J. Membrane Biol. 24, 305–328 (1975)

Potassium-Ion Conduction Noise in Squid Axon Membrane

Harvey M. Fishman, L. E. Moore, and Denis J. M. Poussart

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550,
Department of Physiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106,
Département de Génie Électrique et Centre de Recherches en Bionique, Université Laval, Québec, Canada and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Received 10 March 1975

Summary. Spectral analysis (1–1000 Hz) of spontaneous fluctuations of potential and current in small areas of squid *(Loligo pealei)* axon shows two forms of noise: f^{-1} noise occurs in both excitable and inexcitable axons with an intensity which depends upon the driving force for potassium ions. The other noise has a spectral form corresponding to a relaxation process, i.e., its asymptotic behavior at low frequencies is constant, and at high frequencies it declines with a slope of -2. This latter noise occurs only in excitable axons and was identified in spectra by (1) its disappearance after reduction of K^+ current by internal perfusion with solutions containing tetraethylammonium (TEA^+), Cs^+ or reduced $[K_i^+]$ and (2) its insensitivity to block of Na^+ conduction and active transport. The transition frequency of relaxation spectra are also voltage and temperature dependent and relate to the kinetics of K^+ conduction in the Hodgkin-Huxley formulation. These data strongly suggest that the relaxation noise component arises from the kinetic properties of K^+ channels. The f^{-1} noise is attributed to restricted diffusion in conducting K^+ channels and/or leakage pathways. In addition, an induced K^+-conduction noise associated with the binding of TEA^+ and triethyldecylammonium ion to membrane sites is described. Measurement of the induced noise may provide an alternative means of characterizing the kinetics of interaction of these molecules with the membrane and also suggests that these and other pharmacological agents may not be useful in identifying noise components related to the sodium conduction mechanism which, in these experiments, appears to be much lower in intensity than either the normal K conduction or induced noise components.

In the initial phase of studies of spontaneous fluctuations in three different axon membranes, the predominant noise evident in power-density spectra (PDS) of both voltage and current fluctuations was f^{-1} in form, with an intensity which depended upon K^+ current flow (Derksen, 1965; Derksen & Verveen, 1966; Poussart, 1969; Poussart, 1971; Fishman,

1972). However, it was apparent from a substantial existing physical literature (*see* Kittel, 1958, part 2; Lax, 1960) on fluctuations arising from conduction processes that the ion-conduction "channels" implicit in the Hodgkin-Huxley equations ought to produce noise characteristic of a relaxation process, viz PDS of the form $[1+(f/f_c)^2]^{-1}$. In 1972 Hill and Chen and Stevens independently published derivations of PDS of conductance fluctuation based upon the assumption of discrete two-state K^+ channels in the HH formulation, which produced only relaxation spectra. With the foregoing insight as well as improvements in methods and instrumentation, relaxation noise was observed in addition to f^{-1} noise (Siebenga & Verveen, 1971; Fishman, 1971, *unpublished data*) and appears to be related to K^+ conduction (Fishman, 1973; Siebenga, Meyer & Verveen, 1973).

The present paper describes squid axon K^+ conduction fluctuations which have been reported previously in preliminary accounts (Fishman, 1972; Fishman, 1973; Fishman, 1975*a*; Fishman, Moore & Poussart, 1975*a*). f^{-1} noise occurs in both excitable and inexcitable axons with an intensity which depends upon the driving force for K^+. Relaxation noise dominates f^{-1} in excitable axons and has properties which suggest that it is produced by the kinetic behavior of conducting K^+ channels. Observation of relaxation noise further suggests that f^{-1} noise arises from membrane leakage conduction or perhaps restricted diffusion through conducting K^+ channels. An induced K^+conduction noise by tetraethylammonium and a quaternary-ammonium ion derivative within axons may reflect the "gating" properties imposed upon K^+ channels by the interaction of these molecules with membrane sites.

Materials and Methods

A detailed account of the preparation, isolation of a patch of membrane, and noise analysis instrumentation was presented previously (Fishman, 1975*b*; Fishman, Poussart & Moore, 1975*b*). In this paper, the power-density spectra ($\log \overline{|\Delta X|^2}/\Delta f vs. f$, where ΔX is either a fluctuation about the mean potential or current and f is frequency) were produced from FM tape recordings of the original data. The spectral analysis of each noise record was done in three of several overlapping frequency bands (0.125–50 Hz, 0.5–200 Hz, 1.25–500 Hz, 2.5–1000 Hz, 5–2000 Hz and 12.5–5000 Hz) with the lowest frequency in each band corresponding to the resolution in each 400 point band. A number of real-time spectra produced during each noise record on tape were averaged digitally to obtain an averaged PDS. The number of real-time spectra averaged depended upon the frequency band. Generally, in the lowest band 16 nonredundant real-time spectra were averaged with a record length (sample length) of 129 sec, whereas in the highest frequency band 128 real-time spectra were averaged with a sample length of 21 sec. The spectra in each frequency band were plotted by an X-Y recorder and in Fig. 1, they were assembled and redrawn as continuous spectra. The "noisiness"

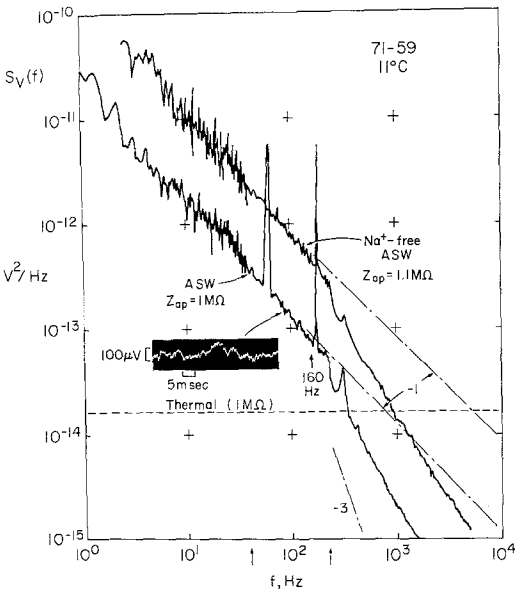

Fig. 1. f^{-1} power-density spectrum (PDS) of voltage noise from a patch of axon at "rest" potential which gave a low (50 mV) spike amplitude upon stimulation. f^{-1} noise is predominant only in inexcitable or poorly excitable axons. (Inset) Short time-segment of fluctuation waveform reproduced from tape (effective noise bandwidth of 1.25 kHz). The curves are composite reproductions of X-Y plots produced by spectral analysis (400 points/band) in three overlapping frequency bands indicated by arrows on the abscissa. Na^+-free ASW is ASW with isosmotic sucrose substituted for Na^+. Z_{ap} is the effective isolation of the patch from the bath solution. Extraneous components at 60 and 180 Hz are power-line related. Dashed line is the calculated thermal power-density from the Nyquist relation

of the spectra is greatest in the lowest portion of the frequency spectrum because relatively fewer real-time spectra were averaged. In all other PDS the same procedure applied; however, smooth curves, which were drawn by eye through the averaged spectra, are presented.

The standard external solutions were filtered Woods Hole seawater (SW) and artificial seawater (ASW) composed of 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 5 mM Tris-HCl, pH 7.4 at 25 °C. All other external solutions were made from the ASW recipe with the substitutions indicated in the text or figure legends. The standard internal perfusate was 0.5 M KF and 5 mM Tris-HCl, pH 7.4 at 25 °C. Dilution of the K^+ concentration was made with isosmotic sucrose solution (0.8 M). Two quaternary ammonium compounds were used as additions to the standard perfusate. Tetraethylammonium, TEA^+, was prepared as a salt from triethylamine and 1-bromoethane. Triethyldecylammonium, $TEDA^+$, was kindly supplied by Dr. Clay Armstrong.

The conventions in this paper follow those of the preceding papers (Fishman, 1975*b*; Fishman *et al.*, 1975*b*). "Rest" potential (indicated as 0 mV) corresponds to the potential (usually +20 to +30 mV) recorded from within the patch electrode (during sucrose solution flow) to the bath ground electrode for zero-mean membrane current. The potentials indicated in the text and figures are the measured *changes* of patch potential from "rest". Positive changes in potential indicate depolarization of the "rest" potential and negative changes indicate hyperpolarization.

