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Effect of the action potential on the Raman spectrum of the pike olfactory nerve

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Raman bands due to the C–H stretching vibrations of the phospholipid acyl chains, as well as those due to resonance enhanced vibrations of carotenoid pigments, were used to probe for conformational changes during the passage of the action potential through fibers of the pike unmyelinated olfactory nerve. Our results show that if there are any spectral changes during nerve excitation, these are less than 0.5% for both the phospholipid and the carotenoid bands.

Several spectroscopic methods have been used for the purpose of obtaining structural information on the membrane of nerve fibers during the passage of the action potential. However, some techniques, such as light birefringence [1], did not provide enough details at the molecular level while others, such as transient fluorescence spectroscopy [2,3], required the use of external probes, which could affect the integrity of the nerve preparation [4]. Nevertheless, some of these techniques have suggested that changes of the fluidity of the nerve-membrane lipids did occur during excitation [1,3].

Vibrational spectroscopy has been particularly successful in the study of the conformational and fluidity of phospholipids of model and natural membranes [5], as well as of intact nerve fibers under steady-state conditions [6–8]. Although small changes have been detected in the infrared spectrum of the phospholipid components of the nerve membrane during the excitation of unmyelinated nerves [9], Raman spectroscopy has been unsuccessful to show such changes for the crab walking nerve [6]. This failure has been attributed to an inadequate signal-to-noise ratio in the spectra, even after long acquisition times, although it may have been related to the low density of excitable membrane in this type of nerve.

In a recent study [8], we have demonstrated that the garfish olfactory nerve is well suited for Raman spectroscopic studies, as it gives well-resolved phospholipid bands. In this communication, we report on the effect of the passage of the action potential on the Raman spectrum of the pike olfactory nerve, which is very similar to the garfish one.

The biological specimens used were the olfactory nerve of the pike *Esox lucius* and the leg nerve of the lobster *Homarus americanus*. Details pertaining to the nerve dissection and the composition of the physiological solutions are given in Ref. 1 and Ref. 8, respectively. To obtain the spectra, the samples were placed in a leucite chamber similar to that described previously [3].

The Raman spectra, recorded on a microcomputer-controlled Spex (Model 1400) Raman spectrometer [10] at a spectral slit width of 5 cm^{-1}, were excited by the 514.5 nm line from a Spectra Physics Model 165 argon ion laser, at an average power of 200 mW at the sample. At this low laser

power, we have found that the functionally of the nerve preparations was not affected by the laser beam after several hours of irradiation. In addition, since the concentration of absorbing species, such as carotenoid pigments, was rather low in the nerve fibers investigated, their Raman spectra were found to be independent of the laser power if the latter was kept under 400 mW.

The width of the action potential, activated by an impulse from a Grass stimulator, was about 100 ms when the temperature of the sample was reduced to 5°C. Observation of the Raman signal was conducted in a repetitive manner, over 50 ms periods coinciding with the action potential. A reference measurement of the same length of time was also made in each case, after the nerve had relaxed to its initial state (approx. 1 s). The spectra were typically recorded over a period of 2 h, the condition of the nerve fiber during this period being monitored from the magnitude of the action potential. Raman spectra over a particular frequency range were obtained by accumulating the signal for a determined number of cycles at each frequency interval of 2 cm^{-1}. Measurements at a fixed frequency permitted long accumulation times, from a large number of recording intervals (400–1800 cycles of up to 80 ms each).

The steady-state spectrum of the resting pike olfactory nerve is shown in Fig. 1. Because of the presence of some carotenoid pigments in the sample, the low-frequency region of the spectrum (500–1800 cm^{-1}) is dominated by strong bands at 1157 and 1522 cm^{-1}, due to resonance enhanced vibrations of these chromophores. Nevertheless, several well-defined peaks due to either the protein or the phospholipid component of the nerve membranes are detected. As expected, the intensities of the phospholipid bands relative to those of the proteins are weaker in this spectrum than in that of a myelinated nerve [8]. For example, the characteristic triplet due to the conformationally sensitive C–C stretching vibrations at 1060, 1080, and 1131 cm^{-1} is rather weak. The band at 1654 cm^{-1}, due to the C=C stretching vibration of the unsaturated acyl chains, is fairly strong but, unfortunately, it is overshadowed by the protein amide I band. The choline band at 717 cm^{-1} is well resolved, but this peak is known to be insensitive to any conformational change. The remaining peaks in the low-frequency region of the spectrum come

