Invited commentary

Probing dendritic function with patch pipettes

Greg Stuart and Nelson Spruston

Max-Planck-Institut für medizinische Forschung, Heidelberg, Germany

Most neurons in the CNS have complex, branching dendritic trees, which receive the majority of all synaptic input. As it is difficult to make electrical recordings from dendrites because of their small size, most of what is known about their electrical properties has been inferred from recordings made at the soma. By taking advantage of the higher resolution offered by improved optics, it is now possible to make patch-pipette recordings from the dendrites of neurons in brain slices under visual control. This new technique promises to provide valuable new information concerning dendritic function.

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Introduction

In an attempt to understand the electrical properties of the dendritic tree, Rall (see [1]) developed theoretical models of dendrites based on cable theory. These pioneering studies led to a basic understanding of the spread of voltage within a dendritic tree. It became clear, however, that an accurate, quantitative understanding of dendritic function would require experimental data from dendritic recordings. The first intracellular electrical recordings from dendrites were made from cerebellar Purkinje cells by Llinás and his colleagues [2,3] using sharp microelectrodes. From this and other experimental work (e.g. [4,5]), the picture emerged that dendrites of CNS neurons do not act as purely passive receivers of synaptic input, but are electrically active.

In addition, differences in dendritic electrophysiology were observed between neuronal types, suggesting that the distribution of various voltage-activated channels in the dendritic membrane differs between classes of neurons [6]. These differences, together with differences in dendritic morphology, presumably play a critical role in shaping the functional diversity of CNS neurons. Differences in the nature of synaptic currents, even at synapses utilizing the same neurotransmitter, have also been identified, due, in part, to the diversity in the properties of the postsynaptic transmitter-activated channels (see [7]).

A full understanding of dendritic function will therefore require a detailed description of the types, distributions, and densities of voltage- and transmitter-activated channels in the dendrites of CNS neurons, together with an understanding of the role these channels play in the initiation and spread of synaptic and regenerative potentials within the dendritic tree. The ability to make patch-pipette recordings from the dendritic membrane now enables cellular neurophysiologists to begin to address some of these issues. Here we highlight some new findings obtained with this technique and suggest other possible applications.

Techniques for making dendritic patch-pipette recordings in brain slices

Using techniques previously developed for making patch-pipette recordings from the soma of neurons in brain slices [8], the first patch-pipette recordings from dendrites in brain slices were made from the proximal dendrites of cerebellar Purkinje cells using standard light microscopy [9]. Improved resolution of dendrites can now be obtained by using an infrared (IR) light source in combination with differential interference contrast (DIC) optics: IR-DIC microscopy [10]. With this increased optical resolution, it is possible to visualize dendrites of small diameter (~ 1 μm) in living brain slices, and to record from them by placing a patch pipette directly onto the dendritic membrane under visual control. This technical advance greatly increases the ability to record with patch pipettes from the dendritic membrane (Figs 1a,2a; for details, see [11,12]).

To make a dendritic patch-pipette recording, positive pressure is applied to the back of a patch pipette as it is advanced through the brain slice. Once the pipette tip is seen to touch the dendritic membrane, the pressure is released and a slight suction is applied. In most attempts,

Abbreviations

AMPA—L-α-amino-3-hydroxy-5-methyl-4-isoazolepropionate; **CNS**—central nervous system; **DIC**—differential interference contrast; **EPSP**—excitatory postsynaptic potential; **GABA**—γ-aminobutyric acid; **IR**—infrared; **NMDA**—*N*-methyl-D-aspartate.

