FIGURES AND TABLE

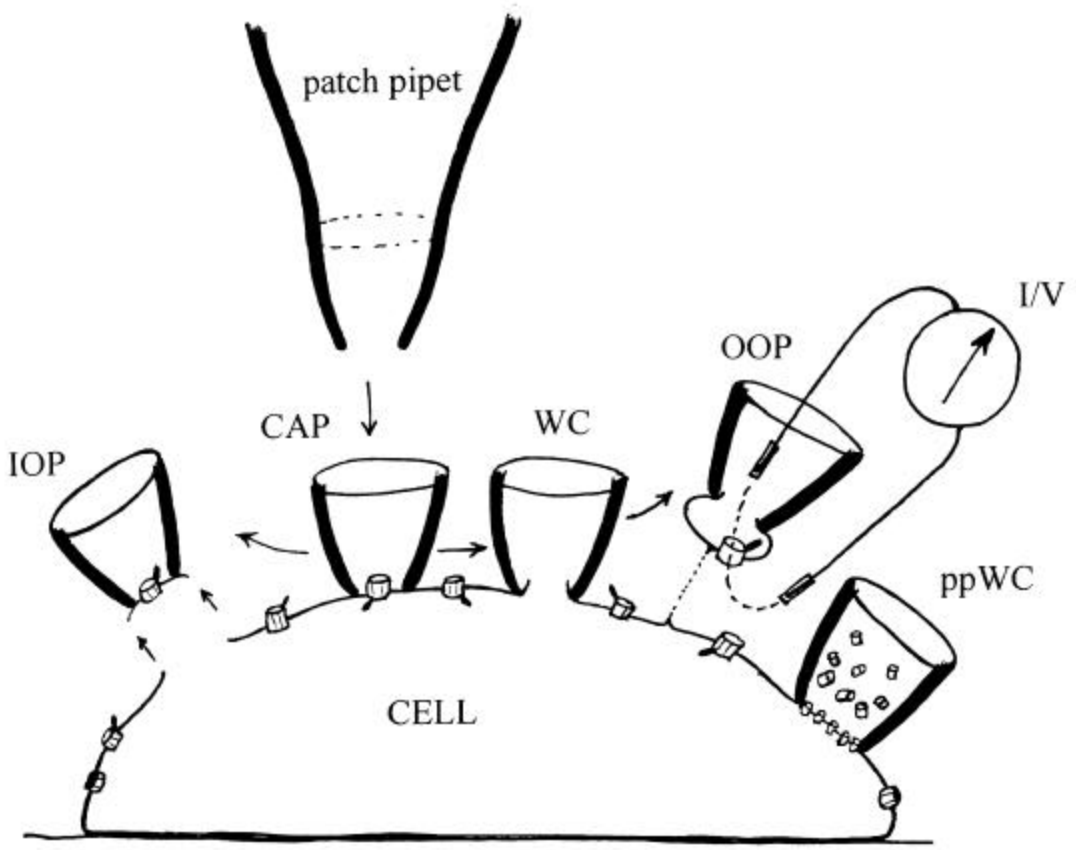

Figure 1.1. Diagram of the five patch-clamp measurement configurations

The figure depicts a living cell seen from the side immersed in extracellular solution and adhered to the substrate. The barrel-type pores in the membrane (some with movable lids or gates) represent ion channels. The five "cups" drawn in semi-perspective close to the cell are the tips of fluid-filled glass micro pipettes connecting the cell to the amplifier. The figure is a composite drawing, as if all five pipettes were placed on one cell. Although this is not a practical possibility, it is possible to make simultaneous two-electrode WC/CAP recordings (DeFelice, 1997) and CAP/CAP would not be out of the question. All five tips are in position to illustrate how the various measurement configurations are derived from the initial cell-attached-patch (CAP) configuration, established after the giga-sealing procedure. The inside-out patch (IOP) is a CAP excised from the cell membrane. The whole-cell (WC) configuration is obtained by rupturing the CAP. The outside-out patch (OOP) is a vesicle (a "tiny cell") pulled from the WC configuration. The permeabilized-patch WC (ppWC) develops from the CAP if the pipette solution contains pore-forming molecules incorporating in the CAP. The measuring patch-clamp amplifier and the connecting electrodes, one inside the pipette and one in the bathing solution, are drawn for the OOP-configuration; however, they apply to the other configurations as well. The amplifier measures current (I) through the membrane or voltage (V) across the membrane.

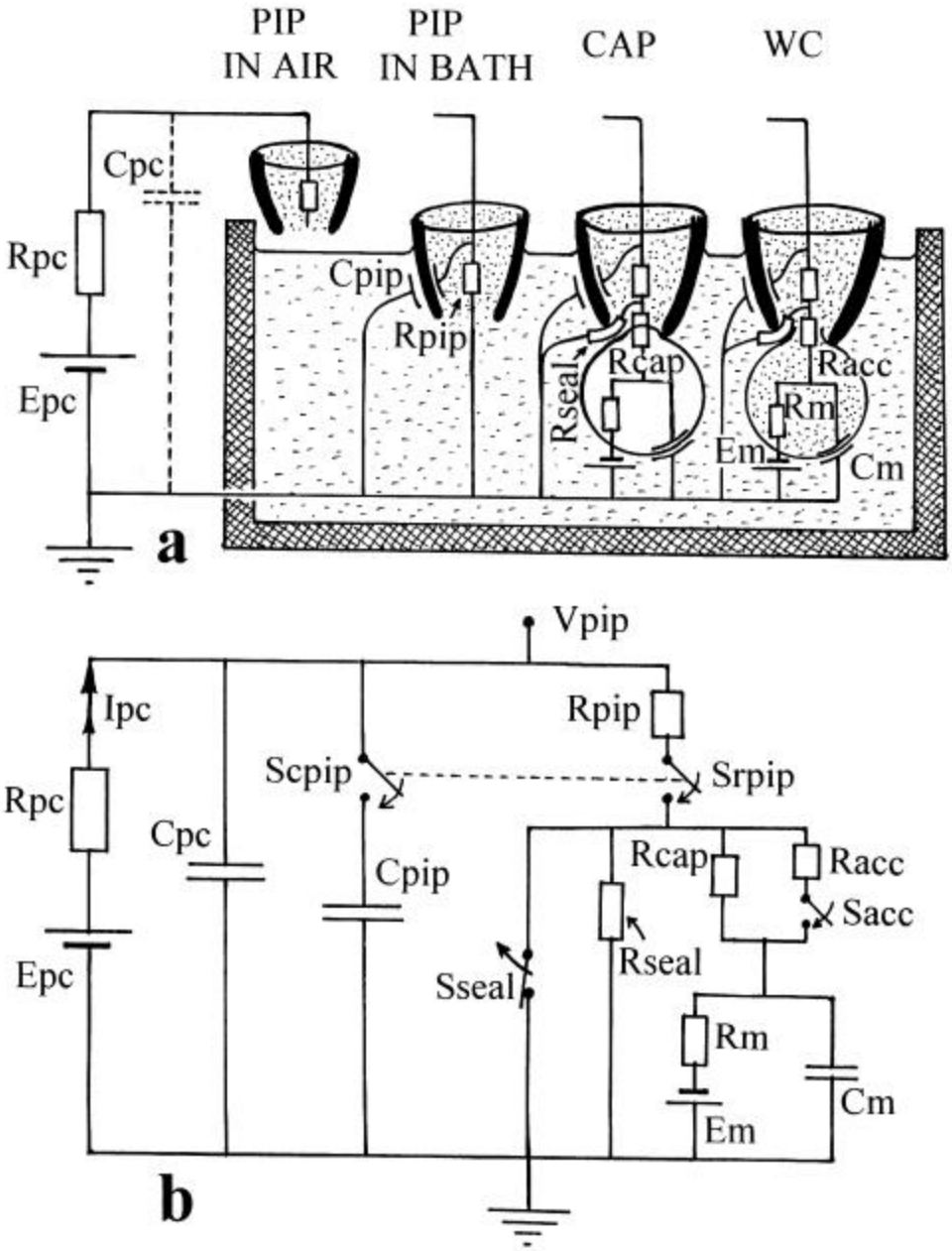

Figure 1.2. A simple electrical circuit modeling the successive patch-clamp procedures for obtaining the whole-cell configuration

A pipette (PIP) entering the bath, forming a giga-seal with the cell, and breaking the cell-attached patch. **Part a** shows how the various components of the circuit can be identified with components of the measurement configuration(s). **Part b** shows the circuit abstracted from the drawing in Part a, including the switches (S) for going through the three successive procedures leading to a WC configuration. The procedures and component names are further explained in the text. During the experiment the quality of the pipette, the giga-seal, and the whole-cell configuration are tested by applying voltage-clamp Epc steps to the pipette and measuring the resulting patch-clamp current Ipc.