Results

Electrically excitable as well as inexcitable membrane patches produce voltage and current noise with a power-density spectrum which is approximately f^{-1}. Spontaneous voltage and current fluctuations in both excitable and inexcitable squid axon membrane are relatively large. Fig. 1 (insert) shows a very short time-segment of voltage fluctuations at the patch "rest"-potential from a patch which gave less (50 mV) than normal (>90 mV) spike amplitude. Spectral analysis of the voltage fluctuations $S_V(f)$, Fig. 1 (lower spectrum), shows (1) frequency components between 1 and 300 Hz which are in excess of the thermal noise expected from the measured patch isolation (1 MΩ) and computed from the Nyquist relation and (2) a spectral distribution in power density which declines as $f^{-\alpha}$ where $\alpha \approx 1$ from 1–160 Hz, and a further decline from f^{-1} at frequencies beyond 160 Hz. In general, spontaneous voltage and current-fluctuations in squid axon are so large that thermal noise levels cannot be measured in the frequency analysis band (1–1000 Hz). The spectral power-density between 1–50 Hz is an order of magnitude or more greater than the noise produced by the patch isolation process (Fishman *et al.*, 1975*b*), which was measured in test membranes. The value of α, which characterizes the low-frequency spectral decline, was not always unity. Instead, α ranged from 0.5 to 1.5. Thus this noise falls into the class of f^{-1} noises. The high-frequency decline, which is noticeable as a distinct break in the spectra of Fig. 1, apparently reflects "filtering" of the spontaneous voltage-fluctuations by the membrane impedance (Fishman, 1975*a*; Fishman *et al.*, 1975*b*), since a resting membrane resistance of 1000 Ω cm^2 and capacitance of 1 μF/cm^2 should produce a corner at about 160 Hz.

The f^{-1} portion of the voltage spectrum was insensitive to modifications in the Na^+-conduction process as indicated by data in Fig. 1 (upper spectrum), in which another patch in the same axon was isolated with Na^+- free ASW in contact with the external patch surface. The f^{-1} spectrum was also unaltered by applications of 10^{-6} M tetrodotoxin (TTX) (Narahashi, Moore & Scott, 1964). In contrast to Na^+-concentration changes, alteration of external K^+ concentration did affect f^{-1} spectra as indicated in Fig. 2. In this axon a control spectrum was obtained (patch 1) in ASW. Another patch (2) was isolated with the inner pipette filled with K^+-ASW [all of the Na^+ (430 mM) was replaced with K^+] and with K^+-ASW flowing externally across the nonisolated portion of the axon. To assure "equilibration" in ion concentrations at the patch, fluctuations were recorded and analyzed 10 min after the change of external solution. The resulting spectrum (Fig. 2) shows significant reduction in the magnitude

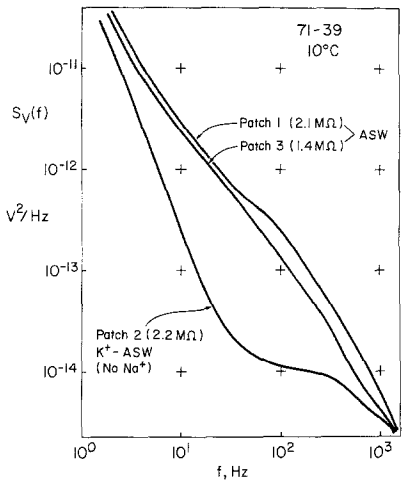

Fig. 2. PDS of voltage noise for a low spiking patch (1) with ASW externally, followed by another patch (2) in the same axon in K^+-ASW (all Na^+ replaced by K^+), and return to patch (3) in ASW. Solutions indicated were those in chamber bath as well as in patch pipette. Numbers in parentheses indicate patch isolation. In these and subsequent spectra smooth curves were drawn by eye through the X-Y plots of spectra to emphasize form

of all spectral components, with the spectrum tending toward a "white" noise character. In most experiments of this kind an f^{-1} spectrum remained, but its intensity was altered. Steady-state polarization of the patch by application of constant current also produced f^{-1} spectra which changed only in intensity. However, a minimum intensity at all frequencies always occurred for patch polarizations to the potassium equilibrium-potential E_K. Fig. 3 illustrates this point. The intensity of the noise component at

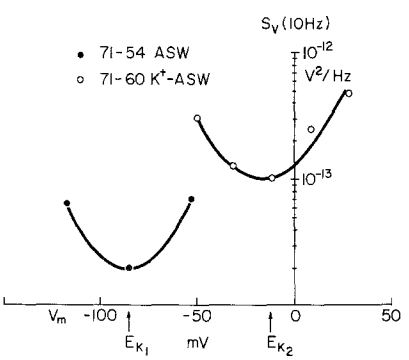

Fig. 3. Relation of voltage noise power density at 10 Hz from a patch exhibiting f^{-1} noise in ASW and for changes in potential from patch "rest" and the same for a patch in another axon in K^+-ASW (no Na). E_K estimated potassium equilibrium potential *(see text)*

10 Hz is plotted for polarization of a patch in ASW and K^+-ASW. E_K was calculated from the ratio of K^+ concentration in the external ASW to an assumed internal value of 400 mM (Keynes & Lewis, 1951). These results on f^{-1} noise are thus in agreement with noise measurements on nodes of Ranvier in single frog fibers (Derksen, 1965; Derksen & Verveen, 1966) and on artificial nodes of lobster axon in the double sucrose-gap (Poussart, 1969, 1971). Also, the presence of a minimum in f^{-1} noise near E_K was recently reported by Conti, DeFelice and Wanke (1975) who erroneously stated that this was in contrast to the findings of Poussart (1971). Thus, the suggestion by Conti *et al.* (1975), that sucrose isolation methods produce excess f^{-1} noise which does not show a minimum at E_K should be rejected.

Relaxation Noise

Another noise component which is most pronounced in measurements from excitable patches has a form which is characteristic of a relaxation process. Fig. 4 shows short time-segments of voltage fluctuations from an excitable patch for changes in patch potential (*a*) and changes in chamber-solution temperature at the patch "rest" potential (*b*). It is apparent from

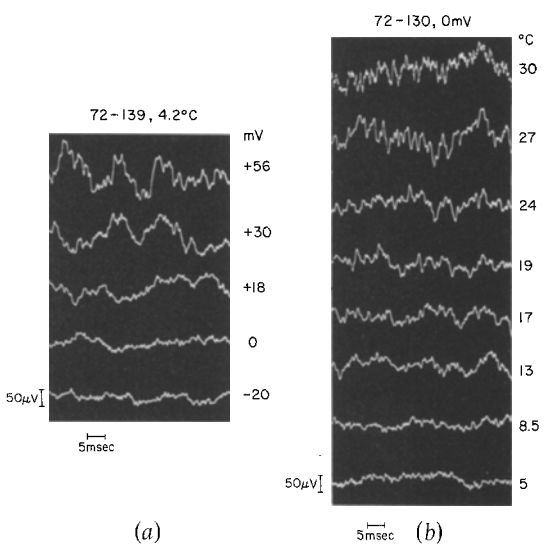

Fig. 4. Voltage fluctuation waveforms, reproduced from tape (effective noise bandwidth 1.25 kHz) of noise from excitable patches (spike amplitude >90 mV). (*a*) Changes of potential from patch "rest" (0 mV) at fixed temperature. (*b*) Another axon patch at "rest" at eight different temperatures. These very short time-segments were taken randomly from 1-min lengths of recorded data

these data that the fluctuations change amplitude and spectral character with potential and temperature changes. Fig. 5 shows power-density spectra of the sample records shown in Fig. 4*a* and spectra of voltage fluctuations from the same patch for potential changes at two higher temperatures. These voltage spectra are about two orders of magnitude in excess of thermal levels. In this respect, the fluctuations which produce this type noise are of the same magnitude as the fluctuations which produce f^{-1} noise (compare Fig. 1, insert). In contrast to the f^{-1} noise, the spectra