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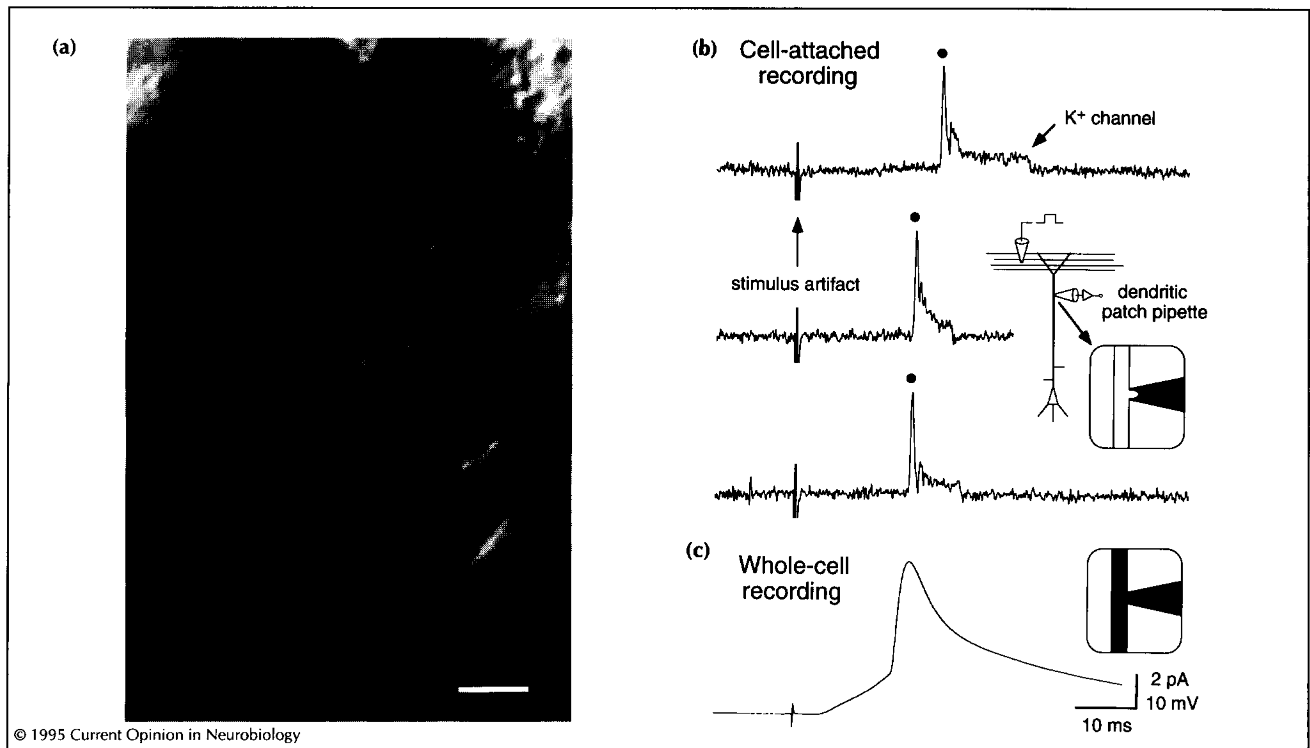

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Fig. 1. Patch-pipette recordings from dendrites of neurons in brain slices. **(a)** IR-DIC image of a neocortical pyramidal neuron dendrite during dendritic patch-pipette recording (290 µm from the soma). Scale bar is 5 µm. **(b)** Cell-attached patch-clamp recordings of single-channel activity induced by synaptically evoked action potentials (the arrow denotes the stimulus artifact). The dendritic membrane patch was held at the resting membrane potential and the pipette solution contained normal extracellular solution. In each trace, an action potential evokes a transient, outward capacitive current (●) that is followed by outward, single-channel potassium currents. Note that the action potential is evoked at different times after the stimulus artifact in the different sweeps. The inset shows a schematic diagram of the experimental arrangement showing a dendritic cell-attached recording. **(c)** An EPSP-evoked action potential recorded after rupture of the membrane patch in (b) to obtain a dendritic whole-cell recording (170 µm from the soma). The dendritic resting potential was −58 mV. The inset shows a schematic diagram of a dendritic whole-cell recording. Scale bar in (c) also applies to (b). Recordings of the type shown in this figure can be used to monitor the activity of dendritic voltage-activated channels during physiological stimuli.

this results in the formation of a high resistance (>5 GΩ) seal of the patch-pipette tip with the dendritic membrane. Using this technique, recordings can regularly be made from dendrites as small as 1 µm in diameter, from a variety of different neuronal types. Currents can then be recorded in cell-attached mode (Fig. 1b), or the membrane patch can be ruptured by a brief pulse of suction to obtain a whole-cell recording (Fig. 1c). Confirmation that the whole-cell recording is in fact from the patched dendrite can be obtained by filling the cell with a fluorescent dye (Fig. 2b). Following the formation of the whole-cell configuration, withdrawal of the patch pipette almost always results in the formation of an outside-out patch (Fig. 2c).

