Identification of Capacitance Distribution in Neuronal Membranes from a Fractional-Order Electrical Circuit and Whole-Cell Patch-Clamped Cells

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Passive electrical membrane properties are key determinants of signaling processes in brain cells, influencing input-output responses as well as action potential firing. We propose a novel interpretation of patch-clamp recordings that brings out the fractional dynamic of the electrical properties of cell membranes and provides a better knowledge of their microscopic behavior. The passive electrical properties of the cell membrane were modeled using an electrical equivalent circuit (EEC) consisting of a constant phase element (CPE) in parallel with a resistor. The Mittag-Leffler function was used to describe the non-exponential behavior of the voltage transients that are attributable to the processes of charging and discharging the membrane capacitance. The procedure proposed, based on circuit theory and fractional calculus, was used to study the voltage transients obtained in response to low-amplitude hyperpolarizing current pulses applied to cultured mice dorsal root ganglion (DRG) neurons under whole-cell current-clamp configuration. To further validate the method, we also analyzed the voltage transients obtained from hippocampal pyramidal neurons and glial cells recorded in mice brain slices in vitro using the short-time behavior of the resulting membrane voltages.

The electrical properties of cell membranes are usually modeled using an electrical equivalent circuit (EEC) consisting of an ideal capacitor $C_m$ in parallel with a resistor $R_m$. This model was initially suggested by classic studies using strength-duration curve techniques. Figure 1a shows a comprehensive introductory EEC that provides a quantitative description of the electrical properties of cell membranes. The capacitance $C_m$ in a cell results from the plasma membrane behaving as an electrical capacitor, because the phospholipid bilayer acts as a dielectric wall that separates the charge that exists in the cytoplasm from that in the extracellular matrix. Precise measurement of neuron capacitance is important not only for determining cell size, due to the linear relationship between the capacitance of a cell and its membrane surface area, but also for modeling of cell basic electrical properties. Some models used to study passive membrane properties combine morphological reconstructions of individual cells, including dendritic spines, with electrophysiological measurements. Proteic pumps and channels located throughout the membrane maintain the electrochemical gradients of charged ions between the inside and the outside. The cell membrane also exhibits an electrical resistance ($R_m$) to the flow of ions, which cross the membrane through the channels and pumps. Thus, $R_m$ is related to the selective permeabilities of the membrane to the different ionic species. A voltage source $V_{rest}$ has been included, in series with $R_m$, to model the resting transmembrane potential.

The membrane potential, which can be modulated actively in excitable cells, is denoted $v_m(t)$ with the polarity indicated in Figure 1a. The current $i_m(t)$ flowing through the cell membrane is the sum of the ionic current $i_m(t)$ and the displacement current $i_d(t)$. Note that when $i_d(t) = 0$, the membrane potential is $v_m(t) = V_{rest}$. It should be mentioned that the values of the conductance $1/R_m$ and $V_{rest}$ in the EEC of Figure 1a will be fairly close to those of the conductance and the Nernst potential, respectively, for the ionic species with the highest conductance (potassium in a typical cell at rest), bearing in mind that the Nernst equation establishes the electrochemical equilibrium value of an ionic ion at both sides of a membrane with selective permeability for that ion, as is the case with the cell plasma membrane. Underlying the condition $i_d(t) = 0$, the cell membrane is in a resting state at which the ionic diffusion (concentration gradient) and the electric field (potential gradient) are in a dynamic equilibrium.

By convention, the current is taken to be that of the flow of positive charge and outward membrane currents (as shown in Figure 1a) are defined as positive. For instance, if a negative (hyperpolarizing) dc current, $i_m(t) = -I_{in}$, is applied to the EEC of Figure 1a, the membrane capacitance begins to charge exponentially from $V_{rest}$ (a negative value) toward $V_{rest} - I_{in} \times R_m$ (membrane potential becomes more negative). Conversely, if $i_m(t) = I_{in}$, that is, a positive (depolarizing) dc current, $v_m(t)$ rises toward $V_{rest} + I_{in} \times R_m$ (and a threshold potential for action potential triggering could eventually be reached). Throughout the paper, we shall use the LTI (linear time-invariant) circuit theory, that is, the $v_m(t) - i_m(t)$ relationship is described by a linear differential equation with constant coefficients. It implies that, during the charging/discharging processes studied, the values of $C_m$, $R_m$, and $V_{rest}$ remain constant.

Neurons can be considered as electrochemical systems due to the electrical properties (voltage, resistance, capacitance) they generate across their plasma membranes through the control of ion concentration gradients at both sides. The membrane capacitance determines its time constant $\tau_m = C_m / R_m$ that, in the case of the neurons, is a crucial parameter for the ability of the neuron to integrate the electrical signals it receives, determining how fast the neuron membrane responds to synaptic inputs. Among the various methods that can be used to experimentally calculate cell capacitance, the analysis of the transient voltage in response to current steps under current-clamp protocols has been shown to be the most accurate method, particularly in the case of non-isopotential cells.