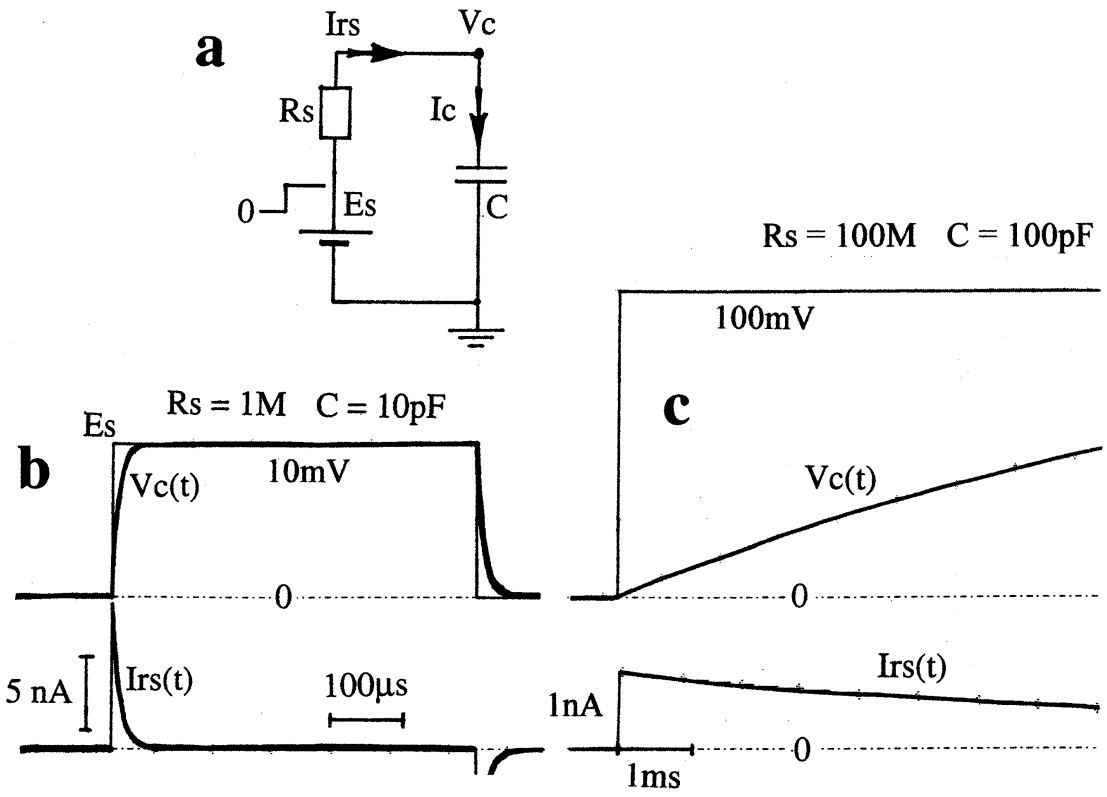

Figure 2.1. Charging a capacitor: ERC-circuit I.

Part **a** shows the ERC-circuit studied, **b** the voltage-clamp behavior upon an Es voltage step at a given (low) value of Rs and **c** the current-clamp behavior of the circuit upon a much higher Es voltage step at a much higher value of Rs (notice time calibration difference). The upper records of b and c show the Es voltage steps applie d (positive, from zero) and the exponential Vc responses. The lower records show the exponential current responses. Notice that the Vc and Irs transients change with the same time constants and that the current-clamp transients are much slower than the voltage-clamp transients. In both cases there is no stationary current after the transient, since Vc becomes exactly equal to Es. Es is the stimulating voltage, Irs is the current through the source resistance Rs, Vc is the voltage across the capacitor C, and Ic is the current flowing into C. The records in panels **b** and **c** have been calculated with the use of a computer model (PSI, see Note 1.3.1) of the circuit in **a** for the component values given in panels **b** and **c**.

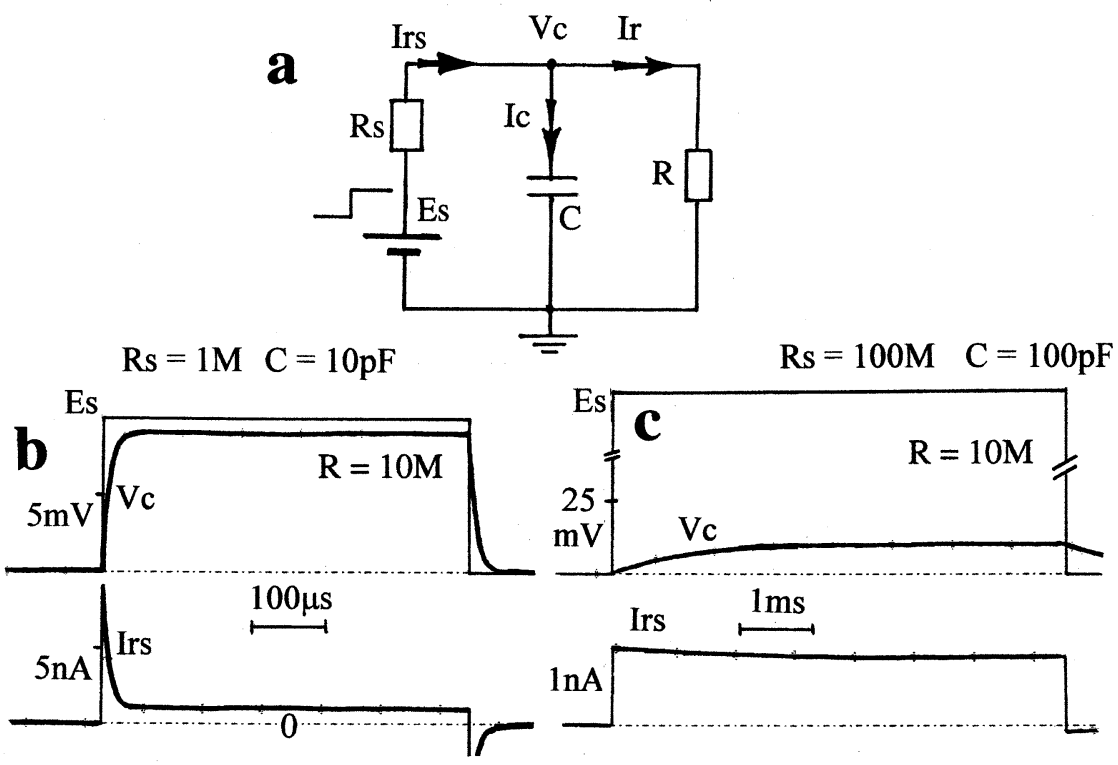

Figure 2.2. Charging a leaky capacitor: ERC-circuit II.

Circuit (**a**), record types (**b,c**) and symbols are as in Figure 2.1 except for an extra component in the circuit, resistance R in parallel to C. It is now the parallel RC-circuit that is voltage-clamped (Rs << R) (**b**) or current-clamped (Rs >> R) (**c**) by the Es/Rs source. The records have been calculated for the indicated component values. The Es step in c is 100 mV. The voltage-clamp records at the given resolution do not look very different from those in Fig. 2.1b, but the significant difference is that Vc never becomes exactly equal to Es and that Irs reaches a stationary value ~Es/R. It is the value after the capacitance transient that is important during a real patch-clamp experiment, because this value reflects the conductance of the membrane if R >> Rs. This implies that the capacitance peak current is much larger than the current after the peak transient. The current-clamp records look very different from those in Fig.2.1c. Irs reaches a stationary value Es/(Rs + R) ~ Es/Rs and Vc shows a stationary displacement ~IrsR. The time constant is now smaller than for ERC-circuit I: ~RC in stead of RsC.

