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ELECTROPHYSIOLOGICAL PROPERTIES OF THE CEREBROVISCERAL CONNECTIVE OF THE FRESH WATER MUSSEL *(ANODONTA CYGNEA L.)*

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The cerebrovisceral connective (CVC) of the fresh water mussel *(Anodonta cygnea* L.) is a fibre fascicle consisting of axons free of myelin and neurylemm in which the thickness of the fibres varies between 0.2 and 1.5 μ (LÁBOS and co-workers 1963). The stimulation of this nerve results, depending on the parameters of the electric impulses, in the contraction of the posterior adductor muscle or leads to its significant relaxation. On the strength of this latter phenomenon PAVLOV (1885) assumed the presence of specialized tone abolishing inhibiting fibres; these studies of PAVLOV were supported by our previous experiments (SALÁNKI and LÁBOS 1963).

ZHUKOV (1946), KAHN and KUZNETZOV (1938) and SVERDLOV (1956) on the strength of their investigations established that in the CVC several fibres of different velocity of conduction are present and on the other hand that these are conducting the nerve impulse with a decrement. ZHUKOV (1956) however denies the existence of inhibiting fibres.

In our present study we are dealing with the excitability of CVC, with the properties depending on stimulation of the action potentials and with the properties of the individual fibre components. The aim of our investigations was to obtain, by applying a more up-to-date technique, a clear picture of the action potentials, excitability, velocity of conduction of the various fibres present in this nerve and perhaps in this connection disclose a relationship between the fibre components of the CVC and the fibres responsible for the contractions and/or relaxation of the adductor muscle.

Method

Experiments were conducted on CVC-s isolated from *Anodonta cygnea* L. specimens kept in an aquarium. The CVC prepared was held in MARCZYNSKY's (1959) physiological solution until the beginning of the experiment. In the course of stimulation and leading off the preparation was in paraffin oil to avoid drying out and losses of potential. The stimulating and leading electrodes

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were made of Pt wire. Stimulation and conduction was monopolar *(Fig. 1)*. For the stimulation square valve impulse generator, with anodic output while in the course of leading off RC amplifyer with 0.75 sec time constant was used

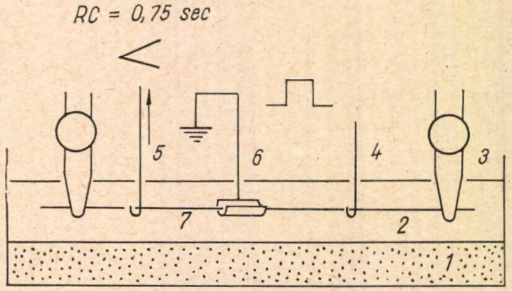

Fig. 1. Methodical picture. 1 = physiological solution, 2 = paraffin oil, 3 = clamp, 4 = stimulating electrode, 5 = electrode leading off, 6 = earth, 7 = preparation
1. ábra. Metodikai ábra. 1 = fiziológiás oldat, 2 = paraffinolaj, 3 = befogó, 4 = ingerlő elektróda, 5 = elvezető elektróda, 6 = földelés, 7 = preparátum

Results

Excitability

Since CVC is combined from different groups of nerve fibres the tension that just elicits the appearance of the action potential characterizes the fibres of minimum stimulation threshold. Thus the dependence of the threshold voltage on the duration of stimulus is characteristic not of the excitability of the whole connective but exclusively of the group of most excitable, thickest fibres. In conformity with our methodical possibilities square impulses of 0.2 to 120 msec duration and 0.1 to 24 V voltage were used for stimulation and with different impulse widths the tension was sought which is necessary to elicit the just visible (about 0.5 mV) action potential (A component) at a given amplification.

Fig. 2 shows such relation the data of which represent the mean of measurements performed on 10 nerves. From the figure it appears that the points of threshold voltage are arranged along a curve which at the beginning, at the increase of impulse duration, is sloping very rapidly and subsequently, from about 1 msec on slowly. The value of rheobasis is less than 100 mV while the chronaxy is between 10 and 100 msec. From *Fig. 3* it appears that increasing the distance between stimulating and leading electrodes from 30 to 40 mm leads to a shift of the curve because with the same impulse period a higher voltage is required to elicit the same action potential of minimum size. The difference is particularly great on the ascending part of the curves while the knee and the descending part exhibit lesser or minimum differences.

The voltage dependence of the properties of the action potential

Increasing the parameters of the stimulus we obtain the action potential of the whole nerve consisting of several components. The components point to the presence of fibre groups supplying action potentials of different velocity of conduction and of different value.

As it may be observed on *Fig. 4* at the increase of voltage with a given width of impulse the number of the components of the action potential increases and the proportion of their size changes. At the lowest voltage the most rapid component appears which up to a certain voltage is of dominant size and subsequently is exceeded by the values of lower components. Using the nomenclature of ZHUKOV (1946), we refer, in the order of velocity of conduction, to fibre groups *A*, *B* and *C*.