Advantages of dendritic patch-pipette recordings

The patch-clamp technique offers a number of advantages over sharp microelectrode recordings as a means of probing dendritic function. As both the tip of the patch pipette and the dendrite to be patched can be seen, recordings can be made under visual control, from identified structures. The high-resistance seal between the patch-pipette tip and the cell membrane both electrically isolates the dendritic membrane patch and reduces the background noise to levels where single-channel events can be detected in cell-attached or excised patches (Figs 1b,2c). In the whole-cell recording configuration (Fig. 1c), the high-resistance seal eliminates problems associated with electrical shunts between the recording pipette and the cell membrane that exist with sharp microelectrodes.

Two basic types of dendritic recordings are possible: those examining the types, properties, and distribution of ion channels in isolated patches; and those measuring current or voltage in the whole-cell configuration. Variations on these possibilities, as well as combining dendritic patch recording with other technology (e.g. fast agonist application to excised patches or fluorescent imaging techniques), promise to yield a wealth of new information concerning the function of dendrites.

Distribution and properties of dendritic channels

One of the obvious applications of dendritic patch clamping is to use it to directly assess the distribu-

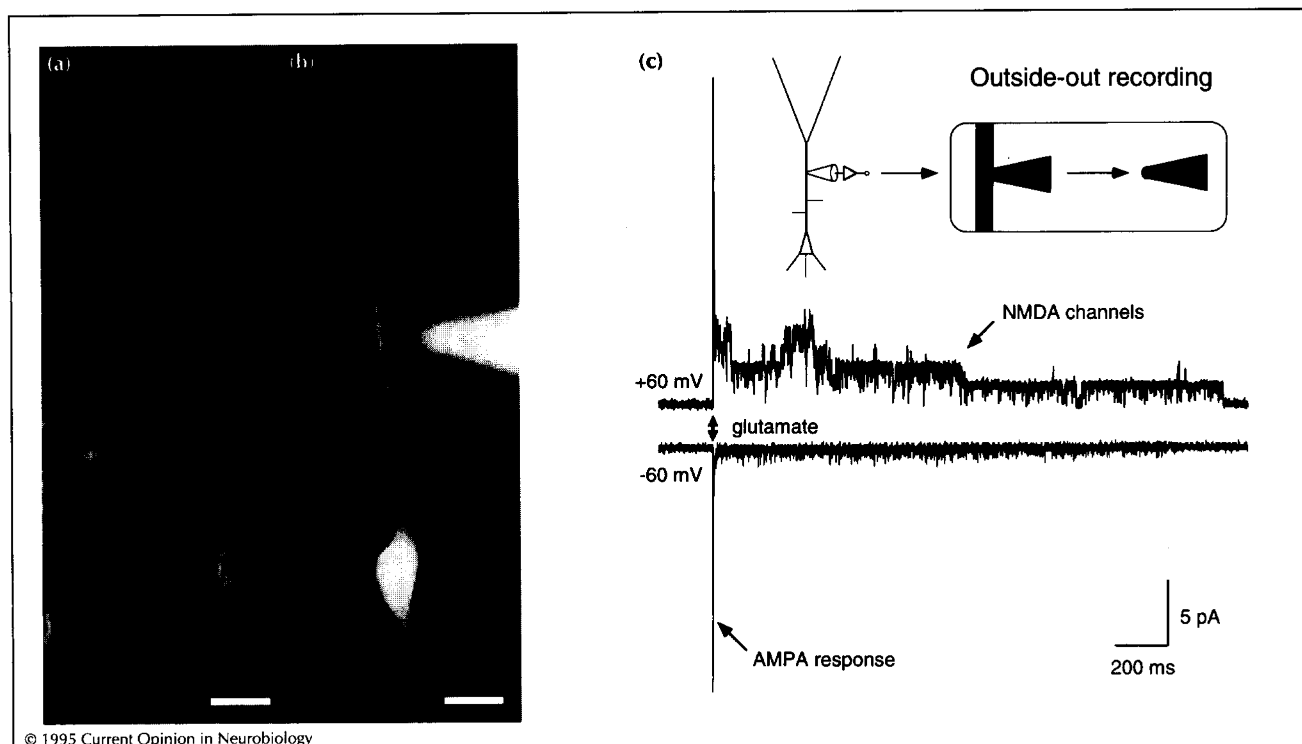

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Fig. 2. Fast agonist application to outside-out patches from the dendrites of hippocampal pyramidal neurons. **(a)** IR-DIC image of a CA1 pyramidal neuron during a patch-pipette recording 70 μm from the soma. Scale bar is 10 μm. **(b)** Fluorescent image of the same neuron filled with Lucifer yellow via the dendritic patch pipette. Scale bar is 20 μm. (a) and (b) adapted from [20]. **(c)** Dendritic AMPA and NMDA receptor channels were activated by 1 ms pulses of 1 mM glutamate (time of application indicated by double arrowheads), at holding potentials of +60 mV and –60 mV, in the presence of 1 mM Mg^{2+} and 10 μM glycine. The dendritic patch was taken 80 μm from the soma of a CA3 pyramidal neuron. The peak of the early transient current, mediated by AMPA