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Mechanical Changes in Crab Nerve Fibers during Action Potentials

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Abstract A nerve impulse travelling along a crustacean nerve was found to be accompanied by a small,rapid movement of the nerve surface.The movement was 10-20nm in amplitude and was concurrent with a rise in the"swelling pressure"of the order of 5mg/cm2 for a nerve bundle.Initiation of an action potential at the site of cathodal polarization was preceded by a small,slow mechanical change in the
nerve fiber.Anodal polarization produced a large mechanical change
of the opposite sign.Tetrodotoxin and procaine suppressed rapid me-chanical changes.

Several previous investigators suggested the existence of rapid mechanical changes in nerve fibers concomitant with the production of action potentials (KAYUSHIN and LYUDKOVSKAYA,1955;FRANK,1958;LETTVIN et al.,1962;HILL et al.,1977).The alleged rapid mechanical changes reported before signal aver-ages became available were all very large and most of those findings could not be confirmed by subsequent investigators(SANDLIN et al.,1968;COHEN,1973). HILL et al.(1977)employed the laser interferometry method combined with signal averaging and reported that electric excitation of a crayfish nerve fiber induced a rapid reduction of the fiber diameter followed by a relaxation.
The present paper describes the results of our demonstration of small and rapid mechanical changes in crab nerve fibers during action potentials by the use of both optical and mechano-electric methods.We found that there is a distinct upward movement of a light-reflecting target placed on the surface of a nerve when action potentials are generated by electric stimulation.Furthermore,we could demonstrate that this movement is produced by a transient swelling of the nerve fiber.Obviously,these rapid mechanical changes are totally unrelated to the slow mechanical changes(KORNAKOVA et al.,1947;HILL,1950;BRYANT and TOBIAS,1955)which are known to be produced cumulatively by a large number of action potentials.
In the present paper,we deal exclusively with the results obtained by using

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crustacean nerve fibers;the results obtained from squid giant axons will be de-scribed later.Preliminary accounts of our results obtained with the same methods are presented elsewhere(TASAKI and IWASA,1980;IWASA and TASAKI,1980).

METHODS

Most of the experiments described below were carried out on claw nerves of
the blue crab,Collinectes sapidus.On several occasions,nerves taken from
walking legs or claws of the lobster(Homarus americanus)or claw nerves of the
crayfish(Procambarus clarkii)were also used for comparison;no clear difference
in behavior was observed among different preparations of crustacean nerves.
The procedure of dissecting claw nerves of the crab is described elsewhere(TASAKI
and Sisco,1975).Electron microscopic examination of the claw nerve of the
blue crab indicated that the fiber diameter ranges between 1 and 12ƒÊm.The

connective tissue and the blood vessels attached to the bundle of these nerve fibers
were removed under a dissecting microscope prior to the following examination
of mechanical properties of the fibers.

Nerve chambers employed were designed to enhance the mechanical stability of the portion of the nerve used for measurements.The bottom surface of the chamber was usually constructed in a convex,semicylindrical form, so that the lower surface of the nerve under tension was pressed against the chamber bottom. A pair of stimulating electrodes(Pt or Ag)were placed near one end of the nerve in the chamber;a pair of extracellular recording electrodes were arranged usually near the other end.
The standard optical probe used in the present studies was a Fotonic sensor
purchased from Mechanical Technology,Inc.,Latham,N.Y.This probe consists
of two bundles of optic fibers mixed at one end.One of the two bundles was
used for transmitting strong white light from a source(Osram quartz-iodine lamp)
to the mixed(i.e.,sensing)end;the other bundle was employed for carrying light
from the sensing end to a photo-detector(Pin-10,United Detector Technology,
Inc.,Santa Monica,Ca).To measure small displacements of the surface of a
nerve,a light-reflecting target was placed above the nerve.Gold dust, a piece of
aluminum foil,a small piece of platinum wire,a small ball of silver or stone,etc.
were used for this purpose;no significant difference was observed in the results
obtained with different kinds of targets.Diagram A,in Fig.1,shows the de-
pendence of the observed light intensity upon the distance between the target and
the sensing end of the Fotonic sensor employed.The output of the operational
amplifier(Analog Devices,Norwood,Mass.)connected to the photo-detector
rises sharply with the distance and reaches a maximum at about 150ƒÊm.As the
distance is increased further,there is a gradual fall in the intensity.After de-
termining the position of the maximum intensity for each target employed,the
tip of the sensor was moved to one of the roughly linear portions of the intensity-
distance curve,either on the"proximal"or"distal"side of the maximum.

