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Membrane Electrical Noise in *Chara corallina*[1]

I. A LOW FREQUENCY SPECTRAL COMPONENT

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ABSTRACT

Some aspects of the membrane physiology of the giant-celled alga *Chara corallina* have been studied using the techniques of membrane noise analysis. Cellular voltage noise was measured by means of a microelectrode inserted in the vacuole of the cell. The significant feature of spectra estimated from voltage noise signals in these cells was a low-frequency component possibly associated with the active, electrogenic proton pumping which occurs in these cells. The effect of high external pH is described.

Membrane noise analysis has become over the past two decades a distinct subfield of biophysics, large enough to merit an introductory monograph (5). This technique has been applied to the study of the membrane physiology of characean giant algal cells (8, 10, 16, 17).

In membrane noise analysis advantage is taken of the fact that the statistics of random currents or PDs[3] across membranes are determined by the statistics of the channels or carriers conducting discrete amounts of charge during their 'conductance fluctuations.'

Over the years a few reports of membrane noise due to active transport have trickled in. Strandberg and Hammer (24) measured voltage noise power over the frequency bands 5 to 10, 20 to 40, and 100 to 200 Hz. They found that membrane noise increased under conditions in which active transport occurred across toad urinary bladders.

Segal (20) found a spectral component produced by active transport of sodium ions across frog abdominal skin. The validity of his results was disputed (9), but Segal (21) has shown convincingly that the spectral component was indeed produced by active transport.

Roa and Pickard (16, 17) reported that there was an effect of various inhibitors on low frequency noise in the alga *Chara braunii*. They postulated that the inhibitors were affecting active transport in these cells. Ferrier *et al.* (8) studied voltage noise spectra in *Chara corallina*, but interpreted their results in terms of passive K^+ transport and did not apply specific inhibitors which might reveal a relationship to an active transport.

Hayashi and Hirakawa (10) published voltage noise spectra taken from cells of *Nitella axilliformis*, another characean alga, in which PSD estimates were extended down to a lower frequency (10^{-2} Hz) than in previous studies. Those authors were interested in the difference between spectra from the cell in its resting state and near the threshold for an action potential. They found that a peak of power spectral density appeared near this threshold, due to large scale fluctuations in membrane potential. They speculated that this peak might be related to the activity of the electrogenic proton pump.

It will be demonstrated in this article that there is a spectral component at low frequencies (<5 Hz) produced by a transport process which the authors propose to identify as the electrogenic proton pump, a type of transport which is known to occur in these cells.

The plasmalemma of *C. corallina* has been shown to undergo a complete shift of transport properties when the pH in the external bathing medium reaches a critical value between pH 9 and pH 10 (2, 3); resting membrane potential follows a proton diffusion potential up to about pH 12. It was decided to compare membrane noise at an ordinary physiological external pH to that at high external pH to examine the effect of this change in membrane properties from the normal state where the transmembrane PD is hyperpolarized electrogenically to the high pH state where cell potential is determined mainly by passive proton diffusion.

MATERIALS AND METHODS

Cells were cultured in the laboratory in fiberglass tanks as described previously (19).

Figure 1 illustrates schematically the experimental arrangement used to sample voltage noise signals. An internode of *Chara corallina* was held in a bath filled with the culture medium described in Hope and Walker (11), which will be henceforth referred to as CPW. The CPW was buffered by adding 5 mM Tes and titrating to pH 7.5 with 1 M NaOH. Other pH values were obtained using 5 mM concentrations of the buffers described in Ross *et al.* (19).

Noise signals were amplified and tape recorded for later analysis on a PDP-11/03 computer system. The tape-recorded noise signals were sampled via an analog-to-digital converter, then power spectra were computed as described in Ross (18). A low-pass frequency filter was used to avoid the problem of aliasing, in which power from frequencies higher than the Nyquist frequency appears spuriously at lower frequencies (18). In all cases the background equipment PSD, produced by the noise from the electronic equipment and the electrodes, was subtracted from spectra sampled from cells. RMS voltage in a frequency band selected by the frequency filter could be plotted as a function of time by means of an electronic RMS converter connected to a chart recorder.

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[3] Abbreviations: PD, potential difference; PSD, power spectral density; CPW, *Chara* pond water; RMS, root mean square; LFSC, low frequency spectral component.

