

Rapid mechanical and thermal changes in the garfish olfactory nerve associated with a propagated impulse

Ichiji Tasaki, Kiyoshi Kusano, and Paul M. Byrne

Laboratory of Cell Biology, National Institute of Mental Health, and Laboratory of Neurochemistry, National Institute of Neurological and Communicative Diseases and Stroke, Bethesda, Maryland, 20892

ABSTRACT Mechanical and thermal changes associated with a propagated nerve impulse were determined using the garfish olfactory nerve. Production of an action potential was found to be accompanied by swelling of the nerve fibers. The swelling starts nearly at the onset of the action potential and reaches its peak at the peak of the action potential. There is a decrease in the length of the fibers while an impulse travels along the fibers. The time-

course of the initial heat was determined at room temperature using heat-sensors with a response-time of 2–3 ms. Positive heat production was found to start and reach its peak nearly simultaneously with the action potential. The rise in temperature of the nerve was shown to be $23 (\pm 4) \mu^{\circ}\text{C}$. In the range between 10° and 20°C , the temperature coefficient of heat production is negative, primarily due to prolongation of the period of positive heat produc-

tion at low temperatures. The amount of heat absorbed during the negative phase varies widely between 45 and 85% of the heat evolved during the positive phase. It is suggested that both mechanical and thermal changes in the nerve fibers are associated with the release and re-binding of Ca-ions in the nerve associated with action potential production.

INTRODUCTION

The olfactory nerve of the garfish, *Lepisosteus osseus*, contains $\sim 10^7$ non-myelinated nerve fibers of $0.2\text{--}0.3 \mu\text{m}$ in diameter (Easton, 1971). The great advantage of using this nerve and the similar pike olfactory nerve (Horwarth et al., 1975) for studies of various membrane phenomena lies in the fact that the ratio of membrane area to nerve volume is extremely large. According to Easton (1971), the membrane-to-volume ratio for the garfish olfactory nerve is 5,400 times as large as that for the squid giant axon of the same diameter.

The present paper describes the results of a study of rapid mechanical and thermal changes in the garfish olfactory nerve associated with a propagated nerve impulse. It has been shown previously that the production of an action potential in non-myelinated nerve fibers is accompanied by both mechanical and thermal changes in the fibers (see e.g., Iwasa and Tasaki, 1980; Horwarth et al., 1975). The present study was prompted by the situation that a new type of thermal detector with a high time-resolution could now be fabricated in this laboratory (see Methods). The use of garfish nerve preparations in the present study greatly simplified analyses of the experimental data acquired. The results described here indicate that mechanical and thermal changes associated with a propagated impulse are nonelectrical manifestations of

the electrochemical events underlying the process of nerve excitation.

MATERIALS AND METHODS

Garfish nerve preparations

Garfish were purchased from Worldwide Scientific Animals Apopka, Florida, and were kept alive in the laboratory by feeding them small goldfish until they were sacrificed. Olfactory nerves, usually 45–60 mm in length, were dissected by the method described by Easton (1971) with a slight modification. The blood vessels and the bundle of myelinated nerve fibers adhering to the olfactory nerve were removed under a dissecting microscope. The composition of the bathing solution (see Ritchie and Straub, 1975) was as follows: 120 mM NaCl, 2.5 mM KCl, 5 mM CaCl_2 , 60 mM sucrose, 24 mM D-glucose, and 5 mM Hepes (pH 7.4). Both mechanical and thermal measurements were performed, unless stated otherwise, at room temperature ($20\text{--}21^{\circ}\text{C}$).

Mechano-electric transducer

A piezoceramic bender (model R0505 G1195 bender element; Gulton Industries, Metuchen, New Jersey) was employed to detect rapid mechanical changes in the nerve (see Fig. 1A). The bender used was 1.5 mm wide and 20 mm long. Near one end of the bender, a 20-mm long stylus weighing ~ 20 mg was glued perpendicularly by using epoxy resin. At the tip of the stylus, a foot-plate with an area of roughly 1.5 mm^2 was attached. The other end of the bender was immobilized and was connected to the input of an operational amplifier (OPA 111) with a high feedback resistance ($10 \text{ G}\Omega$) and a parallel capacitor (500 pF). Small batteries were employed as a power supply for operating the amplifier. The bender, the amplifier, and the power supply were housed in a small metal box. The box was attached to a rack-and-pinion device with $1 \mu\text{m}$ accuracy. The resonant frequency of the bender (with a stylus

Please address all correspondence to Rm 2B-16, Bdg 36, National Institutes of Health, Bethesda, MD, 20892.