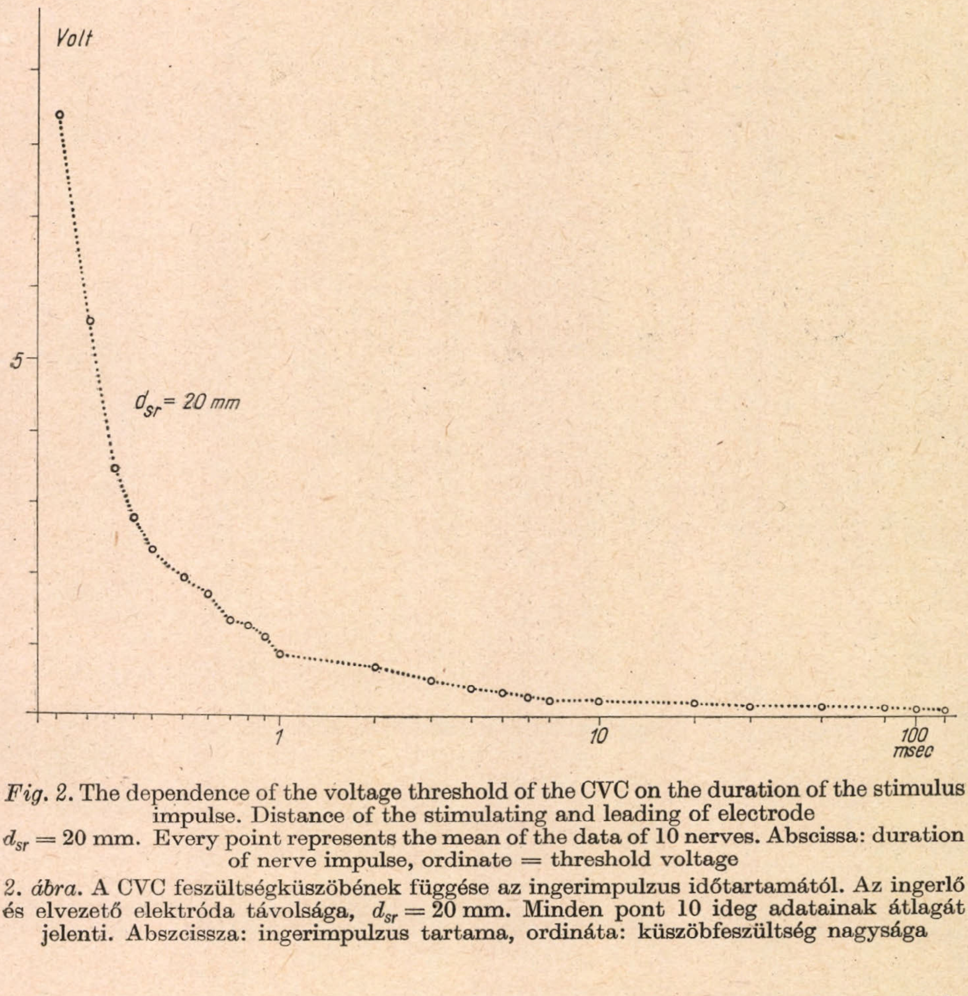

Fig. 2. The dependence of the voltage threshold of the CVC on the duration of the stimulus impulse. Distance of the stimulating and leading of electrode $d_{sr} = 20$ mm. Every point represents the mean of the data of 10 nerves. Abscissa: duration of nerve impulse, ordinate = threshold voltage

2. ábra. A CVC feszültségküszöbének függése az ingerimpulzus időtartamától. Az ingerlő és elvezető elektróda távolsága, $d_{sr} = 20$ mm. Minden pont 10 ideg adatainak átlagát jelenti. Abszcissza: ingerimpulzus tartama, ordináta: küszöbfeszültség nagysága

From *Fig. 5* it appears that at high voltage (20 V) the action potentia engendered by the *B* fibres is highest, which is followed by fibres *C* and *A* On these grounds it can be seen that the fibres of medium velocity of conduction participate with the greatest weight in the determination of the value of the action potential. This is in good agreement with our data obtained in the course of fibre analysis according to which the cross section of the medium about 0.5 μ diameter fibres occupies the most important part of the examined total nerve cross section (LÁBOS and co-workers 1963).

Stimulating with single square impulses of different durations we recorded the action potentials and on *Fig. 6* represented the value of the greatest negative component of the action potential and of the positive after potential as a function of the stimulus voltage. At a given width of impulse, the curves have a characteristic S-shaped run down with an initially slowly ascending, a medium, almost linear, and a subsequent flattening section. The steepness of the medium linear section is explicitly connected with the period of the impulse: when increasing the impulse width, the angle formed with the abscissa grows at first rapidly, than slower.

