Inconsistent Sodium Current Records Derived on Ranvier Nodes with a Commercially Available Potential Clamp Device According to Nonner

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Abstract. We tried to reproduce some basic implications of the Hodgkin-Huxley-Frankenhaeuser formalism by measuring sodium currents in single myelinated nerve fibres with a commercially available version of the potential clamp device according to Nonner. The following contradictory observations were made: 1. The potential dependence of the time to peak sodium currents showed a discontinuity around the sodium equilibrium potential. 2. Defining the sodium permeability P_{Na} by the constant field equation and fitting the peak P_{Na}-voltage relation by a sigmoid function we obtained unbelievable high values of P_{Na} at rest. 3. Testing P_{Na} as calculated by the constant field equation by so-called "sodium tail current" experiments we obtained instantaneous changes of P_{Na}. Summing up, neither the kinetics of sodium currents nor the constant field concept as tested with the equipment used seem to agree satisfactorily with the standard data of sodium currents in Ranvier nodes.

Key words: Ranvier node — Potential clamp — Standard data — Consistency of results — Equipment reliability

Introduction

The first matured method of measuring and controlling the membrane potential of single Ranvier nodes (Frankenhaeuser 1957; Dodge and Frankenhaeuser 1958) was a landmark in modern nerve physiology. It led to the well-known and generally accepted Hodgkin-Huxley-Frankenhaeuser (HHF)-formalism in cluding the so-called standard data of the nodal membrane (see, e. g., Frankenhaeuser and Huxley 1964). The method involves two intermeshed feedback loops and was considered by many experimenters as difficult both to rebuild and to manage. Therefore, the one-loop potential clamp system of Nonner (1969) seemed easier to handle and received worldwide appreciation.

The original HHF-formalism has been extended based on experimental results obtained using the Nonner device (see, e.g., Neumcke et al. 1976). However, one should expect that crucial implications of the formalism are confirmed by this technique. Thus, results should be unquestionably independent of the measuring system used or at least one of the two competing technologies for potential clamping Ranvier nodes would suffer from misleading errors in measurement.

The aim of the present work was to find out whether some basic implications of the Hodgkin-Huxley-Frankenhaeuser formalism could be confirmed by experiments performed with a commercially available version of the potential clamp device according to Nonner.

Materials and Methods

Preparation and measuring device. Experiments were carried out on large (22–28 μm) single myelinated nerve fibres dissected from the sciatic nerve of the toad (*Xenopus laevis*). The dissection procedure employed strictly followed the description given by Stämpfli and Hille (1976) and yielded axons characterized by easily detectable Ranvier nodes. No specific efforts were directed to preserve the reserve length of the axon under investigation (Koppenhöfer et al. 1987).

Ionic currents were measured by means of a commercially available version of the potential clamp system according to Nonner (1969) designed for *Xenopus* fibres. Membrane current records were filtered through a switchable low-pass, fourth order Bessel filter (−3 dB at 40 and 80 kHz, respectively) taking care for the corner frequency being as high as possible and corrected for leakage current assuming a potential independent leakage conductance (Dodge and Frankenhaeuser 1958) as measured by appropriate negative test pulses. No compensation for the influence of the nodal series resistance was employed and no correction for the low-pass filter properties of the current measuring internode (Schumann et al. 1983) was made.

Nomenclature and calibration. Sodium currents were calculated from the output voltage of the clamp amplifier by means of a scaling factor of 22.5 Ωcm^2 given by Dodge and Frankenhaeuser (1959) for the neighbouring nodes being cut. It was assumed that the amplitude of pulses applied to the node under investigation equalled changes of membrane potential, V, given relative to normal resting potential, E_R. Following most contemporary experimenters, the sodium inactivation variable h (Frankenhaeuser 1959) was set at about 0.8 assuming $E_R = -70$ mV. As a rule, positive test pulses were preceded by negative prepulses of $V_{pp} = -40$ mV and 50 ms in duration, thus at the beginning of test pulses h was unity.

Statistics. To our knowledge no detailed analysis of the probability distribution of membrane current data in Ranvier nodes have been published so far. Moreover, "un physicien éminent me disait un jour à propos de la loi des erreurs: «Tout le mond y croit fermement parce que les mathématiciens s'imaginent que c'est fait d'observation, et les observateurs que c'est un théorème de mathématiques.»" (Poincaré 1892). Relative small numbers of observations, e.g. $n < 60$, are characterized more precisely by medians and ranges of individual observations ($\tilde{x}$ and R) rather than by the mean and related parameters (Sachs 1984). Therefore, we decided to give in this paper and in comparable instances medians and ranges.