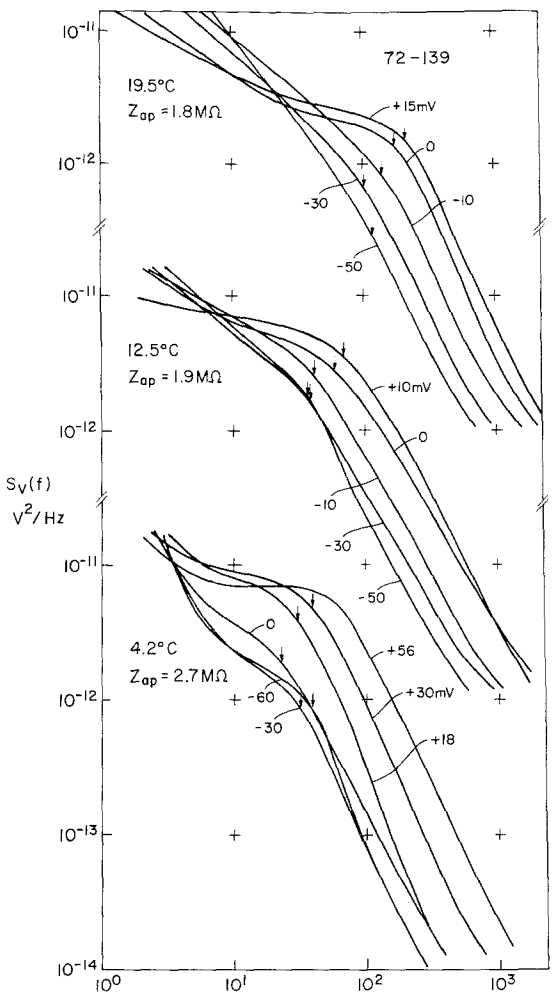

Fig. 5. PDS of the voltage fluctuations represented in Fig. 4*a* and noise from the same patch at two higher temperatures. "Humps" are evident in spectra which decline generally. Arrows indicate corner frequencies determined by intersection of lines drawn tangent to the low and high frequency portions of spectra. Positive potentials are depolarized changes from "rest"

in Fig. 5 show a pronounced hump which, at depolarized potentials, is relatively flat at low frequencies, contains a transition region, and declines approximately as f^{-2} at high frequencies. In the course of an experiment, the humps become less pronounced and eventually disappear leaving only the f^{-1} spectrum. Qualitatively, it appears that these spectra contain a dominant noise component which is first-order in form, i.e., $[1+(f/f_c)^2]^{-1}$, superposed on a lesser f^{-1} component. By extrapolation of the low and high-frequency asymptotes of each spectrum, an intersection point, which is indicated by arrows in Fig. 5, defines a transition or corner frequency f_c in each spectrum. It is apparent that the corner frequencies indicated in the spectra change with potential and temperature.

In previous reports it has been noted that voltage fluctuations reflect both membrane current fluctuations and impedance (Poussart, 1971; Stevens, 1972; Anderson & Stevens, 1973; Fishman, 1973; Wanke, DeFelice & Conti, 1974; Fishman, 1975*a*; Fishman *et al.*, 1975*b*). Since a description of conductance fluctuations is desired, membrane impedance must be taken into account in the use of voltage noise data. By virtue of the constancy of patch impedance from low frequencies to a few hundred Hz, it was shown previously (Fishman, 1975*a*; Fishman *et al.*, 1975*b*) that patch voltage-noise PDS are equivalent in form to current-noise PDS, which reflect conductance PDS directly. Thus the humps which appear in $S_V(f)$ in Fig. 5 are an indication of humps in conductance spectra. The use of patch $S_V(f)$ data as a reflection of conductance PDS was a convenient first step in the analysis of spontaneous fluctuations in squid axon membrane (Fishman, 1973). Subsequently, voltage noise measurements were discontinued in favor of measurements of current fluctuations during potential control, which reflect conductance fluctuations directly and extend observation to 1 kHz. Thus Fig. 6 shows power-density spectra of current noise from a voltage-clamped patch (Fishman *et al.*, 1975*b*) at polarized potentials from "rest" (0 mV) and at two different temperatures. The pronounced humps at depolarized potentials are again apparent and the arrows indicate changes in transition frequency with both potential and temperature.

The transition frequency data obtained from Figs. 5 and 6 are summarized in Fig. 7. Some of these data, which were derived from the voltage PDS of Fig. 5, were presented previously (Fishman, 1973). In addition, the transition frequencies from current-noise PDS (Fig. 6) are included for comparison. The data from current noise substantiate the earlier report, and indicate that the behavior of the corner frequency of patch noise spectra relates to the Hodgkin-Huxley potassium conduction relaxation

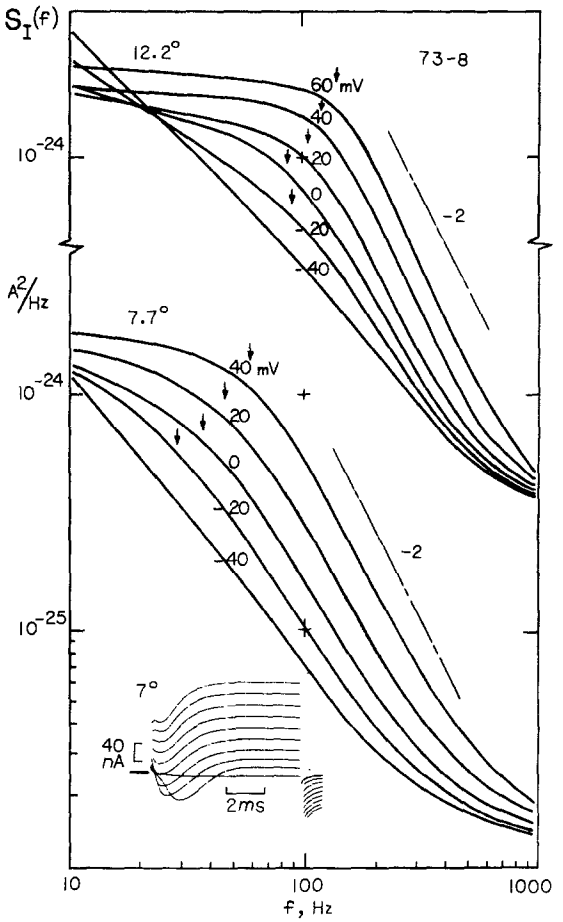

Fig. 6. PDS of current fluctuations, recorded during constant voltage conditions, in the same patch at two different temperatures. The spectral component which occurs as a hump on top of the f^{-1} spectrum (at hyperpolarized potential) becomes, with substantial depolarization, dominant and recognizable as a relaxation spectrum (i.e., asymptotic behavior which is constant at low frequencies and declines with a slope of -2 at high frequencies). (Inset) Clamp-current records in the patch (isolation leakage removed) for step voltages of 20 to 200 mV in increments of 20 mV from "rest" at 7 °C

time τ_n according to $f_c = (2\pi\tau_n)^{-1}$. Implications for some conduction models of an inverse first power relationship between f_c and τ_n have been discussed previously (Chen & Hill, 1973; Fishman, 1973).