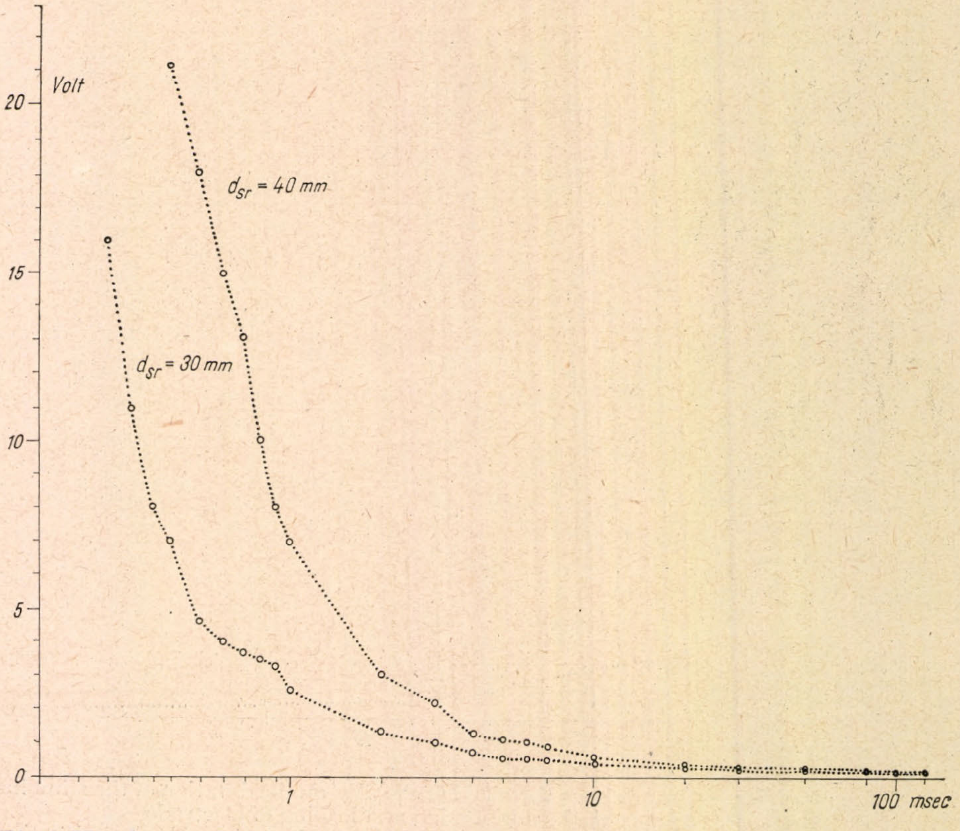

Fig. 3. The change of the impulse width dependence of the voltage threshold at the change of the distance between the electrodes stimulating and leading off (in the case of 30 and/or 40 mm leading off distance)

3. ábra. A feszültségküszöb impulzusszélességfüggésének változása az ingerlő és elvezető elektródok távolságának változásakor (30, ill. 40 mm elvezetési távolság esetén)

The dependence of the negative component and the after potential on the intensity of the nerve impulse is similar, with the difference that the values are substantially lower in the case of the after potential.

The dependence of the properties of the action potential on the duration of the nerve impulse.

The dependence on the amplitude of impulse of the greatest negative component of the action potential and of the positive after potential was examined at different voltages. In the domain of amplitude of impulse and of voltage investigated in linear representation the S-shaped run of the curves could not be observed *(Fig. 7).*

When studying the impulse width dependence of the greatest negative component we obtained curves of a steepness closely proportional to the voltage. (For easy survey of data the time-axis is logarithmic.)