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Fig.1. Calibration of Fotonic sensor(left)and Gulton bender(right) .The diagrams at

the top show the experimental setups schematically(see text).The maximum value of
the light intensity,Io,observed with the Fotonic sensor was 2-4ƒÊA.L stands for light
source;D,detector;d,distance between light-reflecting target and sensor;G
,Gulton
bender;W,weight;V,voltage-output of bender.

Small movements of the target associated with the production of action potentials are calculated by the following linear formula:

△l=△I/(siIO),

where si is the slope of the light intensity-distance curve,•¢I the amplitude of the
change in light intensity associated with the production of action potentials,and
to the maximum value of the light intensity observed.The tip of the sensor was
usually placed at the position of the steepest rise;this"proximal"slope was ap-
proximately(115ƒÊm)-1 when the target was a piece of short platinum wire.To
supplement the data taken at the proximal slope,the position of a linearly declin-
ing portion of the intensity-distance curve was also used;the slope of the curve at
this"distal"position was approximately-(770ƒÊm)-1.We usually measured the
displacement at I/Io=0.6 on the proximal slope and at I/I0=0.7 on the distal
slope.These points are located near the centers of the linear ranges.It is im-
portant to note that we can distinguish up-and-down motion of the target from
other modes of target motion by comparing the mechanical records taken at the
two different slopes.We used this comparison to check our results.

The standard mechano-electric transducer employed in the present investiga-tion was a piezo-ceramic bender(model R050S)kindly supplied by Gulton In-
dustries,Inc.,Methuchen,N.J.An approximately 6mm-long bender was used
in conjunction with a voltage-follower made with an FET operational amplifier
(Analog Devices,Norwood,Mass.).The sensitivity of the bender was calibrated

by hanging known weights from the end of the bender(see Diagram B,in Fig.1). The voltage-sensitivity of the bi-morph bender was roughly 0.5mV/mg.To test

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whether or not the bender can follow rapid changes in tension,the tip of the bender was driven sinusoidally by connecting to a calibrated condenser-micro-
phone by means of a thin thread.We found that the bender could follow up to
about 10,000Hz.In the range below 1,000Hz,there was no distinct peak of

resonance.

To measure pressure change,a stylus was installed close to the free end of the bender,which was mounted on a micromanipulator.A pressure was applied to the nerve surface through the stylus.A detailed description is given in an earlier report(IWASA and TASAKI,1980).
The voltage-output of the FET operational amplifier connected to the Fotonic sensor or to the piezo-ceramic bender was amplified by a factor of 1,000 with a condenser-coupled Tektronix preamplifier.The output of the preamplifier was displayed directly on the screen of a Tektronix oscilloscope(model 502).Under the conditions of the experiments described below,the signal-to-noise ratio at the output of the preamplifier was of the order of 2-10.For a quantitative deter-mination,therefore,signal-averaging over 4-152 trials was adopted.

RESULTS

1. Small rapid movement of the surface of nerve fibers associated with propagated impulse
In preliminary studies,we found that there is a small,transient,upward
movement of a small piece of platinum placed on the nerve when an impulse ar-
rives at the site of mechanical recording.In crab nerves,the magnitude of the
movement was in the range between 10 and 20nm.We made optical targets of
various materials,insulators as well as conductors,and obtained consistent
results.In those studies,the site of mechanical recording was only a few mm
away from the site of electric stimulation.In the following studies, an attempt
was made to determine the velocity at which the locus of the maximum move-ment travels along the nerve.
The diagram on the top of Fig.2 illustrates the arrangement of the two sites of mechanical recording in relation to the stimulating and recording electrodes. The entire 35-40mm-long desheathed nerve was immersed in seawater.The light-reflecting targets were placed at two sites along the nerve,one at a distance of 5mm(proximal target)and the other at 15nm(distal target)away from the
site of stimulation.The separation between the stimulating and the recording
electrodes was 24mm.The stimulating current pulses were 0.3msec in duration and the strength employed was approximately 30times the threshold value.
As expected,the mechanical record taken at the distal target had a latency
longer than that obtained at the proximal target.The velocity of the mechanical
response determined by this procedure was roughtly 4m/sec at 21•Ž.This value
was slightly smaller than the velocity of the fastest dominant peak of the action
potential,approximately 5m/sec.This slight difference may be attributed to a