The experimental determination of the capacitance and resistance of the cell membrane requires taking into consideration the complete electric circuit involved in the measurement procedure. The EEC of Figure 1b is similar to that of Figure 1a except for an added resistance $R_0$ in series with the $R_mC_m$ subcircuit. $R_0$ comprises the series resistance associated with the measurement process. Note that $v(t)$ models the voltage measured from the whole-cell patch-clamp technique, but the parameter of interest is actually $v_m(t)$ which is obtained by subtracting the voltage drop $v_0(t)$ across $R_0$ from $v(t)$, i.e., $v_m(t) = v(t) - R_0 \times i_m(t)$. Let us consider that $i_m(t) = -I_{in}$ is applied to the EEC of Figure 1b. The overall waveform at $v_m(t)$ is then shifted vertically by the constant amount $-R_0 \times I_{in}$. An appropriate series resistance
compensation ($R_0 \ll R_m$) assures that the quantity $R_0 \times I_m$ is then small and difficult to visualize in a complete charge/discharge phase, but not immediately to the step change (see below). As mentioned earlier, the membrane capacitance will begin to charge exponentially from $V_{\text{rest}}$ toward $V_{\text{rest}} - I_m \times R_m$. However, a question arises from the fact that the experimental decays observed are described either by single exponential models, or also by multi-exponential models,\textsuperscript{14–16} depending on whether a cell is isopotential or not. In the latter case, the transient-voltage response is expressed as a linear combination of exponential decays from which the neuron capacitance can be estimated.\textsuperscript{14–16} The superposition of exponential relaxation processes suggests a distribution of time constants underlying the well-known non-exponential relaxation functions.\textsuperscript{17} Although some types of neurons are nearly isopotential, like the case of *C. elegans* sensory neurons,\textsuperscript{18} most neurons are non-isopotential due to their complex, ramified morphology or non-uniform conductance distributions. Therefore, new EECs need to be introduced for an accurate description of the cell membrane dynamics.

To implement this idea, we have set aside the traditional $R_m C_m$ circuit, that assumes an ideal capacitor (EEC of Figure 1b) and proposed a revised EEC where the capacitor has been substituted by a constant phase element (CPE$_m$, Figure 1c) that involves the mathematical tools of the fractional calculus.\textsuperscript{19} Equation 1, relating the CPE$_m$ voltage $v_m(t)$ and current $i_{Qm}(t)$, involves a fractional-order derivative of order $\alpha$.\textsuperscript{20,21}

\[
i_{Qm}(t) = Q_m \frac{dv_m(t)}{dt^\alpha} [1]\]

In the frequency domain, the CPE provides a constant phase angle ($-\alpha \pi/2$, in radians) independently of the frequency.\textsuperscript{21} It gives rise to its name. The current-voltage relationship of an ideal capacitor ($Q_m = C_m$) is obtained from the expression above for the case $\alpha = 1$. A fractional capacitor is obtained for $0 < \alpha < 1$, where $Q_m$ has the units of (farads) $\times$ (seconds)$^{\alpha-1}$ with $i_{Qm}(t)$ in amperes, $v_m(t)$ in volts, and $t$ in seconds.

In circuit theory, lumped elements (such as those, $C_m$ and $R_m$, of the EEC of Figures 1a or 1b) are localized at a point in the space. However, the cell membrane occupies a finite region of the space over which its capacitance is distributed. Thus, from an electrical point of view, the cell membrane can be considered as a distributed-parameter network that the EEC of Figure 1a tries to approximate by a lumped-component circuit. In the literature, the CPE has been essential for modelling capacitive properties of biological tissues\textsuperscript{1,21} or double-layer capacitances.\textsuperscript{22}

In Figure 1c, CPE$_m$ considers the space distribution of the electrical properties of the cell membrane which is in agreement with the patch-clamp recordings obtained experimentally. In fact, our interpretation of the physiological processes involved is the existence of membrane heterogeneities that influence the ion movements responsible for the voltage responses.

The aim of this paper is to study the suitability of the electrical circuit theory and fractional calculus to describe the voltage transients evoked by current injection during whole-cell patch-clamp recordings. First, from the EEC of Figure 1c, we obtain the mathematical expressions for the transient-voltage response to a step current, applied on a baseline current level. The knowledge of the step response allows us to obtain the response to any piecewise-constant signals, which is of interest in neurosciences. A useful approximation for sufficiently short times showing that $v_m(t)$ is related to $t$ by a power law characteristic ($t^\alpha$) is also derived. Note that the analysis of the very early part of the raw voltage transients (charge and/or discharge phase) reduces the probability of synaptic current coincidence. The minimum time necessary to obtain consistent membrane electrical parameters is also found. The consistency of the proposed model is reported by analyzing electrophysiological recordings obtained from cultured mice dorsal root ganglion (DRG) cells and from pyramidal neurons and glial cells recorded in hippocampal slices in vitro. Passive electrical properties of the neuronal membrane interpreted by fractional calculus have been barely reported in the scientific literature.\textsuperscript{20,23,24} To the best of the authors’ knowledge, previous applications of fractional calculus to the interpretation of experimental whole-cell patch-clamp recordings do not exist.