Solutions. The bathing medium for the node under investigation was Ringer solution containing (in mmol/1): NaCl 110.0, KCl 2.5, $CaCl_2$ 2.0, *tris*(hydroxymethyl)-aminomethane HCl-buffer 5.0. The adjacent internodes were immersed in an artificial intracellular fluid (in mmol/1): KCl 105.0, NaCl 13.0, "tris-buffer" 5.0. The pH of the solutions was set at 7.2 ± 0.1. The temperature was kept at constant values (± 0.5 °C) between 11 and 15 °C.

Results

Kinetics of sodium currents. Following the Hodgkin-Huxley-Frankenhaeuser formalism the kinetics of sodium currents in Ranvier nodes are described by the time constants of the activation term, τ_m, and of the inactivation term, τ_h, in the form

$$[1 - \exp(-t/\tau_m)]^a \cdot \exp(-t/\tau_h) \quad (1)$$

where a denotes the power of the activation term (Frankenhaeuser 1960). The potential dependence of both τ_m and τ_h has been demonstrated to yield continuous curves decaying with increasing positive test pulse amplitudes above threshold (see, e. g., Stämpfli and Hille 1976). Therefore, following Eq. (1) the time to peak sodium current, t_{peak}, should change with test pulse amplitude continuously as well. When we applied potential steps close to the sodium equilibrium potential it became evident that this was as a rule not the case. In the experiment illustrated on Figure 1 the time to peak sodium inward current first decreased with increasing pulse amplitude as indicated by the shift of the arrows to the left. However, as soon as the current clearly changed its direction ($V = 134$ mV) a sudden increase of t_{peak} became detectable which declined again with an even stronger pulse ($V = 143$ mV).

For a more detailed analysis of this unexpected finding we applied test pulses up to $V = 190$ mV and plotted t_{peak} versus test pulse amplitude. From corresponding peak sodium current-voltage relations we derived the median of the sodium equilibrium potential, $\tilde{V}_{Na}$, of 6 experiments ($\tilde{V}_{Na}$ and R: $133 \leq \leq 138 \text{ mV} \leq 152$). Individual $t_{peak} - V$ curves were lumped together by correcting their potential axis for the deviation of the respective V_{Na} from $\tilde{V}_{Na}$; the plot of all data (Fig. 2) thus applies for $\tilde{V}_{Na} = 138$ mV. For sodium inward currents ($V < \tilde{V}_{Na}$) t_{peak} decreased with increasing test pulse amplitude as expected from the standard data (Frankenhaeuser 1960). However, with test pulses just exceeding $\tilde{V}_{Na}$, again a sudden increase in t_{peak} becomes evident; it was detectable in each of the $t_{peak} - V$ curves.

Sodium permeability. According to the HHF-formalism for the sodium permeability P_{Na} as calculated from sodium currents I_{Na} holds

