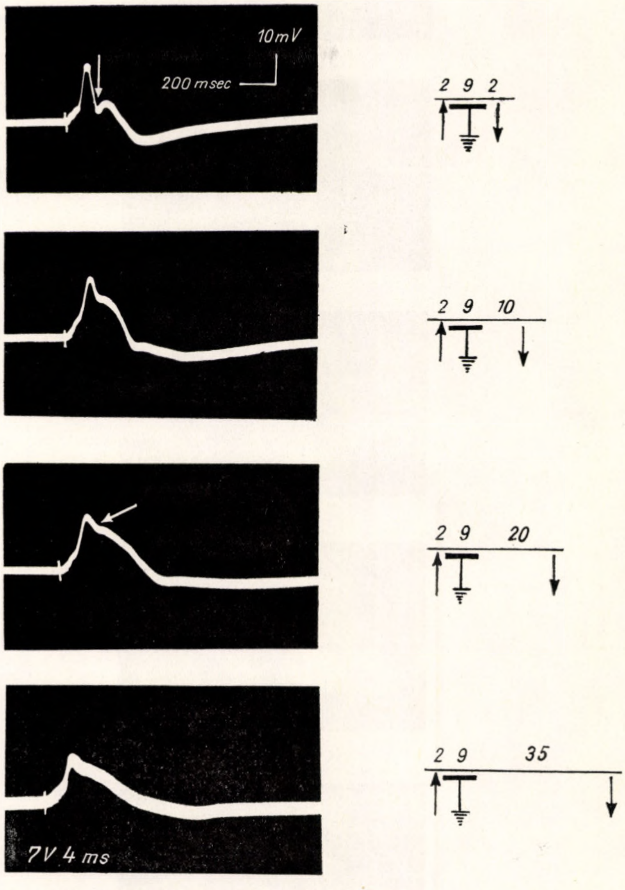


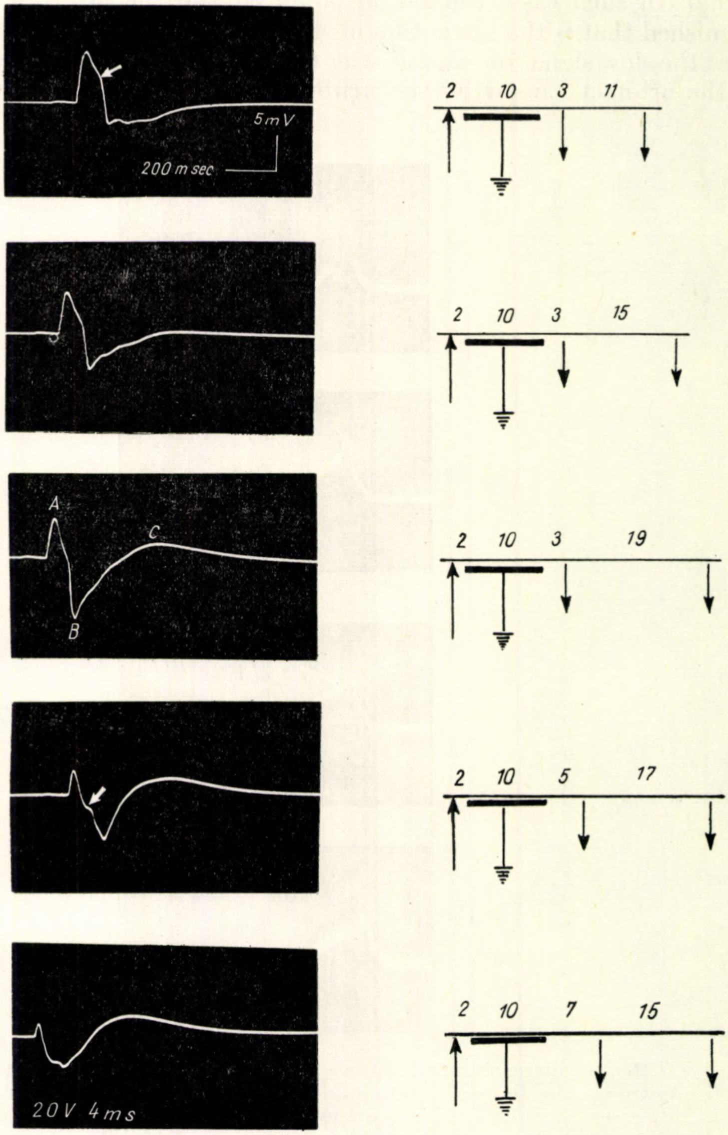
Fig. 5. Effect of increasing the distance between the active electrode leading off and the earth electrode. The figures in mm stand for the data of length. For explanation of the arrow see text