Dependence of Relaxation Noise on K^+ Current

Since the noise which gave characteristic humps in spectra of current fluctuations appeared to relate to the kinetics of K^+ conduction, the

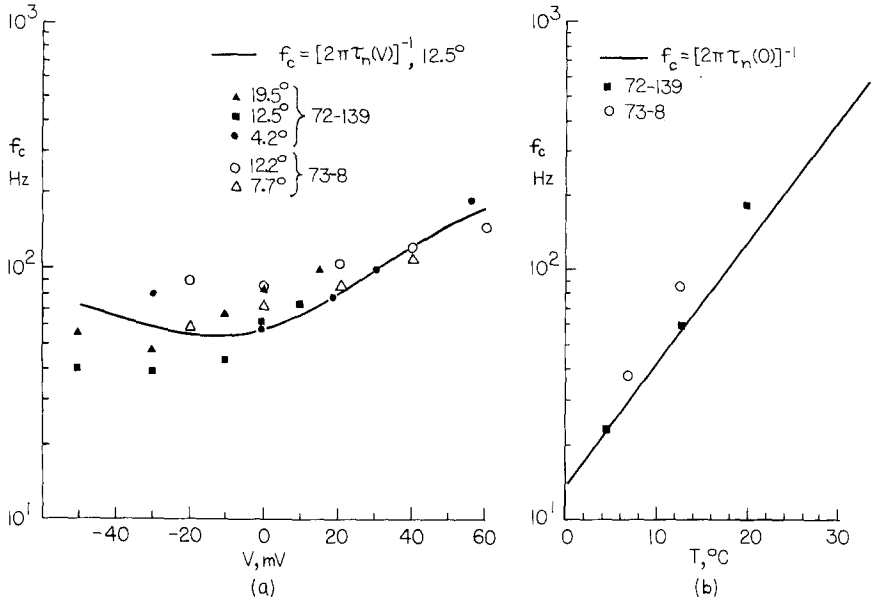

Fig. 7. Comparison of corner-frequency data from Fig. 5 (solid symbols) and Fig. 6 (open symbols) with voltage (*a*) and temperature at "rest" potential (*b*) with the relation (curve) $f_c=(2\pi\tau_n)^{-1}$, where τ_n is the HH relaxation time for K^+ conductance and a Q_{10} of 3 is assumed. In (*a*) the curve is drawn for 12.5 °C and data have been scaled to this temperature. It should be noted that the curve calculated from the HH $\tau_n(V)$ values uses the convention $V=0$ for normal rest potential, whereas the experimental condition of $V=0$ corresponds to a patch of axon slightly hyperpolarized from rest (*see* Materials and Methods, Fishman *et al.*, 1975*b*)

association was explored further in experiments in which K^+ current was blocked with agents or reduced by lowering the internal K^+ concentration by perfusion. Fig. 8 shows current PDS obtained during internal perfusion with standard perfusate at "rest" and at a 30 mV depolarization from "rest" (solid curves). Generally, noise spectra from perfused axons were indistinguishable from spectra of noise from unperfused axons. After perfusion with 10 mM (or greater concentrations) of TEA^+ the humps disappeared at all potentials for which they were present prior to introduction of TEA^+. The effect of TEA^+ within squid axon is known to block outward K^+ current flow (Armstrong & Binstock, 1965). The loss of the current-noise component which produced humps thus suggests a dependence upon K^+ conduction. An additional important and consistent feature in the comparison of current-noise PDS before and after TEA^+ was the crossing of control spectra at high frequencies, as shown in Fig. 8. In other words, although the gross feature of the hump was removed in the presence of 10 mM TEA^+, the noise power at high frequencies actually increased. This point will be discussed in the next section.

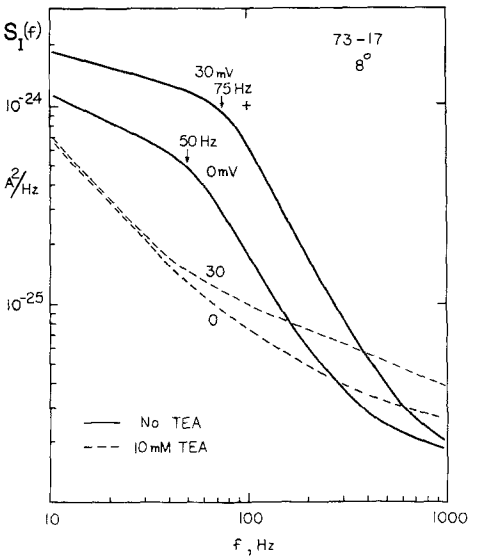

Fig. 8. PDS of current noise from a patch of internally perfused axon. Solid curves are controls obtained with standard perfusate. Dashed curves are the resulting spectra in the same axon and patch after 10 mM tetraethylammonium (TEA^+) is added to the standard perfusate. Note disappearance of relaxation spectrum with TEA^+ present, and the increase in noise power (crossing of dashed and solid curves) at high frequencies

When the internal K^+ concentration was reduced to 50 mM or less while maintaining the osmolarity of the internal perfusate constant with sucrose (0.8 mM), the humps in current-noise PDS again disappeared. Fig. 9 shows an example of these experiments. In the particular experiment shown, the internal concentration was lowered to 50 mM, and the resulting spectra, which were obtained during polarizations from "rest", show only a general decline with a slope slightly greater than unity. The axon, under these conditions, responded to current stimulation with good spikes (>90 mV) and also gave substantial inward current during voltage clamp. The absence of any interesting spectral features again suggests that the relaxation spectra, under standard conditions, relate to K^+ current flow. Furthermore, since the Na^+ conduction system was evident in step-clamp current but not evident in current-noise spectra, it would appear that Na^+ conduction noise is not a substantial part of the spectral feature normally observed in squid axon membrane. An indication of Na^+ noise can be seen in these experiments if difference spectra between depolarized and hyperpolarized runs are calculated. This procedure assumes that Na^+ current noise, in the hyperpolarized state, is nearly zero since, according

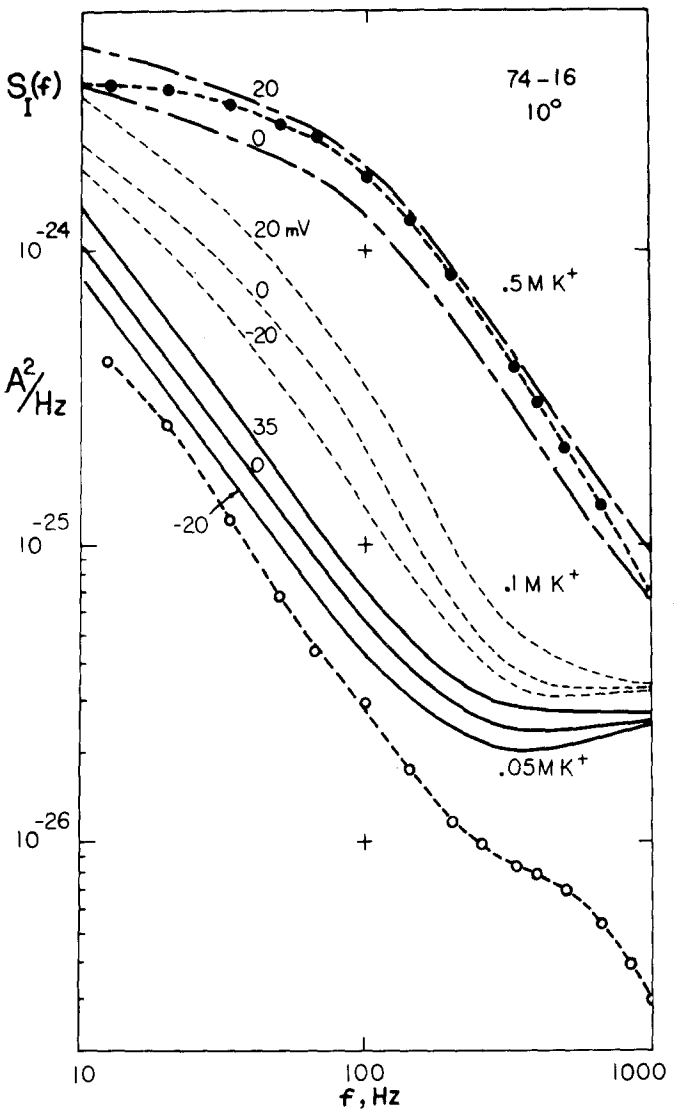

Fig. 9. PDS of current noise from the same patch of an axon internally perfused with three concentrations of K^+ (dilution of standard perfusate with isosmotic sucrose solution) in the perfusate. Relaxation spectra were not observed at concentrations of 0.05 M or below. The filled circles represent a difference spectrum between 500 mM $[K_i]$ at $V=20$ mV and 50 mM $[K_i]$ at $V=35$ mV. The relaxation component becomes apparent at 0.1 M and is obvious at control concentration (0.5 M). The sequence of concentration changes was (1) 0.05 M, (2) 0.1 M, (3) 0.5 M. This axon gave a spike amplitude of 90 mV or greater at all concentrations of K^+, and thus Na^+ conduction was operative. The open circles represent a difference spectrum in 50 mM $[K_i]$ between $V=35$ mV and $V=-20$ mV. The appearance of a curvature in addition to the f^{-1} noise suggests the presence of a Na noise component

to the Hodgkin-Huxley description, the steady-state Na^+ current is insignificant. The curve described by the open circles of Fig. 9 is such a spectrum for 50 mM K_i^+ which shows a Lorentzian-like curve in combination with approximately f^{-1} noise. The observation of Na^+ noise is, perhaps, possible in high $[K_i^+]$ (Conti *et al.*, 1975); however, in our experience it is marginal since the background K^+ noise is relatively high. Because of the small magnitude of this apparent Na^+ noise and other complicating factors related to the use of pharmacological agents (discussed later), we have deferred a fuller presentation of these results until an adequate statistical analysis of the data is completed.

