Dendritic Morphology and Function

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Contents

Abstract

This chapter will cover briefly what we know about the morphological and functional characteristics of dendrites, with a particular focus on the most abundant neuron of the neocortex, the pyramidal neuron.

First, this chapter will describe generally the dendritic diversity within the brain, after which it will focus on the cerebral neocortex and on the major

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neocortical neuronal type, the pyramidal neuron. In particular, this chapter will concentrate on the role of dendritic spines – tiny protrusions that cover the dendrites of pyramidal neurons and which are the receiving site of excitatory transmission – in information processing and storage in the brain. Dendritic input excitability will be discussed at the spine and branch levels in an attempt to describe the diversity in the input/output properties of pyramidal neurons. Some provocative results about the role of spines as biochemical and electrical compartments and the implications for synaptic integration and plasticity will be reviewed. In addition, this chapter will review different forms of dendritic computation and its implication in synaptic plasticity and in the hierarchical processing of information in the cerebral cortex. Finally, some of the theoretical foundations of how passive steady-state and transient electrotonic potentials spread in dendrites, and the biophysical factors that govern the electrotonic spread of potentials in the dendritic arbor of a neuron, will be described.

Keywords

Amygdala • Asymmetrical synapses • Cable theory • Calcium action potentials/ Ca^{2+} spikes • Cerebral cortex • Dendrites • Dendritic arborization • Dendritic excitability • Dendritic spikes • Dendritic spines • Excitatory postsynaptic potentials (EPSPs) • Hippocampus • Impedance mismatch • Interneurons • Neocortex • Neocortical pyramidal neurons • Neuron theory • NMDA spikes • Plateau potentials • Postsynaptic density (PSD) • Purkinje cells • Pyramidal neurons • Reticular theory • Spiny pyramidal cells • Symmetrical synapses • Voltage-gated potassium channels • Voltage-gated sodium channels • Voltage-sensitive calcium channels

Abbreviations

bAP	Backpropagation of action potential
EPSP	Excitatory postsynaptic potential
PSD	Postsynaptic density

Brief History

Introduction

The word dendrite comes from the Latin *dendron*, "tree." Dendrites are extensions of the cell body and the receptive surfaces of a neuron, as was proposed more than a 100 years ago by Santiago Ramón y Cajal. Most synaptic inputs are made onto the dendritic tree, where they are integrated, in some cases leading to the generation of a final output – an action potential – in the axon initial segment of these neurons. The large variability in dendritic shapes and arborization, as well as the presence of various neurotransmitter receptors and voltage-gated channels (active conductances), is an indication of the large variety of functions that dendrites are capable of performing in order to process synaptic information throughout the brain. Recent

work has proposed that dendrites, and in particular the tiny protrusions called dendritic spines, are not static structures but rather plastic devices capable of transforming the synaptic inputs and, thus, the neuronal output.

Dendritic Arborizations

Neuronal dendrites exist in a palette of different morphologies and degrees of ramification throughout the brain (Fig. 1). It is well accepted that the different morphological features are likely to confer different electrophysiological and connectivity properties and as a consequence variations in the input/output properties of these neurons. The classification of neurons based purely on their dendritic structure and density has been proven to be a difficult task. However, one can find neurons that are devoid of dendrites, or *adendritic*, that only have a branched axon (e.g., dorsal root ganglion cells and sympathetic ganglion cells) to neurons with very complex dendritic trees (Fig. 1). Some of the neuronal types with the most characteristic dendritic shapes found in the brain include (a) bipolar cells, where two dendrites

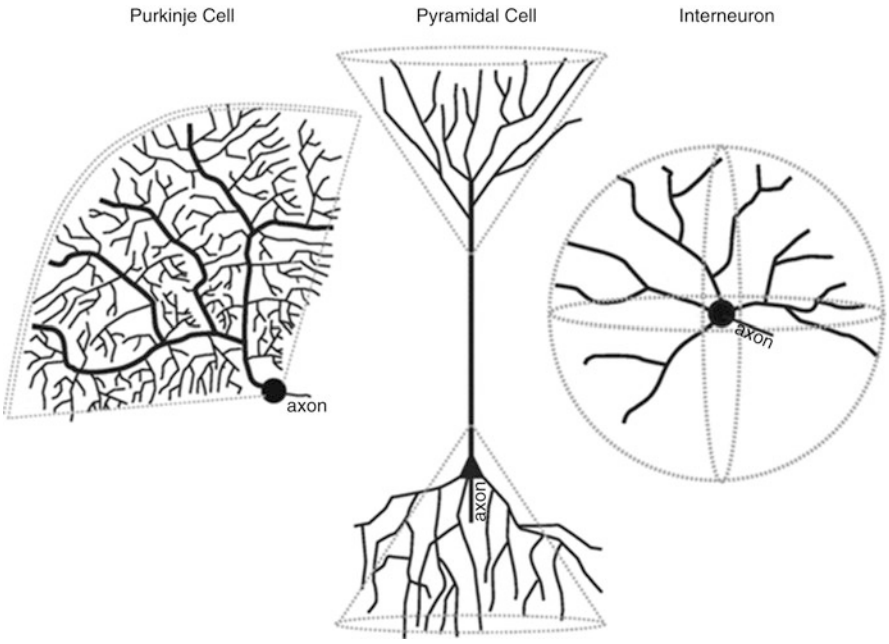

Fig. 1 Diagram of the characteristic dendritic morphologies from three types of neurons in the mammalian brain. Note that in Purkinje cells, dendrites ramify in a fan flat shape; pyramidal neurons often have dendrites emanating from the soma in a conical shape, named basal dendrites, and dendrites emanating in the opposite direction from the apical dendrite, called the apical tuft; and interneurons, where the dendrites emanate in all directions from the cell soma

emerge in opposite directions from the cell soma; (b) several interneurons, in which the dendrites emanate in all directions from the cell soma (Fig. 1); (c) pyramidal neurons, in which dendrites radiate in opposite directions from the cell body, covering two inverted conical areas; and (d) cerebellar Purkinje neurons, in which dendrites emanate from the soma in a flat fan shape (Fig. 1). Many other forms of dendritic arborization are found in the brain, but for simplicity, only some of the most characteristic dendritic shapes are mentioned.

The Neocortex

The neocortex is a thin layer of tissue, composed of millions of neurons, that covers the outer surface of the brain. In its mature form, the neocortex is comprised of six layers (Fig. 2), with information flowing within and between layers in what is likely to be a somewhat stereotypical – yet still not fully appreciated – fashion. Neocortical neurons form complex intra- and interlaminar networks that are ultimately responsible for the production of higher cognitive functions such as sensory perception, the generation of motor commands, thought, spatial reasoning, consciousness, and, in humans, language. Several neuronal types can be found in the neocortex, the most important and obvious distinction being between glutamatergic pyramidal neurons and GABA-releasing interneurons (for further information on neocortical function and organization, see "References" section).

Ramón y Cajal was the first to classify cortical neurons based on the shape of their cell body and the patterns of their dendritic and axonal arborization. Since then, and thanks to the development of molecular, electrophysiological, genetic, and imaging tools, neocortical cells are being classified based not purely on the laminar position of their cell body and the morphology of their soma, dendrites, and axon but also on the axonal target, the type of synaptic terminal, the released neurotransmitter and/or peptides, the presence or absence of dendritic protrusions – the dendritic spines (see below) – and a number of other factors.

A General Classification of Cortical Neurons

In the neocortex, two main types of synapses can be classified according to their ultrastructure, in particular the presence or absence of a prominent and elaborate complex of synaptic proteins called the postsynaptic density (PSD) (▶ Chap. 16, "The Postsynaptic Density" from Rochelle S. Cohen). These two types of synapses are *asymmetrical* and *symmetrical. Asymmetrical*, or excitatory synapses, comprise more than 70 % of the synapses in the brain. They are derived from neurons with dendritic spines (see below and Fig. 5). These neurons are commonly referred as *spiny pyramidal* cells (Fig. 4). In the human, the neocortex has approximately 20 billion pyramidal neurons, each of which can receive up to 10,000 connections,

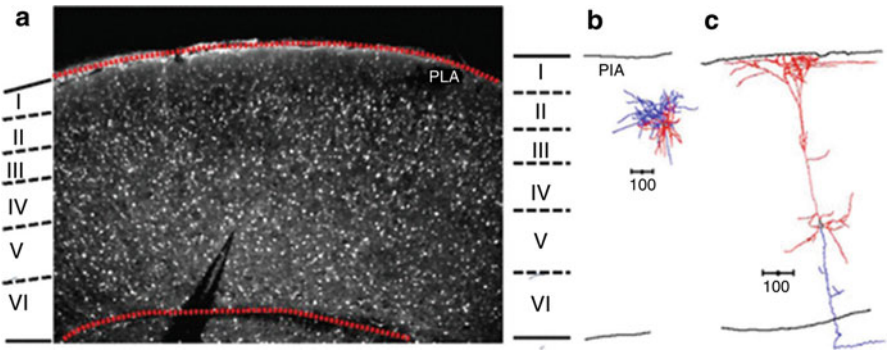

Fig. 2 The neocortex, a thin layer that covers the brain, is composed of millions of neurons that can perform higher functions. The neocortex is made up of six layers. (**a**) A mouse brain slice was labeled with a calcium indicator (mag-indo-1 AM) to locate the cell somas of neurons and measure its activities (Image taken by K. Poskanzer at the Yuste laboratory). (**b**) Neurolucida reconstruction of a basket cell. Axons are shown in *blue* and dendrites in *red*. Note that the dendrites ramify from the soma in all directions and the axon ramifies heavily to form a dense structure that covers an area, in this case, restricted mainly to layer II. (**c**) Neurolucida reconstruction of a layer V pyramidal neuron. Axons are shown in *blue* and dendrites in *red*. These cells are the most abundant neuron in the neocortex (see text). Note the different morphology of the dendritic arbors and axon ramifications when compared with the interneuron in (**c**) (All the Neurolucida reconstructions were taken from the Yuste laboratory database)

making the cortex a highly connected organ with the potential of having up to 200 trillion excitatory or glutamatergic connections. *Symmetrical*, or inhibitory synapses, differ from *asymmetrical* synapses in that they do not contain PSDs and are ultrastructurally symmetrical. These cells are commonly referred as nonpyramidal interneurons (Fig. 2b). These cells are aspiny. The excitatory neurotransmitter is glutamate, and the inhibitory neurotransmitter is γ-aminobutyric acid (GABA).

Inhibitory interneurons and excitatory pyramidal neurons are distributed throughout the neocortex (Fig. 2). The various classes of interneuron types – such as basket cells, chandelier cells, Martinotti cells, and double bouquet cells – are found throughout the cortical layers. Excitatory neurons, by contrast, show significant heterogeneity between layers and are typically divided into two main groups: pyramidal neurons and spiny stellates. Pyramidal neurons are located in layers II to VI (Fig. 4), and spiny stellates are located only in layer IV. Spiny stellates differ from pyramidal neurons in that they do not have an apical dendrite (see below). In layer II/III, there is a variety of cell types, many of which are small- to medium-sized pyramidal cells. The granular layer, or layer IV, is packed with small pyramidal and nonpyramidal cells. Layer V contains mainly pyramidal cells of a larger size than those observed in layer II/III. Finally, layer VI – the layer that borders with white matter – contains cells of varying morphologies, including normal pyramidal neurons, inverted pyramidal neurons, and nonpyramidal neurons.

