Meron Gurkiewicz · Alon Korngreen

Recording, analysis, and function of dendritic voltage-gated channels

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Abstract Ever since the publication of the Hamill et al. [Hamill et al., *Pflügers Arch*, 391:85–100, 1981] paper and the following increase in popularity of acute brain slice preparations, there has been a large increase in the volume of publications investigating voltage-gated channels in the central nervous system using the patch-clamp technique. In the preceding decade, investigations of voltage-gated channels have moved out of the somatic region into dendrites providing much needed information about dendritic voltage-gated channels. In this study, we review some aspects related to the investigation of voltage-gated ion channels in dendrites: recording, analysis, and function.

Keywords Dendrites · Voltage-gated channels · Patch-clamp

Introduction

Three scientific events have combined to generate a small gold rush in the field of neuronal voltage-gated channels. First, the invention of the patch-clamp technique [74] made it possible to record ionic currents from single voltage-gated ion channels. A quick search through PubMed reveals that since 1995, the number of publications containing the phrase “patch-clamp” in their title or abstract has been roughly constant at about 2,000 per year. About one third of these publications are related, according to PubMed, to voltage-gated ion channels. Second, the establishment of the acute brain slice preparation as the

major in vitro preparation for investigating neurons in the central nervous system [17] in combination with differential interference contrast optics and digital imaging [102] enable to selectively and accurately perform repeated measurements from visually identified neurons in many regions of the central nervous system (CNS). It is, therefore, not surprising that the number of papers describing properties of ion channels in the CNS soared in the mid-1990s. Finally, the newfound ability to visualize neurons led to the application of the patch-clamp technique to the investigation of dendritic physiology [100, 102]. This intensive investigation of somatic and dendritic voltage-gated ion channels in the preceding decade has produced a plethora of new and exciting information leading to renewed interest in the computational properties of dendrites [29, 54, 70, 81, 101]. This paper focuses on some technical aspects of this fascinating field of research. We examine the applicability of the patch-clamp technique for recording from dendritic voltage-gated channels. Additionally, we consider the analysis of currents recorded from voltage-gated channels in view of immense increases in computational powers that enable us to perform many calculations (previously requiring heavy supercomputers) using a simple desktop PC. Finally, we point at the increasing difficulty of assigning clear function to a given dendritic voltage-gated conductance in the physiology of any neuron.

Measurement

The patch-clamp technique enables recordings from voltage-gated ion channels via various modes [26]. Nonetheless, not all of these modes are equally applicable to measuring the properties of voltage-gated ion channels in brain slice preparations. The least applicable mode is the whole-cell mode due to the distributed morphology of the neurons. This nonspherical morphology inevitably dictates that the voltage-command applied via the patch-pipette will decay away from the recording site, along the dendrites and axon, leading to incomplete spatial clamping of the

M. Gurkiewicz · A. Korngreen
The Mina and Everand Goodman Faculty of Life Sciences
and the Leslie and Susan Gonda Multidisciplinary Brain
Research Center, Bar-Ilan University,
Ramat-Gan 52900, Israel

A. Korngreen (✉)
Faculty of Life Sciences, Bar-Ilan University,
Ramat-Gan 52900, Israel
e-mail: korngra@mail.biu.ac.il
Tel.: +972-3-5318224
Fax: +972-3-5352184

neuronal membrane. The problems arising from an incomplete space-clamp were addressed extensively [4, 5, 39, 44, 49, 63, 64, 73, 79, 97, 106]. It has, therefore, been widely accepted that voltage-clamp recordings from neuronal somata and dendrites are distorted in a way that makes it difficult, if not impossible, to directly determine the properties of a local voltage-gated conductance even when considering small neurons. Moreover, it is extremely important to stress that even short dendrites can severely distort the currents recorded after the activation of a voltage-gated conductance (see Fig. 1 in [27] for a colorful display of the problem).