Returning to Fig. 9, as the internal K^+ concentration was raised to 0.1 M the hump became more evident in spectra and upon return to 0.5 M the spectrum showed a dominant relaxation component. The appearance

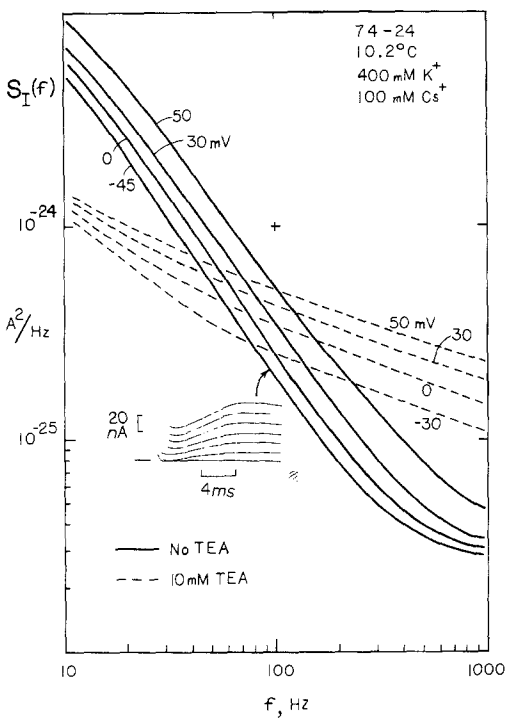

Fig. 10. PDS of current noise from the same patch of an axon internally perfused with a buffered solution containing 100 mM Cs^+ and 400 mM K^+ (solid curves). Again, relaxation spectra, which were present in control spectra during standard perfusion, disappear when K^+ current is reduced. (Inset) Clamp-current records in the patch for step voltages of 50 to 170 mV in increments of 20 mV. Steady-state currents are about half of normal (compare inset of Fig. 6). Dashed curves, spectra after perfusion with the same Cs^+ perfusate with 10 mM TEA^+ added. Note increase in high frequency noise power at all potentials

and obvious increase in intensity of the noise component which produced a relaxation spectrum thus followed the increase in K^+ current observed in steady-state step-clamp current records. At internal concentrations of 1 M K^+, without correction for osmolarity, the relaxation spectrum was of the same form as with 0.5 M K^+ but displaced vertically; i.e., the noise power at all frequencies increased.

One further example of the sensitivity of the relaxation spectrum to K^+ current is shown in Fig. 10. Internal perfusion with a solution of buffered 400 mM KF and 100 mM CsF produced spectra (Fig. 10, solid curves) which resembled those obtained when K^+ concentration was lowered to 50 mM (Fig. 9). The spectra in Fig. 10, however, indicate higher noise power compared to those in Fig. 9. Cs^+ does block K^+ current (Bezanilla & Armstrong, 1972); however, in the presence of 400 mM K^+, the steady-state step-clamp currents were only reduced to half (compare steady-state clamp currents in Figs. 6 and 10) of their control values (without Cs^+). Thus, although the humps disappeared from spectra with only a factor of 2 reduction in K^+ current, the power at all frequency components in the residual noise increased or decreased according to corresponding changes in K^+ current.

K^+ Channel Noise Induced by TEA^+ and a Quaternary Ammonium Derivative

It was noted previously that, although the relaxation noise portion of spectra is eliminated by concentrations of $TEA^+ \geqq 10$ mM inside the axon, there was an increased noise power at high frequencies relative to the noise power measured in control spectra (Fig. 8). This observation was pursued in experiments with TEA^+ for large polarizations of membrane potential. Fig. 11 shows a set of spectra which are representative of results from 15 axons. In the presence of TEA^+, the steady-state patch currents were reduced substantially, as indicated by the step-clamp current records in the inset of Fig. 11 (compare Fig. 6, inset). As a consequence of these reduced currents, it was possible to clamp the membrane potential to 100 mV or more from "rest" without patch destruction, and to observe the high-frequency behavior of patch current noise. Fig. 11 shows the appearance of a new hump (relaxation) at high frequency (1000 Hz). The new hump has several features. (1) It occurred at frequencies about a decade higher than the hump which was attributed to K^+ conduction relaxation (without TEA^+). (2) The hump became more pronounced and the noise power increased with increasing depolarization. (3) The transition

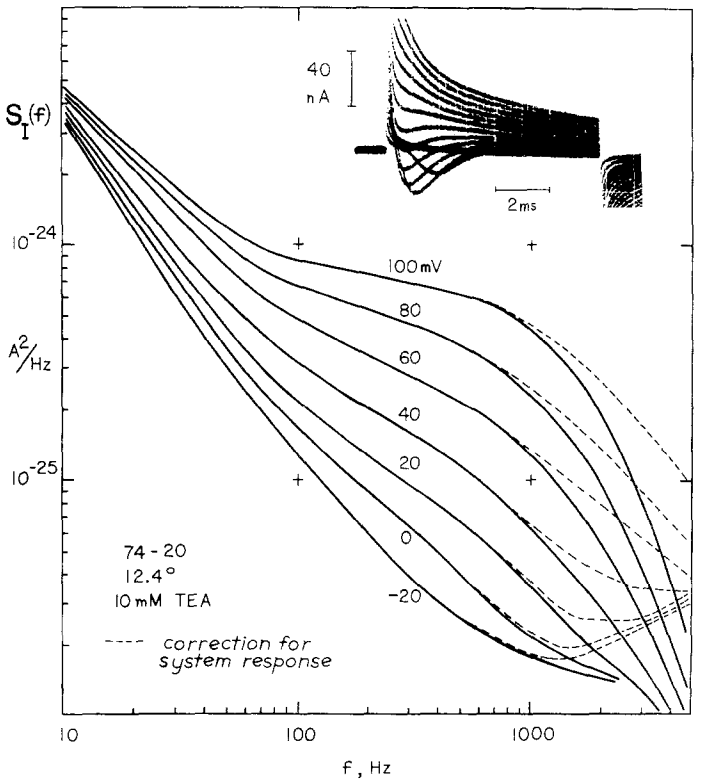

Fig. 11. PDS of current noise from a patch of axon internally perfused with standard perfusate containing 10 mM TEA^+ in addition. Note the appearance of a new hump (at higher frequencies than relaxation spectra without TEA^+-compare Fig. 6) with increasing depolarization. (Inset) Step-clamp currents on the axon patch in the presence of 10 mM TEA^+. Steps of 30 to 150 mV from "rest" in increments of 10 mV at 8 °C

from low to high frequency behavior of the hump appeared to shift to higher frequencies with increased depolarization. Point (3) is not obvious since the transition region occurred at the limit of the frequency response of the low-noise clamp system (Fig. 5*b*, Fishman *et al.*, 1975*b*). Despite corrections for the system response, there is still uncertainty in the spectral behavior above 1000 Hz. Nevertheless, these data clearly indicate a new conductance noise feature in the presence of TEA^+. The induced noise is not associated with Na^+ conduction since it was not present in spectra under conditions in which K^+ conduction was drastically reduced and Na^+ conduction persisted. Instead, it appears to relate specifically to the effect of TEA^+ on K^+ conduction. In particular, the humps did not occur in the presence of TEA^+ if internal K^+ concentration was reduced to 50 mM or less; i.e., the observation of the humps required sufficient internal

K^+ concentration in the same way that the normal K^+ conduction relaxation did. In addition, the spectra produced by TEA^+ are specific to TEA^+ as demonstrated by the data in Fig. 10. Internal perfusion with Cs^+ at high internal K^+ concentrations produced spectra which resembled those obtained during perfusion with low internal K^+ (Fig. 9). However, after addition of 10 mM TEA^+ to the Cs^+ perfusate in the same axon, the noise spectra (Fig. 10, dashed curves) showed high-frequency behavior which was similar to that observed with the addition of TEA^+ alone. These data therefore suggested that the induced noise is associated with K^+ channels, but that its spectral behavior reflects the effect of TEA^+ on K^+ conduction.