**Theory**

In this section, we describe the theoretical underpinnings for the interpretation of the non-exponential voltage transients obtained in response to low-amplitude negative current pulses (so that LTI circuit theory can be applied) under whole-cell current-clamp. As mentioned earlier, under the assumption of LTI circuit theory, the values of $V_{\text{rest}}$, $R_m$, $I_m$, $\alpha$, and $Q_m$ remain constant during the charging/discharging processes analyzed. First, we analyze the behavior of the circuit of Figure 1c in response to an applied current step and, specifically, the resulting membrane potential at sufficiently short times. A method for
obtaining the EEC parameter values of Figure 1c from experimental patch-clamp recordings is also explained. Note that the resistance $R_0$ shifts the level of the potential membrane $v_m(t)$ by the instantaneous voltage $R_0 \times i_m(t)$. We have found the expression for $v(t)$ and sketched its waveform.

Let us consider that a step current from $I_m$ to $I_m + \Delta I_m$, as shown in Figure 2a, is injected to the EEC of Figure 1c, that is,

$$i_m(t) = I_m + \Delta I_m u(t)$$

where $u(t)$ is the unit step function (0 for $t<0$ and 1 for $t>0$). Note that the increment $\Delta I_m$ is negative (hyperpolarizing current step). Equation 2 indicates that $I_m$ has previously been fixed for a “long time” ($t>T$). Thus, a preceding steady-state is approached at time $t=0^-$ (i.e., just before the step change occurs). If the CPEm acts as an open circuit (dc steady-state conditions),

$$v(0^-) = V_{rest} + (R_m + R_0) I_m$$

Voltage $v(0^-)$ is shown in Figure 2b. While $R_m > R_0$, Equation 3 approximates very closely the membrane potential $v_m(0^-)$ (see Figure 2a), which is defined using the gamma function in practice).

$$v(\infty) = V_{rest} + (R_m + R_0) (I_m + \Delta I_m)$$

Again, $v_m(0^-) \approx v(\infty)$ if $R_m > R_0$. This non-exponential decay, as shown in Figure 2b, is given by $v(\infty) + [v(0^-) - v(\infty)] \times E_{\alpha}[-(t/\tau_m)^\alpha]$. That is,

$$v(t) = v(\infty) - R_m \Delta I_m E_{\alpha} \left[-\left(\frac{t}{\tau_m}\right)^\alpha\right], \quad t > 0$$

where $E_{\alpha}[-(t/\tau_m)^\alpha]$ is the one-parameter Mittag-Leffler function,\(^{19}\) which is defined using the gamma function $\Gamma$.

$$E_{\alpha} \left[-\left(\frac{t}{\tau_m}\right)^\alpha\right] = \sum_{k=0}^{\infty} \frac{(-\left(\frac{t}{\tau_m}\right)^\alpha)^k}{\Gamma(k+1)}, \quad \alpha > 0$$

For $\alpha = 1$, $E_{1}[-(t/\tau_m)] = \exp[-t/\tau_m]$ and Equation 6 shows exponential decay (dashed line in Figure 2b), which corresponds to the behavior of the EEC of Figure 1b.

In the frequency domain, Nyquist plot of EEC of Figure 1c sketches a depressed semicircle (the center of its corresponding circle lies below the real axis). This recalls the concept of “distribution of time constants”. In this context, $\tau_m$ is the average time constant and $\alpha$ is a parameter related to the width of the distribution.\(^{25}\) According to the EEC of Figure 1c, $\tau_m$ is equal to the product of the resistance seen by CPEm (in this case, $R_m$) and $Q_m$, raised to the power 1/$\alpha$ to preserve the time constant dimension.\(^{25,26}\)

$$\tau_m = (R_m Q_m)^{1/\alpha}$$

The average time constant given by Equation 8 is equal to that of an ideal $R_m C_{effm}$ circuit.\(^{25}\) It allows us to obtain an effective capacitance $C_{effm} = \tau_m/R_m$, resulting in the following expression

$$C_{effm} = (R_m Q_m)^{1/\alpha}$$

Similar expressions to Equation 9 are found in electrode processes to consider surface or natural time-constant distribution.\(^{27}\)

The Mittag-Leffler function $E_{\alpha}[-(t/\tau_m)^\alpha]$ exhibits two asymptotic approximations (see Equation 10): an initial fast decay described by the Kohlrausch-Williams-Watts (KWW) relaxation law (stretched exponential function) and a long tail (inverse power-law behavior).\(^{28}\)

$$E_{\alpha} \left[-\left(\frac{t}{\tau_m}\right)^\alpha\right] \sim 1 - \frac{(t/\tau_m)^\alpha}{\Gamma(1+\alpha)} , \quad (t/\tau_m) \rightarrow 0^+$$

Note that the value of $(t/\tau_m)$ governs the behavior of the EEC, that is, the transition from the stretched exponential ($t<<\tau_m$) to the inverse power-law ($t>>\tau_m$) voltage responses. As shown in Equation 10, the stretched exponential is preceded earlier by the rapidly decreasing expression $1-(t/\tau_m)^{\alpha}/\Gamma(\alpha+1)$.