receptor channels, is relatively voltage independent (with a reversal potential of –5 mV), whereas the NMDA channel activity occurring later in the traces is blocked in a flickering manner at the negative potential. The inset shows a schematic diagram of the experimental arrangement depicting the formation of a dendritic outside-out patch. Recordings of this type have been used to characterize the properties of glutamate-activated channels in the dendrites of hippocampal pyramidal cell dendrites (see [20]).

tion and properties of channels in the dendritic membrane. To date, both cell-attached and outside-out patch-clamp recordings have been made from the dendritic membrane of a variety of neuronal cell types. These recordings have provided valuable information concerning the distribution and properties of both voltage- and transmitter-activated dendritic channels.

Dendritic voltage-activated channels

The distribution and properties of dendritic voltage-activated sodium channels have now been examined in both neocortical [13,14] and hippocampal [15] pyramidal cells, cerebellar Purkinje cells [16], and substantia nigra dopamine neurons (M Häusser, G Stuart, unpublished data). In neocortical and hippocampal pyramidal neurons, both the density and properties of dendritic sodium channels are similar to those of somatic sodium channels [13–15]. In contrast, the density of sodium channels in the dendrites of cerebellar Purkinje cells is markedly lower than at the soma [16].

In hippocampal pyramidal neurons [15] and cerebellar Purkinje cells [9], the distribution and properties of dendritic voltage-activated calcium channels have also been determined. The soma and dendrites of Purkinje cells appear to contain largely high-threshold P-type calcium channels [9]. In contrast, the dendrites of hippocampal pyramidal neurons contain largely low-threshold T- and R-type calcium channels, whereas at the soma, high-threshold L- and N-type calcium channels predominate [15].

To date, little is known about the dendritic distribution or properties of other channel types, such as voltage- and/or calcium-activated potassium channels. In cerebellar Purkinje cells, however, there seems to be a slight decrease in voltage-activated potassium channel density with distance from the soma (G Stuart, M Häusser, unpublished data).

