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Low-noise amplification of voltage and current fluctuations arising in epithelia

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Two low-noise differential input amplifiers designed for voltage and current fluctuation measurements in epithelia are described. The first one uses a matched pair of low-noise transistors and is particularly suited for low-frequency current and voltage noise measurements in frog skin and other preparations with impedances below 1 kΩ. The second one is designed around a matched pair of JFETs and can also be used for higher source impedance. Performance is demonstrated with Na^+ current power density spectra obtained from frog skin with the transistor-input stage.

I. INTRODUCTION

The statistical analysis of random current or voltage fluctuations, which arise in biological membranes, has become a powerful tool for the investigation of ion transport mechanisms. Current fluctuations recorded under voltage clamp conditions are directly proportional to the conductance fluctuations of the membrane and yield information about the number of channels, the single channel conductance, and the mean lifetime of channel states. In this case a constant voltage can be applied across the membrane to study the voltage dependence of these parameters. The interpretation of the voltage noise recorded under current clamp conditions is somewhat more difficult because it requires knowledge of the impedance of the membrane in the frequency range used. In both cases suitable low-noise amplifiers must be employed. The design of these amplifiers must aim to minimize current and voltage fluctuations arising in the input stages and will depend (a) on the membrane and electrode impedances used, and (b) on the frequency range to be investigated.

In epithelia current and voltage fluctuation measurements[1-3] have shown that the relaxation times of the membrane processes involved are rather long (0.02–2 s). The spectral analysis in the corresponding frequency range (0.01–100 Hz) thus requires special properties of the amplifiers used. Very few data on the noise characteristics of amplifiers, transistors, and JFETs in this frequency range are found in the literature. For work with epithelial preparations of low impedance we developed a four-electrode voltage clamp circuit suitable for recording low-frequency current noise. A special problem was posed by the differential input stage of the feedback circuit. The commercially available differential amplifiers were found to have too much input noise to be used for this purpose. For this application we had to design the input stages with improved low-frequency-noise performance, which are described below.

II. NOISE SOURCES IN A FOUR-ELECTRODE VOLTAGE CLAMP CIRCUIT

Voltage clamps are feedback circuits which vary the current flow through biological membranes such that the membrane voltage remains close to the desired value. The recorded signal is the feedback current (transmembrane current). The circuit used by us for this

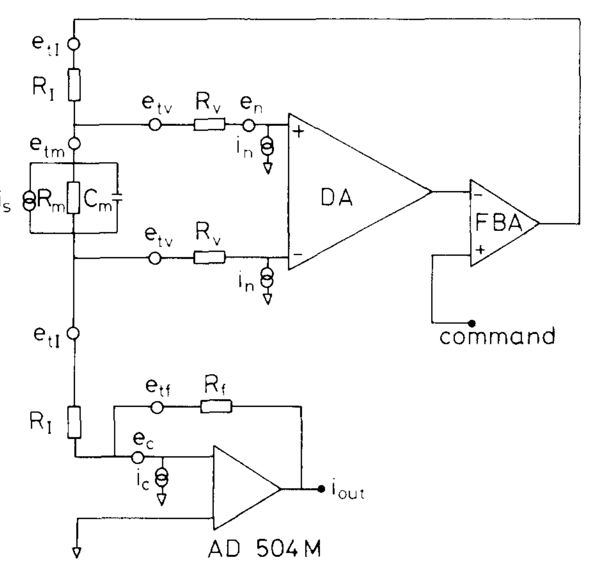

FIG. 1. Basic layout of a four electrode voltage clamp circuit. The equivalent input noise sources of the differential amplifier (DA) are represented by i_n and e_n. The membrane is represented by an equivalent *RC* network (R_m and C_m) of resting potential V_0 in parallel to the current noise source to be investigated (i_S). The resistances of the potential and current electrodes are R_V and R_I, respectively. Their thermal noise voltages are e_{tV} and e_{tI}. Current is recorded with the operational amplifier (AD504M) used as a current to voltage converter. Thermal noise of the feedback resistor (R_f) is e_{tf}, while e_c and i_c designate the intrinsic voltage and current noise at the input stage of this amplifier.