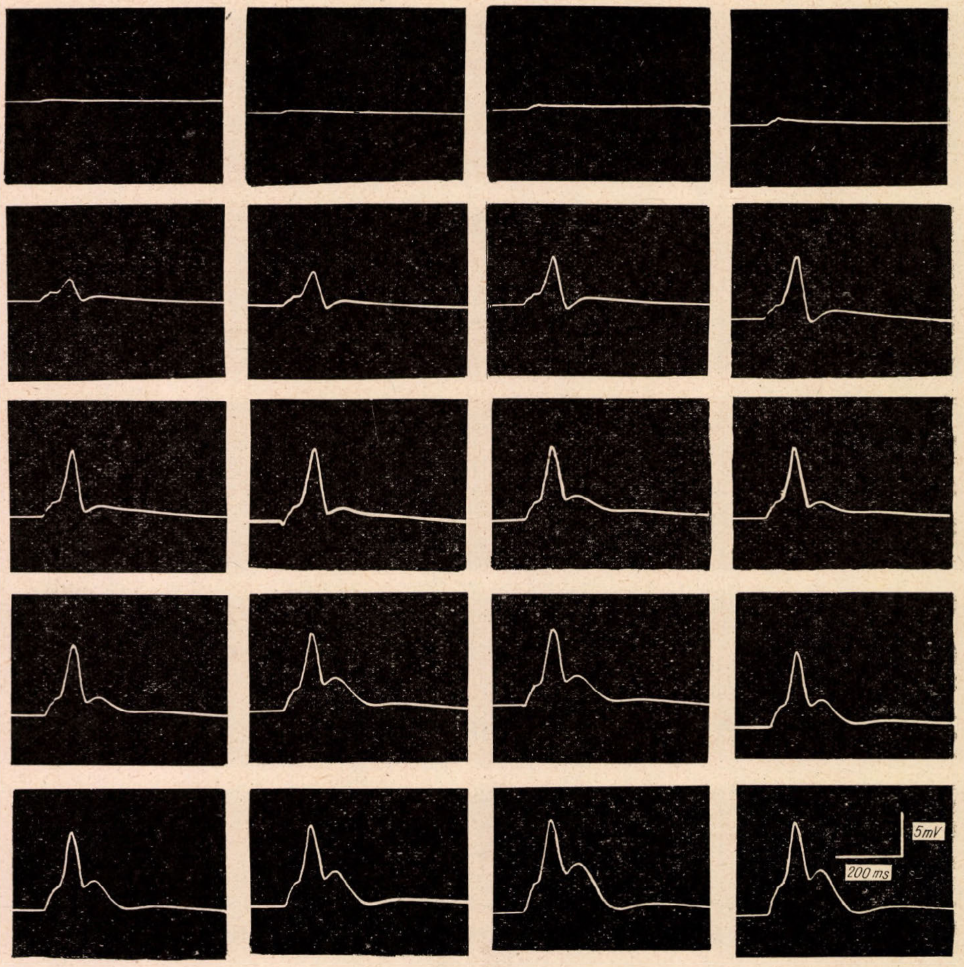

Fig. 4. The action potential of the CVC. Stimulation was performed with single impulses of 4 msec width from 1 to 20 V increasing per Volt

4. ábra. A CVC akciós potenciálja. Az ingerlés 4 msec időtartamú 1 V-tól 20 V-ig, voltonként növekvő egyes ingerekkel történt

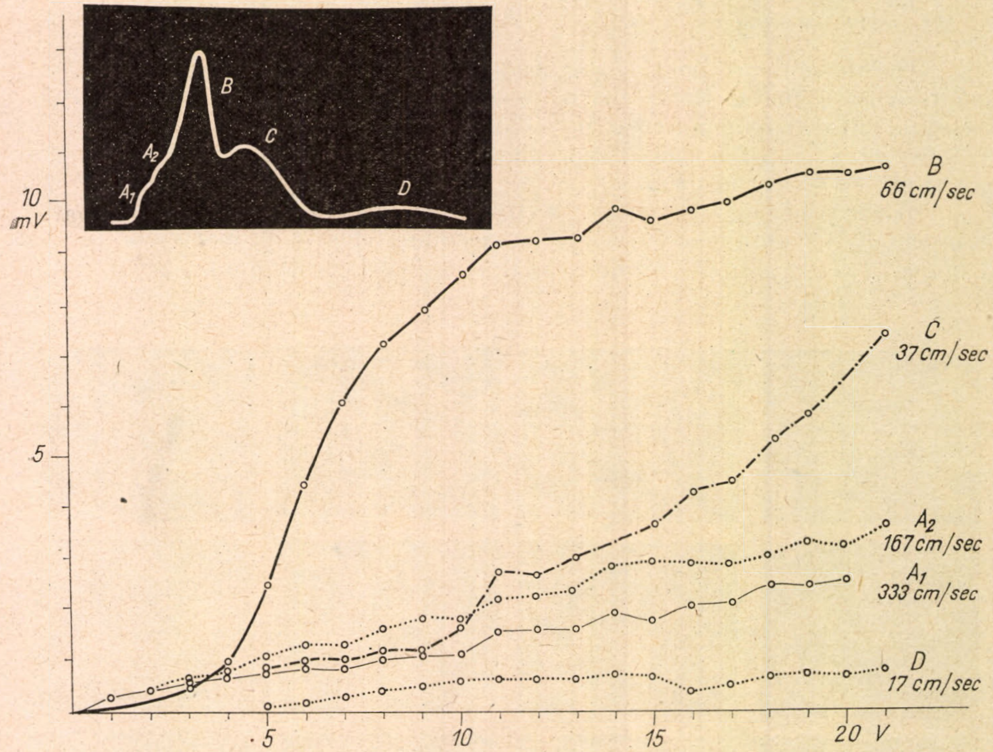

Fig. 5. The dependence of the components of the action potential of CVC on the stimulus voltage. The stimulation was performed with single impulses of 4 msec period. The distance between the electrodes stimulating and leading off was 20 mm. Besides all curves the value of the conduction velocity is indicated

5. ábra. A CVC akciós potenciálja komponensei nagyságának függése az ingerfeszültségtől. Az ingerlés 4 msec időtartamú egyes impulzusokkal történt. Az ingerlő és elvezető elektródák távolsága 20 mm volt. Az egyes görbék mellett a vezetési sebesség értékét tüntettük fel

Analysis of responses obtained on series stimulation

Since the CVC contains nerve fibres of comparatively slow conduction which according to data of literature (Zhukov, Streltzova 1943) have a great refractory stage (of the 100 msec order of magnitude) we examined the measure of the decrease of amplitude observed in the course of series stimulation with square impulses of various voltage and period. Amplitude was measured from the zero line to the maximum of deflection of negative direction.

When applying a series of 4 msec width impulses, the decrease of amplitude observed in the course of the increase of frequency above 50 c/sec is 100 per cent. Regarding the amplitude of action potential obtained in the course of the application of the single impulse as 100 per cent, a 50 per cent depression of amplitude is observed approximately in the case of 20 c/sec *(Fig. 8)*.