In addition to determining the properties and distribution of voltage-activated channels, it is also important to examine under which conditions these channels are activated by physiological events. Somatic action potentials have been shown to activate dendritic sodium channels [14,17,18], calcium channels [18], and potassium channels (see Fig. 1b). Dendritic sodium channels boost the amplitude of action potentials as they 'backpropagate' into the dendritic tree of some neurons (see below). Also, Magee and Johnston [18] have demonstrated that dendritic sodium and calcium channels can be activated by subthreshold excitatory postsynaptic potentials

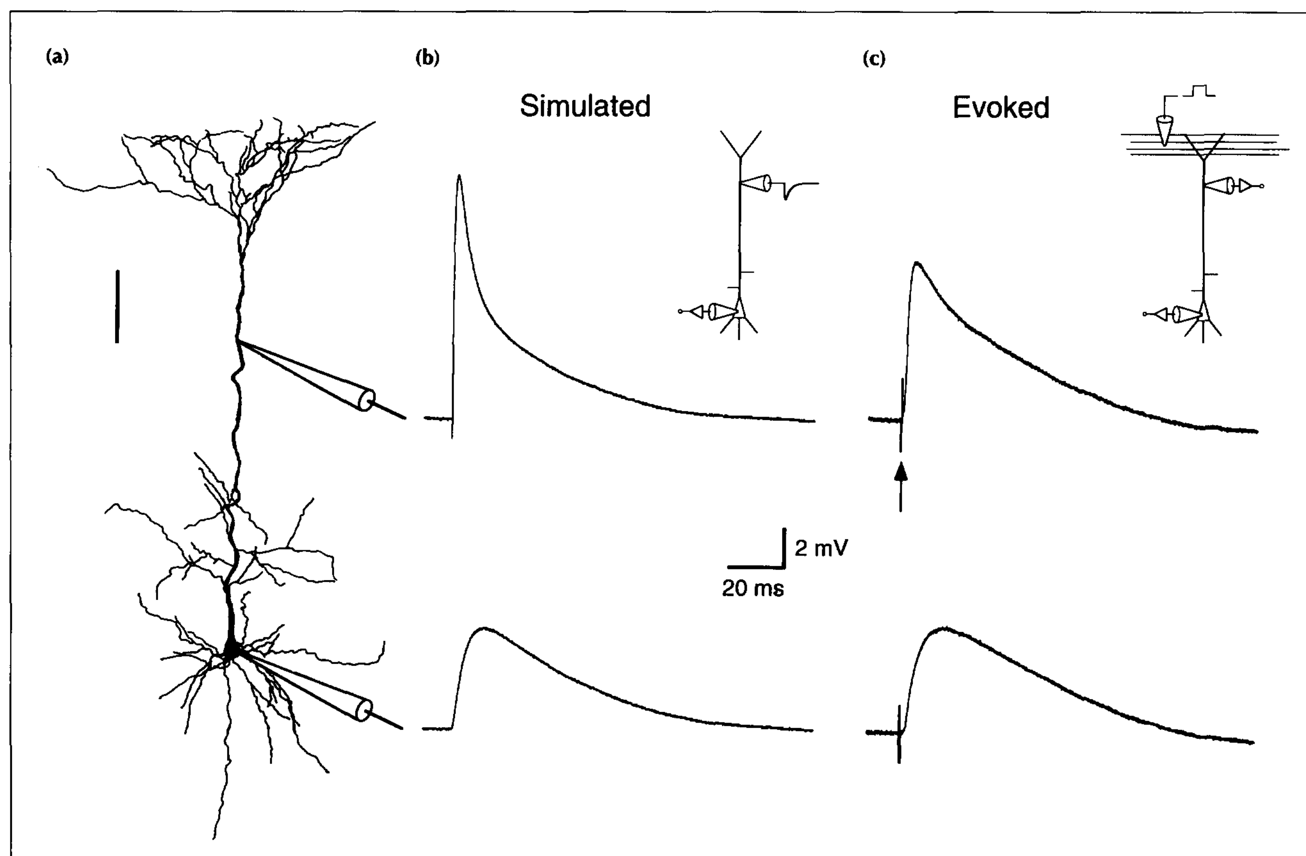

Fig. 3. Spread of simulated and stimulus-evoked EPSPs from the dendrites to the soma. **(a)** Camera lucida drawing of a neocortical pyramidal neuron, indicating the approximate location of the two recording pipettes. Scale bar is 100 μm. **(b)** Simulated EPSPs recorded simultaneously at the soma and the dendrite following current injection through a dendritic electrode (425 μm from the soma) to simulate a dendritic synaptic event (500 pA peak, 0.3 ms rise time constant, 3 ms decay time constant). Note the attenuation of the simulated EPSP as it propagates to the soma. **(c)** Stimulus-evoked EPSPs measured at the same dendritic and somatic locations as in (b) — the arrow denotes the stimulus artifact. Note the similarity between the somatic stimulus-evoked and simulated EPSPs. The somatic and dendritic resting potentials in (b) and (c) were –60 mV. Scale bar applies to both (b) and (c). The insets are schematic diagrams of the experimental arrangements. Experiments of this type can be used to both mimic the voltage change that occurs during an EPSP and to investigate the spread of electrical signals within the dendritic tree.

(EPSPs), suggesting a role for these channels in enhancing the propagation of EPSPs from the dendrites to the soma.

Dendritic transmitter-activated channels

One approach that has been used to study the distribution and properties of dendritic transmitter-activated channels is to mimic synaptic transmitter release by using fast application of agonists (see [19]) to outside-out patches from different dendritic locations (Fig. 2c). This approach has been used to study glutamate receptor channels in both hippocampal pyramidal neurons [20] and cerebellar Purkinje cells [21]. Both studies have found no apparent differences between the properties of somatic and dendritic glutamate-activated channels in these neurons. Interestingly, patches taken from the region of mossy fibre termination on CA3 pyramidal neurons have revealed that, in contrast to previous binding studies [22,23], this region contains NMDA receptor channels, as well as AMPA receptor channels [20].

Mapping receptor and channel distribution by local application of substances to the dendritic membrane