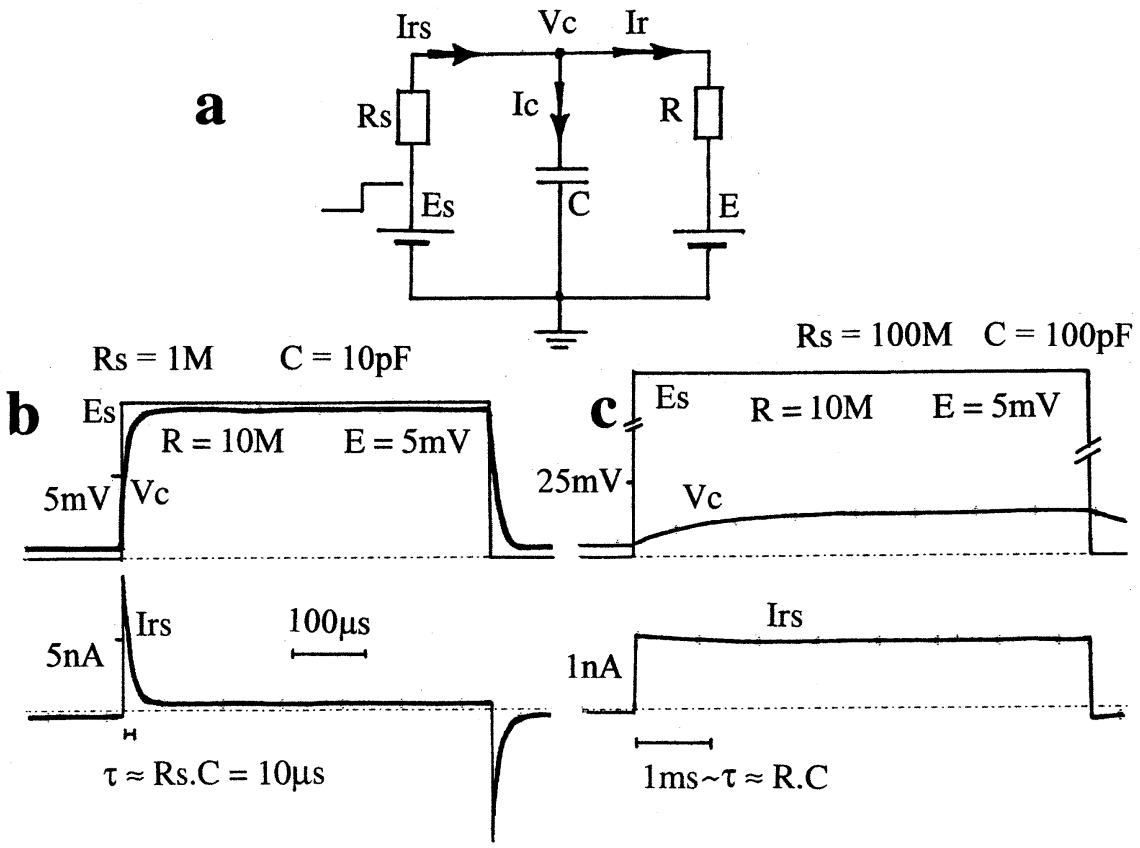

Figure 2.3. Clamping an ERC-model: ERC-circuit III

This circuit (**a**) contains, compared to ERC-circuit II, an extra voltage source E added in series with R. This makes the resulting ERC-circuit resembling a patch pipette with offset voltage or a cell membrane with membrane potential, voltage (**b**) or current (**c**) clamped by the Es/Rs source. The Es step in c is 100 mV. Note the difference in time scale between b and c. The records in b and c have been calculated for the indicated component values and are similar to those in Fig. 2.2b,c, except for the following differences: (1) E causes stationary (inward) current in b when Es = 0 and the stationary current upon the Es step is also different because of the presence of E; (2) the Vc record in c now starts from E (here chosen positive) in stead of from zero.

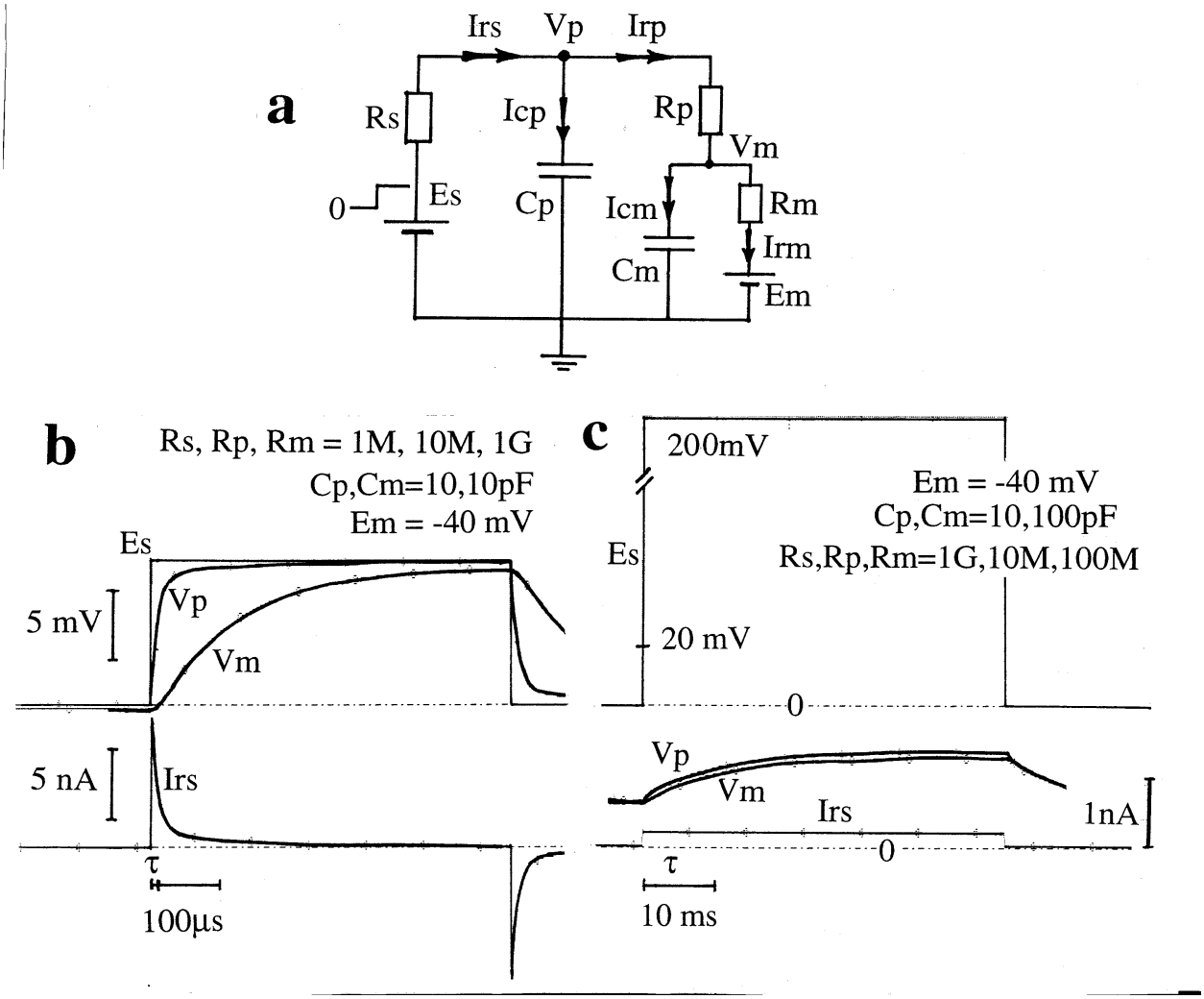

Figure 2.4. Clamping an ERC cell membrane through a patch pipette: ERC circuit IV

The circuit in **a** is Fig.2.3a for a whole-cell configuration, extended with components representing the properties of the measuring patch pipette. The pipet resistance Rp is the access resistance to the Em/Rm/Cm membrane circuit, and Cp shunts the input of the Es/Rs source. The presence of two capacitors coupled by a resistor makes the voltage-clamp (**b**) and current-clamp (**c**) responses biphasic (less clearly visible in current-clamp at the given resolution). Records calculated for the indicated component values.

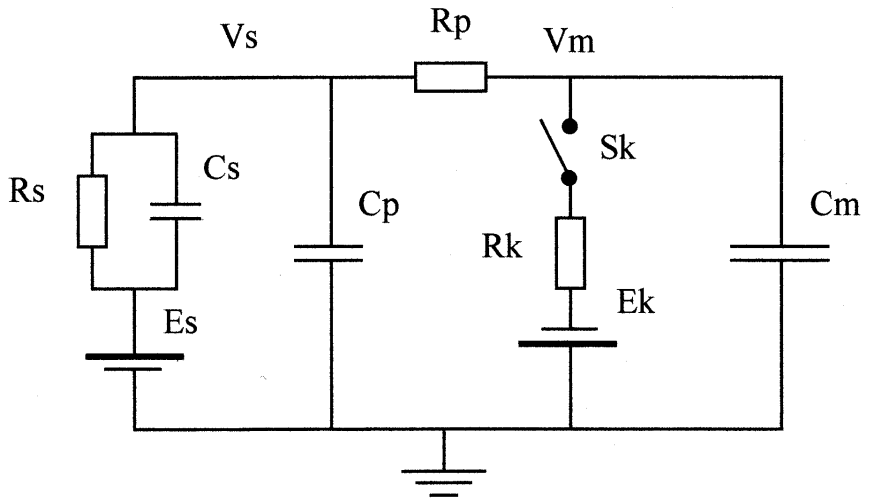

Figure 2.5. Electrical equivalent circuit (ERC circuit V) of a Whole-Cell (WC) measurement configuration to illustrate filtering of WC-current steps

The Es/Rs/Cs branch at the left is the voltage clamp. The Ek/Rk/Sk/Cm circuit at the right represents the whole cell. A current step was evoked by closing switch Sk of the Gk branch. Rp and Cp represent the pipet, connecting the WC with the voltage clamp. The only difference with Fig. 2.4a is the presence of Cs. In the example in the text, Vm is clamped at Es = 0mV, which makes Cp and Cs parallel capacities. Note that Rk >> Rp >> Rs and Cm >> Cp and Cs >> Cp. The first filter acting on Ik upon activation of Gk is the RpCm-filter. The second filter acting on Ik is the RsCs filter. If Rs Cs > Rp Cm, then the RsCs-filter removes a fraction of the high signal frequencies, which passed the RpCm-filter.