$$P_{Na} = \bar{P}_{Na} \cdot m^a \cdot h, \quad (2)$$

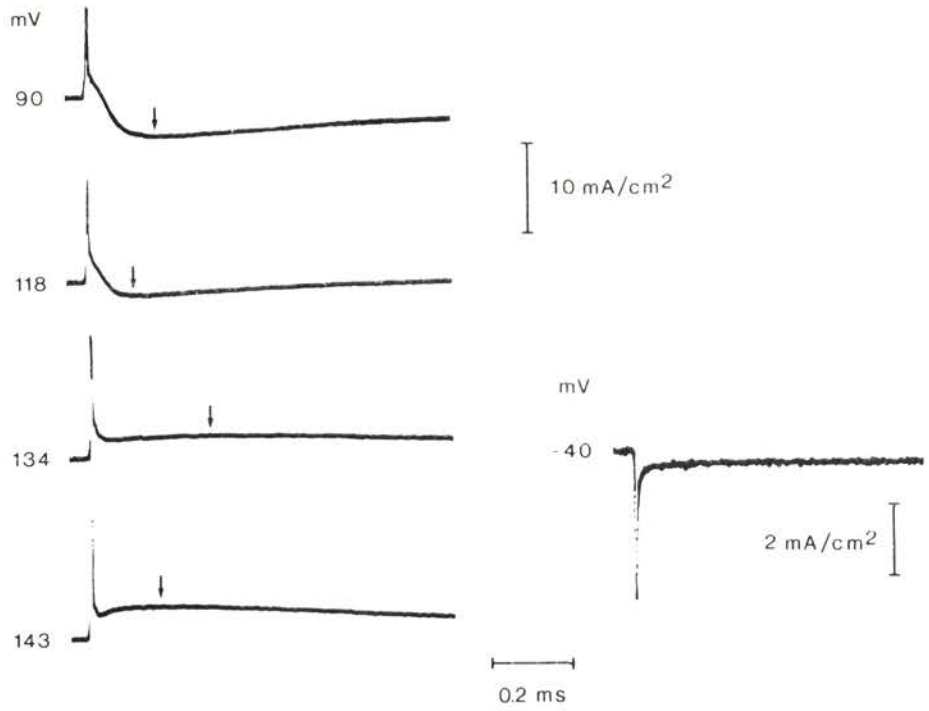

Fig. 1. Current records elicited by positive potential steps around the sodium equilibrium potential (*left*) and by a negative step of −40 mV (*right*). Peak sodium currents are indicated by arrows. The ends of the traces were cut.

where $\bar{P}_{Na}$ represents the maximum sodium permeability constant, and m and h are the potential and time depending activation and inactivation variables, respectively. Moreover, they both are unique functions of membrane potential (Hodgkin and Huxley 1952). Therefore, P_{Na} should be a unique function of membrane potential as well. Further, the definition chosen should account for the so-called "sodium rectification" seen in peak sodium current-voltage relations (see, e. g., Khodorov 1974) in a sense that the peak P_{Na}-V curve should be S-shaped. For this purpose the so-called constant field equation has been introduced as suitable definition of P_{Na} in Ranvier nodes (Dodge and Frankenhaeuser 1959):

$$P_{Na} = I_{Na} \cdot \frac{R \cdot T}{F^2 \cdot E \cdot [Na]_o} \cdot \frac{\exp(EF/RT) - 1}{\exp[(E - E_{Na}) \cdot F/RT] - 1} \tag{3}$$

(E: absolute membrane potential; E_{Na}: absolute sodium equilibrium potential; $[Na]_o$: external sodium concentration; R, T and F have their usual meaning).

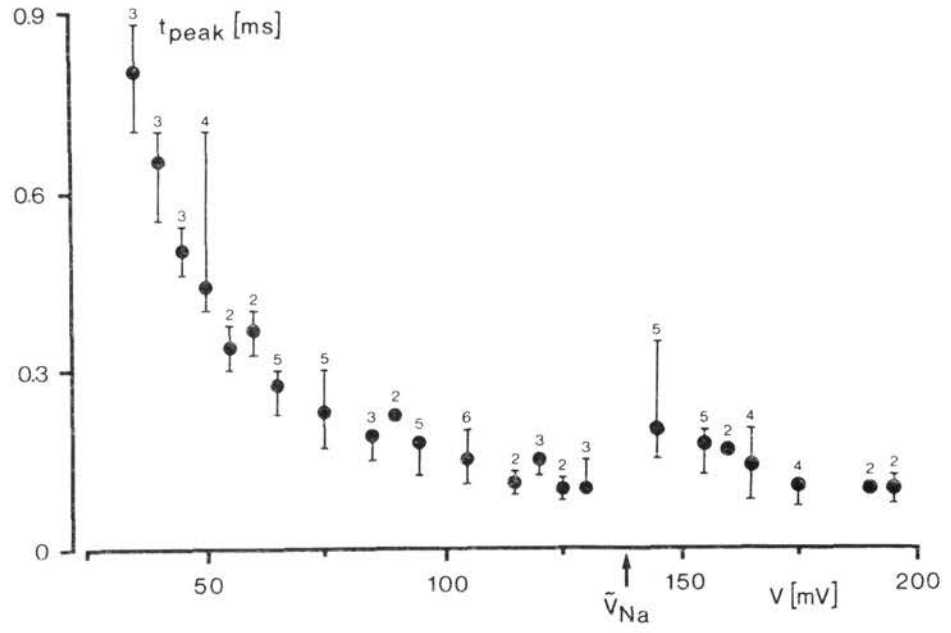

Fig. 2. Time to peak sodium currents, t_{peak}, as function of changes of membrane potential, V. Symbols, bars, and figures denote medians, ranges, and number of measured values respectively on six axons. Note that the potential axis was normalized to the median of the sodium equilibrium potential, $\tilde{V}_{Na} = 138$ mV.