We now analyze the initial fast part of $v(t)$ by substituting the series expansion of Equation 7 into Equation 6. If $(t/\tau_m)^\alpha/\Gamma(\alpha+1)$ is small compared to $(2\alpha+1)/\Gamma(\alpha)$, the infinite series can be truncated after the second term, that is,

$$t < \tau_m \left(0.2 \frac{\Gamma(2\alpha)}{\Gamma(\alpha)} \right)^{1/\alpha}$$

and we can rewrite Equation 6 as

$$v(t) = v(0^+) + \frac{\Delta I_m t^\alpha}{Q_m \Gamma(\alpha + 1)}$$

Note that the portion of the current step at sufficiently short times is much shorter than the electrical inertia exhibited by the EEC and, thus, Equation 12 does not depend on $\tau_m$. The charge injected into the CPE corresponds eventually to a coulometric step.

The inset of Figure 2b shows the short-time power law $t^\alpha$ behavior (solid line) of Equation 12. Note that the asymptotic behavior ($t/\tau_m<<1$) of the Mittag-Leffler function exhibits a faster decay than the exponential one (dashed line).

Let us consider an experimental waveform $v(t)$ obtained from a whole-cell patch-clamp recording. The parameter values of the EEC of Figure 1c can be obtained using the following procedure: (i) $V_{rest}$ is previously obtained under the constraint $i_m(t) = 0$; (ii) $R_0$ is found from the magnitude of the jump discontinuity in Equation 4; (iii) $R_m$ is found from Equations 3 or 5. Note that if $R_m > R_0$, $v_m(0^-) \approx v(\infty)$ in Equations 3 and 5; (iv) $\alpha$ and $\tau_m$ are determined by fitting the experimental waveform of $v(t)$ to Equation 6; (v) $Q_m$ is found from Equation 8. Alternatively, at sufficiently short times (see Equation 11), $\alpha$ and $Q_m$ are determined by fitting the transient-voltage response $v(t)$ to Equation 12 and $\tau_m$ is found from Equation 8. Finally, an effective capacitance of the cell membrane $C_{effm}$ can be obtained using Equation 9.

Note that the above equations are also valid for a depolarizing current-step (amplitude sufficiently low for LTI conditions). In this case, the increment $\Delta I_m$ is positive and Equation 6 will show a non-exponential rise rather than decay. Furthermore, the above equations allow $v(t)$ to be obtained in response to any piecewise-constant current signal. Specifically, Figure 2c shows the low-amplitude negative current pulse of width $T$ and height $\Delta I_m$ used throughout the paper. It can be considered as sum of two steps: a negative one of height $\Delta I_m$ occurring at $t=0$ (i.e., $\Delta I_m \times u(t)$) and a positive one of height $\Delta I_m$ occurring at $t = T$ (i.e., $\Delta I_m \times u(t-T)$). The right-hand side of the waveform $v(t)$ has been labeled using the above equations in Figure 2d (the left-hand side is identical to that of Figure 2b). The dashed line indicates $v(t)$ for the exponential behavior ($\alpha = 1$). Since $T>>\tau_m$, a steady state is reached just before the positive step change. Note that the time constant $\tau_m$ remains unchanged and Equation 6 is satisfied for both the charging ($0<t<T$) and discharging ($t>T$) processes. In the latter case, $r$ is changed to $t-T$, to account for the translation in time.
Figure 2. Response of the EEC of Figure 1c to switching-signal waveforms. (a) Applied step current $i_m(t)$, from $I_m$ to $I_m + \Delta I_m$, at time $t = 0$. (b) Transient-voltage waveform in response to the step current shown in (a). (c) Applied negative current pulse of width $T$ and height $\Delta I_m$. (d) Transient-voltage waveform in response to the current pulse shown in (c). Insets in (b) and (d) show expanded views of transient-voltages responses for sufficiently short times. The dashed and solid lines indicate $v(t)$ at the terminals of the EECs shown in Figure 1b ($\alpha = 1$, exponential behavior) and Figure 1c, respectively. $V_{rest}$, $R_m$, $R_0$, and $\tau_m$ are the membrane resting potential, membrane resistance, the resistance associated with the measurement process and the distributed time-constant of the cell membrane, respectively. $Q_m$ and $\alpha$ are the CPE parameters.

Experimental

For experiments conducted in cultured DRG neurons, three to twelve-week-old male mice were anesthetized by inhalation of CO$_2$ and subsequently decapitated. The dorsal root ganglia were isolated and incubated for an hour in collagenase type XI (0.66 mg/ml) and dispase (3 mg/ml). Thereafter, ganglia were mechanically dissociated and cultured in minimal essential medium with Earle’s balanced salt solution and L-Glutamine (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 1% minimal essential medium vitamin solution (Invitrogen), 100 mg/ml penicillin/streptomycin and 100 ng/ml nerve growth factor (NGF). Cells were plated onto poly-L-lysine-coated glass coverslips and used after 1 day in culture. Recordings were made in a submerged recording chamber, and superfused with a solution containing (in mM): NaCl 141, KCl 2, MgCl$_2$ 1.3, CaCl$_2$ 2.4, HEPES 10, Glucose 10, pH 7.4. The solution was maintained at 36–37°C and perfused at a constant flow rate of 2–3 ml/min.