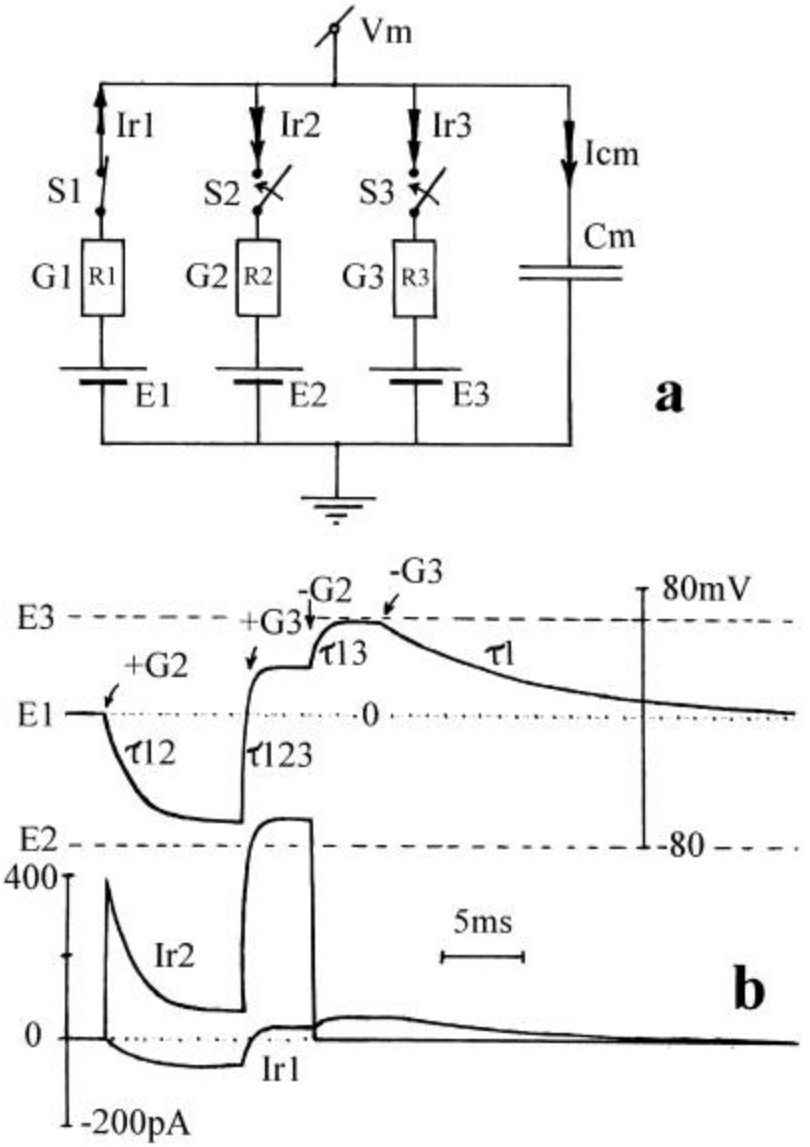

Figure 2.6. Membrane potential and current changes during conductor switching in an ERC-membrane model in current clamp: ERC-circuit VI

The circuit in **a** is a parallel conductance model of three conductances G1, G2 and G3, which can be switched on or off with the three switches S1, S2 and S3. Each of the three conductance branches contains a voltage source E (E1, E2, and E3, respectively). The membrane capacity Cm is parallel to the conductances. The membrane is under zero-current clamp conditions by assuming that the external current Irs = 0. Vm is the membrane potential, and Icm the capacitance current. I1, I2 and I3 are the currents through the conductance branches 1, 2 and 3. The upper record of part **b** shows Vm changes upon switching the conductors for the condition that E1 = 0, E2 is negative and E3 is positive (as indicated) and that G3 > G2 > G1. G1 is switched-on all the time. First G2 turns on, then G3 (first turning off G2). The various time constants are indicated. The currents I2 and I3 are monitored in the lower record. The records were calculated with a computer model (see Note 1.3.1) based on Eq. 53a.

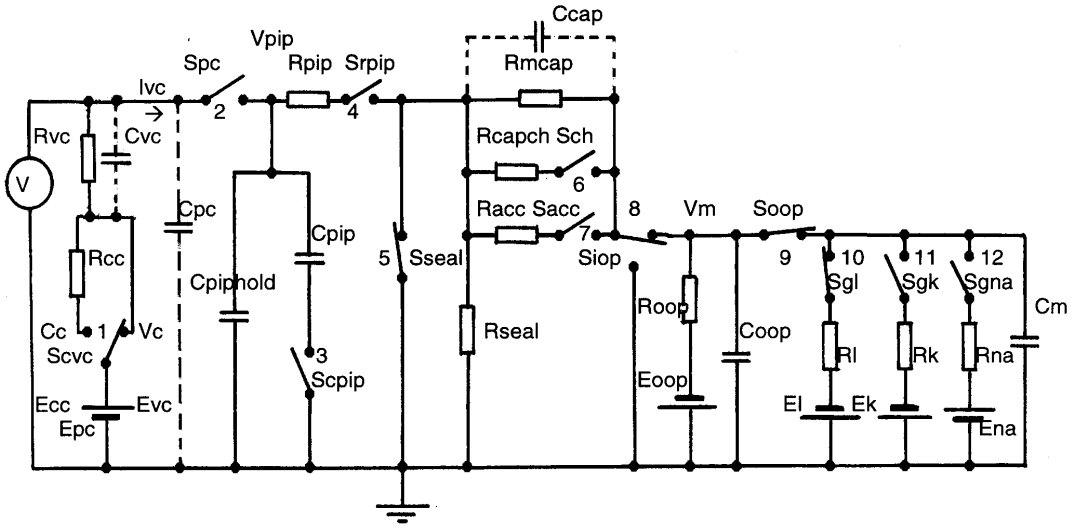

Figure 3.1. Diagram of the electrical equivalent circuit of the model used to exercise patch-clamp procedures and measurements

The dashed capacitors are stray capacitances, not added to the circuit as components. For further explanations, see text and Table 3.1.

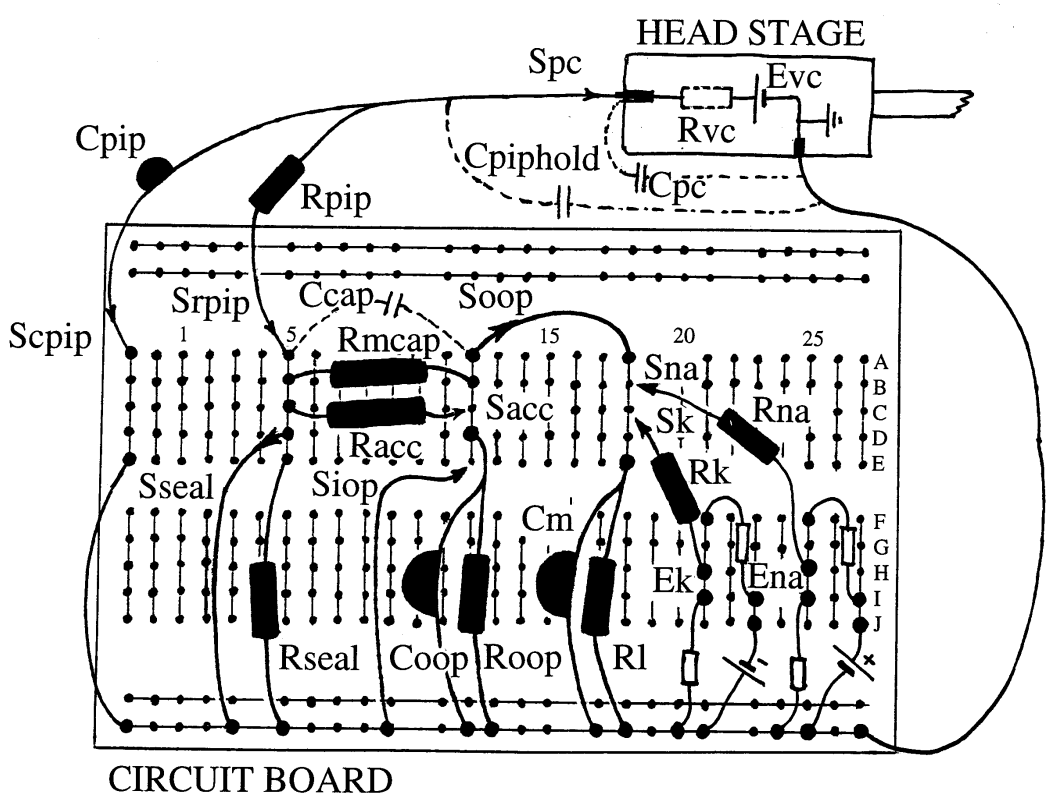

Figure 3.2. Example of equivalent circuit wiring on the breadboard (or circuit board)