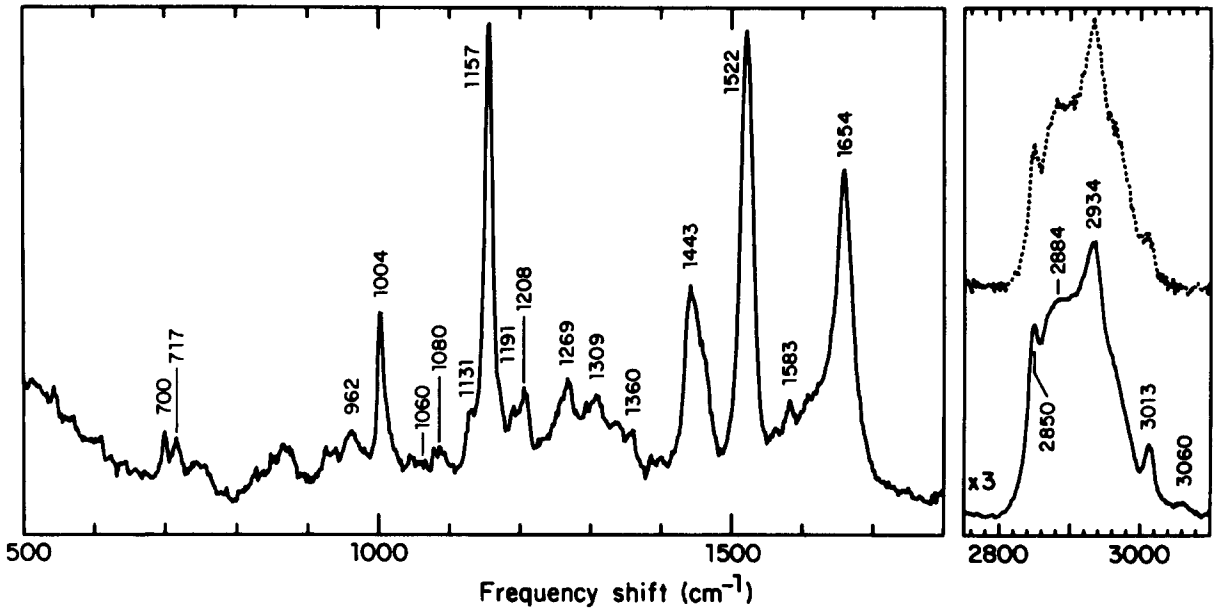

Fig. 1. Steady-state Raman spectra at 10°C of the pike olfactory nerve (solid line, two scans at 2 s/2 cm^{-1} increment) and of the lobster leg nerve (dotted line, one scan at 2 s/2 cm^{-1} increment).

from either the backbone of the membrane proteins, such as the amide III band at 1269 cm^{-1}, or from the side chains of the proteins, like the tryptophan peak at 1583 cm^{-1} or the methionine band at 700 cm^{-1}. Therefore, none of the lipid bands in the 500–1800 cm^{-1} region is strong enough and sufficiently free from protein interference to be useful as a probe for conformational changes during nerve excitation.

In the C–H stretching mode region (2750–3100 cm^{-1}), the two bands at 2850 and 2884 cm^{-1} are, respectively assigned to the methylene symmetric and antisymmetric stretching modes of the phospholipid acyl chains [11]. The 2934 cm^{-1} band results in part from underlying infrared modes, that become Raman active when the intramolecular chain disorder is increased [12], and from the C–H stretching vibrations of the protein component of the membranes and of the axoplasm of the nerve fiber [8]. As seen in Fig. 1 (dotted line spectrum), the protein band at 2935 cm^{-1} is stronger in the lobster leg nerve, as this kind of nerve does not contain as much excitable membranes as the pike olfactory nerve. Since the three bands in the C–H region are particularly strong and also because they are sensitive to both intra- and intermolecular chain disorder, we have chosen this region for the study of the effect of propagation of the action potential on the Raman spectrum of the pike olfactory nerve.

Our first attempts aimed at detecting any change in the Raman spectrum of a fiber during stimulation were based on the complete C–H stretching region of the spectrum. As shown in Fig. 2, the difference between the spectrum taken during the action potential and that of the relaxed fiber shows no change indicative of conformational modifications. Note, however that the background noise in the difference spectrum was quite high, at approx. 10% of the peak heights in this region. In order to improve the accuracy of the results, the measurements were then made at fixed frequencies, corresponding to bands which offered the largest probability of a change during the stimulation. We have thus made up to 1800 consecutive measurements of the Raman signal before and during the action potential, each of a duration of 20 to 80 ms, for a total observation time of up to 135 s at selected points in the spectra.

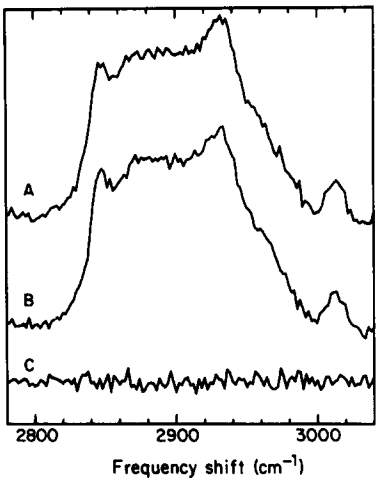

Fig. 2. Raman spectra of a nerve fiber during the action potential (A) and at rest (B). The difference between the two spectra is given in (C). The spectra contain 131 data points (one every 2 cm^{-1}), each resulting from 25 measurements of 20 ms, for a total observation time of 0.5 s at each frequency.