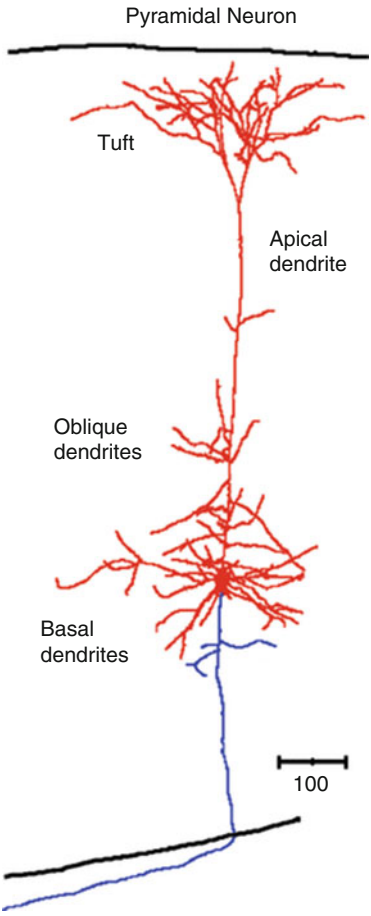

Fig. 3 Pyramidal neurons have extensive dendritic arborizations, and most of the excitatory connections occur in tiny protrusions called dendritic spines (see Fig. 5). These neurons are mainly composed of an apical dendrite, an apical tuft, basal dendrites, and oblique dendrites (see text for details). The soma has a pyramidal shape. The axon emanates from the pyramidal-shaped soma sending axonal collaterals to other regions of the brain

Pyramidal Neurons

Pyramidal neurons are a type of neuron present in many mammalian brain areas including the *amygdala*, the *hippocampus*, and the *cerebral cortex*. These neurons are mainly composed of an apical and a basal dendrite and a pyramidal-shaped soma (Fig. 3). The pyramidal neuron's axon emanates from the pyramidal-shaped soma in a straight fashion for the first 50–100 μm, after which it ramifies extensively in a variety of fashions (Fig. 4). These cells are the most abundant cells in the cerebral cortex, comprising more than 70–80 % of all the neurons in the mammalian neocortex. Layer V pyramidal neurons have received a great deal of attention due to their position within the cortical circuit: they receive highly processed information that has passed through various earlier cortical layers and possess an elaborate structure at the pial surface (termed the *apical tuft*) that receives input from hierarchically separate structures, and they output directly to other cortical and subcortical structures

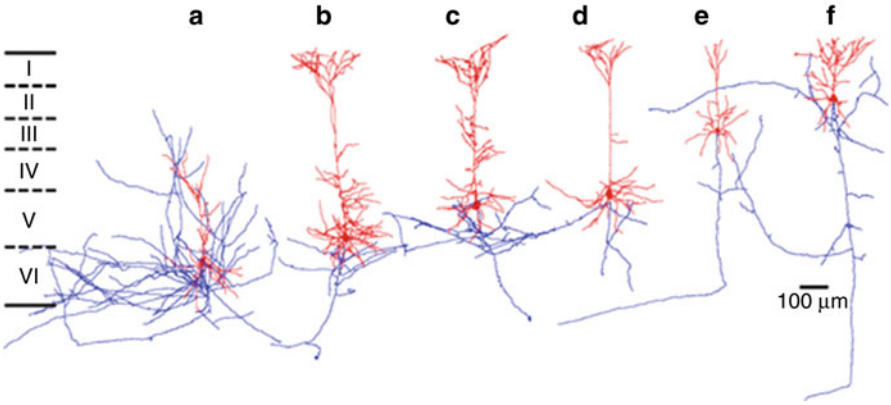

Fig. 4 Morphology and distribution of neocortical pyramidal neurons reconstructed with Neurolucida. Note the variability in size and dendritic arborization as well as the different axonal morphologies with its collaterals. Note that the apical tuft in pyramids from b to f ramifies in layer I independently of their laminar distribution; however, note how in layer VI pyramids the apical tuft projects to layer IV. Different pyramidal neurons project to different regions of the brain (see text) (Unpublished material from Yuste laboratory)

(Figs. 3 and 4). Thus, elucidating the input/output properties of these types of cells is fundamental for the understanding of the function of neocortex and the brain.

Although pyramidal neurons share a general dendritic principle – the presence of apical and basal dendrites – their dendrites nevertheless show a vast variety of shapes and lengths (Figs. 3 and 4). As mentioned before, a general morphological description of these cells often refers to them as biconical (Fig. 1). However, other dendritic ramifications besides basal and apical tuft dendrites are often observed along the main apical dendrite, covering additional areas along the apical dendrite axis. These are termed oblique dendrites and can be seen in Fig. 3.

The length of the apical dendrite, with the exception of layer VI pyramidal neurons, will depend on how distant the cell body is with respect to the uppermost layer of the cortex, named layer I (Fig. 4). Thus, in neocortical pyramidal cells, the apical dendrite of layer II and III pyramidal neurons (Fig. 4e, 4f) is approximately half the length of the apical dendrite of layer V pyramidal neurons (Fig. 4b–4d). Once the apical dendrite reaches layer I, it ramifies in a conical shape in several thin dendrites that together form the *apical tuft*, which receives inputs mainly from other cortical areas and nonspecific thalamic inputs (Fig. 4). Layer II and III and layer V pyramidal neurons have apical tufts that ramify in layer I. However, layer VI pyramidal neurons have apical tufts that ramify in layer IV (see Fig. 4 for comparison). More proximal dendrites – basal and oblique dendrites – generally receive local (intralaminar) input and inputs from other cortical layers.

In neocortical pyramidal neurons, as well as most neurons in the brain, the axon initial segment is the site of action potential (AP) generation (Fig. 16). The AP is an all-or-none response mediated by the opening of voltage-gated Na^+ and K^+ channels and is the final output signal of these neurons.

How Does Excitatory Information Flow in the Neocortex?

Several experiments, in particular those using retrograde and anterograde neuronal tracers, have led to the identification of "feedforward" and "feedback" connections in the neocortex. From these studies, it is well accepted that a simplified excitatory feedforward pathway within a cortical column starts with sensory information from the thalamus entering the primary sensory cortical areas. The major thalamorecipient layer is layer IV, from where information progresses to layers II/III, followed by layers V and VI. From these deeper layers, information is sent either to subcortical regions or alternatively to separate areas of the cortex.

As will be discussed below, the understanding of the structure-function relationships at a dendrite level (e.g., studying the function of single dendritic branches or that of dendritic spines) has proven to be fundamental in understanding pyramidal cell function. In the last 30–40 years, with the development of several techniques such as dendritic patch-clamp recordings, voltage-sensitive dye recording, and the use of two-photon imaging and photoactivation of neurotransmitters to activate single and multiple spines, scientists have begun to uncover the biophysical properties of dendrites and their contribution to the input/output properties of pyramidal neurons, leading to an exquisite knowledge of the biophysical capabilities of pyramidal neuron dendrites.

Dendritic Spines

Dendritic spines are small membrane protrusions that cover the dendrites of excitatory pyramidal cells as well as some inhibitory neurons in the brain (Fig. 5). Spines consist of a small head ($\sim$1 μm head diameter and $<$1 fL volume), separated from the parent dendrite by a slender neck ($<$0.2 μm diameter) (Fig. 5). Spines were first described by Santiago Ramón y Cajal in 1888. He was the first that stated that spines are real structures and not just an artifact of the fixation technique or silver precipitates, as believed by many other scientists at the time. Ramón y Cajal hypothesized that spines serve to connect axons with dendrites and that these structures are the places where synaptic contacts are made rather than directly on the dendritic shaft. This revolutionary idea sets the basis of his *neuron theory*. This theory indicated that neurons are independent units that connect to each other via their axons and spines, instead of a continuous network, as the *reticular theory* stated.

The development of electron microscopy (EM), a microscope with subnanometer resolution, allowed experimentalist to resolve the ultrastructure of dendritic spines. DeRobertis and Palay did the first EM characterization of the spines, and soon after, it was demonstrated by Gray in 1959 that synapses are located in spines. These reports proved that Ramón y Cajal's ideas were right. The use of EM – to reveal the ultrastructure of spines – clearly showed that excitatory synapses occur in spines at specified places in the spine head, at the PSD (▶ Chap. 16, "The Postsynaptic Density"). Spines can be found in several species, ranging from the phylum Annelida to highly evolved species, like mammals.

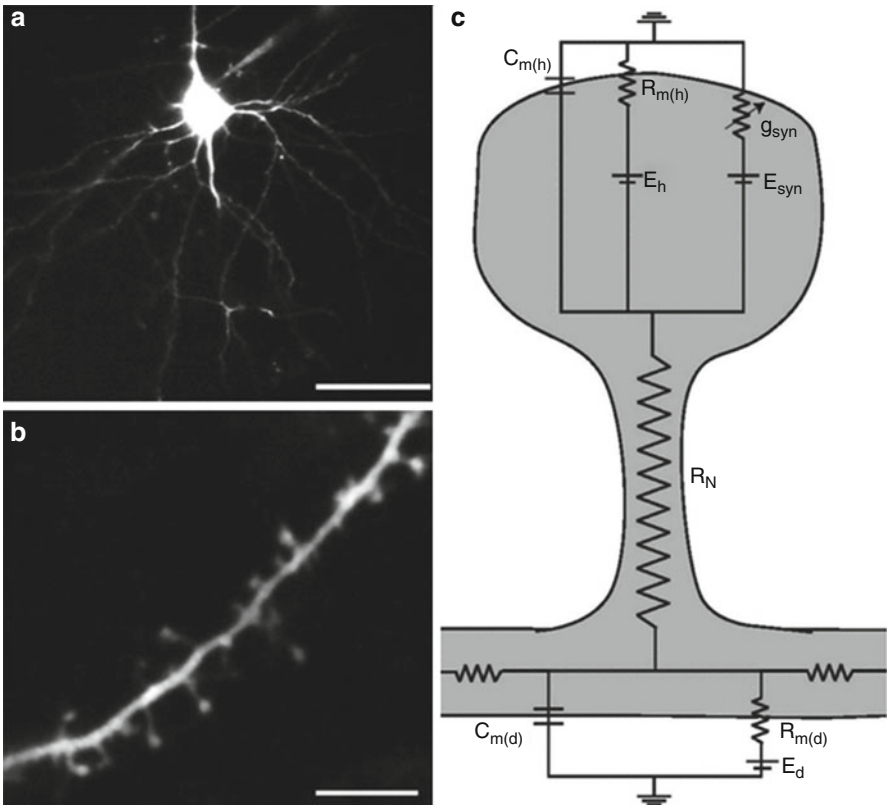

Fig. 5 Dendritic spines are tiny protrusions that cover the dendrites of pyramidal neurons and the places where excitatory connection occurs. (**a**) Two-photon scanning image of a layer V pyramidal neuron filled with Alexa Fluor 488 (scale bar 50 μm). (**b**) Two-photon scanning image showing a representative basal dendrite of layer V pyramidal neurons covered with dendritic spines. Note the variability in shapes of the spines detected (scale bar 5 μm). (**c**) Simplified circuit diagram of a passive dendritic spine. $C_{m(h)}$ capacitance of the spine head membrane, $R_{m(h)}$ membrane resistance of the spine head, E_h reversal potential of the spine head, E_{syn} synaptic reversal potential, R_N neck resistance, $C_{m(d)}$ dendrite membrane capacitance, $R_{m(d)}$ membrane resistance of the dendrite, and $E_{(d)}$ reversal potential at the dendrite

In the neocortex, as well as many other brain areas, excitatory inputs terminate on dendritic spines. Recently, serial EM reconstruction of hundreds of dendritic spines from basal dendrites of neocortical pyramidal neurons, by the DeFelipe and Yuste groups, showed that most of the spines (∼95 %) receive excitatory inputs (Fig. 6). In addition, it was clear from those EM reconstructions that excitatory inputs avoid the dendritic shaft (Fig. 6).

