BRIEF COMMUNICATION

MATERIAL FROM THE INTERNAL SURFACE OF SQUID AXON EXHIBITS EXCESS NOISE

IMPLICATIONS IN MODELING MEMBRANE NOISE

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ABSTRACT A fluid material from a squid (*Loligo pealei*) axon was isolated by mechanical application of two types of microcapillary (1–3-μm Diam) to the internal surface of intact and cut-axon preparations. Current noise in the isolated material exceeded thermal levels and power spectra were $1/f$ in form in the frequency range 1.25–500 Hz with voltage-dependent intensities that were unrelated to specific ion channels. Whether conduction in this material is a significant source of excess noise during axon conduction remains to be determined. Nevertheless, a source of excess noise external to or within an ion channel may not be properly represented solely as an additive term to the spectrum of ion channel noise; a deconvolution of these spectral components may be required for modeling purposes.

INTRODUCTION

The first measurements of ion conduction noise in the nerve membrane gave a $1/f$ power spectrum over a kHz frequency range (Derksen and Verveen, 1966; Poussart, 1971). Subsequent measurements have confirmed these observations in several different nerves (Fishman, 1973; Siebenga et al., 1973; Conti et al., 1975; Fishman et al., 1975; Van den Berg et al., 1975; Conti et al., 1976) and have also indicated Lorentzian $[1 + (f/f_c)^2]^{-1}$ components. The Lorentzian-like power spectral components were shown to reflect the ion conduction kinetics of voltage-dependent ion channels in the membrane, whereas the origin of the apparent $1/f$ component is still unknown, although it clearly relates to restricted diffusion through ion channels, i.e., to ion-channel conductance fluctuations as opposed to their "gating" kinetics.

The purpose of this communication is twofold: (*a*) to report observations of an excess noise in the current through an isolated portion of the material adjacent to the inner surface of squid axon membrane, and (*b*) to point out that the effect of a noise source located outside or inside of the axolemma proper is not validly representable only as a spectral addition to ion channel noise but instead, more properly, includes a convolution of these noises in the frequency domain.

The experiments reported here involve noise measurements on a fluid material that is

isolated and removed from the inside surface of an intact or cut axon preparation by mechanical application of a microcapillary. The current fluctuations in this internal surface material are voltage dependent and the form of the power spectrum is $1/f$ over two decades. The excess noise does not relate to movement of specific ions through ion channels but rather appears to be associated with the general conduction properties of this material. Based on these results, as well as electron microscope evidence (Metuzals and Tasaki, 1978), it appears that the internal surface of an axon may contain structures that extend into the axoplasm to form a matrix that is in series with membrane ion channels. When channels conduct, the current through this matrix produces excess noise that modulates (multiplies in time) the noise produced by changes in the conducting states of channels.

METHODS

Micropipettes were made by drawing glass capillaries in a puller while applying heat from a coil of nichrome wire. Those with a tip diameter of ~1 μm were used unaltered. Another type, with a larger tip diameter, was fired polished at the tip to produce a thick wall with a tip aperture diameter of 1–3 μm similar to that described by Neher et al. (1978, Fig. 4 *c*). The drawn pipettes were filled with 0.5 M KF buffered with 5 mM Tris Cl (pH 7.4 at 25°C). A chloridized silver (Ag-AgCl) wire in contact with the fluid at the wide end of the drawn glass capillary was used for potential measurement and application of current. A 5-mm length of an isolated squid (*Loligo pealei*) giant axon was suspended between two transverse partitions and was bathed externally by cooled, flowing sea water (SW). A Ag-AgCl sheet (4 × 4 mm) served as the ground for the bath and circuit. The measurement arrangement is shown in the inset of Fig. 1. An Analog Devices 43K operational amplifier (Analog Devices Inc., Norwood, Mass.) was used to apply voltage derived from a mercury battery to the microelectrode through a 500 MΩ metal-film feedback resistor. The output of the control amplifier was high-pass filtered and amplified further by a subsequent gain stage (100×), and spectral analysis was performed on a Rockland 512/S 400-point real-time spectrum analyzer (Rockland Systems Corp., Rockleigh, N.J.).

Fig. 1 shows measurements of the current-noise characteristics of an electrode and the control system.