In order to establish that the TEA^+ induced K^+-channel noise is specifically associated with the interaction of TEA^+ on the membrane, it seemed necessary to demonstrate that the spectral character of the noise produced by TEA^+ could be altered by a slight change in the structure of the TEA^+ molecule, which would alter the degree of interaction with membrane sites affecting K^+ conduction. Fortunately, Armstrong (1971) has studied, in detail, the effect of various quaternary ammonium compounds on K^+ conduction. He has shown that substitution of a long hydrocarbon chain, for one of the ethyl groups in TEA^+, produces altered kinetics in the blockage of K^+ channels. One such compound is triethyldecylammonium ($TEDA^+$) which has a 10 carbon chain substituted for one ethyl group in the TEA^+ molecule. Internal perfusion with $TEDA^+$ produced the current-noise PDS shown in Fig. 12. The inset shows a set of step-clamp currents, from which it is obvious that the "inactivation" of K^+ conductance has been slowed considerably compared to the inactivation produced by TEA^+ (Fig. 11, inset). The corresponding noise spectra (Fig. 12) show a pronounced hump which increases in noise power with increasing depolarized potential. The transition-frequency region, however, for the $TEDA^+$ data occurs about a decade below that for TEA^+ (compare spectra of Figs. 11 and 12) and well within the frequency response of the low-noise clamp system. Consequently, the initial premise has been confirmed, viz a change in the structure of the TEA^+ molecule which produces slower kinetics (longer relaxation time) in the inactivation of K^+ conductance also produces a corresponding decrease in the transition-frequency region of noise spectra. Therefore, these data provide additional support for the view that TEA^+ and another quaternary ammonium derivative interact with membrane sites to induce noise in K^+ channels. Furthermore, the spectral behavior of the noise may reflect the "gating" properties imposed upon K^+ channels by the interaction of these molecules with the membrane. It would appear from the substantial increase

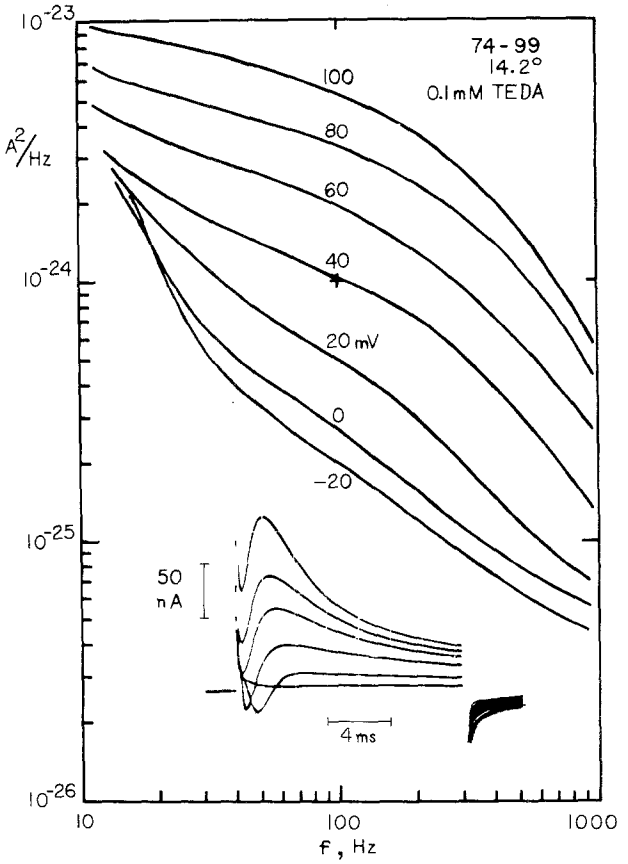

Fig. 12. PDS of current noise from a patch of axon internally perfused with standard perfusate containing 0.1 mM triethyldecylammonium ($TEDA^+$) ion in addition. Note spectral behavior with depolarization similar to TEA^+ (Fig. 11) but transition frequency is a decade lower corresponding to slower kinetics associated with K^+-current inactivation in step clamp currents (inset). Step clamps of 40 to 140 mV from "rest" in increments of 20 mV at 12.3 °C

in noise power with depolarization that the current noise arises from the large transient current in K^+ channels during brief intervals when TEA^+ or its derivative become unbound from sites which control K^+ current flow.

Discussion

f^{-1} Noise

In these experiments, noise of the f^{-1} type occurred under all axon conditions as a background or residual noise. It appears to be unrelated to ion conduction relaxation processes despite its apparent dependence on K^+ movements. Hill and Chen (1972) conclude that there is no way of

obtaining f^{-1} spectra from the channel kinetics expressed in the HH formulation. In most axons, f^{-1} noise is overshadowed by other noise which has the proper qualifications for ion-channel noise. Observation of the other noise is probably the best available argument against an f^{-1} channel "gating" noise. What phenomenon produces f^{-1} noise? The obvious possibility is restricted diffusion in "open" K channels with some contribution from nonspecific leakage pathways (Poussart, 1971). In this connection reports of f^{-1} noise in synthetic porous membranes (Hooge & Gaal, 1971; DeFelice & Michalides, 1972; Fishman & Dorset, 1973) have led to the suggestion that f^{-1} type noises occur whenever there is a physical constraint on current flow (Dorset & Fishman, 1972, 1975).

f^{-2} *Noise*

In contrast to f^{-1} noise, fluctuations which appear to be first-order in form, i.e., $[1+(f/f_c)^2]^{-1}$, satisfy criteria for K^+-channel noise (Fishman, 1973), as follows: (1) The form of conductance spectra corresponds to a relaxation process with corner frequencies which change with potential and temperature and which relate to the HH relaxation time for macroscopic K^+ conductance behavior; (2) the relaxation spectra are insensitive to blockage of Na^+ current, but disappear after blockage or substantial reduction in K^+ current; and (3) inhibition of active transport does not affect these spectra. Although these points were initially apparent from voltage fluctuation spectra (Fishman, 1973), these results have been confirmed here in current spectra obtained during voltage clamp.

The predominant noise sources identified in the PDS of fluctuations presented here are associated with potassium ion conduction. The contribution of the sodium ion conduction mechanism to the PDS is of low amplitude and not easily measurable. Although it is attractive to use blocking agents to attenuate the potassium noise and thus facilitate the observation of sodium noise, the finding of a significant TEA^+ induced noise indicates caution must be exercised in the use of pharmacological approaches to separate ionic processes. In the case of TEA^+, the induced noise (1) is nearly an order of magnitude larger than the presumed sodium noise component (Fig. 9), (2) is of larger amplitude at high frequencies than both the potassium and sodium noise sources (Fig. 8), and (3) disappears when the internal potassium ion concentration is 50 mM or less, despite the presence of an active sodium conduction system. In view of these properties associated with the TEA^+ induced excess noise, the identifi-

cation by Conti *et al.* (1975) of a Lorentzian component related to sodium noise in the presence of 70 mM TEA and high $[K_i]$ is questionable. More likely, the high frequency Lorentzian component is a TEA induced noise similar to that observed in the experiments reported here. Although quaternary ammonium ions are not very useful for observing sodium noise, the spectra of their induced noise provide an alternative means of comparing kinetic schemes (Armstrong, 1971).