We have recently developed a simple numerical algorithm that corrects space clamp distortions of voltage-gated K^+ currents [89]. The method is based on a stepwise estimation of the local conductance density at the site of a local voltage-clamp by fitting space-clamp-distorted currents with simulated ones. We have further shown that the method enables the accurate retrieval of local somatic and dendritic densities as well as the kinetics for voltage-gated K^+ conductances. Additionally, the method could precisely calculate the conductance gradients as well [89]. Nevertheless, it is important to note that our technique does not actually solve the problem of inadequate space clamp, but

rather takes it into account. Consequently, it provides information on the conductance density and kinetics only at the direct vicinity of the recording site. Moreover, because the method cannot account for voltage changes during regenerative events, generated by voltage-gated Na^+ and Ca^{2+} conductances, it is only applicable for nonregenerative voltage-gated currents such as voltage-gated K^+ channels.

Considering all the aforementioned complications, the cell-attached and the excised modes of the patch-clamp technique are still the primary modes of choice for the investigation of dendritic voltage-gated channels in acute brain slice preparations. The previous decade has therefore seen these recording modes being applied in the investigation of dendritic voltage-gated K^+ channels [6, 8, 11, 16, 37, 45, 50, 67, 68], inward rectifier K^+ channels [15, 104], voltage-gated Na^+ channels [11, 19, 50, 56, 58, 59, 61, 82, 83, 93], voltage-gated Ca^{2+} channels [40, 59], and the nonselective hyperpolarization-activated cation channel I_h [10, 57, 108]. Some of these studies revealed non-homogenous distributions of voltage-gated channels along dendrites. These discoveries are in no small part responsible for the recent intense research activity of the computational properties of dendrites [22, 28, 44, 51, 54,

Fig. 1 Diversity of K^+ currents in somatic and dendritic cell-attached patches. **a**, Averaged outward currents in cell-attached somatic patches from large layer 5 cortical pyramidal cells show patch-to-patch kinetic diversity. In these recordings, linear leak and capacitance currents have been subtracted, and the electrode solution contained 1 μ M TTX to block voltage-gated sodium channels. Reproduced with permission from [6], copyright Blackwell Publishing.

b Averaged outward currents in cell-attached somatic patches from the apical dendrite of CA1 hippocampal pyramidal neurons. The ensemble current traces have been recorded from dendritic patches at 220 ± 3 μ m from soma. K^+ currents were activated by a voltage step from approximately -96 mV to $+54$ mV, lasting 400 ms. Reproduced with permission from [16], copyright Blackwell Publishing.

60, 70, 81, 98, 101]. However, in the rush to get results, the applicability of the patch-clamp recording modes to the extraction of dendritic gradients of ion channels was never fully assessed.

The cell-attached configuration enables the recording of currents from ion channels that are still exposed to the cytoplasm, retaining modulations of the channels by intracellular mechanisms intact [26]. However, application of pharmacological agents to the patch is difficult and the absolute membrane potential across the patch is not known [85]. Moreover, because it is not possible to visually measure the patch area, it is practically impossible to provide an estimate of the conductance density of the measured current. This problem is amplified when one attempts to record from dendritic voltage-gated ion channels. Typically, dendritic recordings require using a patch-pipette with a diameter that is similar to or smaller than the diameter of the dendrite. This limits the resistance of the pipettes to 8–15 M Ω that is traditionally used for single channel recordings. It is therefore not surprising that many investigations of dendritic voltage-gated ion channels have reported single channel currents. Thus, in an attempt to improve the signal to noise ratio in cell-attached recordings and provide a quantitative estimate of the current, investigators averaged many repeating sweeps to produce ensemble currents. These ensemble currents have been interpreted as providing an indication of the density of channels at the soma and along the dendrites. Even if one ignores the fact that the surface of the patch is not known, this approach carries several caveats. First, the use of small electrodes considerably increases the scatter of results from patch to patch (Fig. 1). For example, voltage-gated K⁺ currents recorded from somatic cell-attached patches performed at the soma of several layer 5 cortical pyramidal neurons [6] display much kinetic diversity, indicating that each patch contained different numbers of fast and slow inactivating voltage-gated K⁺ channels (Fig. 1a). Similar results have been obtained in dendritic patches from CA1 hippocampal pyramidal neurons (Fig. 1b) where a large variability of the kinetics was observed from patch to patch [16]. This kinetic diversity can and has been used to identify and isolate the types of voltage-gated channels expressed in the soma and apical dendrites of several types of neurons in the CNS [6, 10, 11, 15, 16, 37, 57, 59, 61, 67, 68, 103, 108]. However, this diversity greatly impairs the estimation of the changes of the conductance density along the apical dendrite. Simple simulations, taking into account the diameter of the patch pipette and the membrane channel density, predict that such measurements, in which one or two and occasionally no channels are present in the cell-attached patch, will underestimate the average current from a given membrane area by up to 50%. The scatter typically obtained from cell-attached recordings is shown in Fig. 2a (displaying the peak ensemble currents obtained along the apical dendrite of layer 5 neocortical pyramidal neurons from 2-week-old rats [45]).