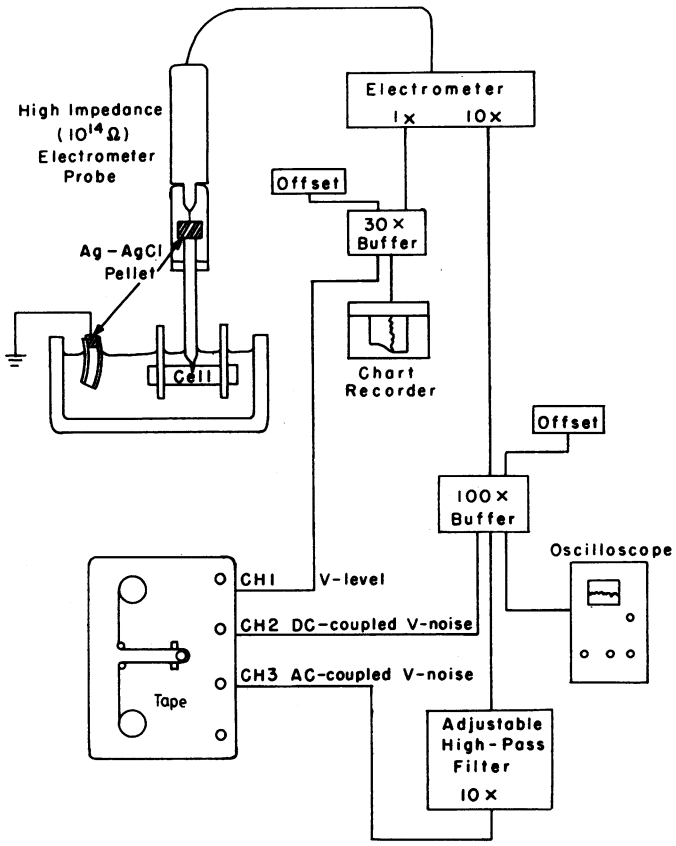

FIG. 1. Schematic diagram of the experimental setup for recording voltage noise signals from internodes of *C. corallina*. A small internode, typically <1 cm in length, was held by a pair of stocks in the bathing solution in a chamber. The sides of the chamber were constructed from microscope slides to allow for good illumination from the rear, and viewing of the cell through a dissecting microscope mounted horizontally. Bath grounding was provided by means of an Ag-AgCl pellet (E. W. Wright model RC-1) inserted into a plastic tube containing 3 M KCl in agar. A glass microelectrode, filled with 3 M KCl solution and mounted in a plexiglass holder with an Ag-AgCl pellet (E. W. Wright model EH-1FS) to provide electrical contact, was inserted by means of a vertically mounted micromanipulator to which the electrometer probe was attached. The voltage signal from the electrometer (Dagan model 8700) was amplified through battery powered 'buffers' which isolated the electrometer from noise produced at the inputs of the tape recorder (Racal model Store 4-D) and was processed as shown, being recorded as three signals: voltage level relative to a recorded reference level, DC-coupled noise recorded with lower amplification, and AC-coupled noise recorded with higher amplification.

In some cases membrane impedance was measured as described in Ross (18) and Ross *et al.* (19). The membrane impedance was then used to convert from voltage power spectral density to current power spectral density as follows (18): when the frequency-dependent membrane impedance, $Z(f)$, is known the magnitude, $|Z(f)|^2$, may be used to derive the current power spectral density, $S_I(f)$, from the corresponding voltage PSD, $S_V(f)$:

$$S_I(f) = S_V(f)/|Z(f)|^2 \quad (1)$$

RESULTS

Low Frequency Noise under Standard Conditions. Under standard conditions of pH 7.5 CPW in the light the cells were found either to have a vacuolar resting potential of approximately −150 mV or, more usually, to be hyperpolarized to more than −200 mV, sometimes reaching a potential of −280 mV. Cells would sometimes spontaneously switch between the two states. Pickard (14) was able to force internodes of *Chara braunii* resting in a 'depolarized state' into a 'hyperpolarized state' by passing a hyperpolarizing current through an inserted pipette; this did not work with the authors' cells, however.