5. ábra. Az aktív elvezető elektród és a földelektród közötti távolság növelésének hatása. A számok mm-ben jelentik a hosszadatokat. A nyíl magyarázatát lásd a szövegben

capacity of the system extends in time to several times 10 msec. This is supported by experiments on died nerves and unliving filaments.

c) The effect of the change of the $\overline{RG_2}$ distance

The effect of changes in $\overline{RG_2}$ distance at a constant value of the earth length and the SG_1 stretch is shown in *Fig. 5.* where the increase of the size of the wave marked with an arrow and the high, elongated character of the late



6. *ábra*. Elvezetés 4 elektródával
Fig. 6. Leading off with 4 electrodes

components is seen. Also the value of the greatest component and the "after potential" is diminishing.

The change marked with an arrow is — in our opinion analogous with the "lead separation effect" described by Gasser. Leading off an action potential from the *n. saphaenus* of a cat Gasser observed the phenomenon within the quickest fibre group. In the present case it is more pronounced, a cause of which in our opinion may be the great resistance of the nerve.

LORENTE DE NO (1947 and 1959) and P. MUELLER (1958) who examined the phenomenon, came to the conclusion that it is the consequence of longitudinal polarisation and may have a role in the normal conduction of impulse. The question evidently requires analysis because it influences many hypotheses built on fibre spectrum analysis.

2. *Leading off with 4 electrodes*

With a bipolar symmetrical lead *(Fig. 1* B) the classic two-phase potential must be obtained. The action potential seen on *Fig. 6* shows the biphase picture of a potential with 2 components. Keeping constant the other electrode distances the abduction of the distal electrode leads to the deepening of the late phase.

At the motion of the proximal electrode the first component diminishes while the other phase shows less change and the after potential significantly increases. The potential essentially gets reversed.

The explanation of the phenomena observed is not simple because the difference in resistance of the stretches between the electrodes leading off spoils the symmetry of the input. Thinking in the usual terms the lowering of the deflexion marked with an arrow and the considerable changes of the proportions of the main waves (A, B, C) can not be explained. The solution must be sought in the different mode of spreading of the components of the impulse wave. At any rate it is beyond doubt that a significant part of the after potential is a consequence of the 2. phase and it may be assumed that the phenomenon manifests itself also in the essentially bipolar asymmetric lead.

Conclusions and summary

1. From the cerebrovisceral connective (CVC) of *Anodonta cygnea* L. action potential was led off with RC amplifier ($T = 0.75$ sec) excited by square impulse.

2. The amplifier used is of symmetric input (2×3.3 MΩ) the impulse of cathode output (~ 1000 Ω) the nerve of high resistance (1 MΩ per cm).

3. Excited with three electrodes the change of the electrode distances had the following effect on the action potential:

a) Upon the increase of the distance of the stimulating pair of electrodes the action potential decreased very strongly and on a stretch linearly. The phenomenon is ascribed to the decrease of the stimulating current.

b) Increase of the earth electrode (plate) length led to the decrease of the initially uniform artefact-action potential complex, to desynchronisation and separation of the artefact. Possible changes in the equivalent of the system were discussed. The phenomenon points to a connexion between the initial component and the stimulus irruption. Uncertainty of velocity measurement is due to the impulse of large extent.

c) Increase of the distance between electrodes leading off points to the "lead separation effect" (GASSER).

4. Data obtained during the course of "symmetric" bipolar leading off seem to point out that symmetry can be hardly realized in the case of a nerve of high resistance. The reversal of the potential and the shifting of the proportions of components point to the complicated mechanism of wave spreading.

5. The factors examined point to some causes of the "variability" of the action potential which can not be left out of consideration when discussing the importance of the components.

