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INTERPRETATION OF LIGHT SCATTERING ASSOCIATED WITH PROLONGED NEURAL ACTIVITY AND TEMPERATURE CHANGES

HOWARD J. BRYANT *, REIN KILKSON, RAPHAEL GRUENER and WILLIAM S. BICKEL

Departments of Physiology and Physics, The University of Arizona, Tucson, Ariz. 85724 (U.S.A.)

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Summary

Changes in light scattering from lobster giant axon which accompany the action potential were observed during periods of prolonged stimulation and as a function of temperature. At an initial temperature of 10°C most (more than 90%) axons produced positive light scattering signals which increased in amplitude when the temperature was lowered. At 2 and 5°C approximately half of the axons produced positive scattering signals. The remaining half produced negative scattering signals which became positive when the temperature was raised to 10°C. The amplitude of the negative signals followed sigmoid transition to positive values as a function of time. The time and temperature dependence of the signal are interpreted in terms of differential changes between the indices of refraction of the membrane matrix and the open or closed early activation channel.

Introduction

Conduction of the action potential is one of the fundamental processes of the nervous system. Observations of physical phenomena correlated with electrical conduction have been reported [1—6]. We have shown that light scattering from axons during propagation of the action potential may provide clues concerning mechanisms of membrane excitation [7]. In that report we correlated the properties of the light scattering with a two-state early (Na^+) activation channel. Studies by Cohen et al., [5] and Keynes [8] have reported that the scattered light intensities from squid and pike axons have large temperature-dependent components. Keynes [8] observed in garfish nerve that the scattered light intensity can reverse in sign (from an increase to a decrease in

* To whom correspondence should be sent at present address: Howard J. Bryant, Ph.D. University of Texas Medical Branch Marine Biomedical Institute 200 University Blvd. Galveston, TX 77550

scattered light) as the temperature is changed. The origin of this sign inversion may be ascribed to species differences, changes in the index of refraction of the bathing solution or structural modifications of the axon membrane components.

In this paper we report a study of the scattering signal from lobster axon as a function of temperature and during long term repetitive firing. Our data show that the sign of the light scattering signal can be positive or negative, depending on the initial experimental conditions. The sign of the scattering signal can be changed by altering the temperature. However, as the preparation was stimulated to exhaustion the sign of the scattering signals invariably became positive regardless of the sign of the initial signal or variations in temperature. We interpret these data in terms of a two-state channel model [7] and differential changes in the index of refraction between the membrane matrix and the channel complex. A preliminary account of this work has been previously reported [9].

Materials and Methods

Giant axons were isolated from the ventral nerve cord of the lobster *Homarus americanus* and mounted in a lucite experimental chamber. The axon was illuminated by a 100 W tungsten-halogen lamp. Changes in the intensity of light scattered from the axon were observed at 90° to the incident light beam. All experimental procedures have been previously described [7]. The temperature was maintained at 10, 5, or 2° C by circulating a refrigerated water/alcohol solution within the chamber walls. (Frequently the bath temperature was below the dew point. Condensation of airborne moisture on the optical surfaces was prevented by enclosing the apparatus in a plastic bag and replacing the entrapped air with dry nitrogen.) The chamber temperature was measured with a YSI thermistor probe located in the bath solution approximately 7 mm from the illuminated portion of the axon. Temperatures were maintained constant within ±0.5° C. Approximately 20 min were required for the system to achieve thermal equilibrium for a temperature change of 5° C.

Normal lobster saline [10] was used for dissection and storage of the preparation. Control measurements were done in a low potassium solution: Na^+, 483 mM, K^+, 1 mM, Cl^- 535 mM, Ca^{2+} 25 mM, and Tris · Cl, 1 mM. Only axons with satisfactory resting potentials (<−70 mV) and action potential amplitudes (>90 mV) were used.