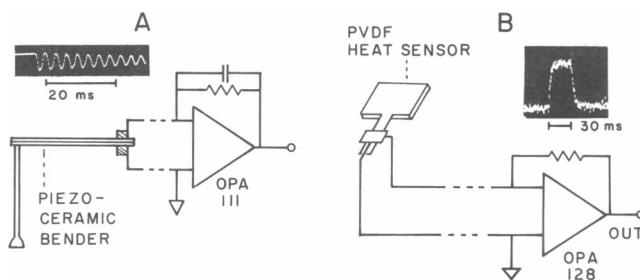


FIGURE 1 (A) Schematic diagram illustrating the mechano-electric transducer employed for detecting rapid mechanical changes in the garfish olfactory nerve. The photographic record on the top shows the output voltage of the operational amplifier (OPA 111) evoked by a sudden release of pressure applied to the stylus. (B) Diagram illustrating the device used for detecting heat production associated with a propagated impulse. The photographic record shows the output of the operational amplifier (OPA 128) produced by a heat pulse of 30 ms in duration and 5.5×10^{-3} deg/s in intensity. See the text for details.

attached) was 310 Hz (see the photographic record in Fig. 1 A). The coefficient of piezoelectric transduction was determined by measuring changes in the output voltage of the amplifier produced by loading and unloading small weights at the end of the stylus; it was ~ 0.0098 V per mg. The output of the amplifier was led to a capacity-coupled amplifier (with a time constant of 5 s) and then to a signal averager (model 1070; Nicolet Instrument Corp., Madison, WI).

Rapid mechanical changes in the nerve were investigated using a bundle of three (sometimes four) olfactory nerves placed on a small, slightly elevated platform in a plastic chamber provided with silver (or platinum) electrodes. The mechanical detector was lowered toward the nerve preparation from above. After the tip of the stylus was brought in gentle contact with the surface of the nerve bundle, the stylus was lowered an additional 20–25 μm . Records of mechanical changes were taken after averaging the signals over 4–8 trials repeated at ~ 4 s intervals. Precautions were taken to guard against extrinsic mechanical disturbances arriving at the detector. The same bender element was used also for recording changes in the longitudinal tension of the nerve associated with a propagated impulse (see Fig. 3).

Thermal detectors

The thermal detectors employed were fabricated by using 6- or 9- μm thick polyvinylidene fluoride (PVDF) film obtained either from Kureha Chemical Industries, Tokyo, or from Pennwalt Corp., PA. The film had already been heated, stretched and poled; it has an ~ 10 -nm thick aluminum layer deposited on each surface.

This highly pyroelectric film is also piezoelectric. In order to suppress the mechanical sensitivity of the detectors, the following design was adopted. A rectangular piece of PVDF film of a desired size, usually 2×4 (sometimes 3×4) mm^2 , was folded in half and its inner surfaces were glued together with a thin layer of epoxy resin. (Note that the piezoelectric effect of bending one layer of such a bi-layer PVDF unit is cancelled by the effect of the other layer). The resulting PVDF unit, 2×2 (or 2×3) mm^2 in size, was covered, in the early stage of the present study, with a layer of 10- μm thick silver sheet. The unit was glued to the aluminum-deposited surface of a 4- μm thick Mylar film stretched over a 4 (or 5) mm^2 hole in a plastic plate. The Mylar film glued to the plastic plate was taut and the PVDF unit glued to the film was located exactly in the middle of the hole in the plastic plate (see Fig. 5, top).

The plastic plate to which the PVDF unit was attached served as a partition, separating the lower (electronic) compartment of the detector from the upper (nerve) compartment. Electrical contacts to the aluminum layers on the PVDF sheet were made by way of an appendix to the PVDF sheet (see Fig. 1 B). These contacts were led to an operational amplifier (OPA 128) with a 3 (or 10) G Ω feedback resistance. The stray feedback capacity of the amplifier was estimated to be < 1 pF. The output of the operational amplifier was led to a signal averager via a capacity-coupled amplifier with a time-constant of 2 s. Usually three (sometimes four) strands of the olfactory nerve were placed above the heat-sensitive area of the detector. The wet weight of the portion of the nerve lying on the heat-sensitive area was in the range between 0.8 and 1.2 mg. The nerve preparation was stimulated with voltage pulses delivered with silver (or platinum) electrodes arranged on the upper surface of the plastic partition. Thermal responses of the nerve preparation were photographed after averaging the responses over 4–32 trials repeated at intervals of 3–4 s. No significant stimulus-artifacts (electrical or thermal) were encountered, when the entire lower surface of the plastic partition was covered with a copper foil.