Based on these observations, two important questions arise:

1. Why excitatory inputs occur in dendritic spines?
2. What is the function of dendritic spines in the processing, storage, and integration of excitatory inputs?

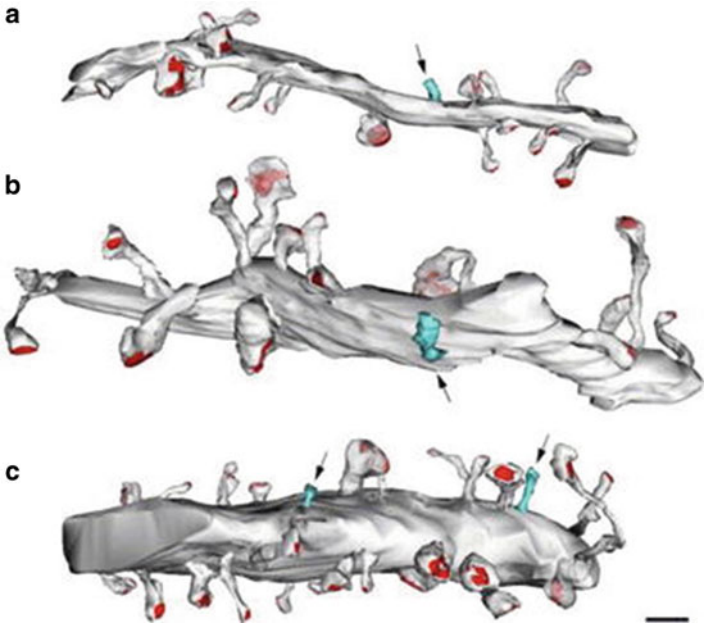

Fig. 6 Synaptic contacts occur in spines. Reconstruction of electron micrographs (*EM*) taken from serial sections of dendritic segments from neocortical pyramidal neurons. Note the distribution of postsynaptic contacts (PSD, *red*). *A* and *B* correspond to basal dendrites and *C* to apical dendrites. Only a few percent of dendritic protrusions are devoid of synaptic contacts (*blue*), and the shaft is devoid of synaptic contacts. Scale bar = 2,000 nm (Modified with permission from Arellano et al. (2007))

Despite their evident importance, the function of spines is not completely understood. Their peculiar morphology, with a small head separated from the main dendrite by a spine neck (Fig. 5), may be responsible for enabling the biochemical and electrical compartmentalization of inputs (see below), which in turn can allow different forms of synaptic plasticity, such as long-term potentiation (LTP). Although there are many studies that support this theory, basic questions regarding the mechanism linking dendritic spines to input integration and plasticity remain unknown.

Spines come in several flavors, and changes in their morphology (e.g., changes in spine head size, neck length, and/or spine neck diameter) and/or its internal biochemistry (e.g., expression and insertion of glutamate receptors and/or voltage-gated channels) are thought to be associated with spine development and synaptic plasticity. Thus, the prediction (based on recent experimental data from several groups; see below) is that different dendritic spine structures will carry differences in their function – as either biochemical or electrical compartments or both – which seems to be relevant in controlling the synaptic weight, synaptic integration and storage, and ultimately the output of a pyramidal neuron.

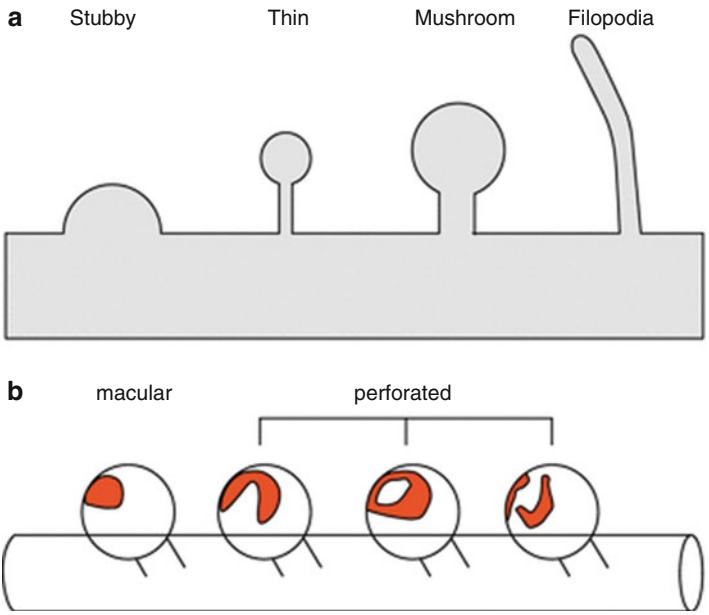

Fig. 7 Types of spines and postsynaptic densities (*PSD*). (**a**) Drawing of the general morphological types of spines. (**b**) Schematic drawings of the types of PSD

In addition, dendritic spines not only can receive excitatory inputs. Indeed, it has been demonstrated that some inhibitory synapses, which normally are directed to the dendritic shaft, the soma, or the axon, can also occur in spines, but their role in synaptic transmission, plasticity, and integration of excitatory inputs in pyramidal neurons remains ill-defined.

Types of Spines

There is a big variety of dendritic spine morphologies. A general classification of spines includes tree morphological group types: *thin*, *mushroom*, and *stubby* spines (Fig. 7). *Thin* spines normally have a thin and long neck and a small head. *Mushroom* spines have a big head and thicker necks and are mainly observed in adult mice dendrites, and finally, *stubby* spines are spines that do not have a neck and are mainly present during early postnatal development but also present in the adulthood (Fig. 7). In addition to these three groups of spines, another type of dendritic protrusion, which is mainly present at early developmental stages, is the *filopodia*. *Filopodia* is a thin and long dendritic protrusion without an evident spine head. The absence of a clear head and PSD suggests that these structures have little if any excitatory synaptic activity.

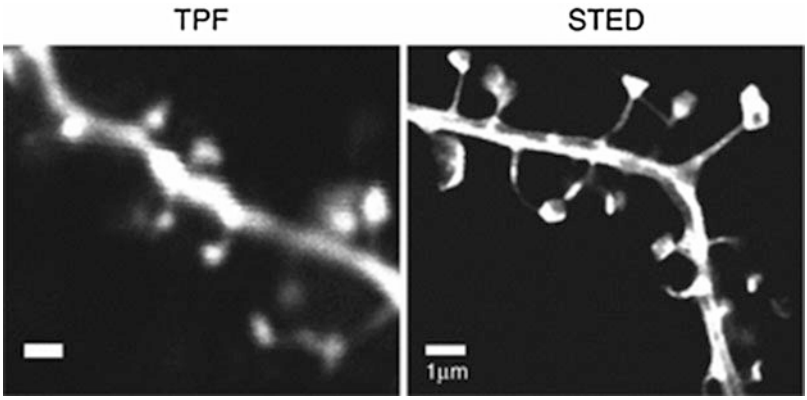

Fig. 8 STED microscopy of dendritic spines. (*Left image*) Two-photon fluorescence (*TPF*) image of a living basal dendrite covered with dendritic spines from a layer V pyramidal cell loaded with Alexa Flour 488 dye. (*Right image*) A super-resolution image of a living dendrite with spines using STED microscopy taken from Nägerl et al. (2008) (With permission from Nägerl UV et al.). Note the significant improvement in the spatial resolution imaging of living dendritic spines by STED microscopy compared to the TPF image

It has been argued by some groups that the distribution of dendritic spine morphologies, instead of a discrete distribution of spine shapes, is a continuum.

Recently, with the use of stimulated emission depletion (STED) microscopy, it has been possible to image live dendritic spines with subdiffraction-limited resolution (Fig. 8). This technique substantially improves the quantification of morphological parameters such as the spine neck length and diameter, allowing a more accurate appraisal of the structure-function relationship with respect to dendritic spines. Therefore, STED and other super-resolution microscopy techniques are likely to become the techniques of choice to investigate the structural and functional properties of dendritic spines.

In addition to the plethora of morphologies of dendritic protrusions in pyramidal cells, and in particular that of dendritic spines, the electron-dense postsynaptic structures at the spine heads, the PSDs, when 3-D reconstructed with EM sections, showed a variety of shapes and sizes that can be classified in two major groups: *macular* and *perforated* PSD. *Macular* PSDs are continuous structures (Fig. 7b), and *perforated* PSDs are larger and irregular structures that can often show a doughnut-like shape, have a continuous and round shape – like a crescent moon – or be composed of two or more patches close to each other (Figs. 7b and 6). The functional implication of the different types of PSD is unknown. However, it has been recently shown by the Spruston group that in hippocampal CA1 pyramidal neurons, the proportion of perforated PSDs increases as a function of distance from the soma (apical dendrites have a higher percentage of large perforated PSD in their spines than those in more proximal spines). In addition, perforated PSDs have a larger amount of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (see below) than those present in macular PSDs. This might indicate that

perforated PSDs could increase the strength of the synapse, thus suggesting that the morphology of the PSD might have an important role in the shaping and decoding of synaptic inputs.

Dendritic Spine Function

Spines as Biochemical Compartments

As pointed out before, spines have a peculiar morphology with a small head connected to the dendrite by a narrow neck. Once glutamate is released from the presynaptic boutons, its action in the dendritic spine will first include the binding of glutamate to both AMPA and *N*-methyl-D-aspartate (NMDA) receptors (Fig. 9). Both receptors are permeable to Na^+ and K^+ ions. Then, the activation of AMPA receptors will result in the depolarization of the spine head (a partial depolarization) due to the influx of Na^+ ions into the spine head (Fig. 9). The binding of glutamate to NMDA receptors at slightly depolarized or resting membrane potentials (~ -65 mV for a layer V pyramidal neuron) will cause a very low conductance through the NMDA receptor pore because the pore is blocked by Mg^{2+} ions that acts to prevent the free flux of ions through the channel. Under these conditions, the excitatory

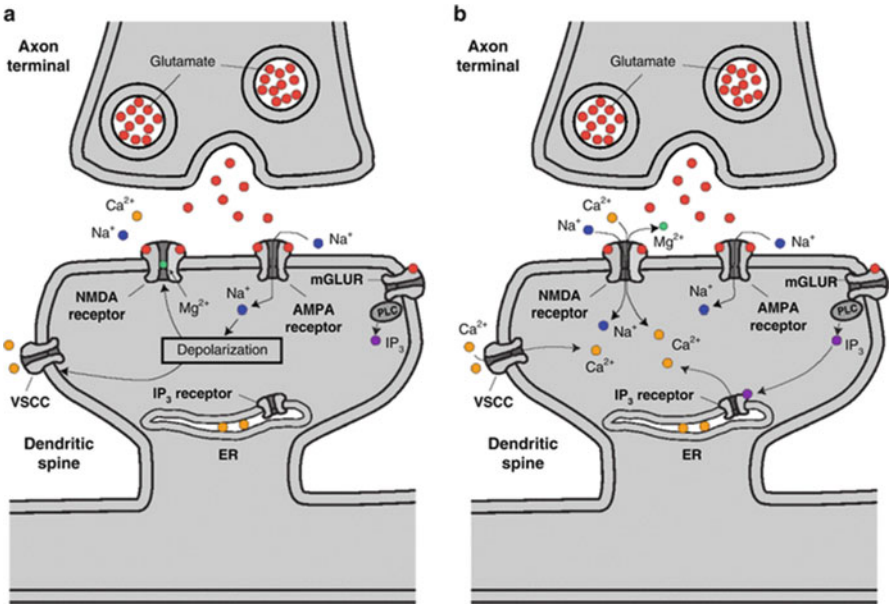

Fig. 9 Schematic representing excitatory synaptic transmission and the sources of calcium accumulations at the spine head. (**a**) Drawing showing how presynaptic released glutamate activates glutamate (AMPA, NMDA, and mGLUR) receptors leading to spine head depolarization. (**b**) Spine depolarization will generate calcium transients at the spine by triggering the magnesium released from NMDA receptors, the activation of voltage-sensitive calcium channels (*VSCCs*), and generation of second messengers like IP_3 (for details see text)