The figure shows a 8x6cm breadboard seen from above. The dots are wire insertion holes for easily making electrical connections without soldering. The straight lines between the dots are the connecting wires between the insertion holes, under the surface of the board. These connections cannot be seen but can be checked with an Ohmmeter. By inserting the leads of the components (resistors, capacitors, batteries) in the holes, one can easily build circuits to study the electrical properties of the various patch-clamp configurations and to exercise the experimental procedures. Resistors (cylinders) and capacities (half-drops) added as components to obtain the equivalent circuit of Fig. 3.1 are black. The switches of the circuit diagram in Fig. 3.1 are just the leads with the arrow points, pointing to or from a connection hole. The enlarged dots show the established contacts. Arrows pointing to close-by holes indicate switch contacts to be made during the proposed series of model experiments. Arrows pointing away from holes indicate contacts to break during the experiments. By comparing this practical breadboard circuit with the circuit diagram in Fig. 3.1, one can identify the various components. In the present circuit wiring, the probe (pre-amplifier) of the patch-clamp amplifier has been connected to the pipette-holder with pipet (switch Spc on), the "pipet has entered the bathing solution around the cell" (Scpip and Srpip on) and the pipet is "touching the cell, ready for giga-sealing". Giga-sealing is established by opening the seal switch, Sseal. This results in the CAP measurement configuration. By connecting Racc to hole 12C (switch Sacc on), one can make a WC from the CAP. By opening Soop one obtains an OOP from the WC. In stead, connecting Siop under CAP conditions results in an IOP. With switches Sk and Sna one can introduce into the WC a Gk and a Gna, respectively. The Nernst potentials have been established with a simple voltage-divider circuit (the small white resistors). The leak conductance has been given a reversal potential El=0mV. The interrupted-line wires are non-existing wires, but indicate the presence of stray capacities (Cpc, Cpiphold, Ccap). Resistor Rcapch with switch Scapch (Fig. 3.1) is not shown on the circuit board.

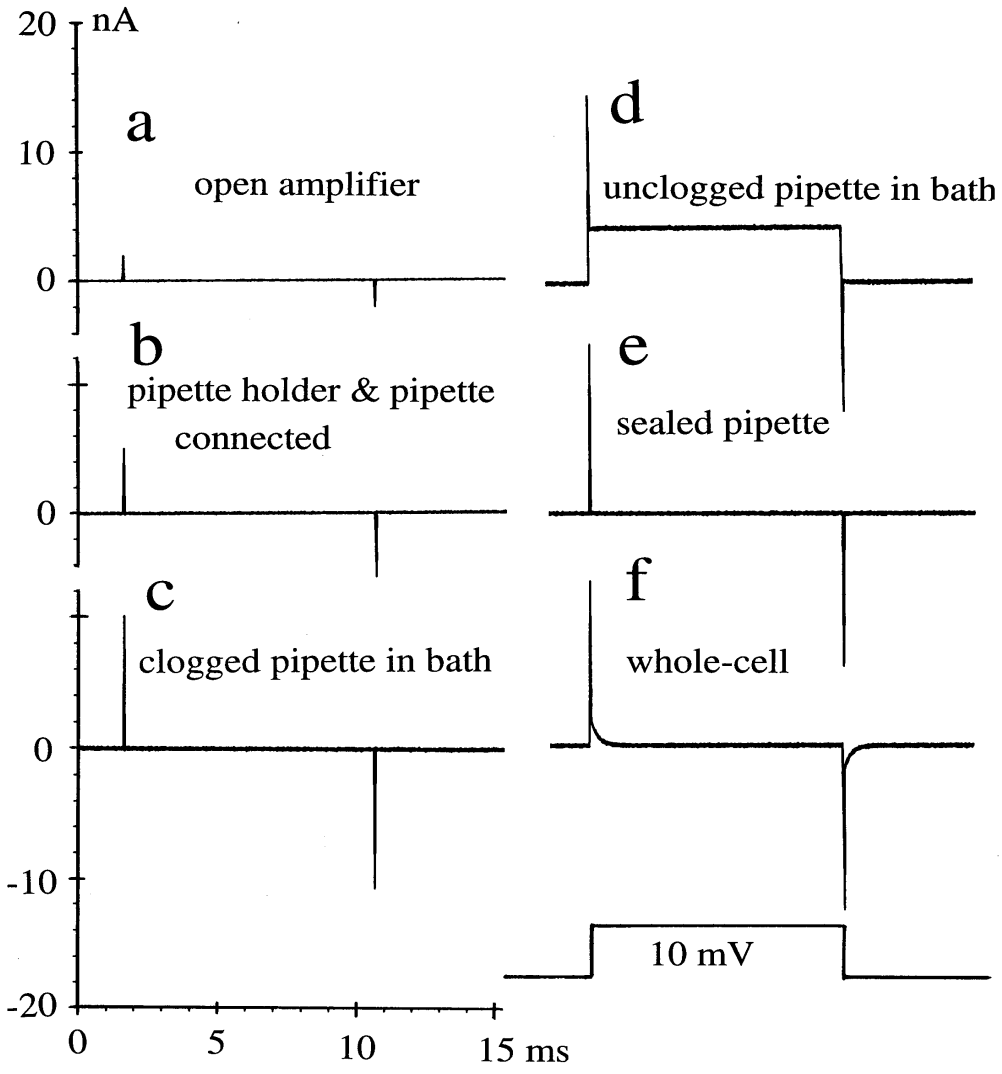

Figure 3.3. Voltage-clamp records obtained during patch-clamp procedures leading to the whole-cell (WC) configuration, all on one scale (lowest gain recording)

The various procedures, described in the text, were carried out while applying voltage-clamp steps of 10mV. The initial model settings were as illustrated in Fig.3.1. Record a applies to these initial settings. Record b is taken after closure of switch Spc, c after closing Scpip, d after closing Srpip, e after opening Sseal and f after closing Sacc. The model components were as in Table 3.1, with Racc=2.2M, El=Eoop=~-60mV. The Gk and Gna branches were not connected (switches 10-12 open). Amplifier settings: 80KHz filtering and no capacitive transient cancellation.

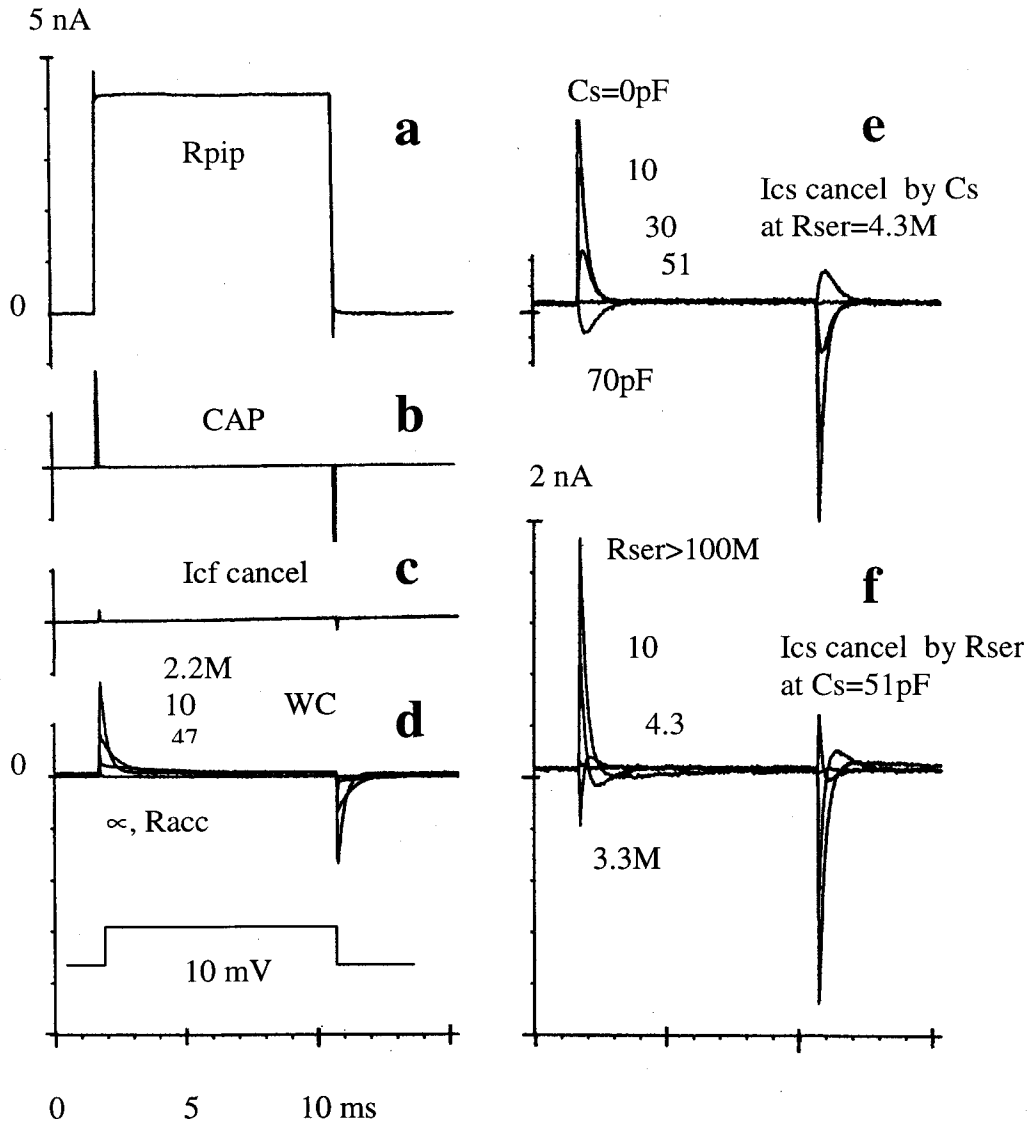

Figure 3.4. Experimental procedures for preparing a conventional whole-cell experiment, including fast capacity current (Icf) and slow capacity current (Ics) cancellation during 10mV voltage step application