Upon the action of a series of electric impulses lasting for a longer period (100 msec) the action potential is about 10 per cent of the original at 10 c/sec

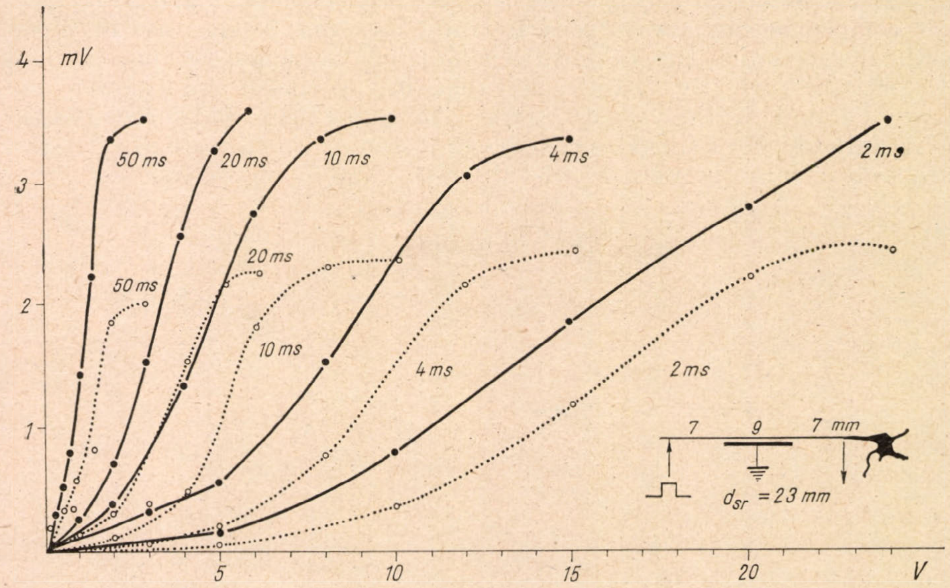

Fig. 6. Dependence of the greatest negative component of the action potential (component B) and the size of the positive after potential on the nerve voltage at 2, 4, 10, 20 and 50 msec impulse width. Abscissa: stimulus voltage, ordinate: value of the potential in mV. Continuous line showing change of the component B, dotted line that of the after potential

6. ábra. Az akciós potenciál legnagyobb negatív komponense (B-komponens) és a pozitív utópotenciál nagyságának függése az ingerfeszültségtől 2, 4, 10, 20 és 50 msec impulzusszélesség mellett. Abszcissza: ingerfeszültség, ordináta: a potenciál nagysága mV-ban. Folytonos vonal a B-komponens, szaggatott vonal az utópotenciál változását mutatja

and a 50 per cent reduction is observed with a value of about 5 c/sec. This frequency characteristic of the action potential depends to a lesser extent on the voltage of the stimulation.

Velocity of conduction on the fibres of CVC

According to the data of Zhukov (1946) the velocity of conduction in the fibres of CVC is 2 cm/sec. Horridge (1958) on the strength of his studies on *Mya arenaria* has found in the CVC of this species also fibres conducting with a velocity of 50 cm/sec. According to our previous data we distinguished fibre groups with a velocity of conduction of 90 to 140 cm/sec, 25 to 45 cm/sec and 5 to 15 cm/sec (Lábos and coworkers 1963). In view of the significant deviation as against Zhukov's data we made repeated measurements in order to control our previous results.

Performing the determination of the velocity of conduction of the components on 30 different nerves it can be stated that 3 to 5 components appear and in most cases four used to distinctly separate. On the strength of velocity determination performed on CVC the following statements can be made:

1. The most initial point of the appearance of the action potential corresponds to a value lower than 2.5 m/sec velocity.

2. The velocity of component A fluctuates between 70 and 140 cm/sec. This component not always separates and its value in the case of supermaximal stimulation lags behind the middle of the highest component. Less frequently, it explicitly separates and splits into a quicker and a slower component (A_1 and A_2) the velocity of which falls into the neighbourhood of the lower and/or higher limit. This component dominates in the case of excitation with 4 ms impulses between 1 and 4 V *(Figs. 4* and *5).*