We repeated the principal experiments of Dodge and Frankenhaeuser (1959) by applying test pulses up to $V = 220$ mV and calculated peak P_{Na} from peak sodium currents by Eq. (3). Assuming that a power of 2 of the activation variable m fits the experimental points (Frankenhaeuser 1960), a sigmoid function corresponding to the empirical equation describing the potential dependence of the inactivation variable h (Frankenhaeuser 1959)

$$\text{peak } P_{Na} = P_{Na\,max} \cdot \left[\frac{1}{1 + \exp[(V_h - V)/k_h]}\right]^2 \tag{4}$$

(Benoit and Dubois 1987) was fitted to the data and yielded numerical values for the maximum peak permeability, $P_{Na\,max}$, for the potential at which peak P_{Na} is half $P_{Na\,max}$, V_h, and for the steepness factor, k_h. Normalizing peak P_{Na} by $P_{Na\,max}$ yielded curves which were lumped together in the same fashion as in the experiments on t_{peak}, i. e. the potential axis of individual experiments was corrected to the median of the respective sodium equilibrium potentials measured ($\tilde{V}_{Na}$

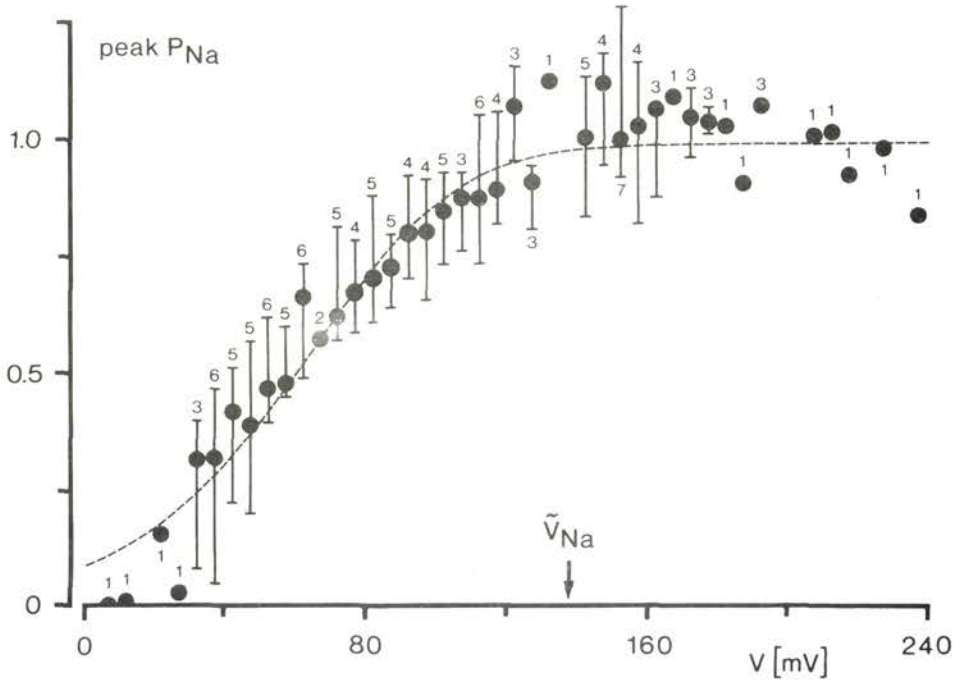

Fig. 3. Normalized peak sodium permeability, peak P_{Na}, as function of changes of membrane potential, V. Symbols, bars, and figures denote medians, ranges, and number of measured values respectively on six axons. Equation (4) was fitted to the symbols (dashed line). Note that the potential axis was normalized to the median of the sodium equilibrium potential, $\tilde{V}_{Na} = 138$ mV. For details see text.

and R: $125 \leq 138\,\text{mV} \leq 145$, $n = 6$). Subsequently, Eq. (4) was fitted to the medians of the normalized P_{Na} values (Fig. 3, dashed line). At the first glance, the calculated curve fits the data reasonably well although a tremendous scatter of the measured values should not be overlooked. At weak positive pulses, however, there are systematic discrepancies between experimental points and the curve.