For experimental recording in brain slices: mice were anesthetized with sodium pentobarbital and decapitated/killed by cervical dislocation. The brain was quickly dissected out, and brain slices (400 μm) were cut in the transverse plane using a vibratome (Pelco, St. Louis, USA). The tissue was always submerged in ice-cold artificial cerebrospinal fluid (ACSF) and saturated with bubbling carbogen (95% O$_2$/5% CO$_2$). Slices were then placed in a holding chamber filled with ACSF for at least 1 hour at RT for recovery. The composition of the standard ACSF was (in mM): NaCl 141, KCl 2.5, NaH$_2$PO$_4$ 1.0, MgCl$_2$ 1.2, CaCl$_2$ 2.5, NaHCO$_3$ 26.2, glucose 11, pH 7.4 equilibrated with carbogen. For recording, individual slices were transferred to the submerged chamber and superfused with ACSF (36-37°C, 2-3 ml/min flow rate).

Cells to be patched were visualized using Nomarsky interference contrast microscopy. DRG cells in culture were easily selected by direct visualization. Once patched, the cells were maintained at a membrane potential between −50 and −60 mV by passing a constant negative direct current of a maximum value of −20 pA. If a higher current was required, the cell was discarded. Spiking activity of selected cells was checked by applying a positive current pulse (+20 pA), which caused action potential shooting, as shown in Figure 3a (gray line). Cells in slice preparations were selected by their morphology and location within the hippocampus. Hippocampal pyramidal neurons to be patched were identified by their pear-shaped somas located in the stratum pyramidale of CA1 region. Such cells showed a membrane resting potential of −50 to −53 mV (no current was used to move their potential) and shoted action potentials when positive current pulses (+40 pA) were applied (Figure 4a, gray line). Putative glial cells were located in the most basal region of the stratum pyramidale and, although precise determination of the glial cell type was not possible, they were characterized by a more negative membrane resting potential and lack of action potential generation when tested using positive current pulses (up to +200 pA, as illustrated in Figure 4d).

Whole-cell patch-clamp recordings were performed using borosilicate micropipettes filled with (in mM): NaCl 2, MgCl$_2$ 2, EGTA 10, HEPES 10, Li-GTP 0.3, Na$_2$-ATP 3, K-glucinate 125, adjusted to pH 7.2. Total micropipette resistance was 3.5–9 MΩ when filled with this solution. Pipettes were coupled to the input stage of a Multiclamp 700B amplifier (Molecular Devices, USA) that was used to record
Figure 3. Experimental and simulated results. (a) Representative cell membrane voltage transient (lower panel, black line) obtained in response to $\Delta I_m = -20$ pA negative current pulse (upper panel, black line) applied under whole-cell current-clamp configuration in a mice cultured dorsal root ganglion (DRG) neuron. Note also the action potential firing pattern (lower panel, gray line) evoked by a positive $\Delta I_m = +20$ pA current pulse (upper panel, gray line) for verification purposes. Patch-clamp analysis of the (b) charge and (c) discharge phases was carried out using the procedure indicated in Figure 2d.

cell activity. Signals were sampled at 50 kHz with a Digidata 1440A converter, and collected and processed using pClamp 10 software (Molecular Devices).

Long current pulses can cause the membrane potential to deviate from its resting levels for longer than short pulses, and thus the former have a higher chance of activating voltage-dependent conductances.\textsuperscript{29} To avoid artefacts from any possible voltage-dependent conductances in our study, only the first 80 ms from the voltage charge phase responses were selected for the DRG analysis; no evidence of hyperpolarization-activated conductances was seen during the selected time window in any of the recorded cells. Additionally, only the voltage responses to small amplitude negative current pulses ($-20$ pA for cultured DRG cells or $-40$ pA for cells in slices) were taken into consideration for our modeling studies. Analysis of voltage transients was also conducted in the discharge phase of the pulse in order to compare the fits of the charge and discharge phases to the model (Figures 3 and 4).

Results and Discussion

We applied square current pulses to whole-cell patched mice DRG neurons in culture and recorded the resultant voltage transients in order to study the kinetics of the membrane electrical capacitance. Although short current pulses are commonly used for this purpose to minimize the activation of voltage-dependent processes,\textsuperscript{7,11} we chose to apply long length-pulses (300 or 500 ms) because we were interested in the analysis of both the charging phase of membrane capacitance at the beginning of the current pulse and the discharge phase at the end. We used hyperpolarizing pulses with amplitudes as low as possible to obtain consistent voltage responses that presented no apparent contamination (i.e., deviation from a horizontal steady state) by voltage-dependent currents and, thus, the LTI circuit theory is applicable. An example of the voltage transient obtained by in a DRG neuron is shown in Figure 3a (black line). A decay phase is observed at the beginning of the pulse, starting from the basal membrane voltage value and reaching a more negative steady-state after some milliseconds. The shape of the decay phase emerges from the combination of a resistive element and a progressively charging capacitive component that form part of the membrane electrical properties.\textsuperscript{30} At the end of the current pulse, a growing phase returned the membrane voltage to the resting values by discharging the membrane capacitance, following an opposite kinetic pattern to that of the charging phase. We purposely avoid defining these phases as exponentials since the aim of this work is to study the feasibility of mathematically fitting their dynamics using mathematical descriptions based on fractional calculus.

