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Title: Advances in Recording Scattered Light Changes in Crustacean Nerve with Electrical Activation

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Advances in Recording Scattered Light Changes in Crustacean Nerve with Electrical Activation

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ABSTRACT

We investigated optical changes associated with crustacean nerve stimulation using birefringent and large angle scattered light. Improved detection schemes disclosed high temporal structure of the optical signals and allowed further investigations of biophysical mechanisms responsible for such changes.

SUMMARY

Most studies of physiological activity in neuronal tissue use techniques that measure the electrical behavior or ionic permeability of the nerve, such as voltage or ion sensitive dyes injected into cells, or invasive electric recording apparatus [1]. While these techniques provide high resolution, they are detrimental to tissue and do not easily lend themselves to clinical applications in humans.

Electrical and chemical components of neural excitation evoke physical responses observed through changes in scattered and absorbed light. This method is suited for *in–vivo* applications. Intrinsic optical changes have shown themselves to be multifaceted in nature and point to several different physiological processes that occur with different time courses during neural excitation. Fast changes occur concomitantly with electrical events, and slow changes parallel metabolic events including changes in blood flow and oxygenation [2, 3].

Previous experiments with isolated crustacean nerves have been used to study the biophysical mechanisms of fast optical changes. However, they have been confounded by multiple superimposed action potentials which make it difficult to discriminate the temporal signatures of individual optical responses. Often many averages were needed to adequately resolve the signal [4]. More recently, optical signals have been observed in single trials [5]. Initially large angle scattering measurements were used to record these events with much of the signal coming from cellular swelling associated with water influx during activation [6]. By exploiting the birefringent properties derived from the molecular structure of nerve membranes, signals appear larger with a greater contrast [5, 7], but direct comparison of birefringent and 90° scattering signals has not been reported.

New developments in computer and optical technology allow optical recording with higher temporal resolution than could be achieved previously [8]. This has led us to undertake more detailed studies of the biophysical mechanisms underlying these transient changes. Optimization of this technology in conjunction with other technical developments presents a path to noninvasive dynamic clinical observation of optical responses [9].

To conduct these optical recordings, we placed dissected leg, claw and ventral cord nerves from crayfish and lobster in a recording chamber constructed from black Delrin. The chamber consisted of several wells situated perpendicularly to the long axis of the nerve that could be electrically isolated for stimulating and recording electrical activation, and a window in the center for optical measurements. To measure the birefringence from the nerve, light from a 120W halogen bulb was focused onto the nerve from below the window through a 10X microscope objective and polarized at a 45 degree angle with respect to the

long axis of the nerve bundle. A second polarizer turned 90 degrees with respect to the first polarizer was placed on top of the chamber and excluded direct source illumination, passing only birefringent light from the nerve. A large area photodiode placed directly on top of the polarizer detected the magnitude of the birefringent light. To measure light scattered 90 degrees by the nerve, a short length of image conduit placed perpendicularly to the nerve directed large angle scattered light from the nerve to a second photodiode. The output of each photodiode was amplified by a first stage amplifier which produced a DC level output, and was coupled to an AC amplifier (0.3 Hz High Pass) with a gain of 1000 to optimally record changes across time.

Our survey of responses from both crayfish and lobster compared the temporal patterns of activation integrated across the whole nerve and a small nerve segment and recorded direct and simultaneous 90 degree scattered light and birefringence signals. During these experiments, we observed a large population of action potentials that varied in amplitude and temporal spacing due to a wide distribution of axon diameters and types within the nerve bundle. Optical responses associated with large population action potentials from both crayfish and lobster nerve bundles (155 nerves recorded) show fast and slow temporal signatures with slight differences between species and type of nerve. Crayfish claw nerves typically produced optical responses that were 25% of the largest lobster leg nerve responses. We are currently performing histological studies to determine the number of axon diameter populations and their relative size within the nerve bundle and to understand the significance of the nerve bundle's anatomical structure to the birefringence response.

To further resolve the optical response, we used a 1 mm pinhole to reduce the temporal integration of the birefringence signal. When the pinhole was placed over the nerve, the birefringence response showed greater temporal structure and was delayed when compared to the response integrated over the whole nerve. Moving the slit 3 mm closer to the stimulation wires moved the birefringence response approximately 3 ms ahead in time and provided an enhanced early component.

Observation of simultaneously recorded birefringence and scattered light revealed differences in temporal structure of the signals. A fast component in the birefringence signal appeared early during electrical activation and a larger slower response persisted for several milliseconds afterward. The change in 90 degree scattered light was 10 times smaller and lagged behind the birefringence by 1.1 ms. It also lacked some of the resolution that was seen in the birefringence signal, possibly due to the lower signal–to–noise ratio.

The nerve bundles consisted of axons of different diameters to facilitate their motor and sensory functions. These axons will have varying propagation velocities depending on their diameter. We and others [7] postulate that the majority of the optical response is derived from smaller axons, at least partially explaining why the response appears after the initial action potentials. The early peak observed in the pinhole appears larger when the pinhole is positioned close to the stimulation electrodes, consistent with the hypothesis that early signals are due to a combination of responses from the larger, faster axons. A hydrostatic compression wave initiated at the site of stimulation may also contribute to the early responses. Further investigation of this phenomenon is required.

The components and timing of the birefringence and 90 degree scattering responses suggest many factors are involved in their production. The three major peaks in the birefringence response may be the result of contributions of different axon diameter populations in the nerve bundle. Histological studies will help to clarify the role these populations have in the response. The delay in the onset of the 90 degree scattered light change compared to the birefringence signal implies different biophysical mechanisms in

these two populations which warrant further studies. We plan to use a battery of neurotoxins to isolate various mechanisms. The change in birefringence is presumably influenced more directly by a change in voltage, causing birefringent voltage sensitive channel molecules and phospholipids in the axon membrane to change their orientation [10, 11]. The scattered light changes are may result from swelling from an influx of water into the cell during activation. Since the change in voltage potential and consequently the reorientation of the voltage sensitive molecules precedes the channel opening allowing ions and water to flow, we expect the birefringence signal to precede the 90° scattering response.

The signals seen in these studies have temporal structure that is lost in typical experiments through spatio/temporal integration of complex action potential volleys. Our approach allows a detailed look at the biophysical mechanisms responsible for scattered light changes in isolated nerves and will assist in developing new strategies for recording such changes non–invasively in humans [9,12]. Noninvasive optical measures could increase the effectiveness of existing techniques, such as EEG and functional MRI by resolving both the fast neuronal responses and slower vascular responses. This method could also lead to fast, cost effective, and noninvasive techniques for pathology and monitoring associated with stroke or head trauma because of its ability to detect intrinsic light changes in many different types of molecules.

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