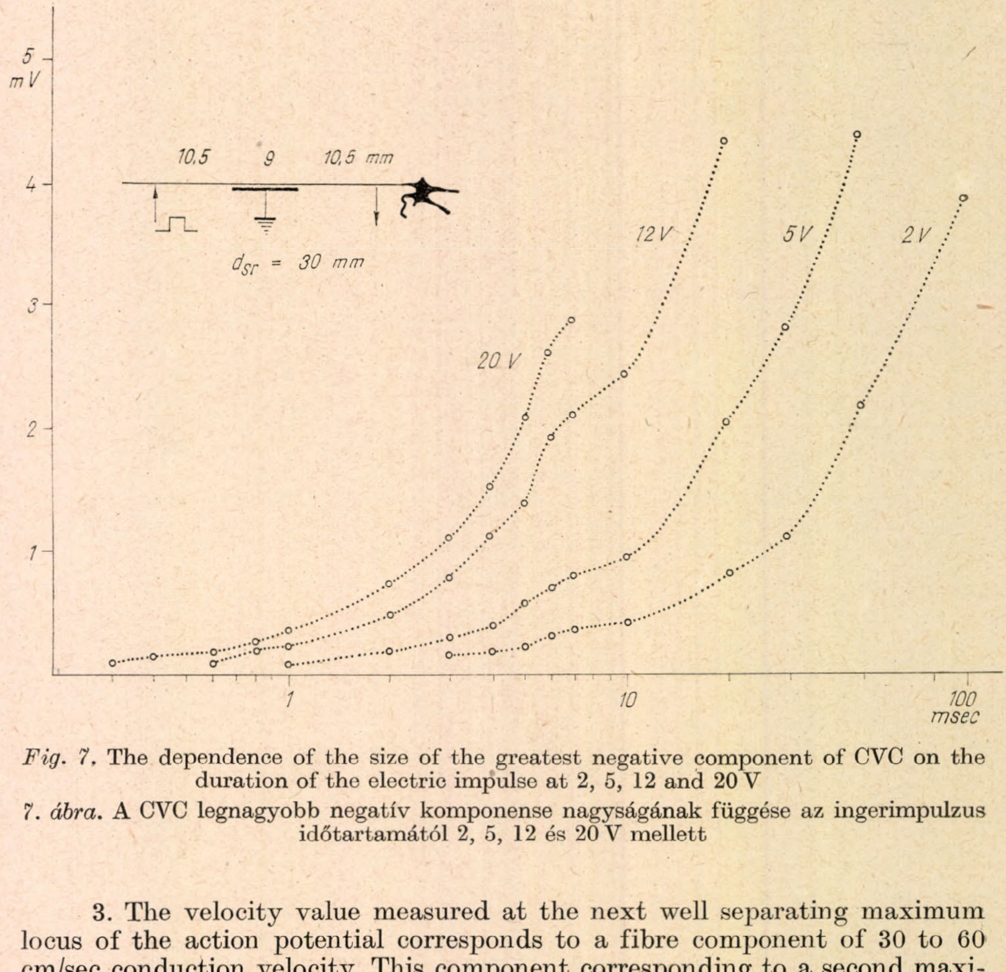

Fig. 7. The dependence of the size of the greatest negative component of CVC on the duration of the electric impulse at 2, 5, 12 and 20 V

7. ábra. A CVC legnagyobb negatív komponense nagyságának függése az ingerimpulzus időtartamától 2, 5, 12 és 20 V mellett

3. The velocity value measured at the next well separating maximum locus of the action potential corresponds to a fibre component of 30 to 60 cm/sec conduction velocity. This component corresponding to a second maximum is marked B. This dominates in the case of the application of supermaximal stimulation. Less frequently it can be observed that two hardly separating components fall between the indicated limits of velocity; this can be marked B_1 and B_2. The value of this component B exceeds the value of component A in the case of the application of a 4 ms width electrode impulse of about 5 V voltage.

4. The fibres conducting between the velocity limits 16 to 30 cm/sec are conceived as a further group of fibres and marked with C. Within these limits

the velocity falling between 18 and 23 cm is more frequent. No further split was observed in connection with component *C*. Its appearance can be comprehended in most cases already at an impulse value of about 5 V (with an impulse width of 4 ms) but in such cases its value still lags behind that of the components *A* and *B*. In the case of supermaximal stimulation the component *C* as a rule exceeds *A* but does not exceed the value of component *B*. In some cases, however, it occurs that in the case of supermaximal stimulation component *C* is higher than component *B*.

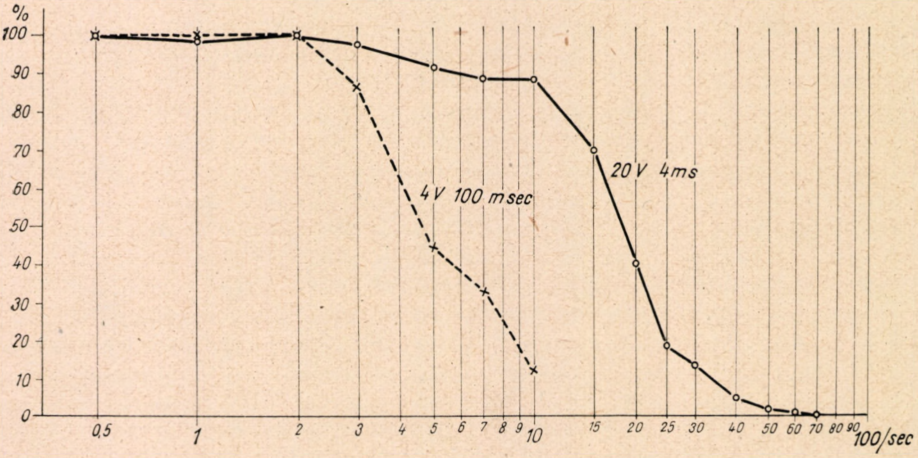

Fig. 8. The frequency dependence of the greatest component of the action potential of the CVC. Abscissa: the frequency of the stimulating impulses. Ordinata: the value of the potential as related to the greatest component of the action potential obtained in the course of stimulation with the single impulse