Below 1 Hz the power spectral density displayed a very steep slope on a log-log plot, −3 to −4, indicating a $1/f^3$ to $1/f^4$ relationship of PSD to frequency (Fig. 2a). However, there was a break in the PSD at approximately 0.2 Hz below which spectral density followed a line of slope −1 on a log-log plot. At frequencies below 1 Hz, impedance magnitude is a fairly flat function of frequency (Fig. 2b). If we divide the voltage spectrum by impedance magnitude as in equation 1, the current PSD illustrated in Figure 2c results. The shallow portion of that spectrum also has a slope of approximately −1 on a log-log plot. If a straight line was fitted to the shallow portion of the spectrum and subtracted, as in Figure 2d, what appeared to be a relaxation, or Lorentzian, spectrum resulted (see Defelice [5] for an explanation and theory of the Lorentzian, or relaxation, spectrum). In measurements taken from 20 of these cells this low frequency spectral component was found without exception.

High Frequency Noise under Standard Conditions. Membrane impedance is strongly frequency dependent in the high frequency range (19) and thus current spectra may be expected to have a significantly different shape from voltage PSDs. Figure 3 illustrates a typical current spectrum in the high frequency range, calculated using equation 1. This type of spectrum was found in most, but not all, cells and always in cells which were in the depolarized state (vacuolar PD about −150 mV as mentioned above). We were unable to extract any interpretable structure from voltage or current noise spectra in the high frequency range on a consistent basis; occasionally, what appeared to be a Lor-

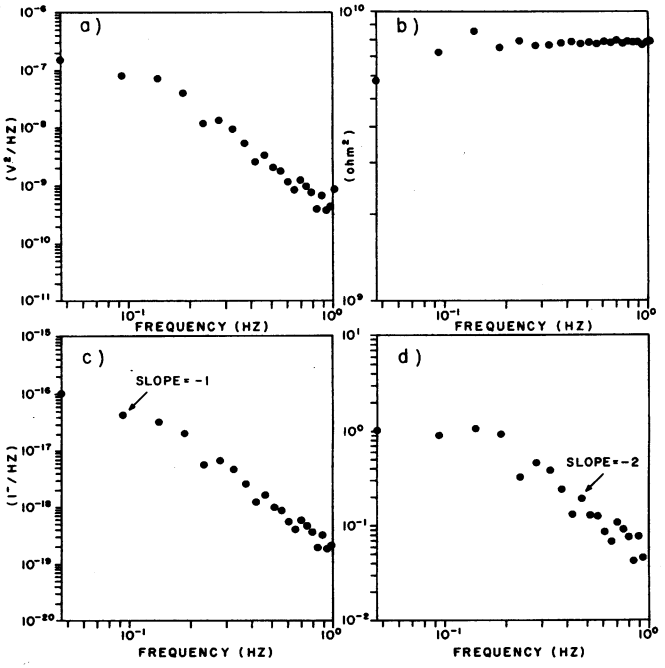

FIG. 2. (a), Voltage power spectral density from an internode of *C. corallina* in CPW in the light; (b), impedance magnitude, $|Z(f)|^2$, *versus* frequency; (c), current PSD obtained by dividing the voltage PSD values of (a) by the impedance magnitude of (b); (d), a log-log line fitted to the first four points has been subtracted from the spectrum of (c) leaving a Lorentzian PSD.

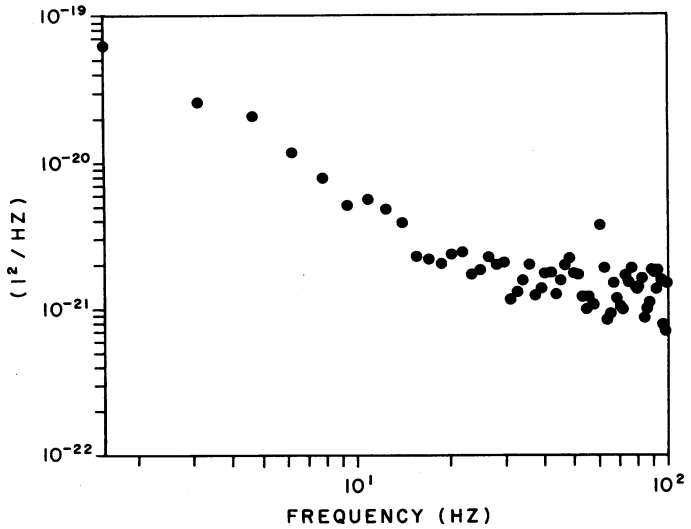

FIG. 3. Current PSD from an internode of *C. corallina* in CPW in the light.