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ELEKTRÓDAMÉRET ÉS TÁVOLSÁG VÁLTOZTATÁSÁNAK HATÁSA VELŐHÜVELYMENTES IDEG ELEKTROMOS INGERREL KIVÁLTOTT AKCIÓS POTENCIÁLJÁRA

Lábos Elemér

Összefoglalás

1. *Anodonta cygnea* L. cerebrovisceralis konnektivumáról (CVC) vezettünk el akciós potenciált RC erősítővel ($T = 0,75$ sec) négyszögimpulzussal ingerelve.

2. A használt erősítő szimmetrikus bemenetű ($2\times3,3$ MΩ), az ingerlő katódkimenetű ($\sim 1000\,\Omega$), az ideg nagy ellenállású volt (1 MΩ cm-ként).

3. Három elektródával ingerelve az elektródatávolságok változtatása a következő hatással volt az akciós potenciálra:

a) Az ingerlő elektródapár távolságának növelésére az akciós potenciál igen erősen és egy szakaszon lineárisan csökkent. A jelenséget az ingeráram csökkenésének tudjuk be.

b) A földelektród (lemez) hosszának növelése a kezdetben egységes artefaktum akciós potenciál komplex csökkenéséhez, deszinkronizációhoz és az artefaktum leválásához vezet. Taglaltuk, hogy a rendszer ekvivalensében ez milyen változást eredményezhet. A jelenség a kezdeti komponens és az ingerbetörés összefüggésére utal. A sebességmérés határozatlansága a nagykiterjedésű ingerlés miatt áll fenn.

c) Az elvezető elektródák távolságának növelése a „lead separation effect"-re utal (GASSER).

4. A „szimmetrikus" bipoláris elvezetés során kapott adatok rámutatnak arra, hogy a szimmetria nagy ellenállású ideg esetén alig valósítható meg. A potenciál megfordulása és a komponensek arányainak eltolódása a hullámterjedés bonyolult mechanizmusára utalnak.

5. A vizsgált tényezők rámutatnak az akciós potenciál „változékonyságának" néhány okára, amelyek a komponensek jelentőségének taglalásakor figyelmen kívül nem hagyhatók.

ВЛИЯНИЕ ИЗМЕНЕНИЯ РАССТОЯНИЯ И РАЗМЕРА ЭЛЕКТРОДОВ НА ПОТЕНЦИАЛ ДЕЙСТВИЯ БЕЗМИЕЛИНОВОГО НЕРВА, ВЫЗВАННЫЙ ЭЛЕКТРИЧЕСКИМ РАЗДРАЖЕНИЕМ

Э. Лабош

1. Автор отводил потенциал действия с церебро-висцерального коннектива (ЦВК) беззубки (Anodonta cygnea L.) при помощи усилителя типа RC (T = 0,75 сек) после раздражения его прямоугольными импульсами.

2. Применяемый усилитель имеет симетричный вход (2×3,3 M), выход у стимулятора — катодный (~1000 Ω.), сопротивление нерва было большим (1 MΩ по 1 см).

3. При раздражении и отведении с тремя электродами изменение расстояния электродов привело к следующим изменениям в потенциале действия:

а) при увеличении расстояния раздражающих электродов потенциал действия сильно снижается, это снижение было линейным на определенном участке. Это явление объясняется снижением раздражающего тока,

б) увеличение длины заземляющего электрода (пластинки) приводит к снижению и к десинхронизации комплекса артефакта потенциала действия, и потом артефакт отделяется от потенциала действия. Это явление указывает на взаимосвязь начального компонента и вхождения раздражения. Неопределенность измерения скорости наступает вследствие большого распрастранения раздражения,

в) увеличение расстояния отводящих электродов указывает на принцип «lead separation effect» (Gasser).

4. Данные, полученные при «симметричном» биполярном отведении, указывают на то, что симметрия на нерве с большим сопротивлением почти неосуществляема. Поворот потенциала и изменение пропорции компонентов указывают на сложный механизм распространения волн.

5. Изученные явления показывают некоторые причины «изменчивости» потенциала действия, которыми нельзя пренебречь при анализе значения отдельных компонентов потенциала действия.