Model Discrimination

A widely assumed class of models for ion-channel kinetics ("gating") is the two-state (open-closed) conductance. Use of these models affords, perhaps, the simplest way to explain macroscopic voltage-dependent conductances. Two-state models have great appeal because of their simplicity, unit channel implication, and because they follow quite naturally from the macroscopic HH equations. Furthermore, the appearance of open-close conductances in lipid films (Ehrenstein, Lecar & Nossal, 1970; Hladky & Haydon, 1972) has nurtured the two-state concept. With the measurement of K^+ channel relaxation noise in axons, it is tempting to assume a two-state model for conduction which permits calculation of important conduction parameters. This has already been done by Katz and Miledi (1970, 1971, 1972) and Anderson and Stevens (1973) in studies of the noise produced by acetylcholine (ACh) interaction with receptors at the frog neuromuscular junction. The model used assumes that ACh rapidly binds to a receptor site and forms a complex. A subsequent slower, voltage-dependent conformational change produces an "open" ion-channel which can then fluctuate between open and closed conformations. As a consequence of two-state simplicity, several ion-channel parameters can be calculated and interpreted from their noise spectra (Stevens, 1972; Anderson & Stevens, 1973).

Implicit in a two-state model is a single value of open-channel conductance irrespective of membrane parameters such as potential, mean current and temperature. Anderson and Stevens (1973) have shown that their data are consistent with a two-state conductance for ACh-receptor noise for their model of the rate process and within the accuracy of their measurements. However, the use of two-state models to interpret ion conduction data in axon membranes is, presently, questionable. The possibility of multi-state conduction is, at this point, equally probable. There is recent evidence of multi-state conduction in lipid films (Eisenberg, Hall & Mead, 1973). Axon noise data appear to provide the kind of

information which may lead to resolution of this and equally important issues.

One of the major problems in the use of axon spontaneous noise data is the presence of significant f^{-1} noise, which prevents accurate determination of relaxation spectra over a wide frequency range. We are exploring ways of unfolding the f^{-1} noise from relaxation noise. Presently, however, we rely upon the dominance of the relaxation noise component over f^{-1} noise in the transition-frequency region of spectra. As noted previously (Hill & Chen, 1972; Stevens, 1972; Fishman, 1973), the spectral form and corner frequency behavior with voltage and temperature in the transition-frequency region alone can be used to compare and relate conduction models. In this respect, the HH formulation is the foremost kinetic scheme from which initial test models can be considered. Hill and Chen (1972) and Stevens (1972) have begun on this course with calculations of PDS based upon two-state channels. The spectra of conductance fluctuations for these models produce a sum of first-order terms, each with slightly different corner frequency (Fig. 13).

The experimental behavior of corner frequency f_c with potential and temperature (Fig. 7) suggests an inverse relation to the Hodgkin-Huxley relaxation time τ_n whereas the two-state models, calculated by Hill and Chen and Stevens, produce spectra with generally larger f_c (Fishman, 1973). However, there are several aspects which must be considered before the data in Fig. 7 may be used to compare models. First, determination of the corner frequencies was made from spectra which contained f^{-1} noise. Consequently, the corner frequencies, which were derived from the intersection of tangents to the low and high-frequency portions of each spectrum, may not be accurate. The amount of error depends upon the relative intensity of f^{-1} noise and its coherence with the relaxation noise. Evaluation of these errors requires a reliable method for unfolding the noises from one another. If, instead, we rely upon difference spectra between 500 mM $[K_i]$ and 50 mM $[K_i]$, i.e., subtraction of the residual noise still present after removal of most of the internal potassium ions, an additional estimate of the corner frequency can be obtained. Fig. 9 shows a difference spectrum (filled circles) taken for the depolarized case. This difference spectrum indicates a near Lorentzian shape and demonstrates that the corner frequencies for both uncorrected and corrected spectra are similar. Difference spectra could also be calculated using TEA^+ instead of the low $[K_i]$ spectra; however, as discussed previously, this procedure leads to substantial error in the derived shape of the spectra due to the induced TEA^+ noise. A further correction to consider in calculating difference spectra is the

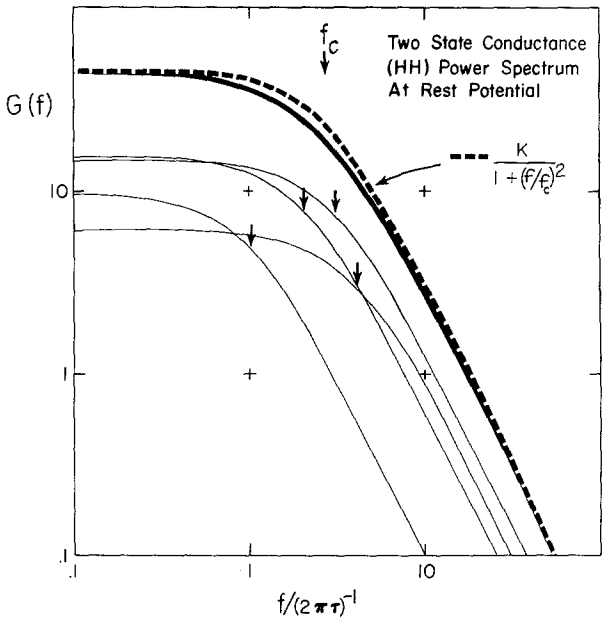

Fig. 13. Power spectrum for two-state channel conductance in the HH formulation at rest potential (0 mV) derived by Stevens [1972, $M_4(f)$, Eq. 16]. $G(f)=M_4(f)/N\gamma^2\times10^{-3}$. At rest $n_\infty(0)=0.318$ and

$$G(f)=9.8[1+(f/1/2\pi\tau_n)^2]^{-1}+15.8[1+(f/1/\pi\tau_n)^2]^{-1}+15.1[1+(f/3/2\pi\tau_n)^2]^{-1}$$
$$+6.1[1+(f/2/\pi\tau_n)^2]^{-1}.$$

Each of the four terms is graphed separately (thin solid curves) with arrows indicating corner frequencies at $k(2\pi\tau_n)^{-1}$ where $k=1$ to 4. The thick solid curve is $G(f)$ and the arrow (f_c) is the frequency at which the low and high frequency asymptotes of $G(f)$ intersect. Dashed curve is a first-order spectrum $K[1+(f/f_c)^2]^{-1}$ drawn for comparison. Note that the "roll-off" of $G(f)$ in the transition-frequency region is not as sharp as a single first-order spectrum

subtraction of the generally observed f^{-1} component. Since this process is dependent on both the membrane potential and the potassium ion concentration, or more directly, the membrane current, it would, in principle, still be present in the above difference spectrum. However, in this particular case the f^{-1} amplitude was insignificant and it was not subtracted.

An additional problem arises from the form of spectra in the transition-frequency region. The corner frequency, as determined from the intersection of low and high-frequency asymptotes, only denotes the half-power point in a spectrum which is first order. Furthermore, the corner frequency from the HH power spectrum for two-state channels can only be compared with spectra which are consistent with first-order rate processes (Fig. 13),

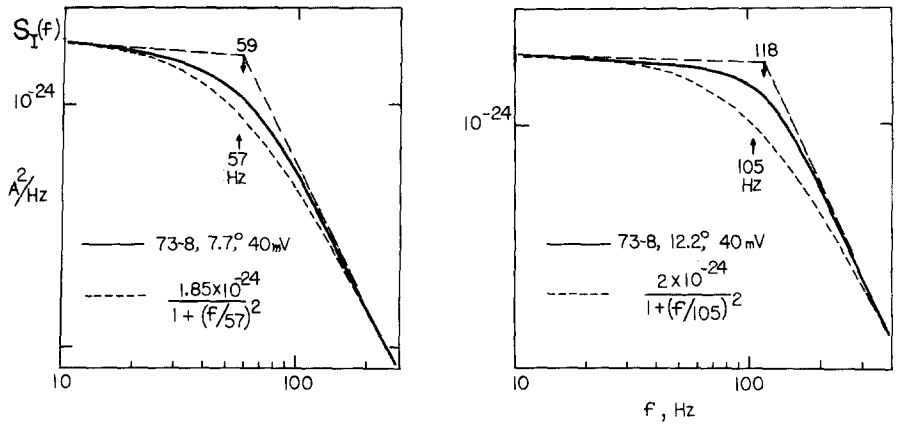

Fig. 14. Current-noise PDS (solid curves) redrawn from Fig. 6 for 40 mV depolarization from "rest" at two temperatures (7.7 ° and 12.2 °C). Dashed curves are the first order functions $1.85\times10^{-24}/1+(f/57)^2$ and $2\times10^{-24}/1+(f/105)^2$. Arrows are corner frequencies from intersection of low and high frequency asymptotes. Note experimental spectra have sharper transition regions than first order

i.e., spectra which show a transition-frequency "roll-off" no sharper than a single first-order process. As indicated in Fig. 13 (compare thick solid and dashed curves), the superposition of two or more first-order spectra with relatively close corner frequencies always yields a spectrum transition region which is not as sharp as a single first-order process, whereas the low and high-frequency portion of the spectrum remains first order. Fig. 14 shows two current-noise PDS taken from Fig. 6 in which the relaxation noise component was clearly dominant over f^{-1} noise. First order spectra (dashed curves) have been computed and graphed based upon a fit of the low and high-frequency asymptote of the experimental spectra. The comparison indicates that the transition region is significantly *sharper* than a first-order relaxation process. Since the kinetics expressed in the HH formulation are first order, it is clear that this behavior cannot be obtained from the HH description. Thus the use of the data of Fig. 7 to test kinetic schemes of the HH type appears to be premature.