Results

Observations at 10° C

At an initial temperature of 10° C, over 95% of the axons (50 axons) produced a positive-going light scattering signal as shown in Fig. 1. The scattering signal ($\Delta I/I_0$) is defined to be positive when the scattered light intensity increases by $+\Delta I$ during excitation over the resting scattering level I_0. At 10° C, the remaining 5% of the axons produced a negative-going scattering signal which was followed by a small, longer-lasting positive tail (Fig. 2). A small number of axons (less than 1%) gave scattering signals which were too small

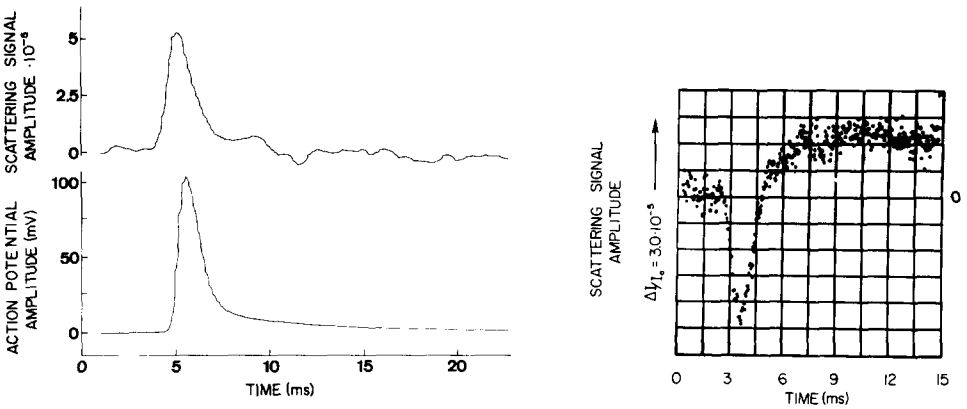

Fig. 1. Representative light scattering signal and action potential at 10° C. Record was taken 10 min after the beginning of the experiment. The axon had propagated 3072 action potentials prior to this record. The scattering signal was averaged over 1024 events. The action potential was averaged over 64 events.

Fig. 2. Typical negative light scattering signal measured at 10° C and averaged over 2002 events. The arrow shows the magnitude and direction of an increase in the light scattering signal.

($\Delta I \simeq 0$) to detect with our apparatus. Axons exhibiting these three types of scattering were indistinguishable from one another with respect to action or resting potential characteristics. Axons which initially had positive scattering signals continued to produce positive signals until excitability was lost. Continued stimulation of axons initially producing negative scattering signals led to scattering signals that went through zero and then became positive. Axons initially having zero signals produced measurable positive-going signals which grew into full sized scattering responses after repetitive stimulation. In all cases, the final positive signals which evolved were indistinguishable for all axons. When excitability failed, the light scattering signal could no longer be measured.

Observations at low temperatures (2 and 5° C)

When the final dissection (desheathing and isolation of a single axon) and the initial measurements were carried out at 2 or 5° C, approximately 50% of the axons produced negative light scattering signals. In time ($\simeq$2 h) these negative signals inverted and became positive. This behavior is illustrated in Figs. 3 and 4. Fig. 4A shows the action potential and light scattering signals in an experiment when the scattering was initially negative. Fig. 4B was recorded from the same axon later after the scattering signal had inverted. The change in the sign of the light scattering signal at constant temperature followed an S-shaped curve resembling a phase transition phenomenon as demonstrated in Fig. 5. While the action potential amplitude decreased slowly with time, there was no significant action potential amplitude variation or discontinuity when the optical signal reversed its sign (Fig. 5).

The remaining 50% of the axons at 2 or 5° C produced a positive light scattering signal which remained positive (constant temperature) as a function of time. The amplitude of these low-temperature positive-going signals, however, was 2 to 5 times larger than that of positive signals measured at 10° C.