postsynaptic potential (EPSP) will be mediated entirely by the AMPA receptors. However, under a normal synaptic stimulus, the activation of AMPA receptors can depolarize the membrane sufficiently to remove the Mg^{2+} from the NMDA channel (Fig. 9). Now, the NMDA channel will permit the influx of not only Na^{+} but also of Ca^{2+}. The activation of AMPA receptors and then that of NMDA receptors will lead to the activation of voltage-gated (or voltage-sensitive) calcium channels (VSCCs) in the spine (Fig. 9). Calcium acts as an important second messenger, activating several intracellular signaling cascades, such as the activation of calcium/calmodulin-dependent protein kinases, or CAM kinases, that act directly on AMPA receptors to modify its conductance to Na^{+} ions and/or act to increase the insertion of AMPA receptor-containing vesicles into the plasma membrane. In addition, Ca^{2+} in the spine head will trigger the production of inositol trisphosphate (IP_3), and the activation of IP_3 receptors and subsequent Ca^{2+} release form internal stores. These events will trigger Ca^{2+} signals at the spine head (Figs. 9 and 10). Due to the peculiar morphology of dendritic spines, it has been demonstrated by several groups that the triggered Ca^{2+} signals are compartmentalized for hundreds of milliseconds at the spine head (Fig. 10). In pyramidal neurons, it has been demonstrated that transient Ca^{2+} accumulation in the spine head (Fig. 10) is responsible for enabling plasticity at a single spine level (Fig. 13), influencing not just the activity of voltage-gated channels and glutamate receptors but also modifying the internal molecular arrangement and composition of spines. The generation of long-term potentiation (LTP) or long-term depression (LTD) at a single spine level has been linked to the presence of large and small calcium elevations at the spine head, respectively (Fig. 13). In addition, it has been demonstrated in pyramidal cells (in the neocortex and hippocampus) that the pairing of synaptic inputs with the backpropagation of action potentials (bAPs), and the subsequent nonlinear Ca^{2+} accumulation at the spine head, might be crucial for triggering the process known as spike-timing-dependent plasticity (STDP). The biochemical compartmentalization observed in spines implies that the narrow neck can act as the main barrier for the free diffusion of molecules from the spine head to the parent dendrite. Thus, changes in the morphology of the spine neck might be expected to have big implication in the biochemical compartmentalization function of dendritic spines.

Spines as Electrical Compartments

As pointed out before, the particular morphology of spines and in particular that of the spine neck lead several theoretical works to suggest that relatively high values of neck resistance can lead to amplification of EPSPs at the spine head and to the attenuation of the EPSP as it passes through the neck, influencing somatic EPSP amplitude. The first to point out that the spine neck might have a high electrical resistance (R_N; Fig. 5), thus attenuating EPSPs from the spine head to the parent dendrite, was Chang in 1952. This idea was further explored by Rall, who pioneered the use of cable theory in neuroscience. Rall developed passive and active multicompartmental models of dendritic spines that showed that the spine neck resistance could indeed be an important variable in sculpting the synaptic weights of each synapse.

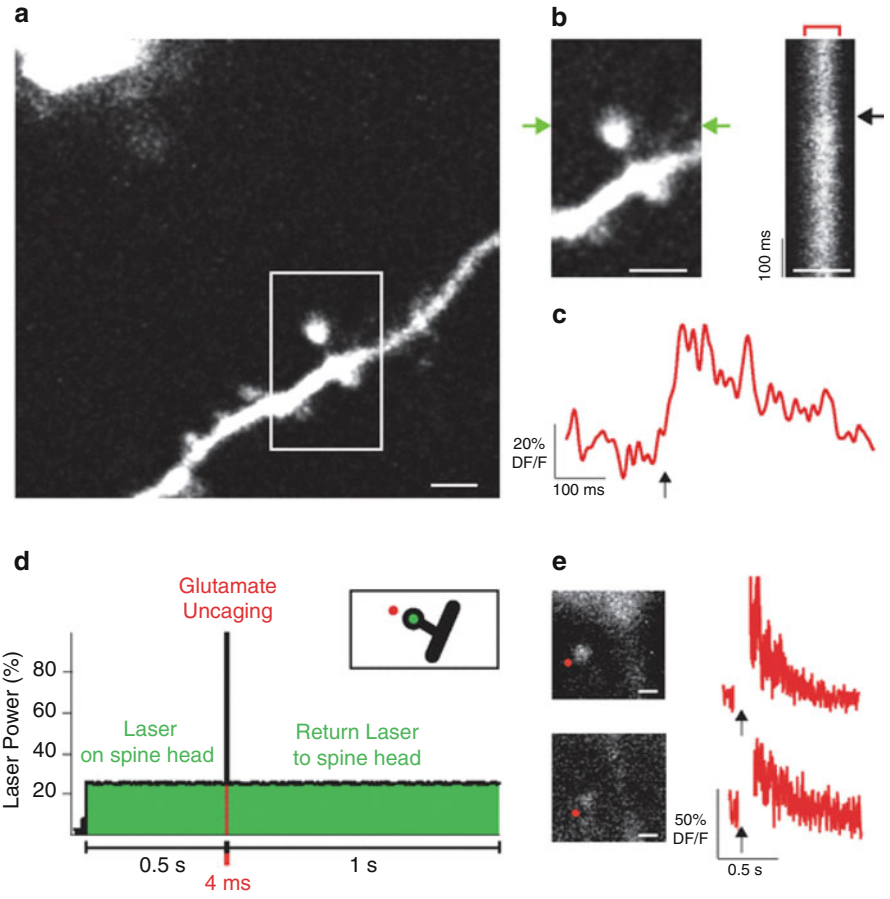

Fig. 10 Two-photon imaging experiments design to measure the synaptically triggered intracellular calcium accumulations at the spine head of neurons labeled with calcium indicators. (**a**) Morphology of a basal dendrite from a layer V pyramidal neuron from a p14 mouse visual cortex loaded with 200 μM Alexa Fluor 488 and 200 μM Fluo-4. (**b**) (*Left panel*) A higher magnification of the region of interest (*boxed area* in **a**). Line scans through the spine head, at positions indicated by the *green arrows*, were recorded. The line scan in the right panel was generated before (above *black arrow*) and after (below *black arrow*) a 20 μs extracellular stimulation delivered through an extracellular electrode (the electrode is shown in the *upper*, *left corner* of **a**). (**c**) A plot from the calcium fluorescence line scan image from **b**. The *black arrow* indicates the time of the extracellular stimulation of the spine. (**d**) The protocol for the measurement of intracellular calcium at the head of the spine before and after two-photon uncaging of glutamate. (**e**) Two examples of spines from basal dendrites of layer V pyramidal cell filled with 200 μM Calcium Green-1. The *red* traces corresponded to the average measurements of intracellular calcium in the head of the spine in response to two-photon uncaging of glutamate. Scale bar, 1 μm (Figure taken from Araya et al. (2011))

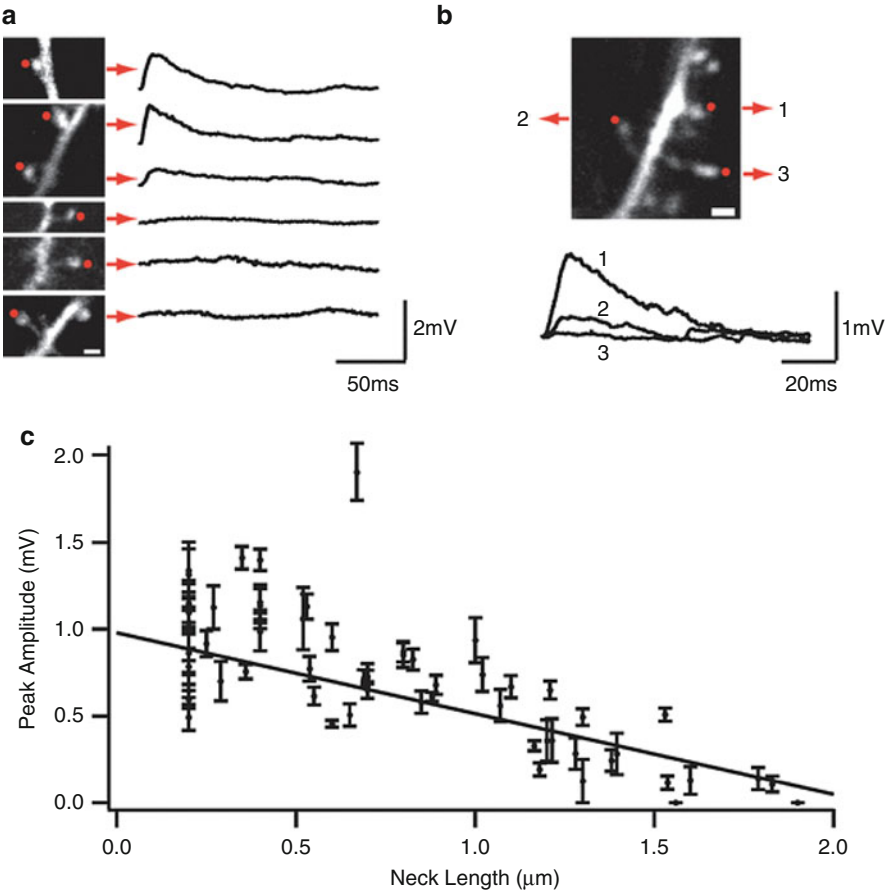

Fig. 11 Inverse correlation between the spine neck length and spine uncaging potentials. (**a**) Examples of two-photon glutamate uncaging potentials in spines with different neck lengths. *Red dots* indicate the site of uncaging, and traces corresponded to averages ~10 uncaging potentials from each spine. (**b**) Three neighboring spines with different neck lengths. Note the different uncaging potentials generated at the soma of the neurons. (**c**) Plot of the peak amplitude of the uncaging potentials versus the neck length. *Line* is the linear regression of the data, with a weighted fit including the standard error of each point (Figure taken with permission from Araya et al. (2006a))

Recent experiments using two-photon uncaging of glutamate showed that the activation of spines with long necks generates substantially smaller EPSPs at the soma than those observed when glutamate was uncaged in short-necked spines (Fig. 11). In addition, calcium imaging at the spine head showed that short- and long-necked spines were equally activated. These results suggest that spines can act as electrical compartments that can influence somatic EPSP amplitude and thereby provide a mechanism for controlling synaptic efficacy. However, our own simulations using morphologically realistic multicompartmental models to explore the

passive spine properties and neck resistance values required to reproduce experimentally the obtained inverse correlation between neck length and somatic EPSP amplitude gave us neck resistance values that are at odds with recent neck resistance estimates. To better understand the electrical properties of spines and the implications for synaptic transmission and plasticity, further experiments devoted to understanding the passive (e.g., spine morphology) and active (activation of voltage-gated channels; see below) mechanisms controlling synaptic efficacy and synaptic amplification at the spine head are required.

Active Spine

The smaller the size of the spine and the narrower (or longer) the spine neck, the higher the input impedance and, thus, the bigger the amplitude of the synaptic potential for a given conductance. Thus, a high neck resistance will trigger a large EPSP at the spine head that could in turn facilitate not just the magnesium release from NMDA receptors but also trigger the opening of voltage-gated channels if present on the spine. It is well accepted that the spine is not a purely passive structure but rather an active device. Voltage-sensitive calcium channels (VSCCs), voltage-gated potassium channels, and voltage-gated sodium channels are among the channels found in spines.