The distributions of the intensity ratio I(excited fiber)/I(fiber at rest) of the individual measurements at some frequencies are given in Fig. 3. The experiment at 2800 cm^{-1} was for control only, as no band is present in this region of the spectrum. The results show that, within experimental error, the intensity of the Raman bands which are sensitive to the state of fluidity of the membrane are not modified during the action potential. The standard deviation on these measurements, as indicated in the figure, was of the order of 0.1%. Taking into account the fluorescent background in the spectra, which was often several times higher than the intensity of the strongest peak, we conclude (with a degree of confidence of 90%) that the intensity of the Raman bands of the membrane lipids in the nerve fiber studied did not change by more than 1 part in 200 during the action potential. This result is in agreement with those of transient pyrene fluorescence of pike nerves which were obtained with noise level of at least one order of magnitude lower than that for Raman spectroscopy during excitation.

Since some changes in the relative intensity of the carotenoid bands at 1157 and 1522 cm^{-1} in the Raman spectrum of a frog sciatic nerve were detected during continuous excitation [13], we have

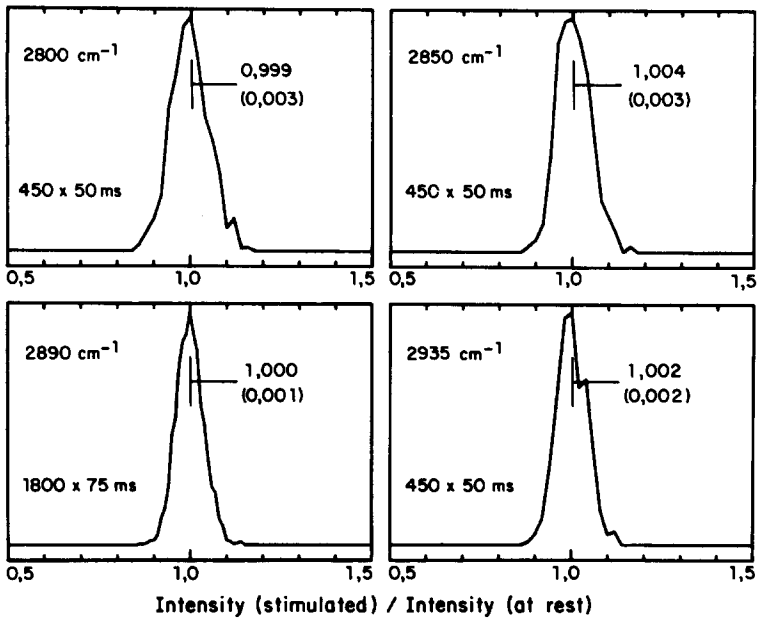

Fig. 3. Distribution of the ratio of the amplitude of the Raman signal for a nerve fiber during the action potential and at rest. Indicated in the figure are: the frequency, the number and duration of the individual measurements, the average and standard deviation on the average (in parenthesis).

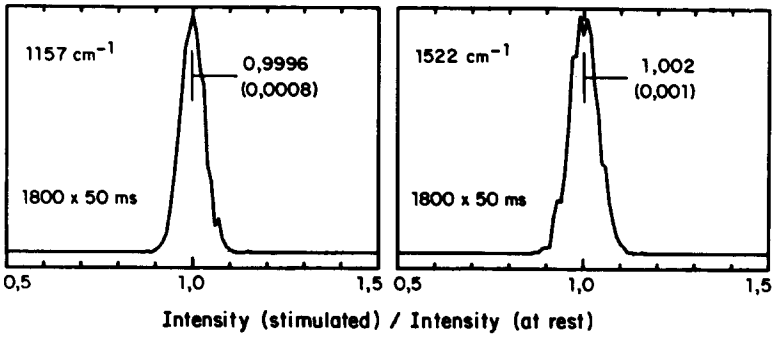

Fig. 4. Ratio of the intensities of Raman carotenoid bands for a fiber in the excited/relaxed states. See Fig. 3 for explanations.

also measured the intensity of these bands in the spectrum of the pike olfactory nerve during the passage of the action potential. As seen in Fig. 4, no change in the intensity of these bands has been detected, even though the signal/noise ratio for these resonance enhanced bands was better than for the C–H bands. Therefore, if the changes observed with the sciatic nerve were not due to an experimental artifact, they were likely related to the presence of the myelin sheath present in this type of fiber.

In conclusion, the above results show that, even for a nerve fiber with a high density of excitable membranes such as the pike olfactory nerve, the changes in the Raman spectra during excitation are less than 0.5% for both the phospholipid and the carotenoid bands. To improve the limit in the detection of such changes, we believe that fibers with a higher density of axons should be used, along with more sophisticated Raman instrumentation, such as with optical multichannel detection and high power pulsed laser source.

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