

Automated and real-time correction of series-resistance errors during membrane capacitance monitoring in the two-electrode voltage clamp mode using a novel hardware device

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We recently presented an improved approach to monitor membrane capacitance (C_m) in large cells such as *Xenopus laevis* oocytes using the two-electrode voltage-clamp (TEVC) technique. This “paired ramps” approach was suited to monitor C_m conveniently, in real-time, and with high temporal resolution, accuracy and precision (Biophys. J. 82:1345-57, 2002). Its performance was limited only by potential clamp errors due to high series resistance (R_s), a general problem in TEVC experiments. At that time, TEVC methods for fast and precise measurement of R_s were unavailable. We proposed, however, that development of a method for monitoring R_s in parallel with C_m should in principle allow to compute the true C_m even when R_s is high, because the observed C_m error strictly followed the theoretical prediction, approximated by $\epsilon = 2R_s/(R_s + R_m)$, where R_m denotes membrane resistance. Meanwhile, a novel hardware device for R_s monitoring in the TEVC mode exists (“ R_s box”, NPI electronic, Tamm, Germany). Using this R_s box, we now developed a strategy for the automated correction of R_s errors and implemented it in the hardware and software setting of our previously described C_m monitoring approach. In brief, this is achieved in a “two-stroke cycle”: First, the R_s box applies an R_s test pulse, samples and holds the R_s value, then the clamp amplifier (TEC-05, NPI electronic) follows with the C_m test pulse; pulse sequence is controlled by a commercial software (PULSE, Heka, Germany). After each “two-stroke cycle”, a second software (X-CHART, Heka) acquires “online analysis” data from PULSE (yielding raw C_m), and the stored R_s value from the R_s box, and uses both for real-time computation of the true C_m according to the indicated formula. Performance of this approach was tested in a calibrated electrical cell model with tunable R_s , R_m , and C_m . Varying the critical ratio $R_s/(R_s + R_m)$ over a wide range at a fixed potential of -50 mV and a C_m of 160 nF was associated with membrane currents between -5 nA and -10 μ A. Under these conditions, uncorrected C_m measurements varied between 115-160 nF, underestimating true C_m by up to 30%. After correction of the R_s error, C_m values accurately reflected the true value within 1 nF ($<1\%$). In spite of an increased cycle duration, a high C_m sampling rate of several Hz could be maintained. In *Xenopus* oocytes, R_s measurements were sensitive to clamp artifacts (non-linear electrode response, capacitive coupling, etc.) and required optimization of R_s stimulus parameters and clamp performance. Taken together, we have established a simple and efficient method to obtain valid C_m measurements even in the presence of high R_s . This method extends the application range of C_m monitoring using the “paired ramps” approach to oocytes with overexpressed and activated ion channels. In a wider context, simultaneous measurements of R_s and C_m may prove useful for elucidating in *Xenopus* oocytes the largely unexplored biological underpinnings of these two electrical parameters.