entzian component appeared in the spectrum, but most often it was absent. Noise was variable in magnitude, as illustrated in Figure 4. RMS power is simply the integral of the power spectrum, and one can see in Figure 4 that there is a large variation with time of total noise in the frequency band from 1 to 30 Hz.

Effects of pH. Over a range of pH values: 5.2, 6.0, 7.0, 7.5, 8.0, and 9.0 there was no significant effect on either low or high frequency membrane noise, and specifically, none on the low frequency spectral component described above. Buffering of the CPW did not seem to have any noticeable effect either. Cells switched from unbuffered CPW, which had a pH of 7.5, to CPW buffered to the same pH displayed exactly the same membrane impedance and produced the same voltage noise PSDs.

Comparisons were also made between membrane noise at pH 7.5 and pH 11, at which the membrane becomes mainly passively permeable to protons (2, 3). There was no significant change in membrane noise in the high frequency range, above 5 Hz. In the low frequency range below 5 Hz, however, the low frequency spectral component disappeared from the current PSD (Fig. 5), indicating that the membrane transport process responsible for the low frequency spectral component in the power spectrum was not operating.

DISCUSSION

The shape of the low frequency spectrum under normal conditions in Figure 2 corresponds to that of spectra measured in characean internodes by other workers. This is gratifying since different techniques were used to measure spectra in each study. While the fast Fourier transform method of PSD estimation (18) was used in this study, Hayashi and Hirakawa (10) used a commercially available spectrum analyzer. Ferrier *et al.* (8) used the Blackman-Tukey method of spectral analysis (4) and Roa and Pickard (16) used a digital bandpass frequency filter method. Hayashi and Hirakawa's results (10) in *Nitella axilliformis* at its resting potential were comparable to those of Figure 2. In their resting cells they found a form of PSD similar to that described above, a power spectrum which followed a straight line on a log-log plot with slope −1 below 0.2 Hz and a line with slope −3 above 0.2 Hz, but they interpreted it as resulting from potassium conductance fluctuations although they could not prove or disprove that idea. Ferrier *et al.* (8) and Roa and Pickard (16, 17) did not extend their measurements to low enough frequencies to show the corner frequency clearly, but they did find a similar steep slope in the frequency range from 0.2 to 5 Hz, confirming