The output of the pyroelectric thermal detector is proportional to the rate of temperature rise (see Roundy, 1975). The output voltage (V in volts) of the operational amplifier is directly related to the rate of temperature rise (dT/dt in deg/s) of the PVDF unit by the following equation:

$$V = a \cdot p \cdot R \cdot (dT/dt)$$

where a is the effective area of the PVDF film (cm^2), p the pyroelectric coefficient of the PVDF film (4×10^{-9} coul $\cdot \text{cm}^{-2} \cdot \text{deg}^{-1}$ for the 9 μm PVDF film), and R the feedback resistance of the amplifier (ohms). The response-time of the thermal detector described above is determined almost exclusively by the time required for conduction of heat from the heat-source above the Mylar sheet to the PVDF unit below. The thermal conductivity of Mylar is known to be 0.91×10^{-4} cal $\cdot \text{s}^{-1} \cdot \text{deg}^{-1} \cdot \text{cm}^{-1}$ (see Encyclopedia Chemical Technology, vol. 13, 1981). The volume specific heat (expressed in cal $\cdot \text{deg}^{-1} \cdot \text{cm}^{-3}$) is 0.60 for PVDF and 0.58 for silver. The thermal diffusivity (expressed in cm^2/s) is 1.4×10^{-3} for water, 0.53×10^{-3} for PVDF, and 1.72 for silver.

During the course of the present study, a number of thermal detectors with a variety of response-times were constructed. In the case of a heat-sensor consisting of four layers of 2×2 mm^2 PVDF film of 9 μm in thickness enclosed in two layers of 10- μm thick silver sheet and glued to a 4- μm thick Mylar film, the temperature of the PVDF unit is expected to rise in a spatially uniform manner. The rate of temperature rise, dT/dt , of the unit is then given by the equation

$$h dT/dt = K(T_0 - T),$$

where T_0 is the temperature of the heat source placed on the Mylar film, h is the heat capacity of the sensor, and K represents the thermal conductivity multiplied by the area-to-thickness ratio of the Mylar film. Because h is $\sim 1.5 \times 10^{-4}$ cal/deg and K is 9.1×10^{-3} cal/s $\cdot \text{deg}$, the time constant h/K is found to be ~ 16 ms.

The response-time of a heat-sensor fabricated by using two layers of 6- μm thick PVDF film glued to a 4- μm thick Mylar film (without inserting silver sheet), the response time may be estimated by the formula $s^2/2D$, s is the distance of the PVDF layers from the heat source and D the thermal diffusivity of PVDF (see Tasaki and Nakaye, 1986). By choosing s to be ~ 16 μm , the response-time is expected to be ~ 2.3 ms.

The responses of the thermal detectors fabricated were calibrated by using Joule's heat as a source. A pulse of constant current was passed through a strip of filter paper moistened with Ringer's solution and placed on the heat-sensitive area of the detector (see the photographic

record in Fig. 2*B*). The results of calibration were in substantial agreement of the expected values.

RESULTS

The action potential of the olfactory nerve

Electrophysiological properties of the garfish olfactory nerve have been thoroughly investigated by Ritchie and Straub (1975). Here, the aspects of the previous findings which have direct bearing on the following mechanical and thermal studies were reproduced. At room temperature (20°–21°C), the average conduction velocity of the olfactory nerve fibers is ~0.2 m/s. Reflecting a small variation in fiber sizes, the foot of the action potential propagates at a rate ~10% higher than the average velocity and the trailing edge travels at a slightly slower rate. As a consequence of this dispersion of velocity, there is a progressive broadening and amplitude reduction of the compound action potential as the impulse travels along the nerve. Extrapolated to zero conduction distance, the duration of the action potential was found to be 10–13 ms. The product of this action potential duration and the conduction velocity is then 2.0–2.6 mm. Near the site of stimulation, therefore, a nerve impulse occupies ~2.3 mm long portion of the nerve. Based on this finding, the sensors employed in most of the following mechanical and thermal measurements were constructed to occupy a portion of the nerve of ~2 mm or less in length.

Mechanical changes in the olfactory nerve

Fig. 2*A*, shows an example of the records of mechanical changes in the olfactory nerve evoked by electric stimulation of the nerve. The footplate of the stylus was placed on a portion of the nerve ~3 mm away from the stimulating cathode in this experiment. The stimulating voltage pulse (0.5 ms in duration) was varied between 5 and 30 V. When the tip of the stylus was in gentle contact with the surface of the nerve, the observed changes in the force were small. As the stylus was lowered, the force developed in response to stimulation increased. The records presented in the figure were obtained with the tip of the stylus lowered ~25 μ m below the point of initial contact. No deleterious effect on impulse conduction was observed at this level of nerve compression.