Voltage-sensitive calcium channels: The activation of glutamate receptors and VSCCs leads to Ca^{2+} accumulations at the spine head (Fig. 9). Several types of VSCCs have been detected in spines from different pyramidal neuronal types; these channels include the following: T-type, L-type, N-type, R-type, P/Q-type, and low-voltage-activated (LVA) Ca^{2+} channels.

Voltage-gated sodium channels: The nine mammalian NaV1 isoforms can be categorized into those that are TTX sensitive versus those that are resistant. In fact, five neuronal subtypes are TTX sensitive (NaV1.1, 1.2, 1.3, 1.6, and 1.7). Previous studies have suggested that dendritic sodium channels could amplify synaptic potentials. Recently, it has been demonstrated that, indeed, spine uncaging potentials are boosted by the activation of TTX-sensitive sodium channels at the spine head (Fig. 12). In addition, the TTX-dependent boosting of the spine uncaging potential lasted for several milliseconds after the onset of the response (Fig. 12), suggesting that a persistent sodium conductance might be present at the spine. These results predicted that spines are indeed electrical compartments endowed with sodium channels at their spine head that can boost EPSPs. A remaining question is the molecular identity, subcellular distribution, and posttranscriptional and posttranslational regulation of these channels in spines and along the dendritic arbor of pyramidal cells.

Voltage-gated potassium channels: Recently, it has been demonstrated that spines are endowed with potassium channels. From the 12 classes of voltage-gated K^+ channels (Kv1-12), the A-type (Kv4.2) channel has been detected in spines. In addition, calcium-activated and inwardly rectifying potassium channels have been detected in spines. The activation of glutamate receptors in spines triggers Ca^{2+} signals that are compartmentalized for hundreds of milliseconds at the spine head. Thus, Ca^{2+}-activated K^+ channels are good candidates to be located in the spine. Indeed, the small conductance Ca^{2+}-activated channel SK has been detected in

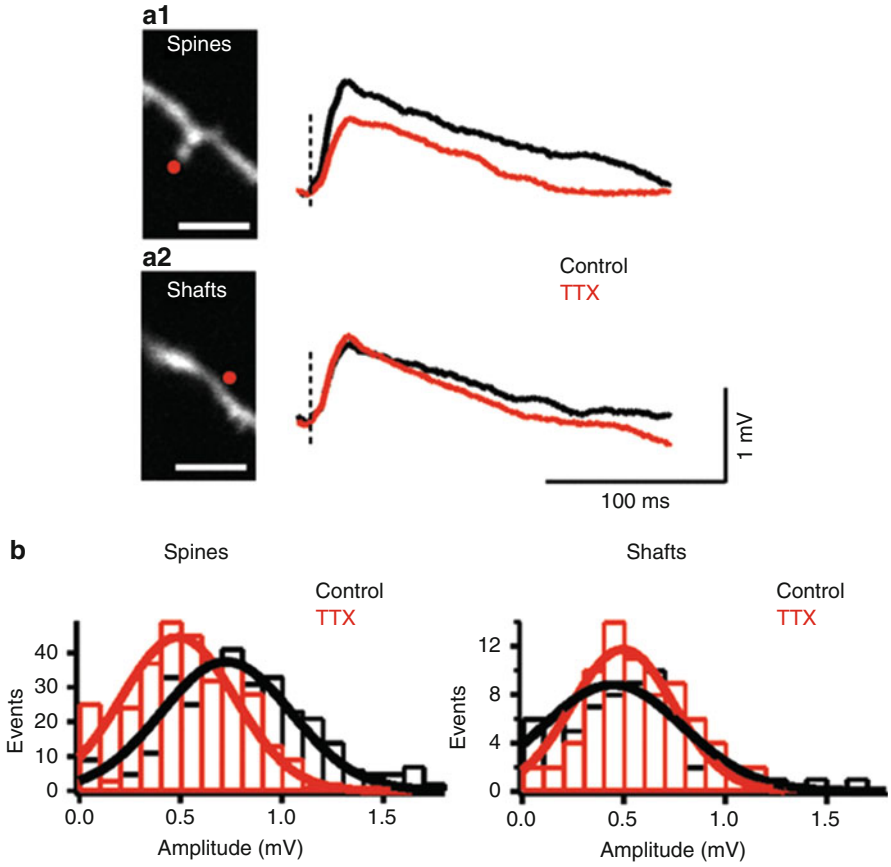

Fig. 12 Sodium channels amplify synaptic potentials. The effect of TTX is postsynaptic and restricted to spines. (**a**) Uncaging experiments in spine (a1) or shaft (a2) locations, under control conditions (*black traces*) and TTX (*red traces*) in current-clamp configurations (scale bar, 3 μm). Note how TTX attenuates spine uncaging potentials, but not shaft potentials. (**b**) Histogram of all individual uncaging potentials on spines and shafts in control and TTX. Note a shift toward smaller potentials caused by TTX in distribution of spine, but not shaft potentials (Image modified from Araya et al. (2007))

hippocampal spines. Three types of SK channel subunits, SK1-3, have been described in the CNS, and recently, it has been demonstrated in hippocampal pyramidal neurons that SK2 is expressed in spines. Furthermore, the G protein-coupled inwardly rectifying K^+ channel (GIRK) has been detected in hippocampal spines. However, the precise molecular identity and role of K^+ channels in the spines of neocortical pyramidal neurons remained ill-defined. It is likely that their activation leads to the regulation of the amplitude and kinetics of EPSP directly at their origin. Thus, the activation of different potassium channels at the spine might be fundamental in regulating the spine output, thus the integrative properties of excitatory inputs in pyramidal cells.

In addition to the specific expression of channels within the spine, it is well known that the expression of active conductances might be controlled by the exact location along the dendrite of pyramidal cells. For example, in CA1 pyramidal neurons, the dendritic expression of the hyperpolarized activated channels HCN [*cationic channels that are activated by hyperpolarization and that in neocortical pyramidal neurons are activated at resting membrane potentials causing membrane depolarization and reduction in the input impedance of the cell*] and that of A-type potassium channels increase with distance from the soma. This location-dependent channel expression has fundamental consequences for the input/output properties of pyramidal cells. For example, the HCN gradient observed in CA1 pyramidal neurons serves to normalize the temporal summation of EPSPs.

Spine as an Electrical Compartment: Its Implications for Synaptic Transmission, Plasticity, and Synaptic Integration

What Is the Purpose of the Electrical Function of Dendritic Spines in the Neocortex?

The electrical role of spines could have important implications for synaptic transmission, plasticity, and synaptic integration. The electrical compartmentalization of spines, proposed by theoretical calculations and supported by recent experiments, implies that the spine neck resistance should be high. The high input impedance, if present, will enhance the EPSP amplitude at the spine head beyond the threshold for sodium, calcium, and potassium channel activation. Several groups, including ours, have argued for the presence of active conductances in spines (see "Active Spine" section). The activation of a single spine with either two-photon uncaging of glutamate or minimal electrical stimulation protocols (using optical quantal analyses) generates small EPSPs at the soma ($\sim$1 mV); however, as pointed out before, the EPSPs at the spine head have to be several orders of magnitude bigger than that recorded at the soma in order to recruit the activation of the active conductances found experimentally at the spines (Fig. 12). In addition, the activation of glutamate receptors at a single spot on the parent dendrite (dendritic shaft) generates similarly small voltage deflections at the soma, but without the recruitment of active conductances, e.g., voltage-activated sodium (Fig. 12) and calcium channels (assuming that these channels are also expressed at the dendritic shaft). These results imply that the voltage deflection at the spine head is large and severely attenuated as it travels from the spine head to the parent dendrite.

Recent experiments have demonstrated that spines rather than static structures can undergo activity-dependent structural changes that can modify the synaptic strength. Indeed, ultrastructural and imaging experiments have suggested that long-term potentiation (LTP) is associated with increases in the size of the spine head as well as changes in the spine neck length and/or diameter, and long-term depression (LTD) has been associated with shrinkage of the spine head size (Fig. 13). Then, it is likely that because of the high input impedance of the spine, they behave as biochemical and electrical compartments, both functions that promote spine plasticity.

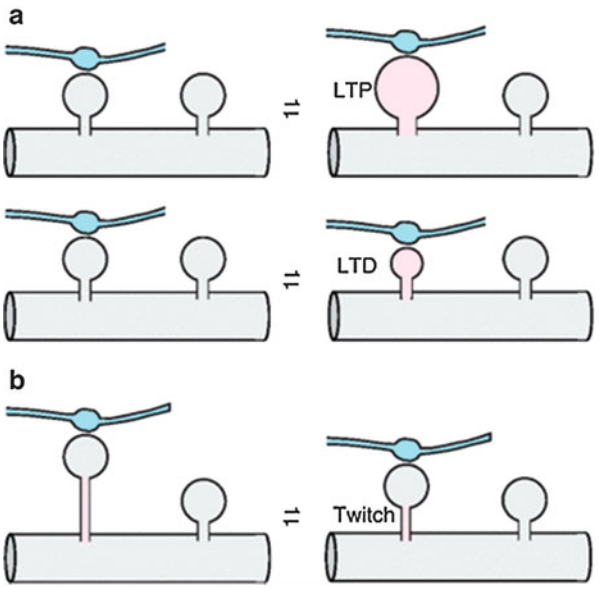

Fig. 13 Activity-dependent structural changes at dendritic spines. (**a**) The generation of long-term potentiation (*LTP*) or long-term depression (*LTD*) at a single spine level has been linked to an increase and decrease of spine head volume, respectively, as well as changes in the spine neck diameter (see text for details). (**b**) Crick proposed that the modulation of the spine neck length or the "twitching hypothesis" could provide fast changes in synaptic efficacy, which can account for the generation of short-term synaptic plasticity. This is an interesting hypothesis that needs to be evaluated experimentally

Since most excitatory inputs occur in spines, a fundamental question is how the electrical function of spines affects the integration of excitatory inputs. Rall, using multicompartmental models, was the first that proposed that the mode of integration of synaptic inputs will depend upon their dendritic location: "....the departure from linearity [linearity meaning the arithmetic sum of the synaptic events] can become quite large when perturbations are superimposed upon the same compartment, the departure from linearity can be surprisingly small when brief perturbations occur in separate portions of the dendritic periphery." In other words, two simultaneous excitatory inputs impinged on the same dendritic compartment will shunt each other (a local decrease in driving force) or have other forms of nonlinear integration; by the same token, if the two inputs are impinged in different compartments along the dendritic tree of a neuron, they will add linearly, without any significant shunting interaction. Rall's prediction was for inputs impinged directly on a cable or dendritic shaft, but what will happen if the excitatory inputs are directed to dendritic spines? Llinás and Hillman (1969) pointed out that if inputs are located on dendritic spines, and if spines have an electrical function (both conditions found experimentally), then the high neck resistance will allow the spines to behave as current injecting devices that prevent the large variation of the input impedance of the dendrite, thus