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purpose is shown in Fig. 1. The membrane under investigation is represented by a membrane resistance (R_m) and a capacitance (C_m) in parallel to a high-impedance current noise source. The excess noise $i_S(t)$, which depends on the membrane voltage and ion activities, is to be measured. It is usually contaminated with the instrumental noise described below.

Instrumental noise in the output of the current amplifier originates (a) from the input stage of the current amplifier itself, (b) from thermal noise in current and voltage electrodes, and (c) from the input stage of the differential voltage amplifier (DA). In our application noise source (c) is of major importance. The input transistors of the DA cause the output voltage of this amplifier to fluctuate. These fluctuations are clamped to zero by the FBA and result in a current noise signal through the current amplifier.

As shown in Fig. 1, the noise generation of the DA can be represented by one equivalent voltage noise source $[e_n(t)]$ in series with one of the inputs, and one equivalent current noise source $[i_n(t)]$ in parallel to each input of the differential amplifier.[4] The thermal voltage noise source of the voltage and current electrodes (resistance R_V and R_I) are represented by $e_{tV}(t)$ and $e_{tI}(t)$, the thermal noise voltage of R_m by $e_{tm}(t)$. The resulting equivalent input noise voltage $e_T(t)$ seen by the differential amplifier under open loop conditions can be computed as a mean square value $E_T^2 = \overline{e_T^2}$, which is the sum of the mean square values of the individual sources if these sources are uncorrelated.[4] Throughout this paper mean square values refer to a bandwidth of 1 Hz. In this case we obtain

$$E_T^2 = \overline{e_{tm}^2} + 2\overline{e_{tV}^2} + \overline{e_n^2} + \overline{i_n^2}(R_{s1}^2 + R_{s2}^2), \qquad (1)$$

where

$$R_{s1} = R_V + R_I + Z_m,$$

$$R_{s2} = R_V + R_I,$$

and

$$Z_m = R_m/(1 + R_m^2 C_m^2 \omega^2)^{1/2}.$$

In Eq. (1) the first two terms can be computed from

$$\overline{e_{tm}^2} = 4kTR_m/(1 + R_m^2 C_m^2 \omega^2), \quad \overline{e_{tV}^2} = 4kTR_V,$$

while $\overline{e_n^2}$ must be determined experimentally by using zero source resistances (R_S), and $\overline{i_n^2}$ by using large source resistances at the input of the DA.

When the feedback loop is closed with zero command voltage, the sum of the equivalent noise voltage $e_T(t)$ and the fluctuating membrane voltage is clamped to zero by current flow through Z_m. The resulting instrumental noise current which originates in the voltage electrodes and the DA-input stage, as seen by the input of the current amplifier, has the mean square value

$$\overline{i_N^2} = \frac{E_T^2 - \overline{e_{tm}^2}}{Z_m^2} = \frac{2\overline{e_{tV}^2} + \overline{e_n^2} + \overline{i_n^2}[(R_V + R_I + Z_m)^2 + (R_V + R_I)^2]}{Z_m^2}. \qquad (2)$$

Consequently, the current noise resulting from E_T depends strongly on the membrane impedance. Due to the capacitance of the membrane preparation the impedance becomes smaller at higher frequencies, resulting in a larger current noise signal. In the numerator of Eq. (2) the contribution of the current noise (i_n) of the DA to $\overline{i_N^2}$ consists of two terms. Both depend on electrode resistances but only one on the membrane impedance. It is clear, therefore, that the i_n-dependent terms will drop to the limiting value $\overline{i_n^2}$ when the electrode resistances are made very small with respect to Z_m. In most practical cases $\overline{i_n^2}$ is negligible so that the signal-to-noise ratio, defined as $S/N = \overline{i_S^2/i_N^2}$, will be independent of the current noise of the input stage. In this case the choice of the input amplifier must only be done on the basis of the voltage noise characteristics, such that JFETs as well as bipolar transistors, which have more current noise but less voltage noise, can be used for the input stage.