Assuming the ratio of the time constants of sodium currents, τ_m/τ_h, to be largely potential independent (Frankenhaeuser 1960) and neglecting the potential dependence of the delay of sodium activation (Neumcke et al. 1976), i. e. the potential dependence of the power of the activation term, a, the peak P_{Na}-curve delivered the underlying activation curve (Fig. 4, continuous curve). Extrapolation to $V = 0$ yielded a puzzling figure, $m_{V=0} = 0.3$, whereas following the HHF-formalism, m at rest ($V = 0$) should be almost zero (Frankenhaeuser and Huxley 1964: $m_{V=0}$: 0.0005) as indicated by the dotted curve in Franken-

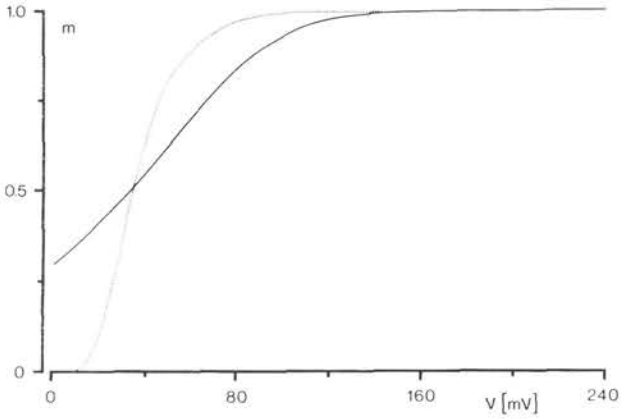

Fig. 4. Activation curves. Continuous line: calculated from the dashed line in Figure 3. Dotted line: activation curve from Frankenhaeuser (1960), redrawn.

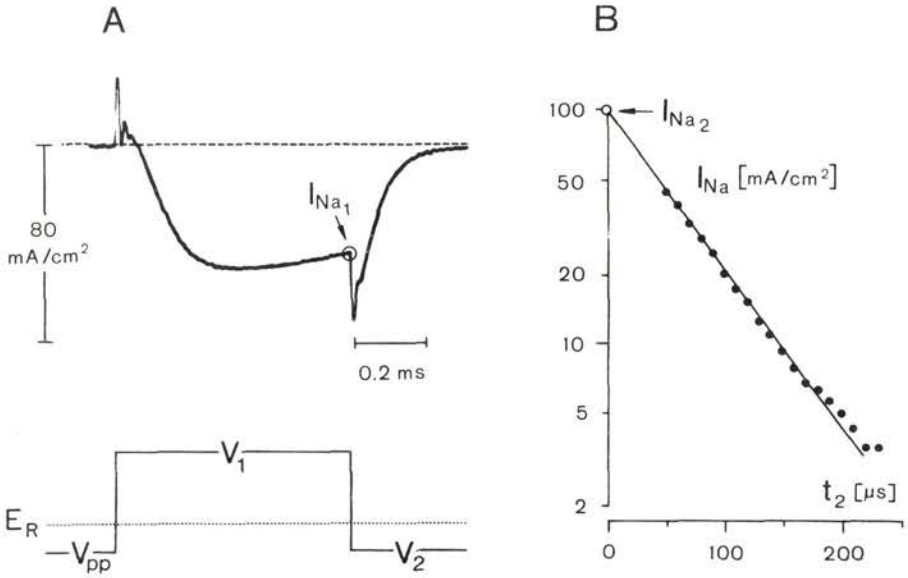

Fig. 5. Membrane currents elicited with a test pulse of $V_1 = 60$ mV interrupted close to peak inward current by repolarization to $V_2 = -40$ mV. *A*: Current record. Note break of V_1 at I_{Na1} and subsequent repolarization current, the so-called sodium tail current. Dashed line: zero current. The time axis of the pulse program (below) drawn to scale. V_{pp}: end of prepulse. *B*: Semilogarithmic plot of sodium tail current. Extrapolation to the beginning of repolarization ($t_2 = 0$) yielded instantaneous sodium current, I_{Na2}.

haeuser (1960). Both curves mainly differ in steepness ($k_h = 27.7$ mV and 12.7 mV, respectively) although the potentials at which $m = 0.5$, V_h, are the same ($V_h = 32.0$ mV). Note that the inconsistency is obviously not due to the type of averaging chosen because each of the peak P_{Na} – V curves shown in Figure 3 yields the same results at rest ($\tilde{m}_{V=0}$ and R: $0.24 \leq 0.31 \leq 0.36$, $n = 6$).