We have not found any aberrations in our methods or technique which could produce transitions sharper than first-order (Fishman *et al.*, 1975*b*). The sharp spectral feature has appeared in both current noise PDS during voltage-clamp conditions as well as in voltage noise PDS divided by the measured square of the magnitude of patch impedance, which is obtained without a voltage clamp. A spectrum characterized by low and high-frequency behavior that is first order with transition behavior that is

not first order implies that a circuit description of the kinetics of the n process contains, in addition to an RL branch, an RLC branch in parallel (*see* Fishman, 1975*c*). Measurements of the "small signal" complex-impedance of squid axon membrane should aid in determining whether an additional circuit branch is necessary.

Note Added in Proof: We have confirmed the existence of a low-frequency feature (1–30 Hz) in both the complex impedance, $Z(f)$, and admittance, $Y(f)$, of squid axon by axial-wire technique. This feature is 1) voltage and temperature dependent, 2) disappears after block of K^+ conduction, and 3) is unaffected by a block of Na^+ conduction or active transport. With respect to a description of K^+ conduction kinetics, the magnitude and phase functions of Z and Y are consistent with an additional RLC branch (non first order) suggested by peaking in patch power spectra of K^+ current noise. Details will be reported at the 1976 meeting of the Biophysical Society and in a future publication.

We thank Dr. Kenneth S. Cole for continued interest and discussions. Dr. Fishman thanks Dr. C. F. Stevens, Dr. T. L. Hill and Dr. Y.-D. Chen for discussions of conduction models and Mr. William Law, Jr. for assistance in instrumentation. This work was supported in part by National Institutes of Health grants NS 09875 (HMF), NS 11764 (HMF), and NS 08409 (LEM); and Canadian National Research Council grant A 5274 to DJMP. Dr. Fishman thanks the SUNY Research Foundation for awards during his affiliation with the Department of Biological Sciences, SUNY at Albany.

References

Anderson, C. R., Stevens, C. F. 1973. Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol.* **235**:655

Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with potassium channels of giant axons. *J. Gen. Physiol.* **58**:413

Armstrong, C. M., Binstock, L. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* **48**:859

Bezanilla, F., Armstrong, C. M. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60**:588

Chen, Y.-D., Hill, T. L. 1973. Fluctuations and noise in kinetic systems. Applications to K^+ channels in squid axon. *Biophys. J.* **13**:1276

Conti, F., DeFelice, L. J., Wanke, E. 1975. Potassium and sodium ion current noise in the membrane of the squid axon. *J. Physiol.* **225**:45

DeFelice, L. J., Michalides, J. P. L. M. 1972. Electrical noise from synthetic membranes. *J. Membrane Biol.* **9**:261

Derksen, H. E. 1965. Axon membrane voltage fluctuations. *Acta Physiol. Pharmacol. Neerl.* **13**:373

Derksen, H. E., Verveen, A. A. 1966. Fluctuations of resting neural membrane potential. *Science* **151**:1388

Dorset, D. L., Fishman, H. M. 1972. Excess noise in electrolytic systems with anisotropic constraints to ion flow. *Biophys. Soc. Abst.* **12**:119a

Dorset, D. L., Fishman, H. M. 1975. Excess electrical noise during current flow through porous membranes separating ionic solutions. *J. Membrane Biol.* **21**:291

Ehrenstein, G., Lecar, H., Nossal, R. 1970. The nature of the negative resistance in bimolecular lipid membranes containing excitability-inducing material. *J. Gen. Physiol.* **55**:119

Eisenberg, M., Hall, J. E., Mead, C. A. 1973. The nature of the voltage-dependent conductance induced by alamethicin in black lipid membranes. *J. Membrane Biol.* **14**:143

Fishman, H. M. 1972. Excess noise from small patches of squid axon membrane. *Biophys. Soc. Abstr.* **12**:119a

Fishman, H. M. 1973. Relaxation spectra of potassium channel noise from squid axon membranes. *Proc. Nat. Acad. Sci. (USA)* **70**:876

Fishman, H. M. 1975*a*. Noise measurements in axon membranes. *Fed. Proc.* **34**:1330

Fishman, H. M. 1975*b*. Patch voltage clamp of squid axon membrane. *J. Membrane Biol.* **24**:265

Fishman, H. M. 1975*c*. Rapid complex impedance measurements of squid axon membrane via input-output cross correlation function. *Proc. 1st Symp. on Testing and Identification of Nonlinear Systems. California Institute of Technology, Pasadena*, p. 257

Fishman, H. M., Dorset, D. L. 1973. Comments on electrical fluctuations associated with active transport. *Biophys. J.* **13**:1339

Fishman, H. M., Moore, L. E., Poussart, D. J. M. 1975*a*. K^+ conductance noise induced by TEA and its C_{10} derivative in squid axon. *Biophys. Soc. Abstr.* **15**:167a

Fishman, H. M., Poussart, D. J. M., Moore, L. E. 1975*b*. Noise measurements in squid axon membrane. *J. Membrane Biol.* **24**:281

Hill, T. L., Chen, Y.-D. 1972. On the theory of ion transport across the nerve membrane. IV. Noise from the open-close kinetics of K^+ channels. *Biophys. J.* **12**:948

Hladky, S. B., Haydon, D. A. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. I. Studies of the unit conductance channel. *Biochim. Biophys. Acta* **274**:294

Hooge, F. N., Gaal, J. L. M. 1971. Fluctuations with a $1/f$ spectrum in the conductance of ionic solutions and in the voltage of concentration cells. *Philips Res. Repts.* **26**:77

Katz, B., Miledi, R. 1970. Membrane noise produced by acetylcholine. *Nature* **226**:962

Katz, B., Miledi, R. 1971. Further observations on acetylcholine noise. *Nature, New Biol.* **232**:124

Katz, B., Miledi, R. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol.* **224**:665

Keynes, R. D., Lewis, P. R. 1951. The sodium and potassium content of cephalopod nerve fibers. *J. Physiol.* **114**:151

Kittel, C. 1958. Elementary Statistical Physics. Wiley, New York

Lax, M. 1960. Fluctuations from the nonequilibrium steady state. *Rev. Mod. Phys.* **32**:25

Narahashi, R., Moore, J. W., Scott, W. R. 1964. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. Gen. Physiol.* **47**:965

Poussart, D. J. M. 1969. Nerve membrane current noise: Direct measurements under voltage clamp. *Proc. Nat. Acad. Sci (USA)* **64**:95

Poussart, D. J. M. 1971. Membrane current noise in lobster axon under voltage clamp. *Biophys. J.* **11**:211

Siebenga, E., Meyer, W. A., Verveen, A. A. 1973. Membrane shot-noise in electrically depolarized nodes of Ranvier. *Pflügers Arch.* **341**:87

Siebenga, E., Verveen, A. A. 1971. The dependence of the $1/f$ noise intensity of the node of Ranvier on membrane potential. *1st Europ. Biophys. Congress, Abstr.* 219

Stevens, C. F. 1972. Inferences about membrane properties from electrical noise measurements. *Biophys. J.* **12**:1028

Wanke, E., DeFelice, L. J., Conti, F. 1974. Voltage noise, current noise and impedance in space clamped squid giant axon. *Pflügers Arch.* **347**:63