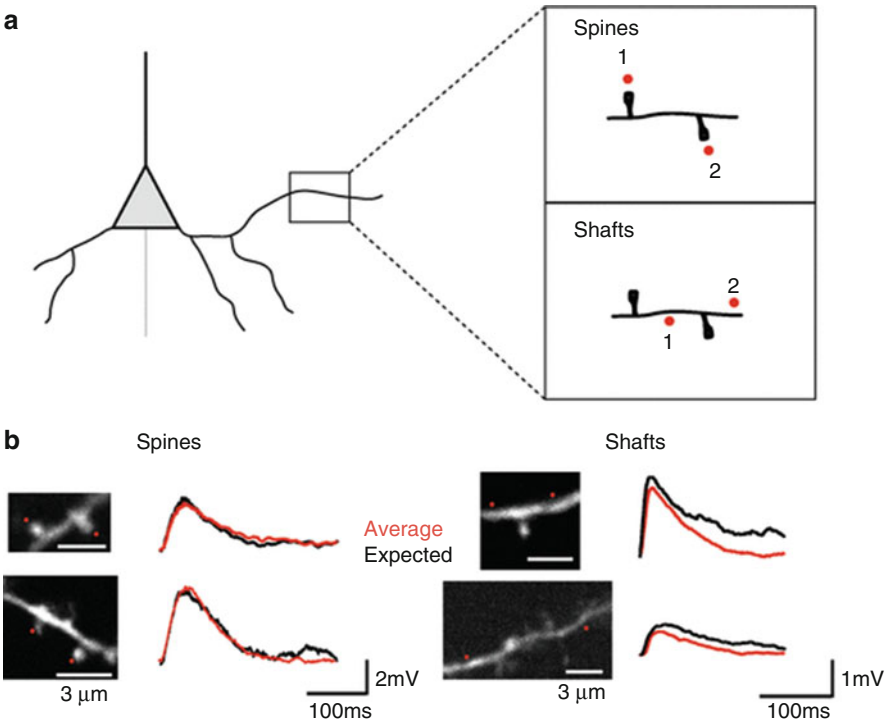

Fig. 14 Summation of excitatory uncaging potentials on spines and dendritic shafts. (**a**) Drawing of a layer V pyramidal cell showing the protocol for testing summation in spines and shafts. *Red dots* indicate the site of uncaging in spines or shaft locations. Voltage responses were recorded with a patch electrode in current-clamp configuration. (**b**) Two-photon uncaging of glutamate was performed first at each spine or shaft location and then in either both spines together or in both shaft locations. *Red traces* correspond to an average of 10 depolarizations caused by uncaging over two spines or shaft locations, and *black traces* correspond to the expected algebraic (linear) sum of the individual events of each spine or shaft locations. Note how the average uncaging response, when spines are activated, is close to expected. However, when inputs are impinged on shaft locations, the integration is sublinear (Image modified from Araya et al. (2006b))

preventing the shunting interactions and promoting linear interaction of inputs in spines that belong to the same dendritic compartment. Then, if two inputs are impinged in the dendritic shaft of a same dendritic compartment, or inputs onto neighboring spines with low input impedance, they will integrate in a nonlinear fashion and shunt each other.

Indeed, it has recently been shown, with the use of nearly simultaneous two-photon uncaging of glutamate over two to three spines located in the same dendritic compartments, that excitatory inputs integrate linearly, whereas inputs delivered to the same compartment but into the dendritic shaft integrate sublinearly, most likely due to shunting interactions of the excitatory inputs (Fig. 14). These results imply that an important reason for spines to behave as electrical compartments, due to its high input impedance, resides in a fundamental biophysical

function: to avoid the shunting interactions of excitatory potentials and to promote a linear sum of the inputs in pyramidal cells of the neocortex. In addition, these results indicate that in most of the spines tested, the input impedance at each spine trespasses the critical threshold for behaving as electrical compartments and, thus, promotes the linear integration of synchronous and clustered excitatory inputs within a dendritic compartment.

Several questions arise from these observations: Firstly, what are the molecular and biophysical mechanisms (passive and/or active) by which spines behave as electrical compartments? Secondly, how are synaptic inputs spatially and temporally distributed along the dendrites of a pyramidal cell? And finally, under what physiological circumstances can spines undergo activity-dependent structural changes that can modify the synaptic weight and thus the input/output properties of pyramidal cells?

To better understand the implications of the electrical role of spines in the input/output properties of pyramidal cells, let us think in two scenarios: if excitatory inputs are impinged on the dendrites of a pyramidal neuron in a (A) clustered or (B) distributed fashion. If simultaneous excitatory inputs are clustered on a single branch of the dendritic tree of a neocortical pyramidal cell, then spines will integrate inputs linearly (as described before) before the generation of a dendritic spike (see below) by avoiding the shunting interactions that will be otherwise expected if inputs are located in the parent dendritic shaft (Fig. 14). Now, if the same excitatory inputs are directed to either spine or shaft locations but in different dendritic compartments within a neuron, then those inputs will be integrated in a linear fashion, without any shunting. In other words, if indeed excitatory inputs are distributed, without any bias toward a particular dendritic zone (as some experimental evidence seems to suggest), and synchronous activation of these excitatory inputs is far enough that the large variation of the input impedance of the dendrite is prevented, then inputs will integrate linearly regardless of whether the inputs are impinged on dendritic spines or shafts locations.

If most excitatory inputs are distributed, then why are they directed to spines? One possibility is that if indeed inputs are distributed, then the main reason of why inputs are directed to spines is because spines are plastic structures that can undergo short- and long-term plasticity and then control the synaptic strength of the circuitry. It will be silly to build rigid circuits. Instead, by modifying the existing connections could be a fast and reversible way to change the neuron's output. In other words, if distributed or clustered inputs are directed to the *plastic devices* of the neuron's dendritic arbor – the dendritic spines – then the modification of those devices will change the synaptic strength and the neuron's output.

It is likely that in a freely moving animal, the dendrites of neocortical pyramidal neurons are receiving both distributed and clustered excitatory inputs (see "Dendritic Excitability" section). If this is indeed the case, then in the clustered input scenario, dendritic spines will be playing a role not only in controlling the synaptic strength of the circuitry but also in promoting the linear integration of inputs. This was indeed observed experimentally for only a few excitatory inputs directed to neighboring spines (Fig. 14).

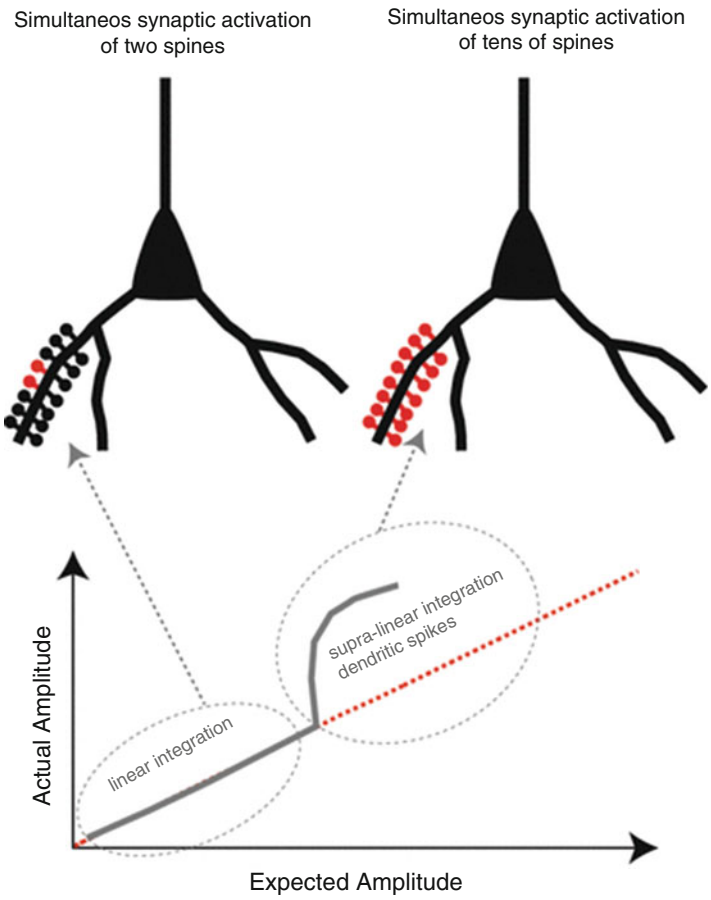

Fig. 15 Representation of the summation of excitatory postsynaptic potentials (*EPSPs*) in the dendrites of a pyramidal neuron. The simultaneous synaptic activation of a few spines within a dendritic branch (*red spines* in the *upper left* diagram) will trigger a voltage response that matches the arithmetic sum of each spine's voltage contribution in a linear fashion (see experiments in Fig. 14 and text). If tens of spines are activated simultaneously within the same branch, then a supralinear response, or a dendritic spike, will be generated. Thus, the voltage response will be bigger than the expected sum of the voltage contribution of each of the spines being activated, generating a supralinear voltage response

Although the data suggested that spines can behave as electrical compartments that promote the linear integration of excitatory inputs, it has also been demonstrated that dendrites are capable of generating nonlinear voltage responses when several inputs are synchronously activated in one dendritic compartment (tens of spines activated simultaneously in one dendritic compartment; Fig. 15). The generation of these nonlinear voltage responses on the dendrites of pyramidal cells will be discussed in more detail in the section "Dendritic Excitability."

Spines as Coincidence Detectors

Another biophysical property of dendritic spines is that due to their morphology and size, it has been suggested that they can sense the information coming from the dendrite with little decrement as opposed to the information coming in the forward direction (from the spine to its parent dendrite) due to the *impedance mismatch*. This biophysical property that will be discussed in more detail in the "Spread of Electrotonic Potentials in Dendrites" section (see below) will allow the spine to follow the potential spread in the dendrite and behave as a "coincidence detector" for backpropagating action potentials (bAP) or subthreshold potentials from nearby synaptic inputs with that of its own synaptic input drive.

Our current understanding of the structural and functional role of dendritic spines comes mainly from experiments in fixed tissue and chronic and acute brain slices. With the use of two-photon microscopes, where you can penetrate deeply into the tissue, several groups have succeeded in imaging individual spines from fluorescently labeled neurons over extended periods of time in intact mammalian brains and visualize live spines from several hundred micrometers deep from the pial surface. This has proven to be a successful tool for studying spine development and the experience-dependent remodeling of dendritic spines in an intact mammalian brain. However, until now, no functional experiments evaluating the role of dendritic spines in the processing, storage and integration of excitatory inputs have been done in the intact brain. Thus, an important goal in the dendrite field will be to understand in an intact mammalian brain the input/output properties of pyramidal cells and to uncover the structural-functional mechanisms of plasticity down to the level of single spines. The development of novel optical, genetic, electrophysiological, and structural tools will likely answer some of these questions in the coming years. Hopefully, these experiments will eventually lead to a better understanding of dendritic spines function and to the development of novel therapeutic approaches for neurological conditions such as fragile X syndrome, where spine structure, density, and function are impaired.