In a frog skin preparation of 3 cm^2 the lower limit of the dc membrane resistance is of the order of 500 Ω. The impedance of the voltage and current electrodes is of the order of 1 kΩ. For a commercially available low-noise amplifier such as the AD606L (Analog Devices) $e_n = 18$ nV/Hz$^{1/2}$ and $i_n = 2$ pA/Hz$^{1/2}$ at 10 Hz. From these data we calculate $i_N = 39$ pA/Hz$^{1/2}$. Noise measurements in frog skin have shown (Sec. 5), that the current fluctuations in this preparation are of the same order of magnitude at the above mentioned frequency. In order to be able to investigate these current fluctuations, an input stage for the DA with a lower voltage noise has to be designed. However, the signal-to-noise ratio can be improved by using smaller areas of the membrane preparation. Due to the fact that Z_m is inversely proportional to the membrane area (A) the contribution of the voltage noise dependent terms $\overline{e_{tV}^2}$ and $\overline{e_n^2}$ in Eq. (2) is proportional to A^2. As discussed before, the influence of the i_n-dependent terms will become negligible when Z_m increases in consequence of the reduction of A to a sufficient small value. R_I and R_V are supposed to be constant. Consequently, in the range of small membrane areas i_N^2 will become proportional to A^2. The investigated excess noise $(\overline{i_S^2})$ is linearly related to A^5 so that the signal-to-noise ratio becomes inversely proportional to A. However, in order to reduce edge damage of our preparation we used relatively large membrane areas.[6]

Noise introduced by the current amplifier itself was extensively discussed by Poussart.[7] Depending on the impedance of the membrane preparation the proper operational amplifier and the optimal feedback resistance (R_f) have to be selected. For low-impedance preparations like frog skin the contribution of i_c is small so that the amplifier with the smallest e_c is to be chosen and to be used with the largest allowable feedback resistance R_f. The maximum value of R_f is determined by the direct current through the amplifier which will bring the amplifier into saturation.

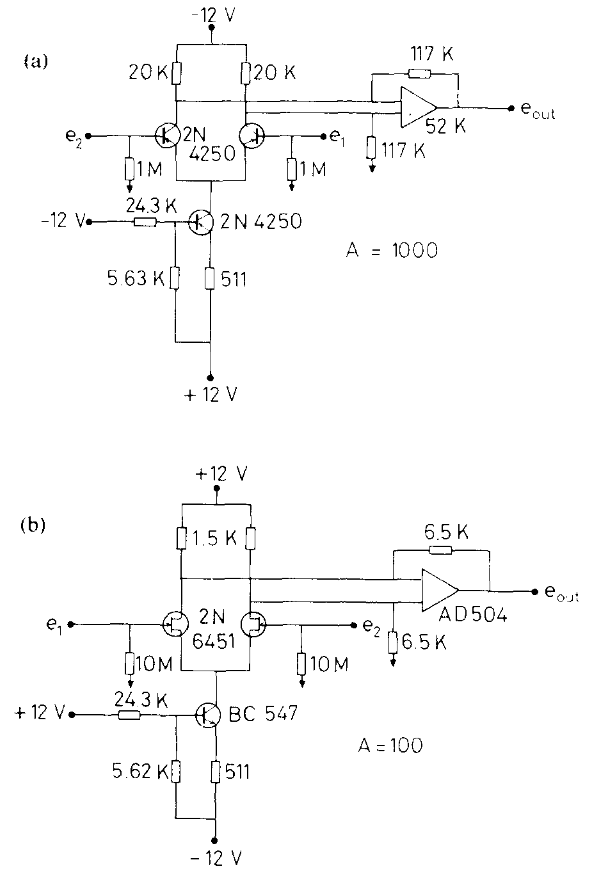

FIG. 2. (a) Differential amplifier with bipolar transistor input stage (TA). (b) Differential amplifier with JFET input stage (FA).