Upward deflections of the oscillograph trace in the figure represent the development of a force which tends to displace the stylus upward; these mechanical responses may therefore be regarded as a sign of swelling of individual nerve fibers associated with the process of excitation. The maximum value of the force observed

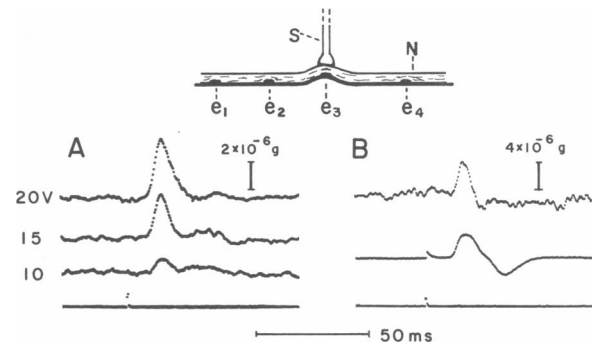


FIGURE 2 (*A*) Mechanical responses of a garfish olfactory nerve to voltage pulses of 0.5 ms in duration. The voltage applied to the nerve using electrodes e_1 and e_2 are given. S represents the stylus for detecting mechanical changes in the nerve (N). (*B*) Records showing the temporal relationship between the mechanical response (*top*) and the action potential (*bottom*). Electrodes e_3 and e_4 were employed for recording the action potential. The observed amplitude of the action potential of the nerve preparation (three strands of wet olfactory nerve bound together at both ends) was ~6 mV.

was ~5 μ g under these conditions. As expected, the latency of the mechanical response increased with the distance between the stimulating cathode and the site of mechanical recording. The velocity at which the mechanical change was propagated was close to 0.2 m/s. As in the case of action potentials, the mechanical changes were found to broaden as they travel along the nerve.

In the squid giant axon, it has been shown that the peak of swelling during excitation coincides accurately with the peak of the action potential recorded intracellularly at the site of mechanical recording (Iwasa and Tasaki, 1980). Here, a similar comparison was made between the mechanical response and the externally recorded action potential (Fig. 2*B*). One of the silver electrodes (0.3 mm in width) for recording the action potential was located ~2.5 mm away from the stimulating cathode. The stylus of the mechanical detector was brought in contact with the portion of the nerve above this electrode. The time-course of the mechanical responses recorded in this manner was compared with that of the action potentials recorded with this electrode referred to another electrode located at a distance of ~3 mm. It was found by this procedure that the mechanical response started roughly at the onset of the action potential. In most of the preparations examined, the time at which the mechanical response reached its peak was found to coincide roughly with the peak time of the action potential.

It was shown previously that swelling of non-myelinated nerve fibers is accompanied by a decrease in the length of the fibers (Tasaki, 1982; Tasaki and Byrne, 1982). Here, a similar observation was made using garfish olfactory nerves. The experimental setup employed

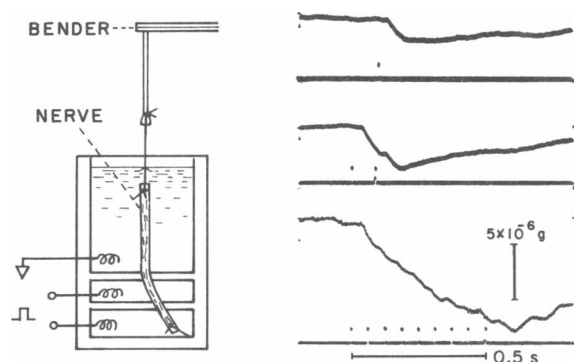


FIGURE 3 (Left) Diagram illustrating the experimental arrangement employed for recording changes in the mechanical force developed in the longitudinal direction of the nerve. The lower portion of the nerve was immobilized by passing it through small holes in the two partitions. The length of the nerve in the upper compartment was ~ 20 mm. The sustained tension keeping the nerve in an upright position is estimated to be ~ 30 mg. (Right) Isometric recording of shortening of the nerve associated with the generation of a propagated impulse. Downward movements of the traces represent the development of a force tending to pull the tip of the piezoelectric bender downwards. The lower traces indicate the electric pulses delivered across the lower partition.

for this observation is illustrated schematically in Fig. 3, left. An olfactory nerve was introduced vertically into a plastic chamber filled with saline solution. The chamber was provided with two partitions with small connecting holes. The lower portion of the nerve was passed through the holes in the partitions. The upper end of the nerve was connected to the stylus of a piezoceramic bender with fine thread. There was a coil of silver wire in each of the three compartments of the chamber. The two silver coils at the bottom were used for delivering brief voltage pulses to the nerve. The silver wire in the upper compartment was grounded.