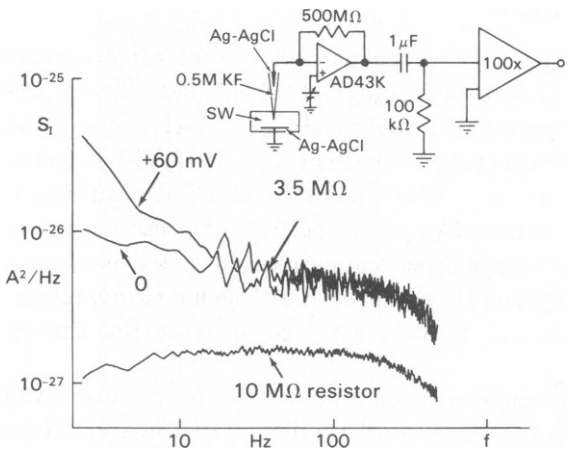

FIGURE 1 The measurement system (inset) and spectra of intrinsic current noise. The spectrum labeled 10-MΩ resistor was measured with a 10-MΩ resistor substituted for the micropipette at the input of the AD43K. The midband power density is essentially that of the resistor thermal noise. The roll-off at low frequencies is due to the high-pass filter before the 100× stage and the roll-off beyond 100 Hz is due to the frequency response limit of the clamp stage with 500-MΩ feedback resistor. Upper spectra are for a fire-polished tip (3-μm Diam) micropipette in the SW bath, with and without an applied potential.

A 10-MΩ resistor substituted for the microelectrode at the input of the control amplifier shows a midband noise that is essentially equal to the theoretical thermal noise of the resistor. The low-frequency decline is due to the high-pass filter. The high-frequency decline beyond 100 Hz is that of the control amplifier. The upper curves in Fig. 1 were measured with a fire-polished micropipette (3-μm Diam) in SW with and without a potential applied. The midband noise is nearly that of the thermal noise of the pipette resistance (3.5 MΩ) with a relatively small amount of excess noise produced at frequencies below 10 Hz.

RESULTS

Material Isolation with Fine-tipped Micropipettes in Intact Axons

The current-noise power spectrum of each micropipette used was measured in SW before insertion into an axon, and the resistance of each pipette was measured. A fine-tipped pipette, with a long taper, was inserted into an intact axon and advanced toward the opposite internal surface of the axon while the open-circuit voltage between the pipette and bath ground was monitored. The usual resting voltage (−60 mV) was measured until the tip of the electrode reached the opposite surface. A small advance of the electrode beyond this point resulted in a drop of the voltage to ~ −30mV, perhaps indicating membrane damage. The drop in voltage always preceded any significant electrical isolation of the membrane surface by the rim of the pipette. Upon further advancement (Fig. 2, inset *1*) a significant dimpling (but not breakout) of the axon was observed and the electrical resistance through the pipette increased by a factor of two or more above the pipette resistance measured in SW (typically 15 MΩ). Spectral analysis after the electrical isolation yielded noise that exceeded the intensity of the pipette noise in SW before insertion and with a $1/f$ spectral form. Upon withdrawal of the pipette from the axon into SW (Fig. 2, inset *2*) the electrical resistance of the pipette fell to its original value, but the $1/f$ noise persisted, as shown in Fig. 2. The intensity of the $1/f$ spectrum is an exponential function of voltage in contrast to a square-law dependence found in patches of squid axon (Fishman et al. 1975).

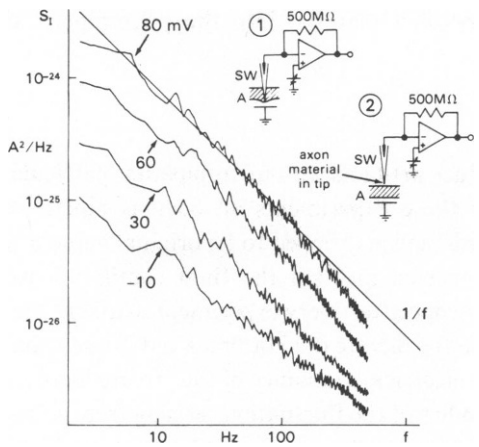

FIGURE 2 Power density spectra of current fluctuations in a material isolated and removed from the internal surface of a squid axon. *1* Micropipette penetrates axon (A) and is forced against internal surface, dimpling it. *2* Withdrawal of pipette from axon into SW with material in tip. Spectra shown are for condition *2* although at *1* they were similar. Spectra are not corrected for frequency response of system shown in Fig. 1.

After each insertion, isolation, and measurement of excess noise upon withdrawal, the tip of the microelectrode was examined with a compound microscope that showed a fluid at the tip with an index of refraction different from that of the fluid further back from the tip. This observation was made repeatedly in several experiments.