III. BIPOLAR TRANSISTOR AND JFET DIFFERENTIAL AMPLIFIER

The transistor amplifier input stage (TA) shown in Fig. 2(A) was built around two matched low-noise transistors 2N4250 (National Semiconductors), the properties of which are extensively discussed in Ref. 4. A first selection of the transistors was done by comparing their noise characteristics at 10 Hz. A Quan-Tech Laboratories transistor noise analyser was used for this purpose. The collector current for which voltage noise was minimal (2 nV/Hz$^{1/2}$) was found to be about 350 μA. Under these circumstances current noise was about 3 pA/Hz$^{1/2}$. These results are in agreement with data discussed in Ref. 4. Two transistors with almost equal current amplification factors were selected. 20-kΩ collector resistances were chosen in order to reduce the collector voltage to a value smaller than the common mode voltage of the amplifier in the second stage. The emitter resistance was omitted in order to obtain the best noise characteristics, although this limited the range of linear amplification to $\pm$50 mV. This range is sufficient in applications where the input voltage is clamped to zero by the feedback circuit. A third low-noise transistor (2N4250) was employed to supply the constant current of 700 μA. The gain of the first stage was 170. Metal film resistors were used throughout. As amplifier in the second stage we used a low-noise FET input operational amplifier (Analog Devices 52 K). The amplification of this stage, determined by the ratio of feedback to collector resistance, was set at 5.88. The small signal frequency response of the whole amplifier is flat from dc to 50 kHz, with a constant roll-off of 6 dB/oct. The dc gain is 1000, which limits the use of this amplifier to input signals of less than 10 mV in current clamp applications.

The JFET amplifier (FA) [Fig. 2(b)] was designed around a matched pair of n-channel JFETs (Texas Instruments, 2N6451). The voltage noise of the JFETs was checked with the Quan-Tech Laboratories transistor noise analyser and was found to be smaller than 5 nV/Hz$^{1/2}$ at 10 Hz, which is very close to the voltage noise of low-noise bipolar transistors. Current noise was less than 1.0 fA. Optimal noise performances were obtained for a drain-source current close to the I_{DSS}, which was about 5 mA. A matched pair of JFETs was selected from a series of 10 specimens. For a constant current source we used a low-noise npn transistor (BC547). The gain of the JFET stage was set to 23 and that of the operational amplifier (AD504M) used as differential amplifier was set to 4.35 to obtain an overall gain of 100. The linear range of the input stage is $\pm$300 mV within 1%. As a consequence of the overall gain of 100 the input signal has to be limited to $\pm$100 mV. Both input amplifiers (and all other amplifiers shown in Fig. 1) are powered by $\pm$15-V batteries. To minimize thermal drift, the input transistors were thermally coupled with a piece of copper and the complete input stage was encapsulated with Eccosil 2CN (Emerson & Cuming).

IV. VOLTAGE NOISE PERFORMANCE OF THE AMPLIFIERS

Voltage fluctuations arising in the input stages of TA and FA were recorded under open circuit conditions while both inputs were connected to ground with equal metal film resistors (R_S). Results were compared with those of the commercially available PAR 113 amplifiers (Princeton Applied Research Corporation). The output signal of the amplifier under investigation was amplified further with a low-noise ac amplifier, to which it was coupled with a simple *RC* network consisting of a polystyrene capacitor of 150 μF and a metal film resistor of 200 kΩ. The first stage of this amplifier consisted of an operational amplifier (52 K, Analog Devices) used as a follower with a gain of 10. Two additional low-noise operational amplifiers (AD504M, Analog Devices) were used to attain an overall amplification of 1000. Observations of the noise signal for relatively long periods (1 h) showed that the output signals of both input amplifiers were "popcorn"-free.

A Fourier analyzer system (HP5451A) was used to sample the data in blocks of 4096 words and to calculate the power density spectra. Two spectra of 2048 frequency lines each were combined to cover the frequency range from 0.01 to 1000 Hz. To prevent "aliasing" the signal was filtered with a 72 dB/oct low-pass Butter-