The behavior of the light scattering signal amplitude as a function of temper-

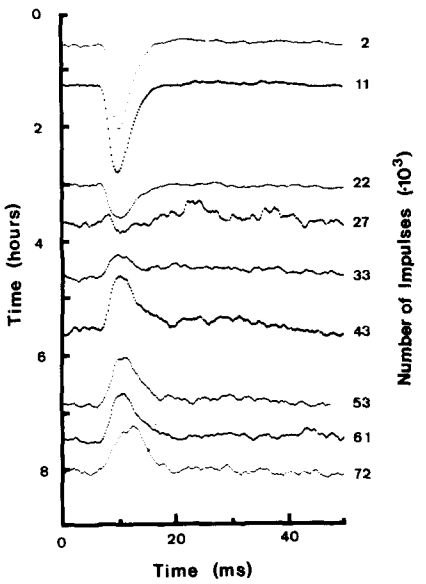

Fig. 3. Time-correlated changes of an initially negative light scattering signal. The signal decreases in amplitude as the number of stimulations increases. At approximately 3.5 h, the scattering signal amplitude is zero. As time and number of stimulations increase, the scattering signal becomes positive and achieves steady state in 6 h. Temperature, 2° C.

ature depended on the initial sign of the scattering. Signals that were initially negative at 5° C could be inverted by raising the temperature to 10° C (Fig. 6). This effect was reversible when the temperature was decreased to 5° C. Further cycling of the temperature resulted in a constant, positive, scattering signal. The maximum number of sign changes observed before the scattering signal

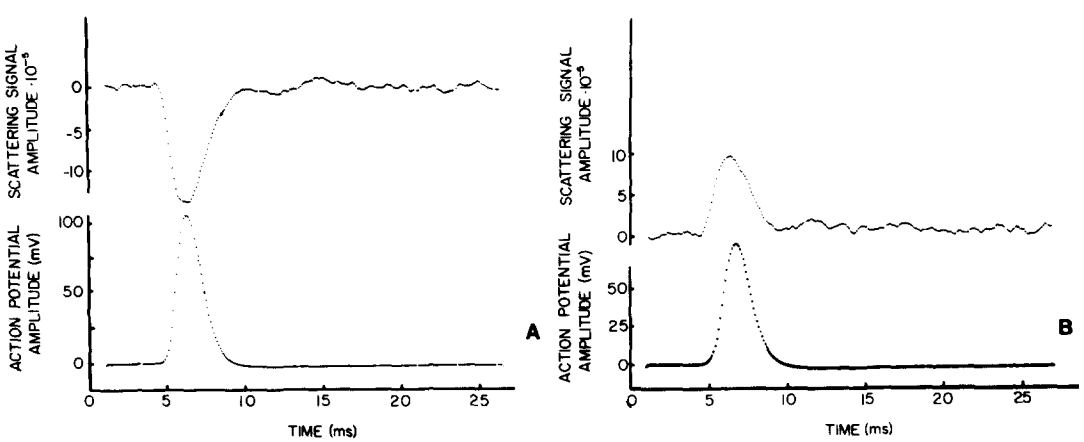

Fig. 4. Light scattering and action potential signals at 2° C. Data were taken from the axon shown in Fig. 5. A, Data obtained 2 h after the start of the experiment, corresponding to $2 \cdot 10^4$ stimulations; B, data obtained 4.5 h after the start of the experiment, corresponding to $5 \cdot 10^4$ stimulations. Optical signals (upper traces in A and B) were averaged over 1024 and 2048 events, respectively. The action potentials (lower traces in A and B) were averaged over 256 events.