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A B

Fig.2. A)Top:Diagram illustrating the arrangement of the stimulating electrodes(S),two
sites of mechanical recording(F1 and F2)and the position of the electrodes used for
recording and the action potential(R).Bottom:Mechanical and electrical responses
obtained with the setup illustrated above.21•Ž.B)Demonstration of swelling of a
crab nerve associated with the arrival of nerve impulses at the site of mechanical record-
ing from two sides.S1 and S2 represent stimulating electrodes located at the same
distance from the recording electrode R at the botton of the slot.G represents a
stylus attached to a Gulton bender pressing the nerve by the mediation of a thin plastic
plate,P,located above the recording electrode R.In the record shown at the bottom,
the upper trace represents the mechanical response recorded with the Gulton bender,
G,and the lower trace the action potential observed with the recording electrode,R.
The downward deflection of the mechanical trace represents a rise in the pressure.22•Ž.

greater contribution of small nerve fibers to the mechanical responses.The
amplitude of the mechanical response recorded at the distal target was smaller
and longer in duration than those taken at the proximal target.This difference
is attributable to an increase in temporal dispersion of nerve impulses in individual
fibers associated with nerve conduction.

2. Increase in diameter of nerve fibers during action potential
We have seen in a preliminary experiment that there is a transient rise in the pressure exerted by a nerve on a stylus on the nerve when an action potential is generated.The surface area of the stylus used was relatively small(approximately 0.1mm2).The possibility could not be excluded therefore that the observed mechanical response was simply a sign of a localized movement of the membrane surface without being accompanied by a change in the volume of individual nerve
fibers.The following observations were made with a view to determining whether

or not the mechanical responses are accompanied by a change in the volume
occupied by nerve fibers.
A nerve chamber provided with a special narrow slot in the middle was used (see Fig.1 B,top).The slot was about 7mm long,roughly 0.7mm wide at the bottom,and approximately 5mm deep.The upper portion of the slot was slightly

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wider so that a desheathed nerve trunk could be introduced into the slot without

difficulty.A thin plastic plate(approximately 0.6mm in thickness and 7mm in width)was introduced above the nerve in the slot(see P in the figure).Finally, a force of 100-500mg was applied to the nerve by means of a Gulton bender(G) pressing the plastic plate.Under these conditions,the space within the slot is packed with nerve fibers;therefore,swelling of individual nerve fibers during an action potential is expected to raise the pressure exerted by the nerve on the bender.

The electric response was recorded with a fine silver electrode,R in the figure,
at the bottom of the slot(referring to the electrode immersed in seawater sur-rounding the slot).The bottom of the slot was usually covered with a thin (roughly 2mm thick)layer of cotton soaked with seawater.The nerve fibers were excited on both sides of the slot so that two nerve impulses reached the slot simultaneously from the two sides of the fibers.The use of this arrangement was intended to prevent the plastic plate(7mm wide)in the slot from tilting upon arrival of a propagated pressure change from one side.
The mechanical record,in Fig.2B,top,indicates that there was a definite rise in the pressure at the time of arrival to two nerve impulses.The small dif-ference in latency between the electrical and mechanical responses may be at-tributed,at least partly,to the inertia and the friction of the mechanical recording system.It seems reasonable therefore to conclude,from these observations,that the cross-sectional area of the nerve fibers increases when action potentials are generated in the fibers.

3. Effects of electric currents

It is well known that a long pulse of electric current produces histological and optical changes in nerve fibers(see BETHE,1920;COHEN,1973).Therefore, it is not surprising to find that mechanical changes in nerve fibers can be generated by application of electric currents.
We employed the following experimental arrangement to examine the effects
of electric currents on mechanical properties of crab nerves.A desheathed nerve was introduced into a plastic chamber consisting of a 6 mm-wide middle pool of seawater separated from large lateral pools by 8mm-wide air-gaps.In order to prevent dessication,the portion of the nerve passing through the air-gap was completely covered with vaseline.By means of a large silver wire electrode,the middle pool was grounded.The two lateral pools were shunted electrically by means of silver electrodes.Pulses of constant currents were applied between the middle and lateral pools,and the mechanical changes of the portion of the nerve in the middle pool was detected either with a Fotonic sensor or with a Gulton piezo-ceramic bender.
Two examples of the mechanical records obtained by the optical method are presented in Fig.3.When the applied current was directed inwards through the