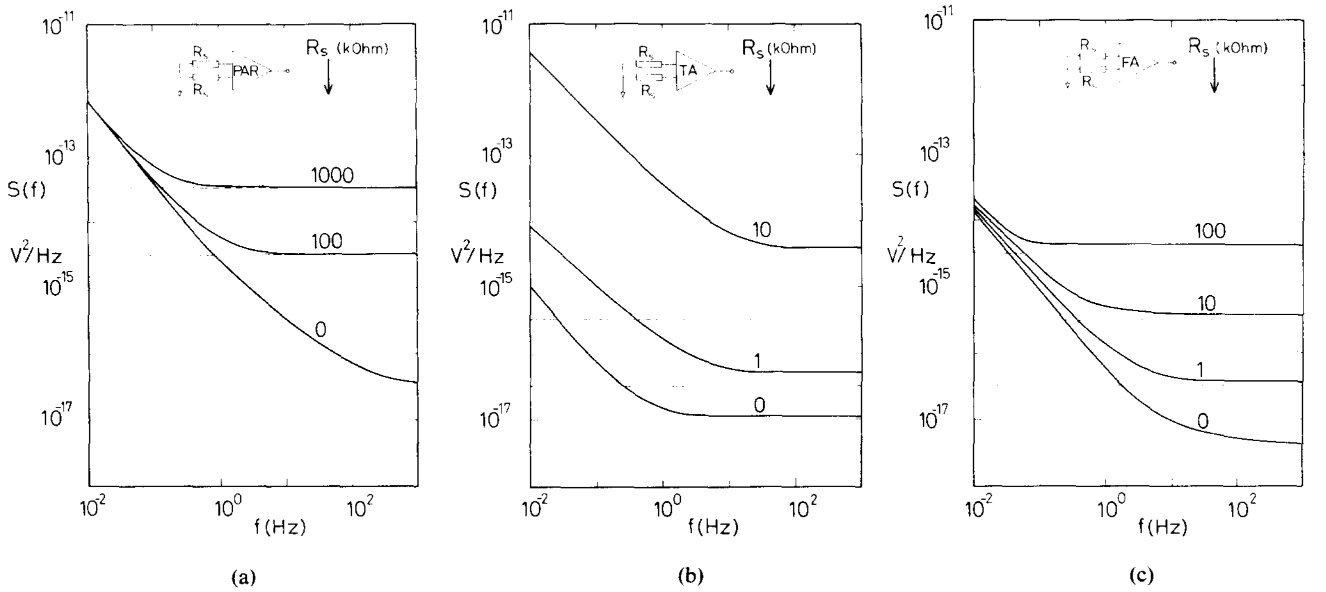

FIG. 3. Power density spectra of voltage noise arising in (a) the commercially available PAR 113 amplifier, (b) the transistor amplifier, and (c) the JFET amplifier. Both inputs of the amplifiers were connected to ground with a source resistance R_s as drawn in the top of the figure. Thermal noise levels were calculated for $2R_s$ and are indicated by the dashed lines. For all three amplifiers, the power density has approximately a $1/f$-dependence in the lower frequency range, which reflects the frequency dependence of e_n.[4]

worth filter. The cutoff frequency was set at 80% of the folding frequency. The final spectra represent an average of 40 data blocks and were recorded in log–log scale with an $X-Y$ plotter. Calibration of power density was checked by analysing with the same procedure the Gaussian output of a pseudo-random noise generator (Hewlett Packard 3772A).

Results of the voltage noise measurements obtained with the three amplifiers are shown in Fig. 3. It can be seen that the power density spectra at low frequencies are of the $1/f$-type. With negligible source resistance ($R_s = O$), e_n will determine the amplifier noise [see Eq. (1)]. In this case we find $\overline{e_n^2}$ to be significantly smaller for the transistor amplifier (TA) below 10 Hz than for the PAR and FET amplifier. For example, at at 0.01 Hz, $\overline{e_n^2}$ is about 10^{-15} V^2/Hz for the TA, 1.3 $\times$ 10^{-14} V^2/Hz for the FA, and 7 $\times$ 10^{-13} V^2/Hz for the PAR amplifier. In the frequency range above 10 Hz the difference becomes smaller. While at 1 kHz, $\overline{e_n^2}$ of the FA is found to be even smaller than that of the TA, $\overline{e_n^2}$ of the PAR amplifier is still five times larger than $\overline{e_n^2}$ of the TA. Thus the low-frequency $1/f$ voltage noise of the TA is considerably smaller than that of the FA and the PAR amplifier, while at higher frequencies the lowest voltage noise is generated by the FA.