The records furnished in Fig. 3, (right), show that a force was developed, tending to pull the stylus downward, when the nerve was stimulated. The onset of this longitudinally recorded mechanical response was shown to reflect the time required for nerve conduction between the stimulating cathode and the upper compartment. The duration of the mechanical responses recorded in this manner were considerably longer than the mechanical changes recorded in the transverse direction. The major source of this difference in duration is considered to be slow conduction of the impulse along the portion of the nerve in the upper compartment. Repetitive stimulation of the nerve under these conditions produced "summation" of mechanical responses. Summation of shortening in the crab nerve, which resembled a tetanic contraction in the muscle, has been reported previously (Tasaki and Byrne, 1982).

Heat production

In the early stage of the present study, thermal detectors constructed with two or four layers of PVDF film covered with two layers of silver sheet (see Methods) were used. It was noted that these detectors were not adequate to obtain faithful recordings of thermal responses of the garfish olfactory nerve. In the later stage, fast-responding detectors were constructed using two layers of PVDF film (see Methods). The time-courses of the heat generated by the nerve in response to electric stimulation were examined by using these detectors.

Fig. 4, (left), shows an example of the records obtained with a 2×2 mm² square thermal detector with a response time of ~ 2 ms (see Fig. 1 B). When a strong rectangular voltage pulse was delivered at a distance of 7.4 mm away from the heat-sensor, a response of the nerve to the stimulating pulse started ~ 33 ms after the pulse. The latency of the response decreased as the conduction distance was reduced (see the records marked 4.3 and 1.2 mm). The dependence of the latency on the conduction distance yielded, in all of this and four other experiments, a velocity of ~ 0.22 m/s. This finding indicates that the observed response was associated with a propagated nerve impulse.

It is seen in the figure that the response evoked by a stimulating pulse delivered close to the heat-sensor was larger in amplitude, shorter in duration, and was more strongly diphasic than those elicited by a pulse delivered at a distance. Evidently, the observed changes in the time-course of the response are brought about by temporal dispersion of nerve impulses in individual fibers.

The downward deflections of the top trace in the left-hand records represent absorption of heat by the nerve. The right-hand records in the figure were derived

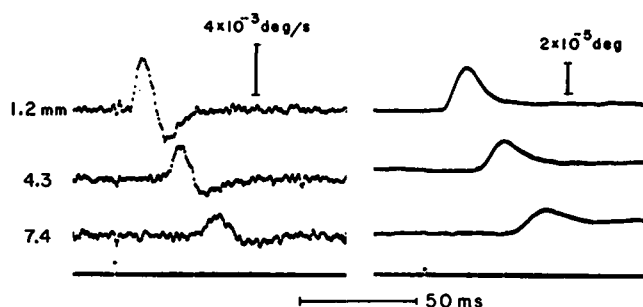


FIGURE 4 Thermal responses of the garfish olfactory nerve at 20°C, demonstrating the variation in the time-course of heat production depending on the distance (given in the figure) between the stimulating cathode and the nearest edge of the heat-sensor. The records were taken after signal-averaging over 16 trials. The records on the right were obtained from the records on the left by integration.

from the left-hand records by integration; they represent the time-courses of the temperature after the delivery of stimulating pulses. By comparing the records in Fig. 4 with those obtained previously by Howarth et al. (1975), it is evident that the properties of the "initial heat" of the garfish olfactory nerve are very similar to those of the pike nerve. In the studies conducted by previous investigators using a thermopile as a heat-sensor, the existence of positive and negative phases of the initial heat was deduced indirectly by the "heat-block" method. Here, on the contrary, the time-courses of the initial heat were determined directly using thermal detectors with a high time-resolution.

The records in Fig. 4 were taken with a heat-sensor which occupied a 2-mm long portion of the nerve. The time required for an impulse to travel across this portion is ~ 10 ms. This time could be reduced effectively to half by the following "collision procedure" (see Fig. 5, *top*).

The thermal detector employed was a 2×2 mm² PVDF unit glued to a 4×4 mm² Mylar film under strong tension. The nerve chamber was provided with two pairs of stimulating electrodes located close to the edge of the Mylar film, one pair on each side of the heat-sensitive area. A 50–60 mm long olfactory nerve was bent twice and a bundle consisting of three strands of the nerve was placed on the heat-sensitive area. Record A in the figure shows the response of the nerve to a strong stimulating pulse delivered using the electrodes on one side of the heat-sensor. Record B was obtained by stimulating the

nerve with the electrodes on the other side; the nerve impulse evoked in this case arrived at the heat-sensor from the opposite direction. Record C was obtained by delivering two stimulating pulses simultaneously to the nerve; collision of two impulses took place in the middle of the sensor in this case. It is seen in the figure the thermal response associated with collision of two impulses (Record C) rose more rapidly and reached a slightly higher peak level than the other responses in the figure. The duration of this response was slightly shorter.