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Fig.3. Mechanical responses produced by long current pulses.The diagram at the top
illustrates the experimental setup employed(see text).The upper trace marked IN
was obtained with a current traversing inward through the nerve membrane in the middle
pool(namely,with a hyperpolarizing current).The trace marked OUT shows the
record obtained with an outwardly directed current(namely,with a depolarizing cur-
rent).The upward deflection of the Fotonic sensor output represents an increase in
the distance between the light-reflecting target on the nerve and the sensor.The sharp,
downward deflection of the mechanical trace represents the response associated with the
propagated impulse induced by the current pulse.The current intensities used were 2
(left) and 4mA(right).21•Ž.

portion of the nerve surface in the middle pool(see the upper trace),there was a gradual increase in the distance between the light-reflecting target on the nerve and the tip of the Fotonic sensor.At current intensities roughly 5-10 times the threshold for excitation,the smooth movement of the target was interrupted by a mechanical response associated with the production of a propagated action potential(Note that swelling of the nerve decreases the distance between the target and the sensor).With pulses of strong currents directed outwards through the nerve surface,a sharp mechanical response representing the initiation of a nerve impulse was superposed on a small and smooth mechanical change.There was a small difference in latency between the sharp mechanical response generated by an inward current and that of the response produced by an outward current. This difference is attributable to the involvement of a finite conduction time in

excitation with an inward current(Note that,when the applied current is inwardly directed through the nerve surface in the middle pool,the nerve impulse is gen-erated in the portion of the nerve in the lateral pools where the membrane current is outwardly directed).The results obtained with a Gulton bender were essen-tially the same as those described above.
Using the same experimental setup,the behavior of the nerve treated with tetrodotoxin(TTX)was examined.When TTX was added to the external medium at a concentration of 20-40 nM,these was a gradual reduction in amplitude of the

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TTX Procaine

Fig.4. Movements of the nerve surface produced by long pulses of electric currents ap-
plied to crab nerves treated with 40nM tetrodotoxin(TTX)or with 4mM procaine.
The experimental setup illustrated in Fig.3,top,was used.The current intensity used
was 4mA.22•Ž.

sharp mechanical response.The record presented in Fig.4,left,was obtained from a bundle of nerve fibers treated uniformly with 40nM TTX.It is seen that TTX completely suppressed the sharp mechanical responses,which are accom-panied by action potentials.The slow changes,on the contrary,were only slightly reduced by the powerful nerve poison.
The effects of local anesthetics were examined by the same procedure.The effect of 4mM procaine was very similar to that of TTX(see Fig.4,right).

DISCUSSION

The main objective of the experiments described under RESULTS was to clarify general physiological properties of the mechanical responses of the crus-tacean nerve.A small target placed on the surface of the nerve was shown to move transiently following electric stimulation.Usually the amplitude of the movement was 10-20nm and was accompanied by a rise in pressure of 5mg/cm2. The mechanical response was shown to travel along the nerve at a rate similar to the velocity of a propagated action potential.When nerve fibers were con fined in a small slot, there was a rise in the pressure exerted by the nerve on the wall of the slot.Consequently,these mechanical responses of the nerve were interpreted as representing swelling of nerve fibers associated with generation of an action potential.
At the site of application of a long pulse of electric current,the mechanical response associated with initiation of a propagated nerve impulse was shown to be preceded by a small,continuous mechanical change.The mechanical re-sponses associated with a propagated nerve impulse were suppressed by TTX,as well as by procaine.The continuous components of the mechanical change generated by electric currents were only slightly affected by anesthetics.Since substantially the same results were obtained by using a Gulton bender instead of a Fotonic sensor,the possibility was precluded that the observed results were produced by a direct effect of the applied current on the optical target.

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The ultimate goal of the present studies is to elucidate the macromolecular
basis of the processes underlying the production of mechanical responses.It is well known that swelling of gels in inanimate colloidal materials is produced by invasion of water.Quite recently,we have obtained suggestive evidence that there is transport of water from outside when a mechanical response is produced in a crab nerve.We saw that the sum of the volume of the surrounding seawater and the nerve remains roughly unaltered when the nerve fibers were excited. Consequently,we are confronted with the following physiocochemical problem: how does a change in the membrane potential induce transport of water from the surrounding medium to the cortical layer of the protoplasm?
For studying this problem,the use of squid giant axons is undoubtedly more beneficial,because the membrane potential can be controlled more readily in giant axons than in crab nerve fibers.The results we have obtained so far indicate

that the mechanical properties of the squid axon membrane are,in many respects, very similar to those of the crab nerve.The results of our studies on squid axons will be presented elsewhere.

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