The charge phase obtained in one representative DRG cell in response to a pulse of $-20$ pA is shown in Figure 3b together with the simulated curve. Similarly, the experimental and simulated data corresponding to the discharge phase of the same cell are illustrated in Figure 3c. Simulated waveforms have been plotted using Equation 6 with the parameter values listed in Table I. An excellent fit between the acquired and the modeled data can be seen in each of the phases. Further, close inspection of the calculated electrical parameters of all the DRG cells analyzed ($n = 6$) revealed a high degree of similarity between the values obtained for the charge ($R_0 = 11.85 \pm 1.84$ MΩ, $\alpha = 0.85 \pm 0.04$, $R_m = 1.57 \pm 0.44$ GΩ, $C_{effm} = 18.05 \pm 2.48$ pF, $\tau_m = 28.67 \pm 9.52$ ms) and discharge ($R_0 = 11.42 \pm 2.23$ MΩ, $\alpha = 0.87 \pm 0.03$, $R_m = 1.54 \pm 0.43$ GΩ, $C_{effm} = 17.96 \pm 2.52$ pF, $\tau_m = 27.49 \pm 8.36$ ms) phases. This result points to the existence of
Figure 4. Experimental and simulated results. Representative example of the membrane voltage transients (lower panels, black lines) obtained in response to $\Delta I_m = -40$ pA negative current pulses (upper panels, black lines) applied under whole-cell current-clamp protocol in (a) a pyramidal neuron and (d) a glial cell in hippocampal slices in vitro. See also the action potentials fired by the pyramidal cell ((a), lower panel, gray line) in response to a positive $\Delta I_m = +40$ pA current pulse ((a), upper panel, gray line) and the absence of spiking in the glial cell ((d), lower panel, gray line) when stimulated with $\Delta I_m = +40$ or even $\Delta I_m = +200$ pA current pulses ((d), upper panel, gray lines). Patch-clamp analysis of the charge and discharge processes ((b) and (c) for the hippocampal pyramidal neurons and (e) and (f) for glial cells, respectively) was carried out using the procedure indicated in the insets of Figure 2d.

Table I. Parameter values of the EEC shown in Figure 1c. Values are extracted from the charge and discharge phases of the voltage transients obtained in the current-clamp experiments performed in cultured mice dorsal root ganglion (DRG) neurons (Figures 3b and 3c). Values for $C_{effm}$ and $\tau_m$ have also been included.

|                  | $R_0$ (MΩ) | $Q_m$ (pF s^2) | $\alpha$ | $R_m$ (GΩ) | $C_{effm}$ (pF) | $\tau_m$ (ms) |
|------------------|------------|----------------|----------|-------------|----------------|--------------|
| Cultured DRG neurons | Charge process | 9.72 | 25.95 | 0.89 | 2.16 | 18.17 | 39.26 |
|                   | Discharge process | 10.21 | 26.99 | 0.87 | 2.07 | 17.54 | 36.31 |

symmetrical membrane electrical behavior and suggests that no rectifying mechanism is present at the recorded membrane potentials over the duration of either phase. Furthermore, $\alpha$ values obtained from the fitting analysis were near identical in all the DRG neurons in the study (almost constant and close to 0.85), indicating that all the cells share quite similar basal membrane electrical properties.

Dissociated neurons in culture are considered a good model for the study of many cell properties. Indeed, dissociated neurons from the DRG and the trigeminal ganglion are assumed to express membrane receptors and channels that are expressed in their peripheral nerve terminals in vivo. However, neurons in culture lack the majority of the synaptic contacts they display in the intact tissue. To obtain some understanding of the membrane properties in a more physiological situation, we proceeded to obtain whole-cell current clamp recordings from pyramidal neurons in a model of in vitro hippocampal slices. Though not identical to the in vivo situation, the slice model maintains most of the synaptic connections that are lost in cultured cells.

Pulses of $-40$ pA were applied to whole-cell patched hippocampal CA1 pyramidal cells, and the voltage transients were recorded as in the case of DRG neurons. An example of the evoked voltage transient in response to a $-40$ pA pulse is shown in Figure 4a (black line). These transients exhibited some distortions along their length, most probably resulting from the arrival of synaptic inputs simultaneously with the current pulse application. As mentioned above, neurons in slices preserve most of the tissue synaptic inputs, which can certainly be active during the recording. Several measures can be adopted to avoid or minimize the effect of synaptic contamination of the voltage transient: one is the addition of pharmacological blockers to inhibit neurotransmitter receptor activation, thus avoiding the generation of the synaptic currents during the experiment; another possibility is mathematically averaging multiple transients generated by repetition of the current pulse, diluting the interference of the individual synaptic inputs. However, these measures would move us away from the physiological situation we are trying to deal with. Our approach consisted in limiting the analysis to the very first part of the raw voltage transients (both in the charge and discharge phases), to reduce the probability of synaptic current coincidence. This technique would eventually indicate the minimum time window that must be analyzed in order to adequately obtain consistent membrane electrical parameters. Figures 4b and 4c show the analysis and fitting with Equation 12 during the first millisecond (thereby verifying the constraint of Equation 11) of the charge and discharge phases, respectively, of a hippocampal CA1 pyramidal cell. Interestingly, the results obtained displayed good agreement with the mathematical model, with $\alpha$ values (see Table II) quite similar in both phases, as in the case of DRG’s (Table I).