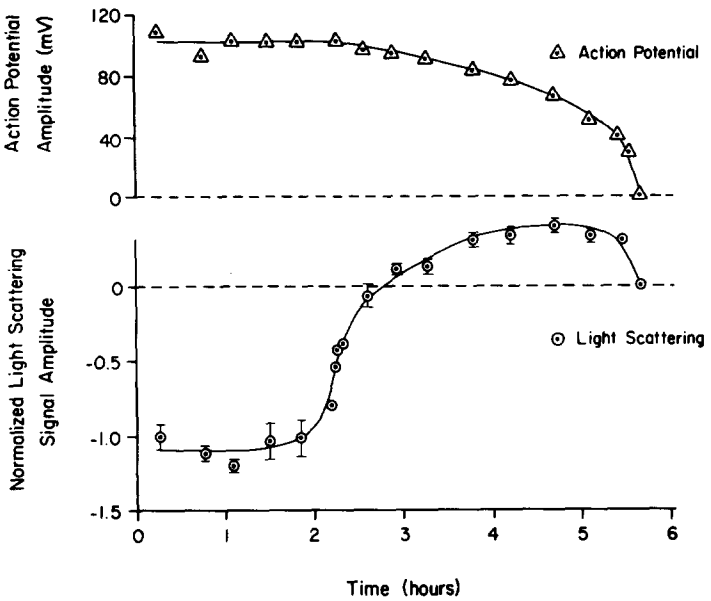

Fig. 5. Normalized light scattering and action potential amplitudes as a function of time. The scattering signal amplitude has been normalized to the initial scattering value. Each datum point with error bars on the light scattering curve represents the mean of 4 measurements taken over a 6 min interval. The 4 measurements shown at approx. 2.2 h were not averaged because of the rapidly changing signal during this transition phase. Light scattering values are the mean ± S.D. Each plotted action potential amplitude is its average value during the 6 min interval. Time zero was taken when the animal was killed. Temperature is 2 ± 0.5°C. Solid lines are drawn between the points to aid the eye.

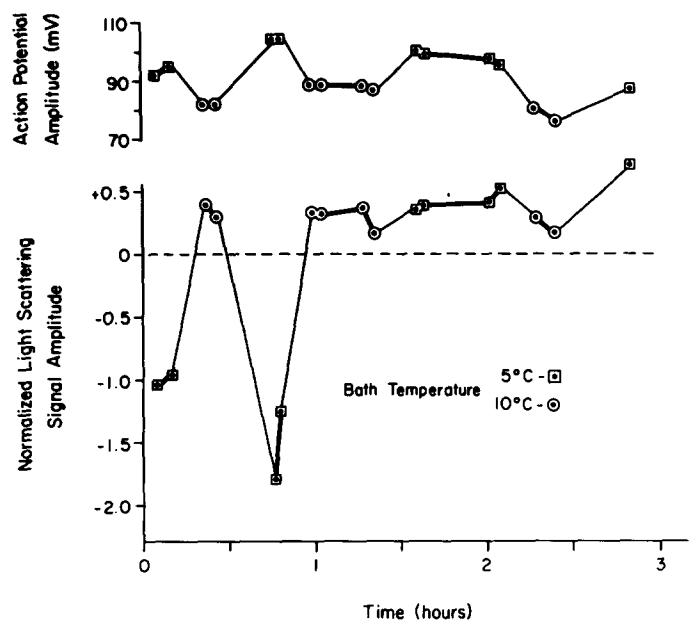

Fig. 6. Temperature dependence of the normalized light scattering amplitudes and action potential amplitudes. Data from one axon. The first measurements were made at 5 ± 0.5°C (squares). Subsequent measurements were made as the temperature was cycled between 10 (circles) and 5°C until the axon became inexcitable. The solution surrounding the axon was changed after every 4 measurements. Solid lines are drawn between the points to aid the eye. The light scattering amplitudes were normalized to the initial value at 5°C.