Dendritic Excitability

Neocortical pyramidal neurons receive thousands of inputs throughout their dendritic trees. The efficacy of EPSPs depends greatly on their synaptic location. Because of dendritic filtering, distal EPSPs (synaptic inputs on distal portions of the dendritic tree) will have little if no direct contribution at the soma and, thus, have only a minor effect on action potential generation (Fig. 18). How, then, can distal EPSPs be relevant for neuronal output? The presence and distribution of voltage-gated channels along the dendrites of pyramidal cells has been demonstrated to be a crucial determinant of the input/output properties of these types of neurons (see "Active Spine" section). In particular, voltage-gated sodium, calcium, and potassium channels have been shown to be responsible for shaping synaptic potentials within individual spines (see below), as well as to support backpropagating action potentials (bAPs) – an action potential that is initiated in the axon and then propagates

back into the dendrites – and the generation of dendritic spikes, a suprathreshold voltage response or spike generated in the dendrites. It is well known that neocortical pyramidal neuron dendrites are capable of triggering sodium, calcium, and NMDA spikes (Fig. 16). It has been shown in layer V pyramidal neurons that these dendritic

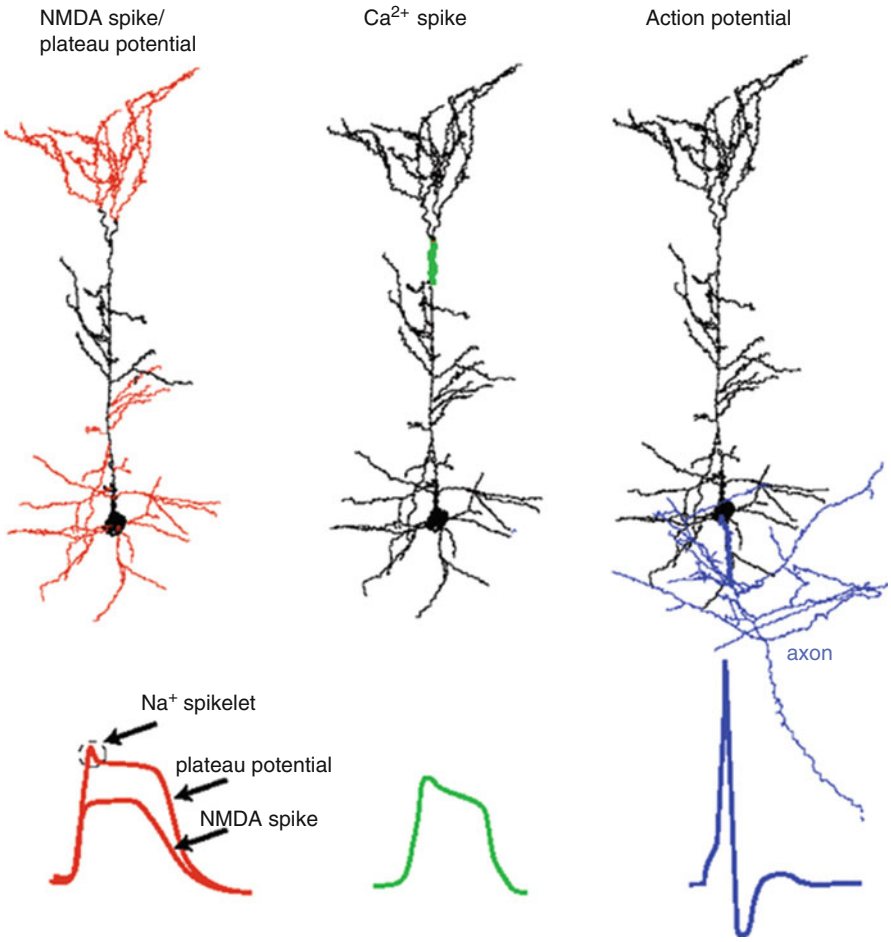

Fig. 16 Types and locations of dendritic spikes. Thin dendrites, basal, oblique, and the apical tuft (*highlighted in red*) are capable of supporting sodium and NMDA spikes (see text for details). Sodium spikes or spikelets (*circle*) are fast events, and NMDA spikes are characterized by a significant voltage deflection lasting hundreds of milliseconds (*red trace*). Plateau potentials are characterized by a rapid onset, a plateau phase, and a rapid end. The major contributor is the NMDA current; however, they are the combination of NMDA receptors and voltage-gated sodium and calcium channel activation. Calcium spikes are normally generated in a restricted zone (*green*) located in the main apical bifurcation. These potentials are generated in an all-or-none manner and last for tens of milliseconds (*green trace*). The axon initial segment is the zone responsible for the generation of the all-or-none action potential, mediated by the opening of sodium and potassium channels. (The axon is highlighted in *blue*, and axon initial segment in *bold blue*)

spikes, if generated by distal inputs, have a transient influence on the neuronal output, as opposed to the filtered and negligible contribution of subthreshold distal EPSPs to the neuron's output (Fig. 18). Thus, the generation of dendritic spikes can serve as a mechanism that can overcome the distance dependency on synaptic efficacy. Indeed, it has been demonstrated that the coincident activation of distal and proximal inputs in layer V pyramidal neurons can be sufficient to trigger dendritic spikes in the more distal locations, thereby having a meaningful contribution at the soma. However, dendritic spikes are not reliably propagated to the soma, and only on rare occasions can they trigger an AP at the soma. Thus, it seems likely that distal synapses must cooperate with more proximal synaptic inputs to produce a somatic action potential. As an example of this scenario, in CA1 hippocampal pyramidal neurons, where distal dendritic spikes do not propagate to the soma, the coincident activation of Schaffer collateral and perforant path synapses is required for the reliable propagation of dendritic spikes to the soma.

As mentioned before, different voltage-gated channels are distributed differently along the dendritic arbor of pyramidal cells, having important consequences for the input/output properties of pyramidal neurons. For example, as described before, in CA1 pyramidal cells, HCN channels are expressed in a somatodendritic gradient (with more channels at distal locations), and in layer V neocortical pyramidal neurons, HCN channels undergo a dramatic age-dependent increase in distal apical dendritic sites. This differential distribution of HCN channels in these neuronal types has important effects on the integration of synaptic potentials.

Types and Locations of Dendritic Spikes

Calcium action potentials or Ca^{2+} spikes: In the apical trunk of neocortical pyramidal cells, in a restricted zone located near the main apical bifurcation, there is a low-threshold zone that, upon strong synaptic stimulation of the apical tuft plus the activation of proximal locations, or local current injections into the apical dendrite, can trigger Ca^{2+}-dependent, regenerative dendritic potentials that can propagate both toward the soma and to the distal tips of the apical tuft (Fig. 16). At the soma, this calcium-dependent potential can interact with somatically generated, sodium action potentials, promoting bursts of somatic spikes. Calcium spikes are initiated in an all-or-none manner, with a clear threshold, and last for several tens of milliseconds (Fig. 16). In in vivo preparations, these potentials have been recorded in layer V pyramidal neurons both spontaneously and after whisker or layer I stimulation. The generation of Ca^{2+}-dependent regenerative potentials in the apical trunk can also be triggered by bursts of somatic action potentials that have surpassed a critical spike frequency threshold or by the coincident arrival of single backpropagating action potentials and distally located subthreshold synaptic inputs. In the neocortex, Ca^{2+} spikes have been detected not only in layer V pyramidal neurons but also in layer II/III and layer VI pyramidal cells, indicating that this form of dendritic computation might be a common integration mode observed under certain spatiotemporal arrangements of excitatory input to pyramidal neurons.

Sodium dendritic spikes: The distribution of Na^+ channels along the dendrites of a pyramidal neuron plays a crucial role in the boosting of synaptic potentials as well as in the backpropagation of APs to distal locations. It has been suggested that the clustering of sodium channels at the synapse can boost excitatory potentials at the spine head, thus reducing the synaptic strength needed to trigger a dendritic spike. Sodium spikes have been observed in both neocortical and hippocampal pyramidal cells. These types of spikes, often called "spikelets," are fast, their duration normally being shaped by the activation of NMDAR and VSCCs (Fig. 16). Such spikes have been observed in basal, oblique, and apical dendrites of CA1 pyramidal cells and in the distal apical tuft and proximal basal dendrites of layer V pyramidal neurons.

NMDA spikes: Recently, some laboratories have been successful in accessing thin dendrites – apical tuft and basal dendrites – from layer V pyramidal neurons by direct patch-clamp recordings. With this technique, the biophysical properties of thin layer V pyramidal neurons have been revealed. These experiments have indicated that basal and tuft dendrites in layer V pyramidal neurons can support Na^+ and NMDA spikes, but not Ca^{2+} spikes, suggesting different excitability in different portions of the dendritic arbor of a single pyramidal neuron (Fig. 16). NMDA spikes are the result of synchronous activation of tens of glutamatergic synapses within a restricted region of the dendrite. These type of spikes are characterized by a significant voltage deflection (40–50 mV) lasting hundreds of milliseconds (Fig. 16). No reports have yet demonstrated the presence of these spikes in vivo.

Plateau potentials: These potentials are seen in cortical and hippocampal pyramidal cells. They can be triggered by glutamate iontophoresis or by one- or two-photon uncaging of glutamate over tens of glutamatergic synapses simultaneously or nearly simultaneously and can last hundreds of milliseconds. These potentials are normally a combination of the activation of NMDA receptors as well as voltage-gated sodium and calcium channels (Fig. 16), although the major contributor is believed to be NMDA receptor currents. These potentials have an initial fast phase, a plateau, and a rapid decay of the signal (Fig. 16). Plateau potentials have been observed under in vitro conditions in layer V pyramidal neurons and in hippocampal pyramidal neurons, although they have not yet been observe in vivo. Further experiments in an intact brain are needed to evaluate whether these events are physiological, and if so, under what condition(s) or stimulation paradigm (s) a dendrite is capable of generating this type of spikes.

It has been proposed by the Larkum and Schiller groups that a unifying integrative principle might exist in layer V pyramidal neurons. This unifying principle highlights the importance of different dendritic subcompartments in the processing of synaptic information: thin dendrites, basal and apical tuft dendrites, the places that receive most of the excitatory synaptic inputs, integrate local inputs by the activation of NMDAR and the generation of NMDA spikes (or the "output" of thin dendrites). NMDA spikes are then passed to either the axon (if the NMDA spike comes from basal dendrites) or to the Ca^{2+} spike generation zone in the apical dendrite (if the NMDA spike arrives from the distal apical tuft) where they interact with signals coming from other parts of the dendritic tree (e.g., interaction between top-down and bottom-up information; see below).

Is there, indeed, one unifying integrating principle in layer V pyramidal neurons (Fig. 16), where thin dendrites – basal, oblique, and the apical tuft – only support sodium and NMDA spikes and thick apical dendrites only Ca^{2+} spikes? This is an interesting idea; however, it has recently been demonstrated by the Larkum group under in vivo conditions that simple sensory stimulation triggers Ca^{2+}-dependent spikes without requiring NMDA spikes in the apical tuft of layer V pyramids. Further in vivo experiments with different and more complex stimulation protocols are needed to fully explore the biophysical properties of thin versus thick dendrites in an intact brain.

What Are the Functional Consequences of Dendritic Spikes?

As mentioned before, some of the important functional consequences of dendritic spikes are that they can serve as a mechanism that can overcome the distance dependency of synaptic efficacy. In addition, it has been suggested that dendritic spikes can be important modes of computation needed for the generation of synaptic plasticity. In particular, it has been demonstrated in pyramidal neurons that either a single dendritic spike or the potentiation of synapses in the apical tuft by dendritic spikes, but not by bAPs, can trigger long-term plasticity (LTP). Moreover, it has been shown that AP burst-evoked dendritic calcium spikes can have an important role in the generation STDP. Therefore, the presence of glutamate receptors and voltage-gated channels in dendrites and the generation of dendritic spikes could be an important signal needed for making different dendritic compartments "relevant" for the cell's output, as well as being a signal capable of triggering plasticity. Thus, dendritic spikes are likely to be important both for shaping the integrative and plastic properties of pyramidal neuron dendrites.

Neocortical Pyramidal Neurons as Associative

Based on these existing data, a provocative general idea for layer V pyramidal neurons has been proposed based on the capacity that pyramidal neurons might have to act as input-associative units capable of integrating feedback inputs coming from higher-order cortical areas directed to the apical tuft (top-down inputs) with "local" proximal inputs (bottom-up inputs) directed to basal and oblique dendrites. Once proximal inputs generate an AP, the backpropagating AP could play a pivotal role in coupling both spike initiation zones, thus changing the contribution of distal inputs on the input-associative function of pyramidal neurons. This function could have important consequence in modifying the neuronal output or spike frequency, thus its function within the microcircuitry it resides on. Thus, the input-associative function of pyramidal neurons might be a fundamental task these neurons implement in shaping complex cortical functions such as consciousness or self-awareness of the external world.