Some investigations of dendritic gradients of voltage-gated ion channels used outside-out patches. This mode of the patch-clamp technique has the advantage that the

Fig. 2 Distributions of dendritic voltage-gated K⁺ channels show similar variability in several cell types. **a** Peak amplitude of the average current as a function of the distance from the soma of layer 5 pyramidal neurons ($\circ$). Voltage steps to +140 mV above the resting membrane potential generated the currents. The smooth line is a linear regression that passed through the data points (somatic and dendritic). Reproduced with permission from [45], copyright Blackwell Publishing. **b** K⁺ peak current in outside-out patches recorded from the apical dendrite of mitral cells in the olfactory bulb plotted against distance from the soma (30 mV). The filled circle indicates the mean K⁺ current at the soma. Reproduced with permission from [11], copyright Blackwell Publishing. **c** Magnitude of peak potassium current (step from -90 to 70 mV) in outside-out patches extracted from Purkinje neurons, plotted as a function of distance from the soma from which the patch was formed (*closed circles*). To facilitate the comparison, only data obtained with 7–11 M Ω pipettes are plotted. The *filled circle* indicates mean $\pm$ SEM for patches from the cell body with the same range of electrode resistances. Reproduced with permission from [68], copyright 2003 by the Society for Neuroscience

external side of the channels is exposed to the bath solution allowing rapid application of drugs and neurotransmitters [26] and absolute control of the membrane potential by the patch clamp amplifier. However, ripping the membrane from the cell often results in a considerable rundown of the currents in the patch and sometimes distortions of the currents due to missing intracellular regulatory pathways [18]. It has been observed that cell-attached and outside-out patches produce similar current amplitudes of dendritic voltage-gated K^+ channels in the apical dendrite of L5 neocortical pyramidal neurons [6], suggesting that both recording modes sample similar membrane areas. Therefore, it is possible to compare distributions of voltage-gated K^+ currents obtained from cell-attached recordings in L5 pyramidal neurons (Fig. 2a) [45] with distributions obtained using outside-out recordings in mitral cells of the olfactory bulb (Fig. 2b) [11] and Purkinje neurons of the cerebellum (Fig. 2c) [68]. The apparent mean current obtained in the recordings increases from L5 pyramidal neurons through mitral cells to Purkinje neurons (Fig. 2). However, it seems that the scatter remained similar. It is therefore possible to conclude that similar inhomogeneity exists on the surface of outside-out patches as in cell-attached patches. Finally, the membrane of dendritic spines is not regularly accessible to the patch-clamp technique.

Therefore, the contribution of spines to regenerative activity in dendrites remains unknown.