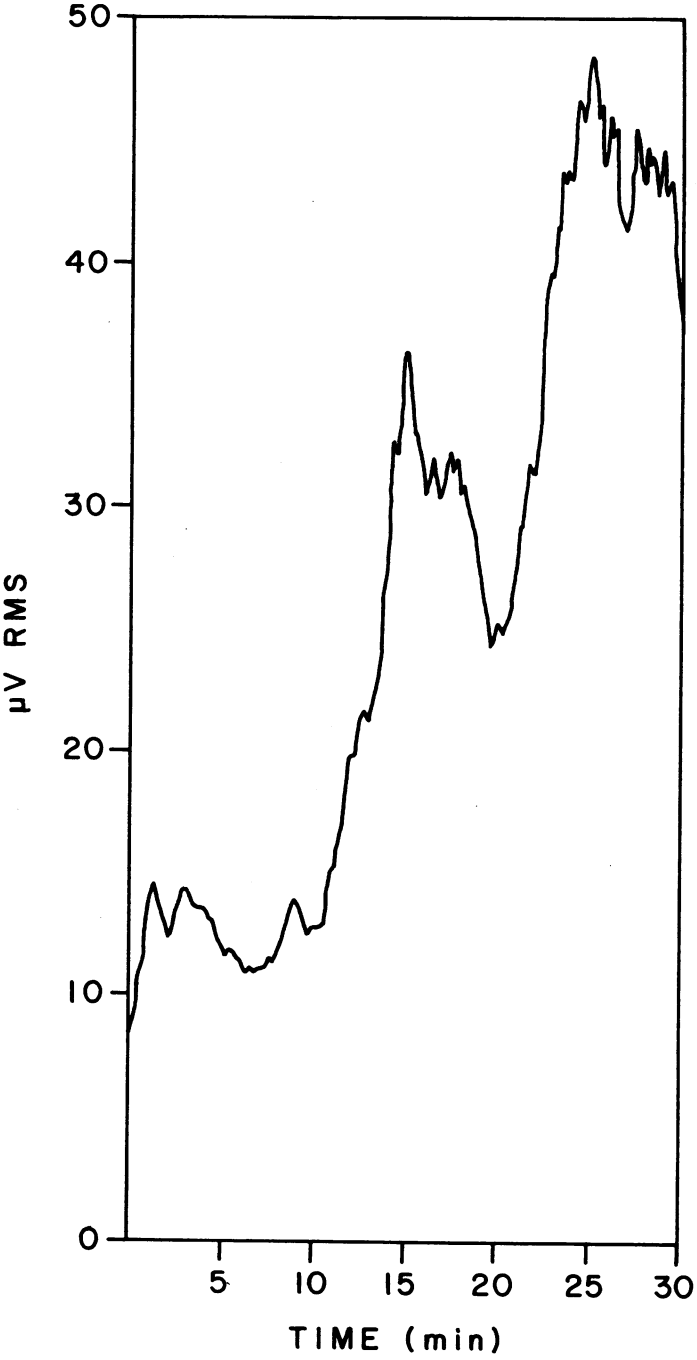

FIG. 4. RMS voltage noise (integrated over the range 1–30 Hz) plotted against time.

these results.

It is apparent that power cannot increase indefinitely toward lower and lower frequencies if the total RMS power in the spectrum is not to be immense. Korff *et al.* (13) observed a peak at 0.0003 Hz in *Nitella* sp. with PSD declining below that frequency. No attempt was made to measure noise in that frequency range for this study, however, due to a limited length of recording tape. To measure spectra at such frequencies one would have to record for about 23 h and ensure that the signal did not go off scale during that period.

In the high frequency range Roa and Pickard (16) found what appeared to be a Lorentzian spectral component in the 10 to 1000 Hz frequency range, with corner frequency at approximately 200 Hz, in voltage spectra measured in *Chara braunii*.

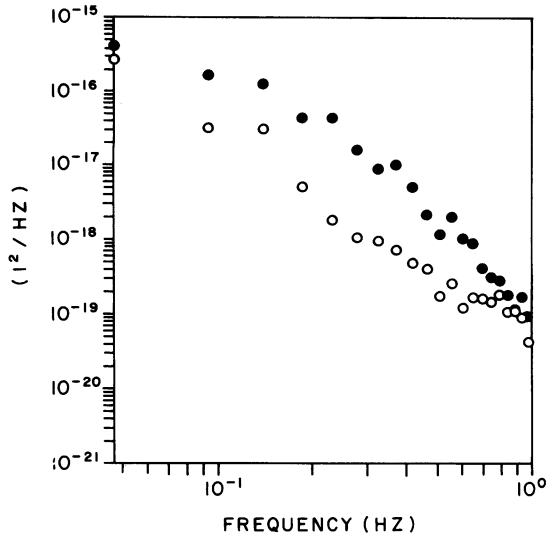

FIG. 5. Current PSD from an internode of *C. corallina* at the following pH values: (●), pH 7.5; (○), pH 11.0.

They did not attempt to study this portion of the spectrum systematically, however, having decided to pursue studies into the effect of various treatments on the low frequency spectrum, which seemed to them to be related to active transport in those cells. In *C. corallina*, Ferrier *et al.* found some structure in that frequency range (8), and were able to obtain a Lorentzian spectral component by subtracting a line on a log-log plot of −2 (equivalent to filtering out a $1/f^2$ spectrum). This relaxation spectrum had a corner frequency at about 55 Hz. The present study did not confirm these results, however. As was noted above, results were inconsistent, they sometimes displayed what appeared to be a Lorentzian component, but most often displayed no structure at all. More work needs to be done to elucidate under what conditions high frequency noise provides significant information in these cells. If one could reliably obtain these Lorentzian spectra one might be able to investigate the attractive hypothesis that they result from potassium transport using the inhibitor tetraethylammonium, which has been shown to block K^+ transport in *N. flexilis* (1, 25).