The effect of collision on the thermal response may be explained in the following manners. When two nerve impulses arrive at the 2-mm long heat-sensor simultaneously from opposite directions, the two halves of the sensor, each 1 mm in length, are heated synchronously. When an impulse reaches the sensor from only one side (Record A or B), the two halves of the sensor are heated successively at an interval of ~ 5 ms. (Note that the time required for an impulse to travel across the 1 mm portion of the nerve is ~ 5 ms.) It is expected, therefore, that Record A (or B) can be reconstructed by adding a half of Record C to another half of Record C displaced by ~ 5 ms. In fact, thermal responses reconstructed by this procedure were found to agree reasonably well with the experimentally observed responses. (See also the records obtained at 10°C described below.)

This agreement between the observed and reconstructed thermal responses indicates that the present method of thermal measurement yields reasonably faithful records of thermal events occurring in the olfactory nerve. It is to be noted here that the duration of the positive heat production observed by the collision technique was between 12 and 15 ms. Obviously, this duration is very close to that of the action potential recorded at a short distance away from the site of stimulation. The average value and the standard deviation of the peak heat production determined by using 16 olfactory nerve preparations were 3.4 ± 0.9 mdeg/s under these conditions. The peak of the temperature rise determined by integration of the response-time curve, was 23 ± 4 μ deg. The ratio of the positive heat to the negative heat varied between 0.45 and 0.85.

A comparison was made at this point between the time-course of the action potential and that of the thermal response. Using the experimental setup diagrammatically illustrated in Fig. 5, (*top*), an additional recording electrode, constructed with a piece of 10 μ m thick silver foil, was brought in contact with the portion of the nerve on the heat-sensitive area of the sensor. The point of contact between the silver foil and the nerve surface was located 0.5 mm away from the center of the heat-sensor. The reference electrode was a large silver wire making contact with the nerve ~ 6 mm away from the silver foil electrode.

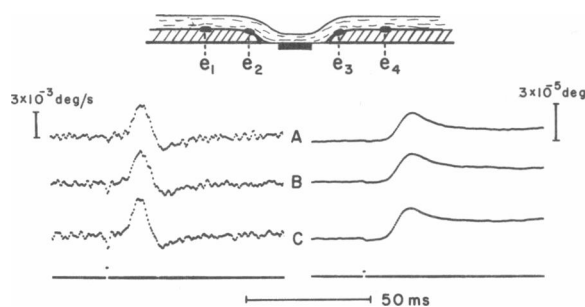


FIGURE 5 (*Top*) Diagram illustrating the experimental setup employed for recording thermal responses of the nerve by using the collision technique. Stimulating voltage pulses were delivered using electrode pair e_1 and e_2 and pair e_3 and e_4 . The distance between the stimulating cathodes (e_2 and e_3) and the nearest edge of the 2×2 mm² PVDF heat-sensor was 1.2 mm. (*Bottom*) Thermal responses associated with collision of impulses (Record C). Record A was obtained by delivering a brief, strong stimulating pulse to the nerve with the electrodes on one side of the sensor. Record B was taken by stimulating the nerve with the electrodes on the other side. Record C was taken by delivering the two stimulating pulses to the nerve simultaneously. The records were photographed after averaging 16 times. The right-hand traces in the figure were obtained by integration of the left-hand traces.

A strong stimulating pulse was delivered at a distance of 1.6 mm away from the silver foil electrode.

The lower trace in Fig. 6 represents the action potential recorded with this electrode arrangement. The upper trace in the figure represents the time-course of the thermal response obtained from the same nerve by the collision procedure. It is seen in the figure that these two traces rise and fall almost simultaneously. In all of the seven records taken in this manner, the peaks of the two responses were found to coincide fairly accurately, the discrepancy being usually <2–3 ms. The negative heat production started near the end of the depolarizing phase of the action potential.

Finally, the results of several measurements of heat production at low temperatures are described briefly. In order to achieve effective cooling of the nerve on the heat-sensor, a special thermal detector in which the lower (electronic) compartment was separated from the heat-sensor (Tasaki and Nakaye, 1986) was used. The thermal detector carrying an olfactory nerve was introduced into a large metal box of which the temperature was kept at 10°C. The temperature of the nerve was determined by using a thermister tip placed in a small space between the heat-sensor and the lower compartment of the detector.