While on the subject of outside-out patches, and although not directly related to dendritic recordings, it is interesting to mention a newcomer to the patch-clamp family: the nucleated outside-out patch [87]. This recording configuration, as its name implies, can be performed only at the soma. Initially, a whole cell recording is established at the soma using the blow and patch technique (Fig. 3a1). Afterwards, negative pressure is applied to the pipette (100–200 mbar above ambient pressure—depending on the diameter of the patch pipette and size of the neuron) which draws the nucleus towards the pipette. A slow and smooth retraction of the pipette then brings with it a large portion of the somatic membrane covering the nucleus (Fig. 3a2). Once the nucleus is free from the cell, the plasma membrane closes behind it (Fig. 3a3) forming an almost spherical and very large outside-out patch (Fig. 3a4). At this point, it is useful to reduce the pressure applied to the pipette to a few millibar above ambient pressure. The nucleated patch usually produces large ionic currents (Fig. 3b,c) that are amenable to pharmacological manipulations and had been successfully used to investigate the properties of various voltage- and ligand-gated channels. Due to the patch's small diameter, there is little filtration owing to the membrane capacitance allowing for very rapid recording

Fig. 3 The making of a somatic nucleated outside-out patch.

a Several stages in the extraction of a nucleated patch from a layer 5 neocortical pyramidal neuron: immediately before release of positive pressure and the establishment of the whole-cell configuration (**a1**), after the whole-cell configuration was established and the retraction of the patch pipette has started (**a2**), the closing of the plasma membrane behind the extracted nucleus (**a3**), and the final nucleated patch (**a4**). The scale bar applies to all four panels.

b Voltage-gated K^+ currents recorded from a nucleated patch extracted from a layer 5 pyramidal neuron. The bath solution contained 100 nM TTX to block voltage-gated Na^+ currents. Sampled at 10 kHz and filtered at 5 kHz.

c Voltage-gated Na^+ currents recorded from a nucleated patch extracted from a layer 5 pyramidal neuron. The pipette solution included CsCl instead of KCl and the bath solution contained 10 mM TEA and 4 mM 4-AP to block voltage-gated K^+ currents. Sampled at 50 kHz and filtered at 10 kHz.

of voltage gated K^+ (Fig. 3b) and Na^+ currents (Fig. 3c). Nonetheless, one should keep in mind that the nucleated patch is an outside-out patch; care should, therefore, be taken to determine the extent of channel rundown. We have observed that the conductance density of voltage-gated K^+ channels calculated from nucleated patches extracted from L5 pyramidal neurons [7, 45] was considerably lower than what is needed for the production of a functional compartmental model of these neurons, suggesting substantial current rundown during patch extraction (S. Bergling and A. Korngreen, unpublished observations).

In summary, dendritic and somatic cell-attached and outside-out patches can be readily used for single channel recording [16] and ensemble current analysis when multiple voltage-gated currents are present in the patch. These recording modes can, however, provide only relative information about the distribution of ion channels along dendrites. Thus, they are probably not a good recording mode for the estimation of conductance density gradients along dendrites due to the large variability in pipette size and, subsequently, in the patch surface area. Furthermore, the scatter resulting from the measuring technique obscures the natural scatter of the conductance density between cells preventing us from obtaining a clear picture of the natural variance.

Analysis

Having worked hard and obtained those precious recordings of voltage-gated currents from dendritic and somatic patches, we may not rest on our laurels—it's time to sit down for analysis. Two general analysis pathways are available to the investigator, the first of which is single-channel analysis. Single-channel analysis has made great advances since the publication of the paper of Hamill et al. [26]. Not only is the theoretical basis for the analysis well established [86], but several analysis tools are freely available for download on the World Wide Web as well. Nevertheless, many investigators of voltage-gated ion channels in acute brain slices shy from performing single-channel recording and analysis for several reasons. First, due to the shallow angle of approach imposed by the water immersion objective, the pipette is usually deeply immersed in the bath solution resulting with a high pipette capacitance that can only be partially eliminated by coating the pipette. Second, acute brain slices require quite rapid fluid perfusion to keep a high level of oxygenated solution around the slice. The mechanical noise introduced by the fluid inlet and suction outlet often makes single channel recording a nightmare. Third, contrary to patch-clamp setups designed for single-channel recordings, the setup designed for brain slice recordings contains a considerable number of electronic noise sources (IR cameras, TV monitors, motorized manipulators, and computers) that must be turned off during the recording. Fourth, while single-channel analysis has been made simpler in recent years, it still carries with it many possible error sources [62]. Finally, single-channel analysis of voltage-gated

channels is somewhat more complex than analysis of steady-state channel activity requiring the production of first latency histograms and other considerations [96].