If the constant field concept were actually an appropriate definition of the sodium permeability in Ranvier nodes P_{Na} should change at potential steps with finite speed only. This was tested by so-called "sodium tail current"-experiments (Fig. 5). Positive test pulses, $V_1 = 60$ mV, were terminated by repolarizing the membrane around peak sodium inward current, I_{Na1}, to $V_2 = -40$ mV. The sudden increase in driving force for sodium ions at the beginning of V_2 caused a sudden increase of sodium current which after the capacitive surge decayed largely exponentially (Fig. 5*B*) as predicted by the Hodgkin-Huxley-Frankenhaeuser formalism. Linear extrapolation of the "tail currents" in a semilogarithmical plot to the beginning of repolarization ($t_2 = 0$) yielded the instantaneous current, I_{Na2}. From I_{Na1} and I_{Na2} we calculated the corresponding permeabilities, P_{Na1} and P_{Na2}, by Eq. (3) using values of E_{Na} as measured on the respective axon. Surprisingly, the ratio P_{Na2}/P_{Na1} was not unity as it should be the case if Eq. (3) would properly account for the discontinuity of sodium currents: in five experiments the median and R amounted to $0.26 \leq 0.49 \leq 0.64$.

Discussion

Kinetics of sodium currents. We have shown that systematic deviations of the time to peak sodium currents from the predictions of the Hodgkin-Huxley -Frankenhaeuser formalism are evidently a peculiarity of the potential clamp device used in our experiments (see, e. g., Neumcke et al. 1987, Table 2). This is supported by the fact that in nine corresponding experiments performed with a setup very similar to the original version of the Frankenhaeuser potential clamp (Dodge and Frankenhaeuser 1958) by one of us (E. K.) no such deviations could be detected. Note, however, that this latter device was definitely not free of systematic errors in measurement (Schumann 1980; Kneip 1987). Nevertheless, our puzzling results might have been due to any specific imperfections of the device tested. Note in this connexion that a deficit in phase reserve of the feedback system under test could be ruled out as indicated by the smooth time course of capacity currents elicited by pulses of either direction (see Fig. 1).

Sodium permeability. In our experiments peak P_{Na} as defined by the constant field equation was in fact a unique function of membrane potential. However, the increase in peak P_{Na} with increasing test pulse amplitude was characterized

by an unduly high scatter. This could be understood if parameters governing peak P_{Na} were not controlled by the measuring system with sufficient accuracy.

Trusting in the formalism chosen for the potential dependence of peak P_{Na} (Eq. (4)) the main problem arises from our observation that at $V = 0$ the activation term is apparently not zero, i. e. that P_{Na} is already turned on at rest although Frankenhaeuser's experiments "did not give any indications in this direction" (Frankenhaeuser 1960). This cannot be explained by inherent uncertainties in assuming $E_R = -70\,mV$ throughout because the sodium equilibrium potential, $\tilde{V}_{Na}$, we measured was not very far from the corresponding figure given by Dodge and Frankenhaeuser (1959).

Our observation of instantaneous changes of P_{Na} as defined by Eq. (3) in "sodium tail current"-experiments are supported by evaluation of corresponding records shown by Nonner (1969) and by Nonner and Stämpfli (1969). Therefore, this kind of experiments casts some doubt on the reliability of the equipment we used. Unfortunately, to our knowledge no detailed data on instantaneous values of P_{Na} in "sodium tail current"-experiments have been published so far.

Equipment reliability. Both the intricated kinetic pattern of sodium current records seen around V_{Na} and inconsistencies between peak $P_{Na} - V$ curves and instantaneous P_{Na} should be seen in the light of numerous sources of errors in measurement of the tested equipment: 1. The so-called air gap reduces the accuracy of membrane current measurements as a whole by slowing down the resolution in time (Sommer et al. 1982). 2. Current load of the potential measuring internode distorts any membrane current records (Steinmetz 1979; Steinmetz et al. 1980). 3. Low-pass filter properties of the current measuring internode clip systematically fast components of current records (Schumann et al. 1983). 4. The absence of compensated feedback prevents decent potential clamp conditions (Duchâteau and Koppenhöfer 1984; Koppenhöfer et al. 1984).

Therefore, we doubt that results derived by aid of this technology could in fact ruin the standard data of Ranvier nodes and the underlying formalism. In this connexion an extended version of the potential clamp device according to Nonner (Albers 1987) will be described and tested in a subsequent paper.

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