Record **a** serves to calculate Rpip (here 2.2M), record **b** shows Icf after giga-sealing, thus in the cell-attached-patch (CAP) configuration. Record **c** shows Icf after almost complete cancelling. The records in frame **d** show the sudden appearance of Ics upon establishing a WC from the CAP. The better the acces to the WC (the lower Racc), the higher and faster Ics (examples Racc= , 47, 10 and 2.2M). Frames **e** and **f** illustrate the changes in Ics during Ics cancellation, in frame e for Cs adjustments (0-70pF) at the right Rser value (4.3M) and in frame f for Rser adjustments (>100M-3.3M) at the right Cs value (51pF). Low-pass frequency filter setting is at 10KHz. Other general conditions are as in Fig. 3.3.

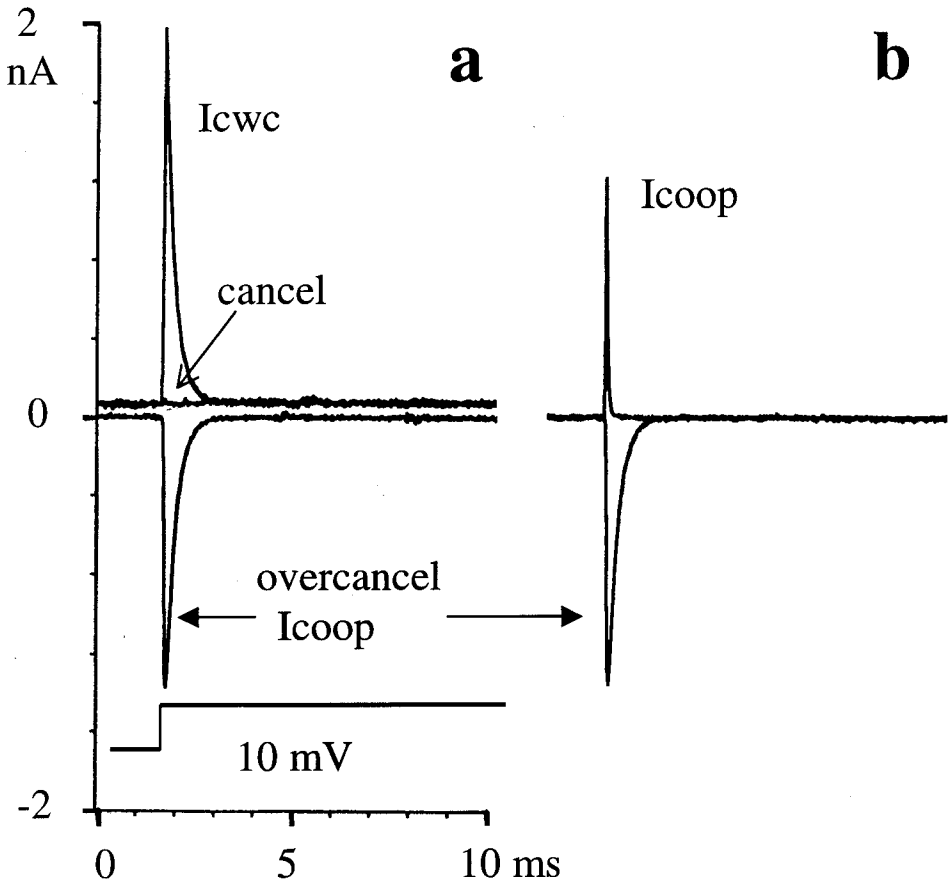

Figure 3.5. Slow capacity current (Ics) changes upon establishment of the outside-out-patch (OOP) measurement configuration

The model cell and the test pulse are the same as those in Fig. 3.4. Icwc is the uncancelled Ics of the WC (frame **a**) and Icoop is the uncancelled Ics of the OOP (frame **b**). The flat record in frame a is the perfectly cancelled Icwc, while the negative records in frames a and b, labeled overcanc(eled) Icoop, are the records obtained just after OOP formation from the condition of perfect Icwc cancellation (flat record in frame a). The sudden appearance of the large inverse Icwc-like current transient is indicative for the formation of an OOP.

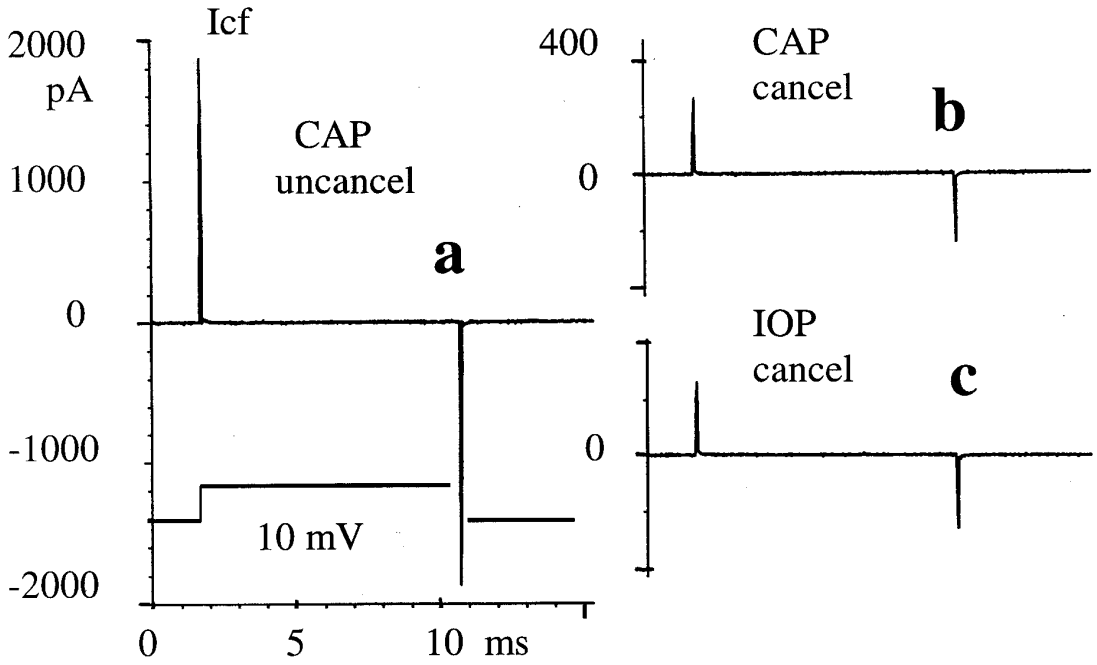

Fig. 3.6. Fast capacitive current (Icf) transients of the cell-attached-patch (CAP) and inside-out-patch (IOP) compared.

The uncancelled CAP-Icf transients in frame **a** are the same as those in Fig. 3.4b for the same experimental conditions. Largely cancelled CAP-Icf transients are shown in frame **b**. Essentially the same Icf transients are seen in frame **c** after excision of the CAP to obtain an IOP (by switching Siop, switch 7 in Fig.3.1, to ground connection). Icf was largely cancelled in frames b and c, in order to be able to better observe changes in Icf upon uncoupling the cell from the CAP.