8. ábra. A CVC potenciálja legnagyobb komponensének frekvenciafüggése. Abszcissza: az ingerimpulzusok frekvenciája. Ordináta: a potenciál nagysága az egyes impulzussal történő ingerlés során kapott akciós potenciál legnagyobb komponenséhez viszonyítva

5. The group of the fibres of slowest conductivity is marked *D*. The separation of this group and the measurability of its velocity of conduction are not good. The error of measurement is much greater than in the case of the components *A*—*C* the cause of which are the flat run-down, not explicit maximum, and the fact that this component disposes of a peak falling into the period of the afterpotentials of the previous quick fibres and is thus heavily distorted. Its velocity of conduction falls between 5 and 15 cm/sec in such a way that within this intervall all values are of about the same probability. This component in the case of 4 ms impulse period becomes visible in stimulations of about 10 V. In the case of supermaximal stimulation it rises above the zero line which in view of the fact that it falls to the period of the after potentials points out that the number of fibres responsible for this component is comparable about to that of group *A*.

Discussion

Examining the impulse threshold of the most excitable fibres in connexion with the duration of the single square impulse we found that the curve thus obtained has two main sections:

a) from 0.2 to 1 msec steeply falling

b) from 1 to 120 msec not changing significantly. This as to run-down does not imply a substantial difference as against similar curves of other nerves.

The exact measurement of the rheobasis could not be carried out but it can be established that it corresponds to a value of about 100 mV.

The chronaxy of the most excitable fibres is between 10 and 100 msec which as to order of magnitude coincides with the data of ZHUKOV and STRELTZOVA (1943).

Since the CVC conducts the impulse with decrement, it was important to keep the distance between the electrodes exciting and leading off at a constant value. The above relation is valid in the case of conduction to a distance of 20 mm, with symmetrical arrangement of the earth electrode. When maintaining the earth symmetry and increasing the distance of the electrodes exciting and leading off, the threshold curve runs down at higher voltages. On the rapidly ascending section of the curve the change is significant while insignificant in the slowly ascending section.

Thus the fact of decrement conduction is accompanied by the consequence that the chronaxy values related to the fibre groups among others depend also on the value of the pathway of conduction. Therefore the chronaxy of the CVC can not be given without indicating the distance between the stimulating and leading electrodes.

The individual components of the action potential, the voltage-dependence of their value has an S-shaped run-down. Beyond that it is asymmetrical. Plotted in an identical coordinate system with a greater impulse width (50 msec) the curve is steep while with lower width of impulse (2 msec) more prolonged.

One of the possible causes of the S-shaped and asymmetrical rundown is that the impulse threshold distribution of the fibres of identical velocity is asymmetrical and disposes of a maximum. Since the dimension of the fibres (diameter, cross section) is in connection with the excitability of the fibres, their velocity of conduction and the value of their elementary action potential, our earlier results, according to which the dimension of the fibres shows an asymmetrical distribution with a maximum (LÁBOS and co-workers 1963) support this assumption.

Therefore we attempted to approach our S-shaped curves with the integral curves of the POISSON distribution. This, however, proved unsuccessful.

An other possible explanation of the S-shaped rundown may be found in the fact that the voltage dependence of the single fibres themselves is such. Proceeding from the gradual theory of NASONOV (1959), LEV and co-workers (1959) demonstrated that even in the case of a single axon of a crab the voltage dependence of the action potential gives an S-shaped curve. A similar possibility must be taken into consideration also in case of the fibres of CVC the more since non myelinized fibres with decrement conduction are involved.

A remarkable occurrence was observed in connection with the rundown of the above curves, *i.e.* that in the domain of higher voltage after a certain flattening increase is again steeper *(Fig. 5)*. This phenomenon can be brought into relationship either with the increase of the response of the elementary fibres or of the number of fibres entering into excitation. In the former case the law of all or nothing is not valid while the latter case can be brought in relationship with methodical conditions since the electrode surrounding the nerve as a hemicircle excites the fibres in the nucleus of the nerve only at higher voltages. The assumption seems unlikely that two groups of fibres with entirely different threshold would belong to a fibre group of a unique velocity. A further assumption could be that upon the action of the higher voltage the fibres would influence the impulses of each other synergetically.

Studying the dependence of the action potential on the duration of stimulation in the 0.2 to 120 msec domain we did not observe the S-shaped rundown found in the course of voltage dependence. The dependence of the dimension of a chosen component of the action potential on the impulse width was nearly linear at the values examined (on *Fig. 7* the linearity is not conspicuous on account of semilogarithmic representation).

The shape of the action potentials obtained shows, however, that when the period of the impulse is increased beyond a certain limit, it begins to act as a direct current and forms the basis of qualitatively different phenomena. From what has been said above it is clear that the duration of the impulse and its voltage act on the excitation of the nerve in a not interchangeable way.

The voltage and duration dependence of the value of the positive afterpotential at constant duration and voltage is of the same rundown as the relations referring to the action potential. The proportion of the action potential and positive after potential changes depending on the voltage and impulse width. With growing voltage and width of impulse the proportion grows to the benefit of the afterpotential.