According to Eq. (1) the spectra recorded with large source resistances (R_s) permit us to estimate the contribution of the input current noise (i_n). Figure 3 show that in the higher frequency range the recorded noise power for the FA and the PAR amplifier is at $R_s > 0$ only slightly larger than the calculated thermal noise for $2R_s$. In the lower frequency range the power density shows the $1/f$ dependence of $\overline{e_n^2}$. This indicates that the contribution of i_n of these amplifiers can be neglected in the range of the source resistances investigated. In contrast, in the TA the contribution of the current noise is considerable. Here the recorded noise levels clearly exceed the calculated thermal noise in the higher frequency range and $\overline{e_n^2}$ in the lower frequency range.

We conclude that for low-frequency voltage noise measurements with low-impedance preparations the transistor amplifier is to be preferred, while for source resistances larger than 1 kΩ, or for measurements in the frequency range above 10 Hz, the FET amplifier is better suited than the transistor amplifier. It is noteworthy that at source resistances above 1 kΩ the low-frequency noise of the FA is much smaller than that of the PAR 113, while the high-frequency noise is only slightly larger.

V. CURRENT NOISE RECORDED UNDER VOLTAGE CLAMP CONDITIONS

For this application the TA and FA were used as input stages in the complete voltage clamp circuit depicted in Fig. 1. The biological membrane was replaced by a metal film resistor (R_m). The resistances replacing the voltage electrodes were of 1 kΩ and those replacing the current electrodes of 500 Ω. Current noise was recorded with the current amplifier (AD504M, Fig. 1) and amplified further with the ac amplifier described in Sec. IV. Spectra were recorded for R_m values ranging from 332 Ω to 3.3 and 10 Ω. Figure 4 shows that below 1 Hz the power density drops toward higher frequencies by about one decade per decade of frequency, reflecting the frequency dependence of e_n. The current noise power increases with decreasing membrane resistance, as predicted by Eq. (2).

The influence of the current noise of the transistor input stage becomes clear by comparing the spectral density curves for TA and FA. For the smallest mem-

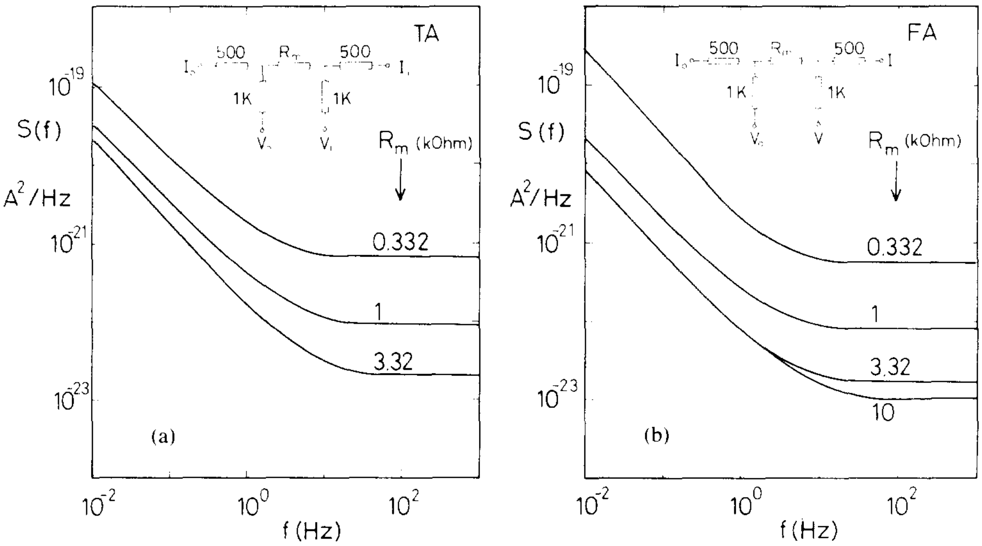

FIG. 4. Power density spectra of current fluctuations arising in the voltage clamp circuit. Input stages were (a) the transistor amplifier and (b) the JFET amplifier. Membrane and electrodes were replaced by a metal film resistor network drawn at the top of the figure. Noise spectra were recorded for different membrane impedances (R_m).