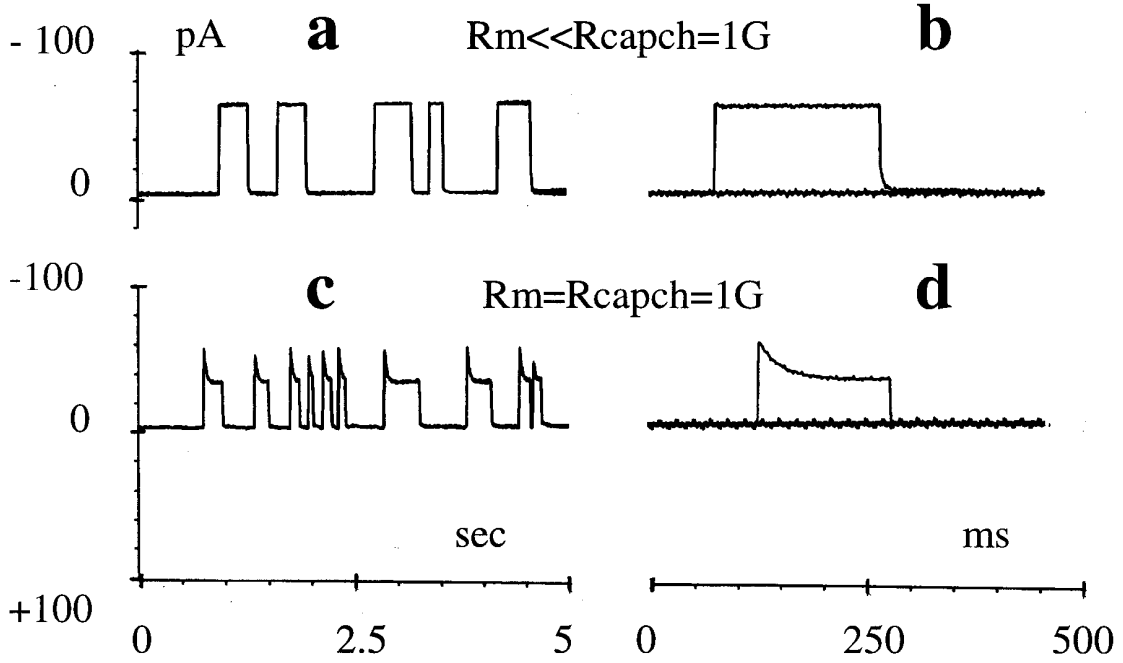

Figure 3.7. Single-channel current recordings from a cell-attached patch under perfect intracellular voltage clamp (square currents in frames a and b) and imperfect intracellular voltage clamp (decaying currents in frames c and d)

The records of a and c are single sweeps on a slow time scale to show repeated channel opening. The records of b and d are 4 superimposed sweeps on a 10x faster time scale for a better resolution of the single channel current shape. In only 1 of the superimposed sweeps of frames b and d a channel current was captured. According to conventions in electrophysiology, inward current through channels is negative. Thus, the current into the CAP-channel from the pipet is negative, but the amplifier shows current flowing out of the pipet as positive, consistent with positive current flow during outward WC-currents. Therefore, the currents have been plotted upward, as produced by the amplifier, but the indicated current polarity is convential. Conditions: Rm=Rl=90M (<<Rcapch) in frames a and b, Rm=1G (=Rcapch) in frames c and d. General conditions: Rcapch=1G, (Ecapch=0mV), Rmcap=20G, Rseal=20G, Vm=El=-62mV (measured in current-clamp whole-cell with Rser=4.4M), Cm~50pF, OOP-components removed and sodium and potassium branches disconnected, Rpip=2.2M, Cpip~5pF, low-pass filter setting at 1 KHz. The CAP-channel was opened and closed by opening and closing a magnetically operated switch (reed contact) in series with the channel by moving a small magnet to and from the switching contact. The small ripples in records b and d are due to 50Hz noise, induced by exposure of the set-up to the experimenter. The movements of the magnet sometimes caused small artifacts in the records, e.g. small waves in the baseline, not significant in these records, except maybe in the tail of the off-current in figure b.

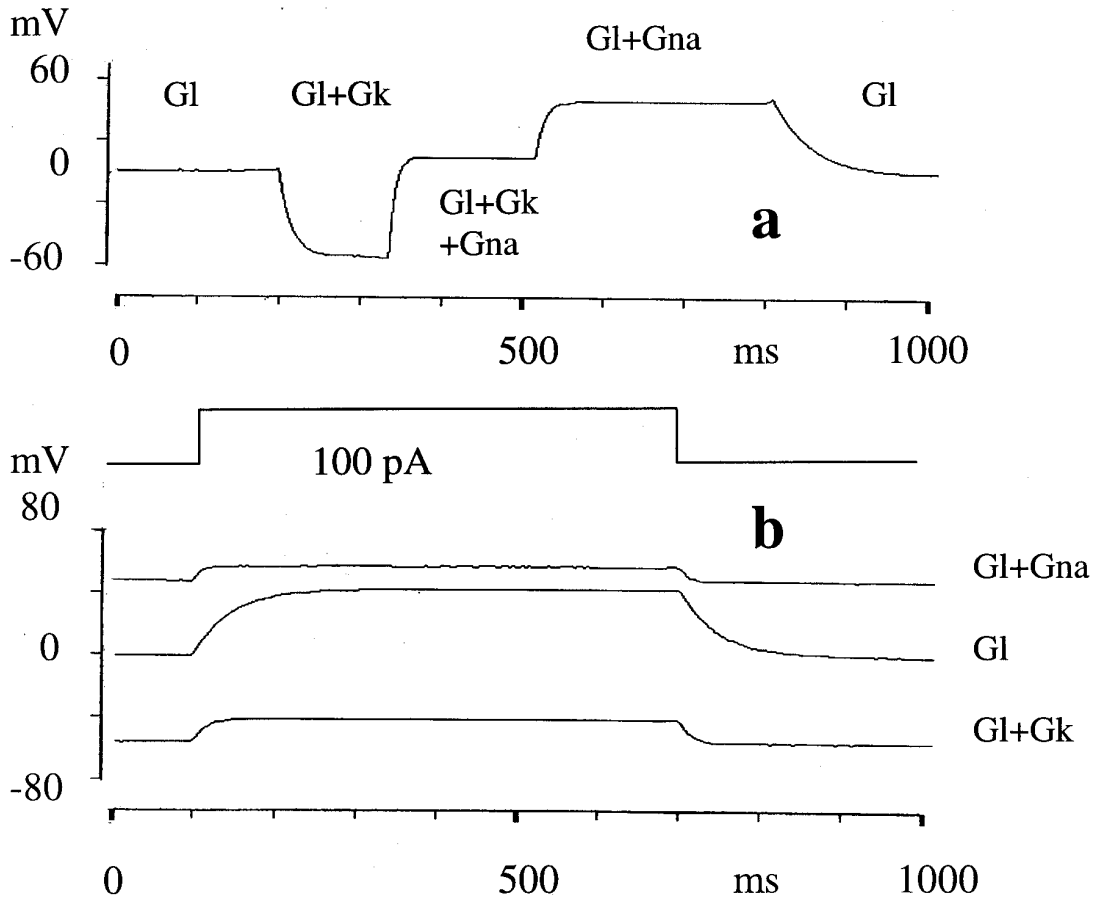

Figure 3.8. Whole-Cell membrane potential changes upon sudden conductance changes (a) and upon current-step stimulation (b)

Gk and Gna were switched on and off with the use of a reed contact in series with Gk and Gna, which can be operated by approaching it with a small magnet. In the initial condition of the experiment of figure **a** only the Gl branch of the 3 conductance branches Gl, Gk and Gna was switched on (Sgl on and Sgk and Sgna off, see Fig. 3.1), which caused the initial Vm=El=0mV. At ~200ms Sgk was also switched on, which resulted in a hyperpolarization towards Ek=-86mV. At ~340ms Gna was added, causing a depolarization towards Ena=+60mV. At ~500 ms Gk was switched off, causing a further depolarization towards Ena.When finally Gna was switched off at ~800ms, Vm returned to the initial Vm=El=0mV. In figure **b** the cell was stimulated by a 100pA block-pulse current in the 3 conductance states of figure a indicated. The responses reflect the RC-properties of the membrane in the 3 conductance states. Other circuit model properties: Rpip=Racc=2M, Rl=400M, Rk=200M, Rna=100M, Rseal=20G, Rcap=20G, Cm~86pF and Cpip~5pF (measured by cancellation).