It is pertinent at this point to calculate those contributions to the spectrum which will be made by sources of noise which occur in all current conducting systems. Thermal noise is voltage noise produced by thermal agitation of charge carriers. Nyquist's formula for power spectral density due to thermal noise is:

$$S_v(f) = 4\ kT\ Re[Z(f)] \qquad (2)$$

where k = Boltzmann's constant = 1.38×10^{-23} joule/°K, T = absolute temperature = 298°K, and $Re(Z[f])$ = the real portion of the complex, frequency-dependent impedance of the conducting system ≃ 100 kΩ. Substituting values we obtain $S_v(f) \simeq 1.6 \times 10^{-15}$ V²/Hz, which is more than two orders of magnitude smaller than the measured voltage spectral density from the cell even at high frequencies.

When current flows across a membrane there will be shot noise produced by the transit of charge carriers. Stevens (23) has shown that shot noise will produce a current PSD, $S_i(f)$, which is constant out to frequencies which are very high by physiological standards, about 500 kHz for a typical membrane. This constant current spectral density will be given by the following expression (15):

$$S_i(f) = 2\ \bar{I}q \qquad (3)$$

where $\bar{I}$ = average membrane current and q = charge magnitude.

If we allow for a large membrane current of 1 amp/m² and assume transport mainly of univalent ions so that $q = 1.6 \times 10^{-19}$ coulomb, then for a cell 5 mm long and 0.5 mm in diameter we would expect a current PSD of approximately 2.5×10^{-24} amp²/Hz, which is much smaller than current PSDs measured in the cells (Fig. 2c). It is apparent, therefore, that thermal noise and shot noise make relatively insignificant contributions to the spectral density measured in cells of *C. corallina*. The spectral density is dominated by larger scale noise generating processes.

The low frequency spectral component was the only feature which was reliably and universally associated with power spectra measured under 'standard' conditions of physiological pH in the light in the authors' experiments. This LFSC is not unprecedented, however. As was noted above, it has been found in a number of characean species; Segal (20, 21) found the same component, with the corner frequency at approximately 0.25 Hz in frog abdominal skin and showed that it was associated with active transport of sodium. Ferrier *et al.* (7) also found the LFSC in a line of osteoblast-like cells cloned from rat bone. It may be that the LFSC is produced generally by one or more active transport processes and will be found in many types of cells as more work is done in the field.

There is good evidence that primary active transport of protons occurs in characean algae (22) and it seems reasonable to postulate that the LFSC is being produced by this transport process. This identification is further supported by the fact that the LFSC was removed by transition from external pH 7.5 to pH 11. This corresponds to a transfer from conditions under which electrogenic proton transport is expected to be maximal, to conditions under which electrogenic proton transport is known to be inhibited (2, 3).

It is unlikely that the LFSC is caused by open-closed transitions of channels, since these usually occur on a time scale much shorter than the 0.8 s time constant implied by a corner frequency of 0.2 Hz. Kolb and Laüger (12) showed that a carrier such as valinomycin under zero net current conditions produces a spectrum which is very different from the LFSC. That spectrum is constant at low frequencies, then rises to a higher constant level at high frequencies. Perhaps there is another type of regulation of populations of channels, or of carriers carrying a net current, which operates approximately on a 1-s time scale.

The lack of effect on the PSD of pH changes over the range pH 5.2 to pH 9 is interesting in that it indicates that the low frequency spectral component is not caused by active transport of bicarbonate ions. Below pH 6 dissolved carbon is almost entirely in the CO_2 form while at pH 8 it is almost entirely in the bicarbonate form. It seems unlikely that if the LFSC were produced by HCO_3^- transport it would be unaffected by the absence of the transported species at pH 5.2. Thus it seems likely that if bicarbonate transport is occurring it is either nonelectrogenic, or very small in magnitude relative to proton transport. It is not necessary to postulate direct transport of the bicarbonate ion to explain carbon uptake in *C. corallina* (6, 26) and the result described here lends some indirect support to the hypothesis that local acidification from proton extrusion results in production of CO_2 which diffuses into the cell (6, 26), although it certainly does not prove it.

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