Thus, most kinetic analyses of dendritic voltage-gated ion channels in the preceding decade have been performed on ensemble currents obtained from cell-attached or outside-out patches. In some cases, like in layer 5 pyramidal neurons from the somatosensory cortex (Fig. 2a), the currents are small requiring considerable averaging [6, 45]. In other cell types, for example in Purkinje neurons (Fig. 2c), the currents are large enabling analysis even without averaging repeating traces [68]. Disregarding the current amplitude issue, almost all investigations have applied the analysis paradigm established by Hodgkin and Huxley [34]. At the practical level, this celebrated analysis methodology proceeds in several simple steps. First, one must construct steady-state activation (and inactivation if required) curves of the investigated conductance. Then, these curves are used to estimate the number of activation gates in the Hodgkin–Huxley-like model that best fits the investigated conductance. Armed with the number of gates, one can now fit the current traces, one by one, to the model and secure the activation (and inactivation) time constants. This procedure has not changed considerably for some decades. Indeed, on many occasions, we have often found that the original papers by Hodgkin and Huxley [31–35] are a better information source than modern papers. However, this analysis procedure may introduce severe errors in the estimation of the kinetic constants of the investigated channel, especially if the activation and inactivation time constants are not sufficiently different [110]. The source of the problem resides in the separate (or disjoint as suggested in [110]) analysis of the steady-state and kinetic properties of the current. This severe error source can be overcome by simultaneous curve fitting of a Hodgkin–Huxley-like model to a data set including current traces recorded at several voltages [109, 110]. Direct fitting of a model to a set of current traces was also recently introduced into the single-channel analysis software QUB [71] allowing fitting of Markov chain models to whole-cell currents. Still, it is important to remember that by fitting a full model to a set of data the number of free parameters increases dramatically. To complicate the problem, standard curve fitting algorithms (such as the Simplex and gradient descent algorithms among others) have the inclination of settling into a local minimum when dealing with many parameter problems [77]. Thus, to verify that the obtained solution is not a local minimum, it is important to select initial values that are sufficiently close to the global minimum. Alternatively, instead of guessing a set of initial values that may lead the minimization algorithm to a local minimum, it is possible to apply stochastic minimization algorithms to search a predefined parameter space. Several popular stochastic minimization tools such as genetic algorithms [72] and simulated annealing [42] have been successfully applied to many problems of nonlinear curve fitting in biology. These algorithms usually perform tens to hundreds of thousands of iterations to find the global minimum. A few years ago, such calculation

would have required the services of a small supercomputer. Nowadays, such calculations can be performed within a reasonable time frame on almost any desktop computer.

Function

Why are we so insistent on accurate recording and analysis of dendritic, somatic, and axonal voltage-gated channels? The legacy of Hodgkin and Huxley dictates that only detailed kinetic analysis, extraction of conductance densities, and construction of a numerical model can provide a biophysical explanation of the function of voltage-gated ion channels. The success of the Hodgkin–Huxley model is due in no small part to the simplicity of the biological system because the squid giant axon expresses two voltage-gated ion channels in its membrane. However, neurons in the CNS express often more than ten voltage-gated ion channels making the extraction of a clear function for each of these conductances a daunting task [53]. Furthermore, channel kinetics are not fixed but can be modulated by neurotransmitters and second messenger systems and have, therefore, different properties in different locations along the dendrite [36]. Moreover, the renewed interest in the active properties of dendrites has led to a reassessment of the computational properties of dendrites as detailed in several excellent recent reviews [29, 54, 70, 81, 101, 105]. This has greatly enhanced a problem investigators of neuronal excitability have been facing for some time now—the proper construction of a numerical model for central nervous system neurons.

