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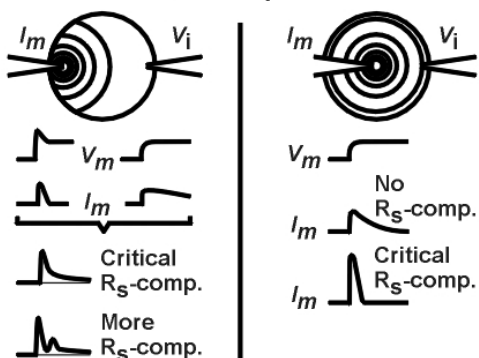
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Practical hints for fast two-electrode voltage-clamping of *Xenopus* oocytes

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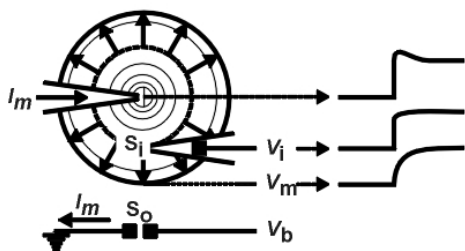
The *Xenopus* oocyte has become a widely used workbench to study heterologously expressed ion-channels of wild-type and mutated cRNA. For the rapidly gating sodium channels we have developed a fast two-electrode voltage-clamp system (TEVC) and used it successfully for the recording of fast gating currents as described^{1,2}. Some methodical aspects had been presented in a workshop and compared to other approaches³. Here, I detail on electrodes, series resistance and means to achieve and check a high speed clamp. We used a TEC-05 (npi-electronics) with current-source and adjusted R_s -compensation⁴.

Fig. 1



As shown in Fig 1, the penetration-depth of the current electrode is of great importance for homogenous charging of the membrane capacitance. At small depth (left panel) the current injection close to the membrane will charge this part of the oocyte more quickly than the other side and there, the voltage V_m which is controlled by the clamp is very different. This results in a capacitance transient with a typical slow tail (see left and right I_m and total recorded I_m). Conventional R_s -compensation (adding a fraction of the recorded current to the command voltage) will speed up the fast component mainly, and an over-critical compensation leads to a ringing around the slow tail. In contrast, central current injection (right panel) charges the membrane homogeneously and the one-component transient current can be critically compensated without leaving a slow tail due to slowly charged membrane compartments.

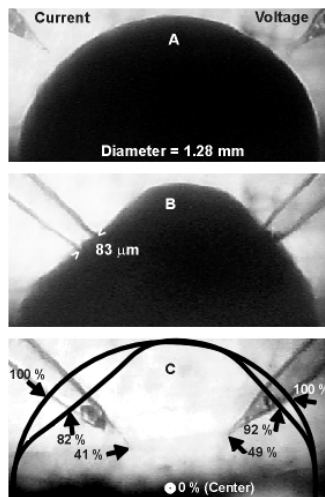
Fig. 2



Clamp speed is limited by the time-constant of R_s and C_m . C_m is typically around 250 nF for 1.3 mm oocytes. R_s depends on the position of the voltage sensing electrodes, i.e. on the resistance which has to be traversed by the current I_m causing a voltage error. In Fig. 2 the clamp would control the voltage at the two sensing points S_i and S_o and the actual voltage at the cell membrane V_m will be in error by $R_s \cdot I_m$. Calculations and measurements show that the bath has some negligible 50 Ω to 100 Ω , unless Agar-bridges (1-2 k Ω) would be brought into the current path between S_i and S_o . We, in fact, use simple Ag-AgCl wires directly in the bath for ground and V_b . The main remaining R_s is in the shell of cytoplasm given by S_i and the very

membrane. Depending on the position of S_i this part of R_s amounts to several 100 Ω up to 1 k Ω and was further reduced by compensation.

Fig. 3



To demonstrate the achieved electrode positions, a sequence of microphotographs shows side views of the tips coming 45° from above just before touching the oocyte (A) and at the final position (B). The reconstruction (C) gives the position relative to the center. To allow fast clamping, the tips were broken to about 5 to 10 μ m dia. and back-filled with agarose⁵ (ca. 1%) to prevent leakage of the 3-M KCl filling solution. The resulting 60 k Ω - 100 k Ω electrodes did not cause leak due to rupture probably due to a good adaptation of membrane to glass.

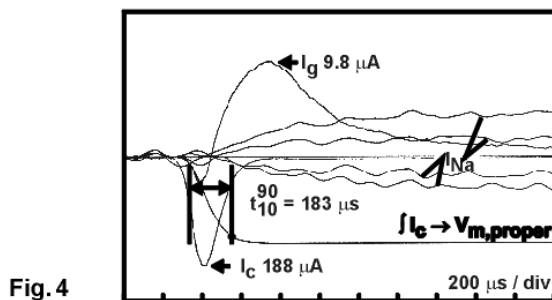


Fig. 4

The achieved performance allows charging of the membrane as fast as 60 μ s but then non-linearity in subtraction of the huge capacitance transients occurs which we currently reduce (Fig. 4, initial downward spike in I_m) by slowing the command voltage step with a filter. As seen, a good compromise is achieved for the recording of sodium channel gating- and ionic-current around the reversal potential. In conclusion, the most important signal to judge the clamp speed is the capacitance transient which should settle without slow tail or ringing; its integral gives the proper voltage time-course at the membrane.

References:

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