To test for the possibility that the $1/f$ fluctuations might relate to ion channels, tetrodotoxin (1 μM), which blocks Na conduction, was added to the SW so that its effect on the $1/f$ spectra of the isolated material could be observed. No effect was observed. Similarly, 4-aminopyridine (10 mM), which blocks K conduction, when added to the SW also produced no effect on the spectra. The fluctuations thus do not appear to relate to any particular ion-channel in the membrane.

Material Isolation with Fire-polished Micropipettes in Cut Axons

A second set of experiments was done with a cut-axon preparation using the fire-polished micropipettes. Because the tip diameters of these pipets were too large for direct penetration of the axon, a small cut was made on the top surface of an axon with micro dissecting scissors to allow entry, while the axon was bathed in a buffered sucrose solution (0.8M). After a patch isolation, flowing SW was restored in the external bath to a level just below the cut. Except for the fact that cutting reduced the internal potential to -10mV, all the observations with fine-tipped micropipettes in intact axons were reproduced with the fire-polished tip pipettes. In addition, in a separate experiment it was possible to suck axoplasm into the larger aperture fire-polished electrode to examine its noise behavior. Axoplasm in the tip did not produce excess $1/f$ noise.

A further observation suggests that the isolated material is closely associated with the internal surface of the axon. Axons were internally perfused with the 0.5M KF buffered solution containing, in addition, the enzyme pronase (1 mg/ml) for 3 min before electrode insertion and isolation. After substantial clearance of axoplasm, as indicated by a good perfusion flow rate, application of the microelectrode to the internal surface of an axon again produced the forced entry of a material into the micropipette that gave $1/f$ noise upon withdrawal into SW.

DISCUSSION

It is relatively easy to produce artifacts with micropipettes, particularly as a result of clogs and air bubbles. However, in these experiments an obvious pipette aberration seems unlikely because: (*a*) The pipette resistance dropped to its original value after removal from the axon while the excess noise persisted and (*b*) the fluid continuity over the entire pipette was examined under the microscope after every experiment to assure the absence of discontinuities within the pipette. Because the pipette did not break out of the axon in both the intact and cut axon preparations and the electrical resistance of the pipette increased during contact with the internal surface, the possibility of the fluctuations arising from a "cored" section of membrane seems remote. In view of the fact that axoplasm does not produce the observed excess noise and that its clearance from the axon does not prevent it, these results appear to imply the existence of a source of excess noise in a material closely associated with the internal surface of the squid axon.

It is of interest to note that Fricke and Parker (1940) showed for a gelatin-water system

that the dielectric constant follows a power law of frequency ($f^{-\alpha}$, $0 < \alpha < 1$) in the range 0.5–1,000 kHz and reaches values many times that of water at low frequencies. This anomalous dispersion (energy absorption) is believed to be due to a concerted orientation of the polymolecular layer of loosely bound water molecules at internal surfaces of the gelatin rather than due to the polarizability of the gelatin molecules.

Although these experiments do not demonstrate that the observed excess noise is identical with the apparent $1/f$ spectral component that occurs in spectra of functional axons, such a source of excess noise, in series with the membrane, would modify power spectra of ion channel noise. However, this modification would properly be represented in a way that is not presently being considered.

In most analyses of nerve membrane current fluctuations, the spectral form of the noise departs significantly from a Lorentzian function, particularly in the high-frequency region. Rather than approaching an asymptotic slope of -2 (log-log plot), the absolute slope is usually much less than 2 (see, for example, Fig. 1 of Conti et al., 1975). The presence of an additive $1/f$ noise to a Lorentzian has been the usual assumption (Siebenga et al., 1973; Conti et al., 1975) in accounting for the slope departure; that is, power spectra are fit by the following function:

$$S(f) = \frac{A_1}{f} + \frac{A_2}{1 + (f/f_c)^2}.$$

However, in light of the above observations on $1/f$ noise, there is another possibility which could have substantial effects on the outcome of model fits to spectral data.