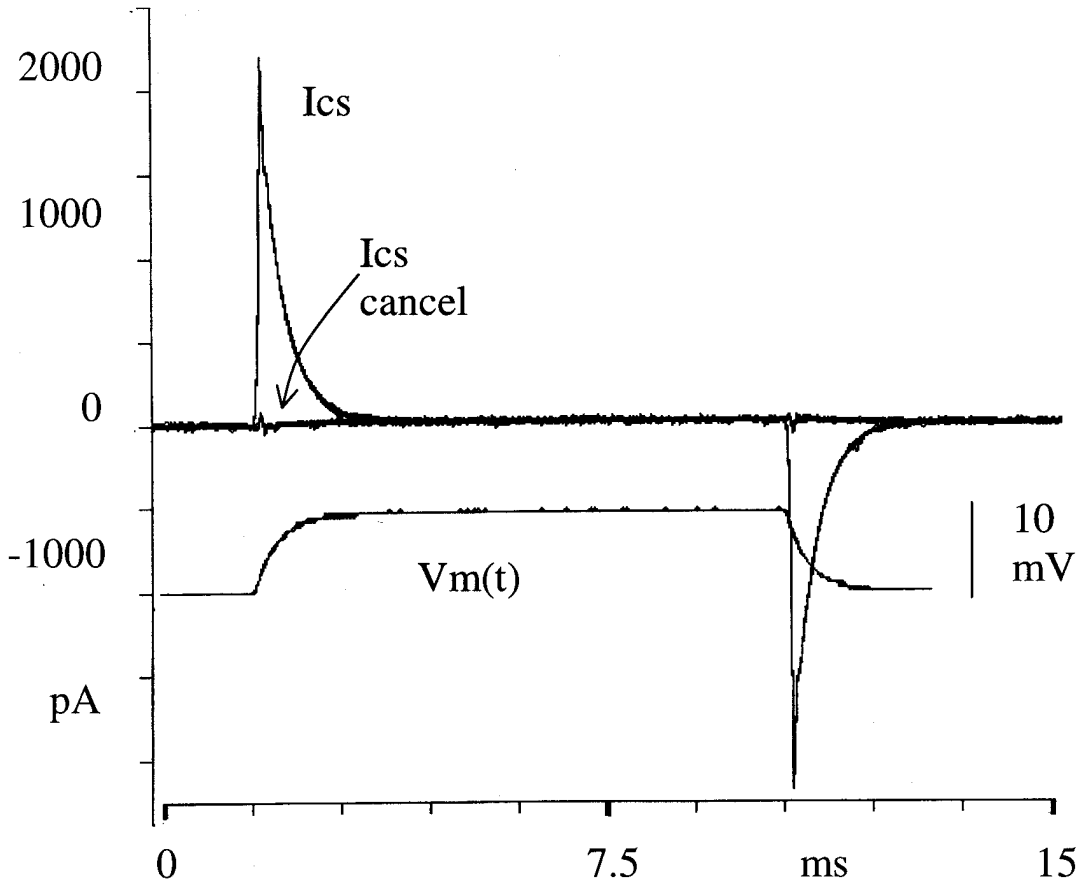

Fig. 3.9. The membrane potential transient, Vm(t), during the slow capacitive current (Ics) transient upon voltage-clamp WC-stimulation and the lack of an effect of Ics transient cancellation on Vm(t)

The records shown are ~30 superimposed sweeps. Experimental conditions (see circuit of Fig. 3.1): Rpip=2.2M, Cpip~5pF, Rseal=20G, Rmcap=20G, Racc=2.2M, Rm=Rl=1G, Cm~94pF (nominal value), resting Vm=El=0mV, Sgk and Sgna are off, the OOP-segment of the membrane is removed, patch-clamp amplifier filter setting on 10KHz. An intracellular microelectrode (current-clamp) amplifier was directly coupled to Vm in the circuit to measure Vm(t) and Icf was completely cancelled at a Cf=4.5pF (at the best fitting τf dial position). Complete Ics cancelling occurred at Cs=84pF and Rser=4.3M (see flat record indicated by arrow), close to the nominal values of the used components (Cm=94pF, Rser=4.4M). The basic observation is that the Vm(t) record did not change upon Ics cancellation.

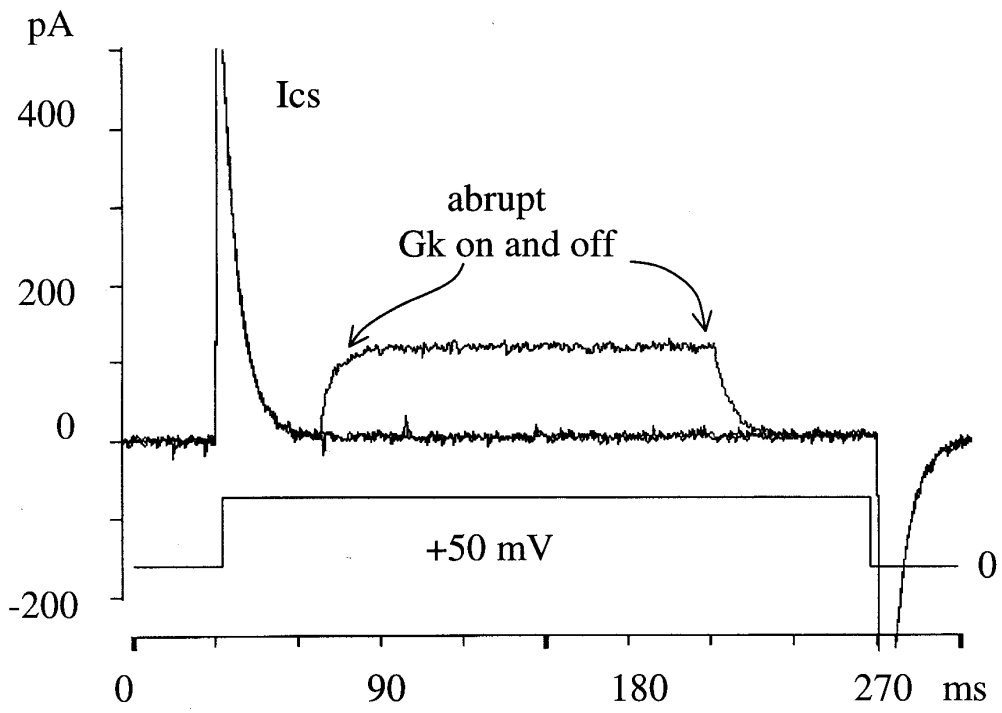

Figure 3.10. Whole-cell current filtering by the Rser.Cm time constant

A Gk=1nS (Rk=1G) is abruptly activated (on) and deactivated (off) by closing and opening, respectively, a series reed contact by a magnet movement upon a depolarization step from 0 to +50mV. Three superimposed records, 2 controls and 1 with Gk activation. Timing of Gk switching was obtained by trial and error. The on and off time constants of Ik are approximately the same as the time constant of the Ics decay (~5ms) . Experimental conditions (cf. Fig. 3.1): no other membrane conductances than Gk (Gl and Gna off, OOP uncoupled), Ek=-62mV, Rpip=2.2M, Racc=50M, Cm=94pF, Rseal=Rmcap=20G, filter setting at 10KHz. The Ik on and off response are not perfectly exponential (too steep initial slopes) probably due to non-ideal switching, but at these time constant values (~5ms) these irregularities are not too disturbing.

Table 3.1. Component abbreviations, used or recommended component values (nominal or estimated) in the model cell experiments and full names of the components

The components are listed in groups and, within the groups, from left to right and clock-wise in the circuit. The abbreviations CAP, WC, OOP and IOP are explained in Fig. 1.1. In some experiments magnet operated reed contacts were used for the switches S6, S11 and S12.

COMPONENT	VALUE(S)	NAME
Rcc	>> 100G (amplif. specif.)	current-clamp Resistance
Rvc	<< 1 M (amplif. specif)	voltage-clamp Resistance
Rpip	2 or 2.2 M	pipette Resistance
Rseal	10 or 20 G	seal Resistance
Rcapch	1 or 2 G	CAP channel Resistance
Rmcap	10 or 20 G	CAP membrane Resistance
Racc	2, 2.2, 5, 10 M	access Resistance
Roop	10 or 20 G	OOP Resistance
Rl	400M, 1 G	leak Resistance
Rk	200 M	potassium Resistance
Rna	100 M	sodium Resistance
Rser	Rpip+Racc	series Resistance
Gl	1/Rl	leak Conductance
Gk	1/Rk	potassium Conductance
Gna	1/Rna	sodium Conductance
Cpc	~1 pF	patch-clamp Capacitance
Cpiphold	~1 pF	pipette-holder Capacitance
Cpip	4.7 pF	pipette Capacitance
Ccap	~ 1 pF	CAP (stray) Capacitance
Coop	3.3 pF	OOP Capacitance
Cm'	47 or 94 pF	membrane' Capacitance
Cm (=Cwc=Cm'+Coop)	~ 50 or ~100pF (excl. Ccap)	WC membrane Capacitance
Cvc		Internal vc Capacitance
Epc		patch-clamp Voltage source
Evc	-100 to +100 mV	voltage-clamp Potential
Ecc		current-clamp Potential
Eoop	0 or ~-60 mV	OOP membrane Potential
El	0 or ~-60 mV	leak reversal Potential
Ek	-60 to –90 mV	potassium Nernst Potential
Ena	~ +60 mV	sodium Nernst Potential
Scvc (S1)		cc-to-vc Switch
Spc (S2)		patch-clamp Switch
Scpip (S3)		pipette capacitance Switch
Srpip (S4)		pipette resistance Switch
Sseal (S5)		seal Switch
Scapch (S6)		CAP channel Switch
Sacc (S7)		access resistance Switch
Siop (S8)		IOP Switch
Soop (S9)		OOP Switch
Sgl (S10)		Gl Switch
Sgk (S11)		Gk Switch
Sgna (S12)		Gna Switch