brane resistance (R_m = 332 Ω) the recorded spectral density of the TA is smaller in the lower frequency range and slightly larger in the higher frequency range than those of the FA. This observation is in accordance with the measurements of the voltage noise spectra (Fig. 3) for R_s = 0 kΩ discussed in the preceeding section. If the membrane resistance is increased to R_m = 3.32 kΩ the recorded spectral densities for the TA become larger than those for the FA over the entire frequency range. This makes both amplifiers equally well suited for an input stage of a voltage clamp circuit with relatively low electrode resistances and used to clamp preparations with a low resistance. However, Eq. (2) predicts that for larger electrode resistances the FA, which has a smaller i_n, will give better performance than the TA. Another advantage of the FA is that thanks to its larger range of linear amplification it permits clamping of the membrane potential to voltages of up to ±100 mV.

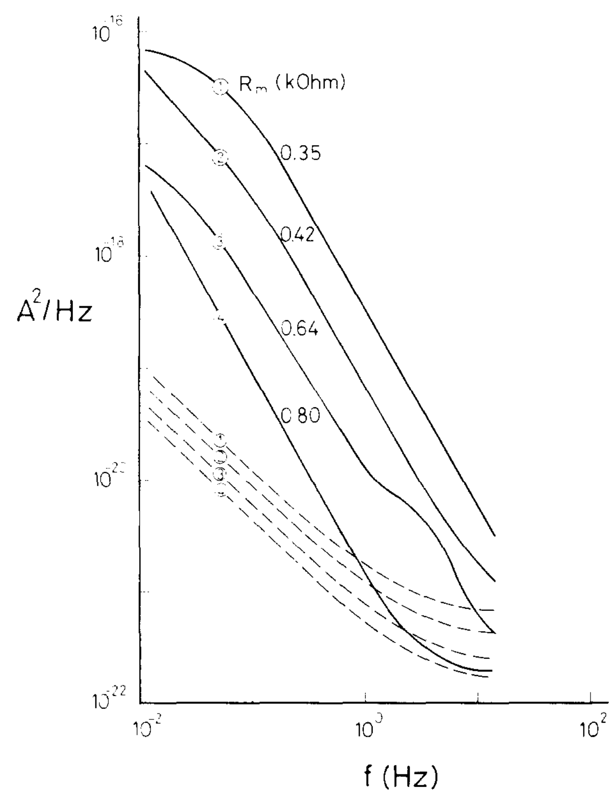

FIG. 5. Power density spectra of current noise in frog skin recorded with different sodium activities in the outer bathing solution: $(Na)_0$ = 60, 30, 12, 0mM. The dashed lines represent the amplifier noise (compare Fig. 4), recorded with R_m values equal to the dc resistance of the skin which was observed with the different $(Na)_0$ values.

VI. APPLICATION: SODIUM CURRENT FLUCTUATIONS IN FROG SKIN

Measurements of current fluctuations in frog skin were done using the FA for the input stage. Isolated abdominal skin of *Rana esculenta* was used at room temperature. It was mounted in a Lucite chamber which left 3 cm² exposed to the bathing solutions. The inner solution was K-sulfate Ringers, which can be expected to depolarize the inward facing membranes of the epithelium and to increase their conductance. Thus transepithelial resistance and potential were largely determined by the apical membrane of the *Stratum granulosum*. The outer bathing solution, too, contained as anions merely the impermeant sulfate. The spectra, shown in Fig. 5, were recorded with different sodium activities in the outer solution [$(Na)_0$ = 60, 30, 12, and 0mM), sodium being replaced by potassium. The amplifier noise obtained with R_m values equal to the dc resistance of the skin at different Na activities is drawn in the same figure with dashed lines. It can be seen that if Na is present in the outer solution the power densities obtained from the epithelium are well above those arising from the voltage clamp itself.

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