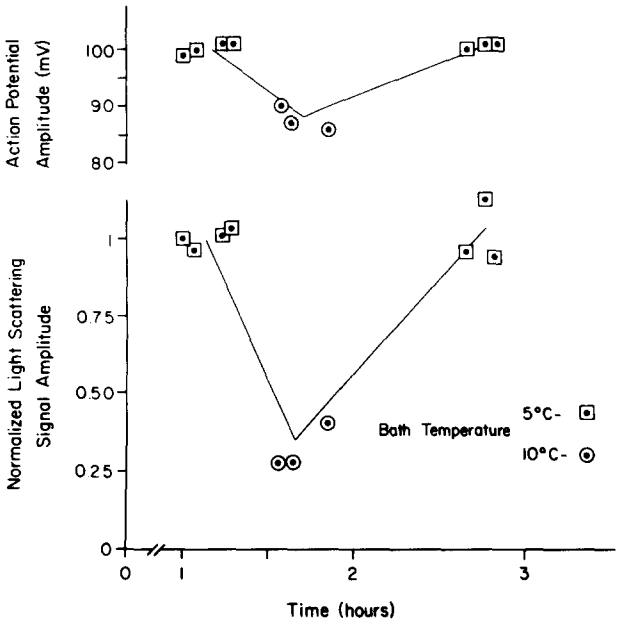

Fig. 7. The effects of temperature on the light scattering and action potential amplitudes. The first measurements were made at 5° C (squares). Subsequent measurements were made as the temperature was raised to 10° C (circles) and then returned to 5° C. The light scattering amplitudes were normalized to the initial value at 5° C.

became irreversibly positive was seven. Scattering signals that were initially positive at 5° C decreased in amplitude, but remained positive as the bath temperature was raised to 10° C (Fig. 7). This behavior was reversible as long as the axon remained excitable. Temperature changes also affected the amplitude of the action potential, although to a much smaller degree, compared to the light scattering signal (Figs. 6 and 7, upper traces).

Discussion

Light scattering occurs as the result of spatial variations in the refractive index of the transmitting medium. Changes in light scattering arise from changes in this refractive index or from changes in its spatial distribution. We assume that the axon surface consists of a matrix structure of refractive index n_m and early activation channel complexes of refractive index n_{ch} embedded in this matrix. Our two-state model proposes that the channel complexes exist in either an open (conducting) or a closed (non-conducting) configuration. Each of these states is characterized by an index of refraction n_o or n_c, respectively. We suggest that the scattering signal measured during excitation arises from the transitions n_c to n_o.

The steady state scattered light intensity (I_0) is

$$I_0 = I_b + F(n_m - n_{ch}) \tag{1}$$

where I_b is the background scattered intensity. The term I_b includes scattering and reflections due to differences between the inner and outer refractive

indices of the axon as well as contributions from other passive elements not directly involved in excitability such as connnective tissue and Schwann cells. The channel complexes scatter due to the difference $(n_m - n_{ch})$. During excitation only n_{ch} changes resulting in a change in $F(n_m - n_{ch})$ and a corresponding change ΔI in the scattered light intensity I_0

$$\Delta I = \frac{dF(n_m - n_{ch})}{d(n_m - n_{ch})} (n_c - n_0) \tag{2}$$

where the change in n_m has been set equal to zero since the membrane matrix is assumed not to be involved in the conduction process and the change in refractive index of the channel complex n_{ch} has been set equal to $(n_o - n_c)$. The observed signal $\Delta I/I_0$ is then

$$\Delta I/I_0 = \frac{(n_c - n_0)}{I_b} \frac{dF(n_m - n_{ch})}{d(n_m - n_{ch})} \tag{3}$$

The term $F(n_m - n_{ch})$ has been neglected in the denominator since $I_b/F(n_m - n_{ch}) \simeq 10^5$. To study the behavior of $\Delta I/I_0$, the channel contribution to the scattering signal $F(n_m - n_{ch})$ may be expanded in a power series about the average channel index of refraction $\bar{n}$ and differentiated to yield

$$\frac{dF(n_m - \bar{n})}{d(n_m - \bar{n})} = a_1 + 2a_2(n_m - \bar{n}) + 3a_3(n_m - \bar{n})^2 + \ldots \tag{4}$$