The ability to visualize dendrites using IR-DIC videomicroscopy provides the possibility to locally apply substances, via iontophoretic or pressure application, to defined regions of the dendritic tree [24]. The use of a dye, such as fast green, in the locally applied solution can be used to monitor the extent of this application [25]. In general, local application of agonist or antagonists offers the advantage, over bath application, that the location of receptors or channels contributing to an event can be determined. Application of neurotransmitters could be used to examine their ability to directly depolarize or hyperpolarize the dendritic membrane, or to modulate voltage- or transmitter-activated channels following the activation of receptors coupled to intracellular second-messenger systems. Alternatively, application of blockers of voltage- or transmitter-activated channels to specific regions of the dendritic tree could be used to restrict the contribution of synaptically or voltage-mediated responses to a specific region of the neuron.

Improved recording of dendritic synaptic currents

Dendritic whole-cell patch-pipette recordings could be used in the voltage-clamp mode to determine the kinetics of synaptic currents generated very close to the site of the dendritic recording electrode. Unfortunately, however, dendritic whole-cell patch-pipette recordings often have higher access resistance than can be obtained by using larger patch pipettes at the soma, thus limiting the speed of the voltage clamp. In addition, it is not yet possible to know where on the dendrite to record in order to be in close proximity to stimulated synapses. One possibility, however, is to record spontaneous dendritic synaptic currents and to select the ones with the fastest rise-times, under the assumption that they originate close to the dendritic recording electrode (M Häusser, Soc Neurosci Abstr 1994, 20:372.7). Another possibility is to examine the properties of synaptic currents during somatic voltage clamp while using dendritic patch-pipette recordings to evaluate the quality of the clamp in the dendrites. For example, the degree of voltage escape in the dendrites during somatic voltage clamp of a synaptic event can be directly measured by a dendritic pipette. In addition, the clamp error at a given dendritic location can be directly assessed by injecting a simulated synaptic current with a known amplitude and time-course via a dendritic pipette and measuring the amplitude and kinetics of this current with a somatic voltage-clamp (M Häusser, Soc Neurosci Abstr 1994, 20:372.7).

The spread of electrical signals within the dendritic tree

As the soma, axon and dendritic tree of the same neuron can be visualized using IR-DIC videomicroscopy, simultaneous patch-pipette recordings can be made from different sites on the same cell. This allows the direction and nature of spread of different electrical signals to be examined in a single neuron. To date, simultaneous recordings from the soma and dendrite, or soma and axon, of the same neuron have been used to demonstrate that action potentials are usually initiated close to the soma or in the axon of several types of neurons, including neocortical [14] and hippocampal [17] pyramidal neurons, as well as cerebellar Purkinje cells [16] and substantia nigra dopamine and GABA neurons (M Häusser, C Racca, G Stuart, unpublished data).