Fig. 7 shows an example of the records obtained. The records on the top were taken from the same preparation by the use of the collision technique before the preparation was introduced into the cooling box. At ~10°C, there was usually a very small (~20% or less) decrease in the amplitude of the thermal response. Because the duration of the positive phase of the initial heat was markedly prolonged by cooling, the peak level of temperature rise evoked by nerve stimulation was 35–45% higher than that observed at room temperature. In other words, the temperature coefficient of the total heat production by the

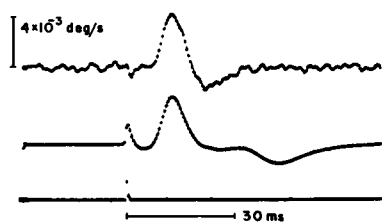


FIGURE 6 Comparison of the time-course of the thermal response (*top*) with that of the action potential (*bottom*). The thermal response was recorded by the collision technique using the electrode arrangement illustrated in Fig. 5, *top*. The action potential was recorded by using a silver foil electrode making contact with the surface of the nerve at a point ~0.5 mm off the center of the heat-sensor; the reference electrode was on the nerve at ~6 mm away from the silver foil electrode. The stimulating cathode was located at 1.7 mm away from the silver foil electrode. The amplitude of the action potential, ~8 mV.

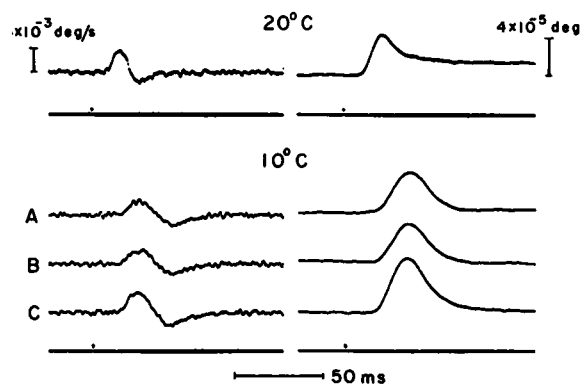


FIGURE 7 Effects of cooling on the thermal response of the olfactory nerve. The records on the top were taken at room temperature using the experimental setup illustrated in Fig. 5, *top*. The records at the bottom were taken ~25 min after introducing the nerve in a metal box kept at 10°C. Records A and B show the responses to stimulating pulses delivered to only one side of the heat-sensor (as in the records shown in Fig. 5). Records C show the thermal responses associated with collision of nerve impulses in the middle of the heat-sensor.

nerve was negative (see Discussion). As has been reported already by Howarth et al. (1975), there was an increase at low temperatures in the amount of heat absorbed during the negative phase. The size of the negative heat was variable; but, the total amount of heat absorbed was usually smaller than the amount of heat generated.

DISCUSSION

The main objective of this paper is to present the results of quantitative determinations of mechanical and thermal changes associated with nerve conduction in non-myelinated fibers. The data described above, demonstrating nonelectrical manifestations of excitation processes, are expected to provide some clues as to the nature of the physicochemical changes taking place in the nerve fibers.

The mechanical properties of these non-myelinated fibers do not appear to be fundamentally different from those of the squid or crab nerve fibers investigated previously by using both isometric and isotonic measuring devices (Tasaki, 1982; Tasaki and Byrne, 1982). The swelling observed in the squid giant axon was shown to be more than two orders of magnitude greater than the value expected from the process of $\text{Na}^+ - \text{K}^+$ ion-exchange during excitation. It is well-known that ion-exchange processes involving divalent and monovalent cations are accompanied by pronounced swelling or shrinkage in artificial gels (see e.g., Katchalsky and Zwick, 1955). The proposal that the swelling observed in non-myelinated nerve fibers is associated with the release of a portion of

the bound Ca^{2+} in the nerve (Tasaki, 1982) appears to account for the mechanical phenomenon observed in the olfactory nerve. (Note that nerve fibers are capable of maintaining excitability for a long time in saline devoid of Mg^{2+} [see Methods], but not when Ca^{2+} in the medium is completely replaced with Mg^{2+} and EGTA.)

The decrease in the length of the garfish nerve during excitation may be regarded as an expression of "swelling-retraction" known to occur invariably in fibrous biopolymers (see e.g., Hermans, 1949). As a consequence of this decrease in the length, the change in the total volume of the nerve fibers is expected to be very small.

The time-courses of the heat produced by non-myelinated nerve have been the subject of repeated discussions in the literature (see Abbott et al., 1968; Howarth et al., 1968, Ritchie, 1973). In the past, thermal responses of the olfactory nerve were recorded by using thermopile-galvanometer detectors of which the response-time was ~60 ms or longer. Because of the limited time-resolution of the detectors employed, the thermal events in the nerve had to be slowed down by cooling. The time-course of heat production was determined indirectly by the sizes of "heat blocks" which could reproduce the observed output of the galvanometer (see Howarth et al., 1975). Nevertheless, many of the previous results have been confirmed by the present study.