If, as is usually assumed, an ion channel has discrete conduction states with relatively fast transitions occurring randomly between these states, the conduction events are, from the point of view of stimulus-response characteristics, highly nonlinear. In general, multiple sources of noise associated with current through an ion channel interact in a multiplicative manner whenever the fundamental conduction process is nonlinear. If two voltage sources of noise $e_1(t)$ and $e_2(t)$ are in series with a conductance that has a nonlinear $i(v)$ characteristic, the functional relation between i and v may be expressed as a power series, i.e., $i = a_0 + a_1 v + a_2 v^2 + a_3 v^3 + \ldots$. For simplicity, assume that the linear term $a_1 v$ and terms above second order are negligible and that the dc term a_0 is removed from the analysis. Thus if a voltage clamp is applied to the terminals of the circuit, the mean-square current fluctuation $\langle i^2 \rangle$ is given by

$$\langle i^2 \rangle = a_2^2 [\langle e_1^4 \rangle + \langle e_2^4 \rangle + 4 \langle e_1^3 e_2 \rangle + 6 \langle e_1^2 e_2^2 \rangle + 4 \langle e_1 e_2^3 \rangle].$$

The above equation shows that the mean-square current fluctuation reflects not only the sum of powers of the individual noise sources e_1 and e_2 but also products of powers of e_1 and e_2. This result, for the simplest nonlinearity, a square-law device, is independent of the physical location of sources e_1 and e_2 (i.e., whether they are within or outside channel), insofar as each affects the current through the nonlinear conductance. In the case of $1/f$ current fluctuations, which have long correlation times, its modulation of discrete conductance-state fluctuations should not be ignored because the contribution of the multiplicative terms to the power density is not likely to be negligible.

To simulate this effect (Fig. 3), white noise was applied to a low-pass filter (single pole) to produce the Lorentzian power spectrum $S_1(f)$. The time-domain function $f_1(t)$ from the

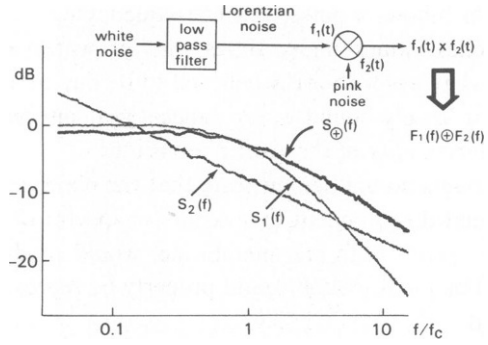

FIGURE 3 Power spectrum, $S_{\bullet}(f)$, of the noise produced by the convolution, $F_1(f) \circledast F_2(f)$, of pink ($1/f$) noise and Lorentzian noise. The convolution spectrum $F_{\bullet}(f)$ was obtained by Fourier transform (large arrow) of the modulation (multiplication) of $f_1(t)$ by $f_2(t)$, which was produced as indicated in the inset. Note that the power spectrum of the convolved functions has a shape that approaches the Lorentzian at low frequencies, but at high frequencies declines at a rate that is greater than the $1/f$ function but less than the Lorentzian. This shape spectrum is similar to that obtained for the squid axon (see Fig. 1, Conti et al., 1975) and frog node (Neumcke et al., 1980).

Lorentzian-producing noise source was then multiplied by the time-domain output $f_2(t)$ of a noise source that produced a $1/f$ power spectrum, $S_2(f)$. This modulation process in the time domain corresponds to a convolution of the Fourier transforms of $f_1(t)$ and $f_2(t)$ in the frequency domain. Consequently the convolution, $F_1(f) \circledast F_2(f)$, is obtained by Fourier transformation of the resulting time function produced by the product of the time functions of the noise sources. As indicated in Fig. 3, the power spectrum $S_{\bullet}(f) = |F_{\bullet}(f)|^2$ of the convolved functions shows a low-frequency form that is like the Lorentzian and a high-frequency asymptotic decline that is less than the Lorentzian but greater than the $1/f$ function. The shape of the convolution spectrum (cross-power spectrum) is similar to that obtained recently for K conduction in frog nerve (Neumcke et al., 1980). $S_{\bullet}(f)$ is unaffected by changes in the multiplicative intensity factors A_1 and A_2, and the low-frequency asymptote is flat, as is that of the Lorentzian. The shape of the additive spectrum, however, does depend on the relative intensity factors A_1 and A_2.

In most membrane noise measurements, spectral analysis is confined to a frequency range of only two to three decades because of instrumentation noise, limited bandwidth, or preparation stability considerations. This means that the data pertinent to spectral shape and the approach to asymptotes is further restricted to, at most, one to two decades. Consequently it may not be possible to choose unambiguously between an additive spectrum and a convolved spectrum. This problem is even more acute when spectra appear to have more than one Lorentzian component in the limited spectral range. Nevertheless, the above analysis indicates that the convolution operation should not be ignored whenever there are multiple sources of noise associated with current through an ion channel. A deconvolution of $1/f$ noise, as opposed to a simple subtraction of it from spectral data, can have an influence on the selection of candidate models for comparison with the data or, for a particular model, alter the parameters obtained by a fit to the data.

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