These experiments have also revealed interesting differences in the way that action potentials spread from their site of origin into the dendritic tree. In Purkinje cells, sodium action potentials spread passively into the dendrites [16], whereas in the other cell types studied, action potentials backpropagate into the dendrites in an active, but decremental manner ([14,17]; M Häusser, G Stuart, unpublished data). In CA1 pyramidal neurons, simultaneous somatic and dendritic recordings and dendritic calcium imaging have revealed that action potentials backpropagate into the dendrites in an activity-dependent manner; action potentials occurring later in a 'train' propagate less reliably than those occurring earlier, partly as a result of the failure of action potentials to actively propagate through some dendritic branch points [17].

In addition to using whole-cell recording, the spread of electrical signals can be examined extracellularly by measuring capacitive currents in cell-attached patches (Fig. 1b; [17]). This approach eliminates any possible effects of washout of intracellular contents or capacitance loading of the dendrite by the patch pipette. The possibility also exists to make extracellular recordings from very fine dendrites or axons that can be visualized with IR-DIC videomicroscopy, but are too small to be patched.

Dendritic patch-pipette recordings can also be used to investigate the role of voltage-activated channels in the boosting of subthreshold EPSPs. As mentioned above, subthreshold EPSPs can activate sodium and calcium channels in the dendritic membrane of CA1 pyramidal neurons [18]. The extent to which such channel activation boosts the amplitude of EPSPs, however, remains to be determined. One approach to address this question is to use a dendritic patch pipette to simulate a synaptic event by injecting current with a time course similar to the synaptic current, and to measure the resulting voltage change at the soma with a second patch pipette (Fig. 3). The effects of blockers of voltage-activated channels on the resulting dendritic and somatic potentials can then be examined.

The electrotonic structure of the dendritic tree can also be examined with dendritic patch-pipette recording, for example, by recording the spread of membrane potential changes between different sites in the same neuron. Combining such experiments with a compartmental model of the same neuron will facilitate more reliable estimates of the passive electrical properties of the dendrites, including their intracellular resistivity (G Stuart, N Spruston, unpublished data).

Combining dendritic patch-clamp recording with imaging experiments

Another useful application of dendritic patch-pipette recording is to combine it with imaging experiments using calcium- or sodium-sensitive fluorescent dyes. The advantage is that the dendritic voltage change can be directly determined at the site imaged. In addition, once filled with fluorescent dye, fine details of dendritic or axon structure are revealed that are not visible using IR-DIC microscopy. Dendritic recording may also facilitate loading of the dendritic tree with dye, as well as reducing possible artifacts due to spatially non-uniform dye loading [26]. In experiments with voltage-sensitive dyes,

dendritic recordings could be used to calibrate these dyes by determining the absolute voltage change at a site in the dendritic tree. To date, dendritic recording has been combined with calcium imaging in both neocortical [24,26,27] and hippocampal pyramidal neurons [17].

Concluding remarks

The ability to probe the properties of the dendritic membrane using patch-pipette recording offers an opportunity to address the function of the dendritic tree more directly than has been previously possible. Already this technique has yielded valuable information concerning the properties and distribution of dendritic voltage- and transmitter-activated channels, and their role in the initiation and spread of electrical signals in the dendritic tree. In conjunction with other techniques, dendritic patch-pipette recording offers a valuable tool to help probe dendritic function via direct observation, with millisecond time-resolution, from living neurons.

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G Stuart and N Spruston, Abteilung Zellphysiologie, Max-Planck-Institut für medizinische Forschung, Jahnstraβe 29, D-69120 Heidelberg, Germany.

E-mail: stuart@sunny.mpimf-heidelberg.mpg.de

E-mail: nelson@sunny.mpimf-heidelberg.mpg.de