One prominent example (which is very close to our hearts) of the extent of the problem is personified by layer 5 pyramidal neurons of the somatosensory cortex. In addition to sustaining active backpropagation of the action potential regenerative Ca^{2+} spikes have been recorded from the apical dendrite of L5 pyramidal neurons [1, 46–48, 91, 112]. In these neurons, when a backpropagating action potential coincides with distal synaptic input, a dendritic Ca^{2+} spike is generated, which leads to the generation of a burst of action potentials at the soma [48]. Under some conditions, Ca^{2+} spikes may be isolated to the dendrites, while under others, the spikes may spread to the soma [46, 91, 92, 94, 95]. It is also important to mention that action potential bursts can be triggered by the proximal apical dendrite as well as the distal apical dendrite [47, 107]. Moreover, depolarization of the proximal apical dendrite facilitates the forward propagation of dendritic Na^+ and Ca^{2+} spikes [47]. Thus, regenerative dendritic events change the output of L5 pyramidal neurons from a single to a burst of action potentials inducing a change in the informational content of the neuronal output [52].

The aforementioned studies have shown that the initiation and propagation of backpropagating action potentials and dendritic Ca^{2+} spikes in L5 neurons are complex events that are probably the result of the synchronized activation of several types of voltage-gated channels. In the previous decade, much has been learned about the types and distributions of voltage-gated channels in the apical

dendrite of L5 pyramidal neurons. The density of voltage-gated Na^+ channels was reported to be constant along the apical dendrite [103]. We have observed three types of voltage-gated K^+ channels and have shown that the total density of voltage-gated K^+ channels decreases along the apical dendrite of L5 pyramidal neurons [45]. Stimulating information indicated that the density of an A-type K^+ conductance increased along the apical dendrite [6]. Inward rectifier K^+ channels have been observed in the apical dendrite but their spatial distribution is currently unknown [104]. The density of the nonselective cation current I_h was shown to increase as a function of the distance from the soma [10, 108]. Large conductance Ca^{2+} dependent K^+ channels have been observed to have a homogenous distribution in somata and apical dendrites of L5 neurons [9]. In spite of the prime importance of understanding dendritic Ca^{2+} spikes, little information is currently available on the dendritic distribution of voltage-gated Ca^{2+} channels along the apical dendrite.

Much of what is known about the types of voltage-gated Ca^{2+} channels in neocortical pyramidal neurons comes from whole-cell recordings of dissociated neurons. These recordings revealed that neocortical pyramidal neurons express at least five high-voltage-activated Ca^{2+} conductances: L-, N-, P-, Q-, and R-type [13, 14, 20, 21, 25, 55, 69, 99, 111] and one low-voltage-activated Ca^{2+} conductance [25, 88]. Imaging of intracellular Ca^{2+} concentration in combination with pharmacological blockers has shown that similar types of voltage-gated channels are expressed in the proximal apical dendrite of L5 pyramidal neurons [65, 66]. More recently, Ca^{2+} imaging experiments in combination with glutamate ionophoresis have indicated that the density of voltage-gated Ca^{2+} channels along the apical dendrite may be constant [75]. Finally, the basal dendrites of L5 pyramidal neurons have been shown to produce NMDA-induced regenerative activity [90] that may dynamically segment these dendrites into multiple summation elements [76].

Thus, there are about a dozen voltage-gated ion channels expressed in L5 pyramidal neurons, some displaying varying conductance densities along the apical dendrite. In the spirit of the Hodgkin and Huxley model, having obtained a kinetic description of the conductances, one should now perform numerical simulations to reproduce the physiological system and extract predictions of the function of the various voltage-gated conductances. This can be performed with relative effectiveness using compartmental modelling [78]. There are several freely available simulation packages that combine the physical rules of passive cable theory [38], the chemical kinetics rules of ion channels, and morphological description of neurons. The two most popular simulation environments are NEURON [30] and GENESIS [12]. Repeating the simulations of the action potential propagation in the giant squid axon is extremely easy with these simulation tools. However, constructing a detailed model of any neuron in the central nervous system, especially a neuron with dendritic conductance gradients where some of the parameters of the model are still not constrained by experiment, is a very daunting task. All of

us that have used compartmental modelling are familiar with the huge frustration accompanying this work. It often takes weeks and months of intense work to construct a functional model of any neuron. One sits at the computer trying seemingly endless sets of parameters until something reminiscent of the physiological response emerges. At this point, researchers are so sick to their stomachs with the whole affair that they are willing to accept any semi-reasonable backpropagating action potential or dendritic Ca^{2+} spike.