In the range of temperature between 0° and 10°C, Howarth et al. (1975) have shown that the positive initial heat of the pike olfactory nerve has a positive temperature coefficient. The present study has demonstrated that, in the range between 10° and 20°C, the temperature coefficient is negative. It was noted during the present study that, at temperatures below ~7°C, both electrical and thermal responses of the garfish olfactory nerve were severely depressed. It is known that the nerve membrane becomes depolarized at very low temperatures (see p. 248 in Tasaki, 1982). Excitation of a nerve in a partially depolarized state is expected to be accompanied by a small heat production. The marked depression of electrical excitability at very low temperatures may also be explained on the same basis.

The thermal responses recorded with detectors with a reasonably high time-resolution have indicated that their duration is very close to that of the action potential. Furthermore, when the action potential was recorded from the center of the area of thermal recording, the time at which the thermal response reaches its peak coincides approximately with the peak time of the action potential. Because the positive heat production was shown to last during the entire period of the action potential, it is improbable that the free energy stored in the membrane capacitor (see e.g., Ritchie, 1973) makes a significant contribution to the observed heat. It is also unlikely that

much of the observed nerve heat production can be attributed to mixing of cations (Na^+ and K^+) across the membrane (Howarth et al., 1975). In a variety of cation-exchangers, replacement of monovalent cations for divalent cations involves an enthalpy decrease of 1.5–3.0 kcal/equivalent (see Coleman, 1952; Flett and Meares, 1966; Sherry, 1968). It is suggested, therefore, that positive and negative heat production described in this paper are associated with a reversible ion-exchange process involving monovalent and divalent cations. The smallness of the olfactory nerve fibers appears to facilitate detection of the signs of cooperative interactions between nerve membrane and the superficial layer of the axoplasm.

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REFERENCES

- Abbott, B. C., A. V. Hill, and J. V. Howarth. 1968. The positive and negative heat production associated with a single impulse. *Proc. R. Soc. Lond. B. Biol. Sci.* 148:149–187.
- Coleman, N. T. 1952. A thermochemical approach to the study of ion exchange. *Soil Sci.* 74:115–125.
- Easton, D. M. 1971. Garfish olfactory nerve: easily accessible source of numerous long, homogeneous, nonmyelinated axons. *Science (Wash. DC)*. 172:952–955.
- Flett, D. S., and P. Meares. 1966. Thermodynamics of cation exchange. Part 4. Uni-, bi- and tervalent ions on Dowex 50. *Faraday Soc. Trans.* 62:1469–1481.
- Hermans, P. H. 1949. Reversible systems. In *Colloid Science II*. Chapt. XII R. H. Krut, editor. Elsevier-North Holland, Inc., New York. 637.
- Howarth, J. V., R. D. Keynes, and J. M. Ritchie. 1968. The origin of the initial heat associated with a single impulse in mammalian non-myelinated nerve fibres. *J. Physiol. (Lond.)*. 194:745–793.
- Howarth, J. V., R. D. Keynes, J. M. Ritchie, and A. von Muralt. 1975. The heat production associated with the passage of a single impulse in pike olfactory nerve. *J. Physiol. (Lond.)*. 249:349–368.
- Iwasa, K., and I. Tasaki. 1980. Mechanical changes in squid giant axons associated with production of action potentials. *Biochem. Biophys. Res. Commun.* 95:1328–1331.
- Katchalsky, A., and M. Zwick. 1955. Mechanochemistry and ion-exchange. *J. Polymer Sci.* 17:221–234.
- Ritchie, J. M. 1973. Energetic aspects of nerve conduction: the relationships between heat production, electrical activity, and metabolism. *Prog. Biophys. Mol. Biol.* 26:147–187.
- Ritchie, J. M., and R. W. Straub. 1975. The movement of potassium ions during electrical activity, and the kinetics of the recovery process, in the non-myelinated fibres of the garfish olfactory nerve. *J. Physiol. (Lond.)*. 249:327–348.
- Roundy, C. B. 1975. performance and uses of high speed pyroelectric detectors. *Proc. Soc. Photo-optical Instr. Eng.* 62:191–198.

Sherry, H. A. 1968. The ion-exchange properties of zeolites. IV. Alkali earth ion exchange in the synthetic zeolites Linde X and Y. *J. Phys. Chem.* 72:4086-4094.

Tasaki, I. 1982. *Physiology and Electrochemistry of Nerve Fibers*. Academic Press. Inc., New York. pp. 348.

Tasaki, I., and P. M. Byrne. 1982. Tetanic contraction of the crab nerve evoked by repetitive stimulation. *Biochem. Biophys. Res. Commun.* 106:1435-1440.

Tasaki, I., and T. Nakaye. 1986. Heat produced by the dark-adapted bullfrog retina in response to light pulses. *Biophys. J.* 50:285-293.