The proportion of the individual components of the action potential changes with the increase of impulse voltage. In a low domain of voltage (1 to 5 V) the quickest component is the highest. With growing voltage, component *A* increases less than *B* and thus on this section the size of component *B* dominates. With further increase of voltage also component *C* exceeds the value of component *A* but lags behind component *B*. From all this it appears that the increase of voltage is accompanied by the increase of the relative value of the slow components. This can be brought in connection with the fact that at in situ stimulation of the CVC the character of the posterior adductor muscle responses changes in the course of the increase of voltage. The adductor muscle contraction belonging to low voltage thus would pertain to the excitation of the components with more rapid conduction while relaxation which can be engendered with the application of higher voltage were brought about by the impulse of the fibres of low conduction and high nerve threshold. This would prove by implication that in the CVC inhibiting fibres are found (PAVLOV 1885, SALÁNKI and LÁBOS 1963). Of course not all components are related to the function of the posterior adductor muscle.

The frequence characteristics of CVC point to the comparatively long refractory stage of the fibres. The observation that excited by 4 msec impulses at 20/sec frequency the amplitude of the greatest component of the action potential diminishes to its half points to the fact that about half of the fibres disposes of a refractory stage shorter than 50 msec and/or that a second impulse following at an interval shorter than 50 msec leads to a response the size of which is at most 50 per cent on the average. There is no possibility under the present experimental conditions to decide which part the absolute or relative refractory stage of the individual fibres plays in the case of frequency characteristics. In the course of increased frequency on the other hand it can be observed that there are fibre groups which are able to pass on impulse for a prolonged period with a frequency of 20 per second.

Summary

The present study has dealt with the excitability of the cerebrovisceral connective (CVC) of *Anodonta cygnea*, with the properties depending on stimulation of the action potential and with the features of the individual components. It was found that

1. in case of stimulation with adequate parameters the action potential consists of four, in some cases of five components which can be readily separated,
2. that the order of the components according to velocity of conduction differs from the order according to size,
3. the indices of excitability depend on the distance of leading off; led off to 20 mm from the stimulation the rheobasis is 0.1 V while the chronaxy between 10 and 100 msec,
4. the dependence of the value of the components shows an S-shaped rundown on the voltage, of the stimulus,
5. in repetative stimulation we found that at 20/sec frequency the reduction of the action potential amounts to 50 per cent (in the case of 4 msec impulse period),
6. conduction velocity of the fibre components is to be found between 2.5 and 0.05 m/sec.

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TAVI KAGYLÓ *(ANODONTA CYGNEA* L.) CEREBROVISCERÁLIS KONNEKTIVUMÁNAK ELEKTROFIZIOLÓGIAI SAJÁTSÁGAI

Salánki János, Lábos Elemér és Nán István

Összefoglalás

Vizsgálataink során *Anodonta cygnea* L. cerebroviscerális connectivumának (CVC) ingerlékenységével, az elvezethető akciós potenciál ingerléstől függő sajátságaival és az egyes komponensek tulajdonságaival foglalkoztunk. Azt találtuk, hogy

1. megfelelő paraméterekkel történő ingerlés esetén az akciós potenciál négy, esetenként öt jól elkülöníthető komponensből áll,
2. a komponensek vezetési sebesség szerinti sorrendje eltér a nagyság szerinti sorrendtől,
3. az ingerlékenység mutatói az elvezetési távolságtól függenek; az ingerléstől 20 mm-re elvezetve a rheobasis 0,1 V, a chronaxia 10 és 100 msec között van,
4. a komponensek nagyságának függése az ingerfeszültségtől S alakú lefutást mutat,
5. sorozat-ingerléskor azt találtuk, hogy 20/sec frekvenciánál 50%-os az akciós potenciál csökkenése (4 msec-os impulzusidőtartam esetén).
6. a rostkomponensek ingerületvezetési sebessége 2,5 m/sec—0,05 m/sec közé esik.

ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЕ СВОЙСТВА ЦЕРЕБРОВИСЦЕРАЛЬНОГО КОННЕКТИВА БЕЗЗУБКИ *(Anodonta cygnea L.)*

Я. Шаланки, Э. Лабош, и И. Нан

Были изучены возбудимость, свойства отводимого потенциала действия в зависимости от раздражения и характер отдельных компонентов биопотенциала. Установили, что:

1. при применении соответствующих параметров раздражения ток действия состоит из 4 или 5 хорошо отдифференцированных компонентов.
2. Порядок компонентов по скорости проведения отличается от их порядка по величине.
3. Характеристика возбудимости зависит от расстояния проведения; при отведении на 20 мм от раздражения реобаза равняется 1 V, а хронаксия —10—100 мсек.
4. Зависимость величины компонентов от напряжения раздражения соответствует s-образной кривой.
5. Установили, что при серийном раздражении при частоте раздражения 20/сек ток действия снижается на 50 процентов (при продолжительности импульса 4 мсек.).
6. Скорость проведения отдельных компонентов волокон располагается между 2,5—0,05 мсек.