As a further step, we applied the same approach to the mathematical modeling of the voltage transients obtained in glial cells recorded in the hippocampal slice preparation. Glial cells are not electrically...
excitable by synaptic inputs in the way that neurons are. The responses of glial cells to neurotransmitters and neuromodulators are mediated mainly by calcium increases that depend on ionotropic or metabotropic receptors. Pulses of $-40 \text{ pA}$ were applied to patched glial cells in the current clump configuration, obtaining voltage responses as the one shown in Figure 4d (black line). Note the absence of action potential firing even during depolarization caused by positive current pulses (+40 and $+200 \text{ pA}$, gray lines). Fitting the negative voltage transient using Equation 12 during the initial millisecond of the transient reported consistently similar $\alpha$ values between charge (Figure 4e) and discharge phases (Figure 4f).

Tables I and II show that the parameter values obtained using the fractional-order EEC model for the charge phase are remarkably close to those found from discharge measurements using both methods (analyzing the complete processes or using the expression for sufficiently short times) for all cells under study. Our experimental results confirm the previous theoretical studies that address non-ideal membrane capacitive behavior and fractional-order $v(t)$ dynamics, following a Mittag-Leffler pattern. This experimental finding is also in agreement with the fractional leaky integrate-and-fire models of neuronal activity.34

What could be the physiological substrate underlying these fractional-order dynamics? Because the ionic concentrations at both sides of the membranes are regulated by ion channels and transporters, membrane potential and excitability are dependent on the presence and distribution of ion channels over the neuron membrane surface. Some ion transporters and channels have been shown to be differentially distributed in lipid rafts (sphingolipid- and cholesterol-rich, specialized membrane microdomains) and non-lipid rafts membrane fractions. Such segregation may have a functional role since lipid raft disruption alters the activity of some ion transporters.35 It is also known that local variations of membrane thickness at lipid rafts can produce changes in cell capacitance.36 In fact, differences in lipidic (sphingomyelin and cholesterol) content in models of phosphatidylycholine-based monolayers produce capacitance changes that are detectable by capacitance measurements, and suggest the existence of microdomain-type heterogeneities that resemble the lipid rafts of bilayer membranes.37 It has also been suggested that the neuron can behave as a set of interacting computational models due to the existence of subneural systems of membrane lipid microdomains that regulate ion channel dynamics; such microdomains would constitute the smallest unit of nervous system signaling.38 The interpretation of the fractional dynamic of neuron membrane capacitance that we propose in this study may reflect the existence of such microdomains. In fact, classical simple and multiple exponential fittings consider a neuron membrane as an individual resistance-capacitor system, while our fractional model supports the idea of an (in)finite succession of resistance-capacitors, each with its own characteristic time constant. The classical view would represent a particular, simplified case in which the cell membrane, despite its possible morphological complexity, is held to exhibit homogeneous behavior. On the other hand, the fractional-order model described here would broaden this concept, proposing a general model encompassing many possible membrane heterogeneities caused by the existence of microdomain areas with different biomolecular composition. The fractional-order circuit model does not contradict or overlap the classical exponential model, and indeed the former supports the validity of the latter by considering it as a particular case ($\alpha = 1$). This new circuit model constitutes a relevant contribution for the development of new paradigms on neuron computation capabilities, like those suggesting that neurons consist on a number of spatially independent threshold units,39 or proposing the existence of different current modules, segregated by voltage range, that define particular sets of membrane passive properties and firing patterns.40 Additionally, the capacitance estimations using this model can be complementary to other methods used for hippocampal glial and neural cells.41

### Conclusions

In this paper we have shown that a novel method of analysis for whole-cell patch-clamp recordings, based on circuit theory and fractional calculus, can be used to characterize the passive properties of cell membranes. The procedure described has been used to study the voltage transients obtained in response to low-amplitude hyperpolarizing current pulses applied under a whole-cell current-clamp configuration in cultured mice DRG neurons. The EEC parameters extracted from charge and discharge processes were remarkably close, indicating symmetric electrical behavior and little or no involvement of voltage-dependent currents or synaptic inputs. An excellent agreement between the experimental and simulated results was observed. In order to assess the suitability of this novel finding, we analyzed voltage responses similarly obtained in hippocampal pyramidal neurons and glial cells from mice brain slices in vitro, using a new technique based on the study of the short-time behavior of the resulting membrane voltages. Comparison of the simulated results with the experimental data showed consistency with the interpretation proposed for both methods. A membrane structural heterogeneity hindering ion movement might underlie the physiological processes associated with the proposed membrane capacitance distribution.