The first term in this series, a_1, is not a physical solution since it is independent of n_m. The second term is a linear function of n_m that changes sign at $n_m = \bar{n}$. Higher order terms are neglected since $|n_m - \bar{n}| << 1$. Substituting Eqn. 4 into Eqn. 3 the scattering signal becomes

$$\Delta I/I_0 = \frac{2a_2}{I_b}(n_c - n_0)(n_m - \bar{n}) \tag{5}$$

We define the factor $(n_m - \bar{n})$ in Eqns. 4 and 5 as the bias of the system. It is this bias that changes the sign of the scattering signal. For example, in Fig. 8A the bias is assumed to be negative. As the temperature changes or as the axon is stimulated to exhaustion n_m, and consequently the bias, may change. The bias may go through zero (Fig. 8B) and become positive (Fig. 8C) resulting in a change in sign of the scattering signal.

The main point is that any variation in n_m will be reflected in the light scattering signal as shown in Fig. 8. The value of n_m determines a bias value with respect to n_o and n_c. As the bias value changes sign, the negative scattering signal can go through zero to positive. Thermal variations of n_m are presumed to be reversible for short periods of time (Fig. 6). This probably reflects phase transitions in membrane-bound lipids [11]. Since the sign of the scattering signal may be reversibly inverted by cycling the temperature (Fig. 6), the behavior of the sign of the bias is most likely not related to long term variations in axon size or in periaxonal volume due to prolonged stimulation. Time and continued stimulation could, however, produce irreversible degradation of membrane structural components and consequently lead to irreversible changes in n_m. The channel complex (and $\bar{n}$) appear to be relatively resistant to this degradation since the action potential decays at a relatively low rate (Fig. 5).

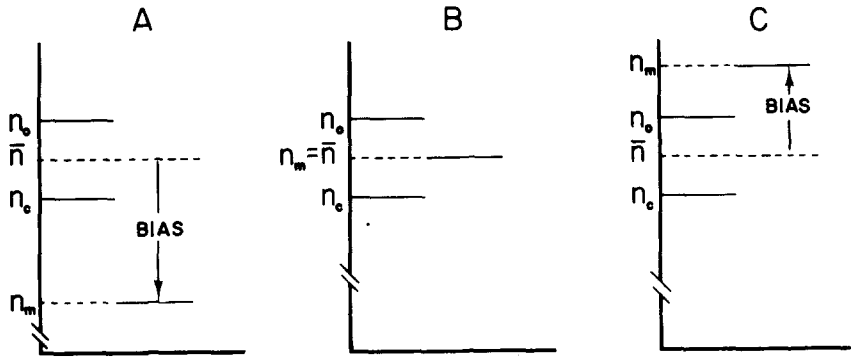

Fig. 8. Relationhip between the various indices of refraction of the axon system. The indices of refraction of an open channnel and a closed channel are n_o and n_c, respectively. The quantity n_m is the index of refraction of the membrane matrix. The average of the open and closed channel index of refraction is $\bar{n} = (n_o + n_c)/2$. The bias of the membrane is defined as $(n_m - \bar{n})$. During conduction, the index of refraction of a channel is assumed to increase from n_c to n_o. The bias, however, can be negative (A), zero (B), or positive (C) depending on the value of n_m. Note: the differences and variations in the refractive indices $|n_o - n_c|$ etc. are small compared to the absolute values of the indices. The vertical scale has been expanded (as indicated by the break in the axes) to illustrate the relative positions of the various indices.

Keeping the axons cold may slow the degradation process and, thus, at 2 or 5°C, a much larger percentage of axons yield initially negative light scattering signals. The initial zero signal is given by axons which are observed in the process of reversing the sign of their negative scattering signals.

In summary, an analysis of the light scattering process during excitation leads to an explanation for the light scattering signal amplitude and sign changes as a function of time and temperature. This analysis can also explain the reversible and irreversible changes observed. The results and the analysis we report here are consistent with our proposed two-state model of the conduction mechanism.

Acknowledgements

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