This procedure, that is, a person performing iterations using heuristics to modify the free parameters of the model, is highly problematic. To begin with, humans tire, change their view of the problem from one iteration to the next and eventually abandon the optimization process before its completion. Secondly, even if a strict score procedure is maintained, there exists no proof that the human eye and judgment are adequate tools for the optimization of compartmental models. Indeed, it has been recently shown that action potential trains, most investigators would have approved of, can be produced from parameter sets that are far removed from the true parameter set [41]. In short, your eyes can and will deceive you.

With this problem in mind, we have recently applied a genetic minimization algorithm and a reduced compartmental model of a layer 5 neocortical pyramidal neuron and compared the efficacy of several fit functions to constrain the model [41]. Using only somatic recordings of the membrane potential was not a sufficient condition for constraining compartmental models containing nonhomogeneous distributions of ion channels. Many of the minimizations carried out using only somatic target data sets displayed considerable deviations in the values of parameters related to the axonal initiation zone in some cases and to the dendrite in others [41]. It is interesting to note that although the parameters deviated from the true parameters, the membrane potential traces at the soma displayed a nice fit emphasizing again that visual fit of somatic traces is not a good criterion for constraining compartmental models. Only when dendritic and axonal recordings of the membrane potential were added to the data set was it possible to obtain a good approximation of the target parameter set [41]. Admittedly, this approach requires recordings from many locations along the dendritic and axonal tree and carries with it a heavy computational toll. Nevertheless, dendritic and axonal measurements of the backpropagating AP have been performed for over a decade [101]. However, simultaneous recordings of the membrane potential from five locations, including axonal recordings, using patch electrodes have yet to be performed. A solution to recording the membrane potential from many locations may lay in voltage-sensitive dyes [2, 3]. However, voltage sensitive dyes possess several downfalls including toxicity, lack of sensitivity (e.g., cell staining often leads to ambiguity as to which cell population generates the registered signal), and problematic quantification (that is, poor signal-to-noise ratio from individual cells). To counter these problems, a new method, similar in many respects to voltage-sensitive dyes, has been developed in recent years

—genetically encoded voltage-sensitive fluorescent proteins [23, 24, 43, 80, 84]. This method is beneficial for voltage-sensitive fluorescent proteins can be attached to voltage-dependent ion channels and fluoresce as the channel changes its conformation in response to changes in membrane potential. However, this method holds several pitfalls. To begin with, VSFPs are very sensitive to changes in intracellular pH (GFP and to a lesser extent YFP) and the hopes of deliverance from DsRed, which is virtually insensitive to pH, have yet to be met. Secondly, voltage-sensing fluorescent proteins exhibit low voltage sensitivity ($\sim 1.5\%/100$ mV). Finally, it is not known what kind of interactions do voltage-sensitive fluorescent proteins have with endogenous proteins or channel subunits. As for the computational overhead, within 15 years, desktop computers have advanced from a clock speed of 33 kHz to 3.3 GHz. With the advent of parallel computing, there should be no problem to perform quite massive simulations for constraining compartmental models in the very near future.

Concluding remarks

As reductionists, we strive to explain the computational powers of the nervous system from the bottom up. For us, this means first to understand the computational properties of single neurons. In recent years, it has become clear that single neurons are much richer in their computational properties and cannot any longer be regarded as simple integrate-and-fire devices. We will be making real progress once we will be able to relate a given configuration of dendritic/somatic/axonal voltage- and ligand-gated channels to a given type of logical operation occurring in that segment of the neuron. In this review, we attempted to point at some of the technical and methodological considerations that stand in our way to this goal. Still, the road goes ever on and there is much more recording, analysis, and functional simulations all of us need to perform before we can understand the computational abilities of single neurons.

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