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### References

1. K. S. Cole, Membranes, Ions, and Impulses: A Chapter of Classical Biophysics, University of California Press, Berkeley (1972).
2. G. Weiss, Arch. Ital. Biol., 35, 413 (1901).
3. L. Lapicque, J. Physiol. Phsyiol., 9, 620 (1907).
4. W. Nernst, Arch. Ges. Physiol., 122, 275 (1908).
5. R. T. Kado, Methods Enzymol., 221, 273 (1993).
6. A. U. Larkman, G. Major, K. J. Stratford, and J. J. B. Jack, J. Comp. Neurol., 323, 137 (1992).
7. G. Eyal, M. B. Verhoog, G. Testa-Silva, Y. Deitcher, J. C. Lodder, R. Benavides-Piccione, J. Morales, J. DeFelipe, C. P. de Kock, H. D. Mansvelder, and I. Segev, *Elife*, 5, e16553 (2016).
8. A. L. Hodgkin and A. F. Huxley, *J. Physiol.*, 117, 500 (1952).
9. M. van Dongen and W. Serdijn, *Design of efficient and safe neural stimulators: A multidisciplinary approach*, pp. 11–24, Springer, Cham (2016).
10. W. Rall, *Science*, 126, 454 (1957).
11. C. Koch, M. Rapp, and I. Segev, *Cereb. Cortex*, 6, 93 (1996).
12. J. Golowasch, G. Thomas, A. L. Taylor, A. Patel, A. Pineda, C. Khalil, and F. Nadim, *J. Neurophysiol.*, 102, 2161 (2009).
13. A. Molleman, *Patch clamping: An introductory guide to patch clamp electrophysiology*, John Wiley & Sons, England (2003).
14. W. Rall, *Biophys. J.*, 9, 1483 (1969).
15. W. R. Holmes, I. Segev, and W. Rall, *J. Neurophysiol.*, 68, 1401 (1992).
16. G. Major, J. D. Evans, and J. J. B. Jack, *Biophys. J.*, 65, 423 (1993).
17. C. F. Lindsey and G. D. Patterson, *J. Chem. Phys.*, 73, 3348 (1980).
18. M. B. Goodman, D. H. Hall, L. Avery, and S. R. Lockery, *Neuron*, 20, 763 (1998).
19. I. Podlubny, *Fractional differential equations*, Academic Press, San Diego (1999).
20. R. L. Magin, *Crit. Rev. Biomed. Eng.*, 32, 105 (2004).
21. E. Hernández-Balaguera, E. López-Dolado, and J. L. Polo, *RSC Adv.*, 6, 22312 (2016).
22. E. Barsoukov and J. R. Macdonald, *Impedance Spectroscopy: Theory, experiment, and applications*, John Wiley & Sons, New Jersey (2005).
23. S. H. Weinberg, *PLoS One*, 10, e0126629 (2015).
24. B. N. Lundstrom, M. H. Higgs, W. J. Spain, and A. L. Fairhall, *Nat. Neurosci.*, 11, 1335 (2008).
25. E. T. McAdams and J. Jossinet, *Bioelectrochem. Bioenerg.*, 40, 147 (1996).
26. E. Hernández-Balaguera and J. L. Polo, *Electrochim. Acta*, 233, 167 (2017).
27. B. Hirschorn, M. E. Oraziem, B. Tribollet, V. Vivier, I. Frateur, and M. Musiani, *Electrochim. Acta*, 55, 6218 (2010).
28. F. Mainardi, *Discrete Contin. Dyn. Syst. Ser. B*, 19(7), 2267 (2014).
29. D. Durand, P. L. Carlen, N. Gurevich, A. Ho, and H. Kunov, *J. Neurophysiol.*, 50, 1080 (1983).
30. E. R. Kandel, J. H. Schwartz, T. M. Jessell, S. A. Siegelbaum, and A. J. Hudspeth, *Principles of neural science*, McGraw-Hill, Health Professions Division, New York (2012).
31. M. Kress and P. W. Reeh, *Proc. Natl. Acad. Sci. U. S. A.*, 93, 14995 (1996).
32. A. Schurr, *Brain slice preparation in electrophysiology*, No. 15, David Kopf Instruments, p. 1, Tujunga, California (1986).
33. D. S. Auld and R. Ribaillie, *Neuron*, 40, 389 (2003).
34. W. Teka, T. M. Marinov, and F. Santamaria, *PLoS Comput. Biol.*, 10, e1003526 (2014).
35. A. Pristera and K. Okuse, *Neuroscientist*, 18, 70 (2012).
36. B. Alberts, J. H. Wilson, and T. Hunt, *Molecular biology of the cell*, 5th edn., Garland Science, New York (2008).
37. M. F. Lecompte, G. Gabelet, C. Lebrun, F. Tercé, X. Collet, and S. Orloowski, *Langmuir*, 31, 11921 (2015).
38. R. Wallace, *Biosystems*, 87, 20 (2007).
39. S. Sardi, R. Vardi, A. Sheinin, A. Goldental, and I. Kanter, *Sci. Rep.*, 7, 18036 (2017).
40. A. Alturki, F. Feng, A. Nair, V. Guntu, and S. S. Nair, *Neuroscience*, 334, 309 (2016).
41. T. Zhou, Y. Ming, S. F. Perry, and S. Tatic-Lucic, *J. Biol. Phys.*, 42, 571 (2016).