Electrotonic Properties of Dendrites: A Theoretical View

The simplest analytical model defining the voltages and currents through conductive cables has several properties in common with the passive or electrotonic spread of signals through dendrites. The assumptions implicit in "cable theory" are that each dendritic segment is a cylinder with constant radius through which the electrotonic potential – or the product of the change in membrane potential – spreads through.

The application of "cable theory" to complex dendritic trees can be achieved by compartmental computational models that can simulate the electrotonic spread of synaptic signals through complex dendritic arborizations by adding together cylinders of different radii. This has proven to be a fundamental tool in understanding the rapid spread of electric current in dendrites and for the understanding of the biophysical factors that determine this spread.

The electrotonic spread of current in a dendrite is well described by the simple Ohm's law linear equation

$$E = IR$$

where E is the potential, I is the current, and R is the resistance. An important consideration is that the electrotonic current experiences two distinctive resistances: the internal (or axial) and the membrane resistances. In the simplest representation of the passive spread of the electrotonic potential, in a steady-state input to a cable or dendrite with uniform resting potential (E_r), the E_r and the capacitance (C_m) can be disregarded. Thus, current only divides itself at any point of the process through two resistance paths: the axial and membrane resistance.

In addition, since parallel resistances add each other to reduce the total resistance, the axial resistance is inversely proportional to the diameter of the cable or dendrite:

$$r_i \ \alpha \frac{1}{A}$$

where r_i is the axial resistance per unit length (in Ω cm of axial length) and A is the cross-sectional area (πr^2). Thus, a thicker cylinder or dendrite has a lower axial resistance than does a thinner process. Hence, assuming that the axial resistance is uniform throughout the cable or dendrite, it can be represented as

$$r_i = \frac{R_i}{A}$$

where r_i is the axial or internal resistance per unit length (in Ω cm of axial length), R_i is the specific axial or internal resistance (in Ω cm^{-1}) of axial length, and A is the cross-sectional area (πr^2).

It is important to mention that dendrites are not empty cylinders; the presence of organelles, cytoskeletal elements, etc., will increase the effective internal resistance, or axial resistance, thus affecting the spread of electrotonic signals (e.g., the spread

of synaptic potentials from the spine head to the parent dendrite through the narrow spine neck).

In addition, the current through the membrane is inversely proportional to the membrane surface area. Thus,

$$r_m = \frac{R_m}{c}$$

where r_m is the membrane resistance for unit length (in Ω cm), R_m is the specific membrane resistance (in Ω cm^2), and c (2 πr) is the circumference.

Another important assumption in cable theory is that the resistivity of the external medium is negligible, due to the large volume of the extracellular medium.

Based on these assumptions, we can now describe the electrotonic spread of potentials in dendrites. Under steady-state conditions, the spread of signals through a cable is described by

$$\frac{\mathrm{d}^2 V}{\mathrm{d}x^2} = V * \frac{r_i}{r_m}$$

The steady-state solution of this equation for a dendrite of infinite length is

$$V = V_o e^{-\frac{x}{\lambda}}$$

where V is the electrotonic potential, V_0 is the value of V at x = 0, and λ defined as

$$\lambda = \sqrt{\frac{r_m}{r_l}}$$

when $x = \lambda$, the ratio of V to V_0 is $e^{-1} = 0.37$. Thus, λ is the length constant or space constant that represents the length at which the electrotonic potential decays to a value of 0.37 of the value at the point of origin. Thus, the higher the value for r_m, the larger the value of λ, and the higher the value of r_i, the smaller the value λ.

The solution for the steady-state spread of electrotonic potentials for a cable or dendrite of length L is defined by

$$V = V_o * \frac{\cosh \frac{x - L}{\lambda}}{\cosh \frac{L}{\lambda}}$$

In addition, the space constant, λ, depends not only on the membrane and axial resistance but also on the diameter of the dendrite, and it can be expressed as

$$\lambda = \sqrt{\frac{r_m}{r_i}} = \sqrt{\frac{R_m}{R_i} * \frac{d}{4}}$$

where d is the diameter of the process. There is a huge variability in dendritic diameters within one neuron. For example, in layer V pyramidal neurons, there are

thin dendrites <1 μm diameter (basal, oblique, and apical tuft dendrites) and thick dendrites ~2 μm diameter (apical dendrites). Moreover, the average diameter of the apical dendrites varies significantly between layers II and III, layer V, and layer VI.

Until now, we have considered the passive spread of steady-state inputs. However, what is the case for the electrotonic spread of transient signals along dendrites? The spread of rapid signals depends not only on the factors described before but also on the membrane capacitance (C_m). In an equivalent circuit, the analysis of a transmembrane current pulse (I_m) is described as

$$I_m = \frac{V_m}{R_m} + C_m * \frac{dV_m}{dt}$$

where the I_m is in A/cm, R_m is in Ω cm, and C_m in F/cm.

Rearranging this equation, we have:

$$I_m R_m = V_m + R_m C_m * \frac{dV_m}{dt}$$

where $R_m\, C_m = \tau$ and τ is the time constant of the membrane.

Solving this equation for $t \to \infty$ dV/dt = 0, $V_\infty = I_m R_m$, thus,

$$I_m R_m = V_\infty = V_m + R_m C_m * \frac{dV_m}{dt}$$

where V_∞ is the steady-state potential. The solution of this equation to a step-current injection is

$$V_m - V_\infty = [V_0 - V_\infty] e^{-t/RC}$$

or

$$V_m - V_\infty = [V_0 - V_\infty] e^{-t/\tau}$$

where V_0 is the potential at time zero of the current pulse.

Thus, τ is the time required to reach 0.37 of its final value. The specific time constant of a patch of membrane will define the transient voltage responses to a current step for a neuronal process. In a multicompartment model, when current is injected into one compartment, the proportion of charge that is divided between C_m and R_m will determine the time constant, τ, or the rate of charge of the membrane. Then, charge will start to flow from the initial compartment to the neighboring ones through the R_i.

Spread of Electrotonic Potentials in Dendrites

Most neurons in the central nervous system have extensively branched dendrites. The spread of signals through dendrites depends greatly on their degree of branching. Imagine a case where the electrotonic potential is traveling from a

piece of dendrite that is connected to (a) either a thicker dendrite (e.g., a signal coming from a basal dendrite directed to the soma or from the spine neck to the parent dendrite) (b) or a thinner dendrite (e.g., a backpropagating signal coming from the apical dendrite to the apical tuft dendrites). In both cases, this will produce an *impedance mismatch*. For the case where the signals traveled from a thin dendrite to a thicker lower impedance dendrite, the impedance mismatch will reduce the electrotonic potential faster than would be expected by just λ, due to the current. On the contrary, if a thicker piece of dendrite connects to a thinner higher-impedance one, there will be a favorable impedance mismatch, and the electrotonic potential will propagate effectively. In addition, impedance is matched, either when (a) connecting pieces of dendrite of the same caliber or (b) by matching the input conductances of the stem with that of its branches following Rall's 3/2 rule, where there is continuity or impedance matching at a branch point if

$$d_0^{3/2} = \Sigma d_l^{3/2}$$

where d_0 is the diameter of the cable along which the signal is propagating and d_l are the diameters of the daughter branches where the signal propagates into. If this rule is obeyed, the entire dendritic tree can be collapsed into a series of equivalent cylinders, assuming uniform R_m, R_i, and C_m. Under this scenario, there is electrotonic continuity (Fig. 17). If we now trigger synaptic conductances at different locations along the dendritic tree and record them at the soma, it is evident that the spread of synaptic potentials along dendrites is accompanied by a reduction in speed and amplitude (Fig. 17) as has been observed experimentally in neocortical pyramidal neurons (Fig. 18).

Outlook

In conclusion, the study of dendrites, and in particular that of dendritic spines, has proven to be fundamental not only for understanding the input/output properties and information storage capabilities of single pyramidal neurons but also to understand the function of pyramidal neurons in the context of the network they reside in.

Further research is needed to map the morphological and functional connectivity map or "connectome" of a single pyramidal cell. This approach, to my mind, should include as a first step the unraveling of the structural and spatial organization of a single pyramidal neuron's presynaptic partners, followed by mapping the identity of its neighbors and the neuron's functional connectivity (at the level of individual spines) under various sensory stimulation paradigms. I believe that with the technologies available today, in particular with the transneuronal tracing capabilities of neurotropic viruses together with functional imaging of spines and dendrites, as well as with optogenetic, electrophysiological, and ultrastructural techniques, it will be possible to uncover and probe the circuitry that relates to an individual cell, or the "single-cell connectome." This task, as opposed to the less approachable, more

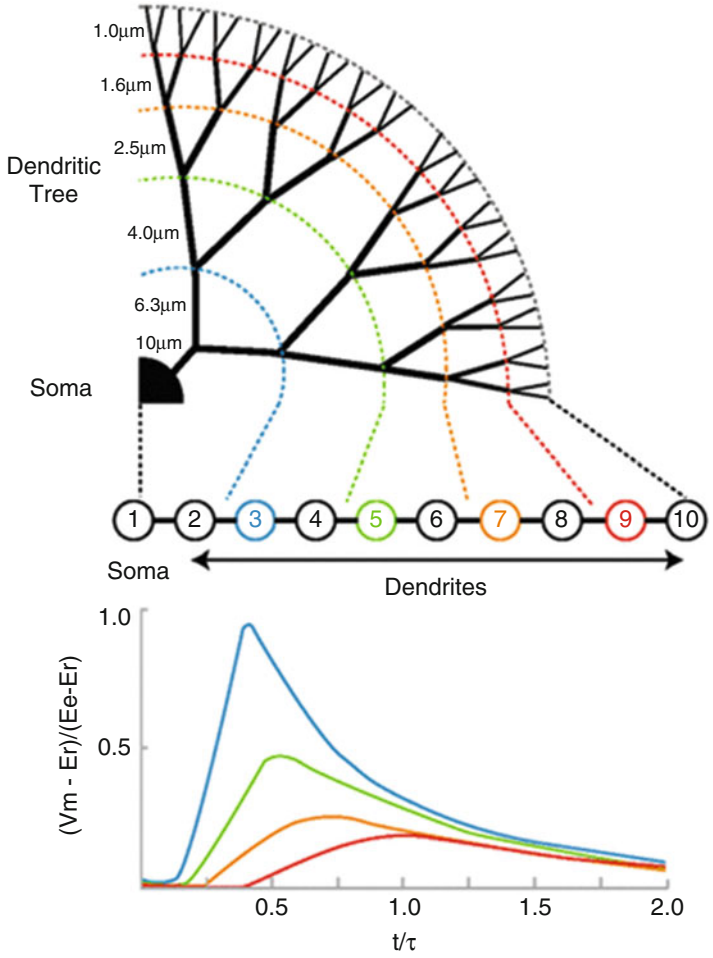

Fig. 17 Electrotonic spread of synaptic potentials in a dendritic tree that follows the d3/2 rule and, thus, can be transformed into an equivalent cylinder. An EPSP is generated in compartment 9, 7, 5, or 3 and recorded at the soma (compartment 1). The graph shows the voltage responses recorded at the soma when the EPSP was generated in the different dendritic compartments (color in voltage traces corresponds to the color or the different dendritic compartments) (Modified from Rall (1964))

complicated, time-consuming, and more expensive "multicellular whole brain connectome" project, is more likely to provide us with the basic rules governing pyramidal cell function, as well as giving us solid hints on how the neocortex works. In addition, I foresee that with the use of subdiffraction limit microscopes (such as STED microscopes), it will be possible to uncover the dendritic spine structure-function relationship with exquisite detail, thereby giving us more precisely detailed information regarding the factors that govern neuronal output under physiological and pathological conditions.

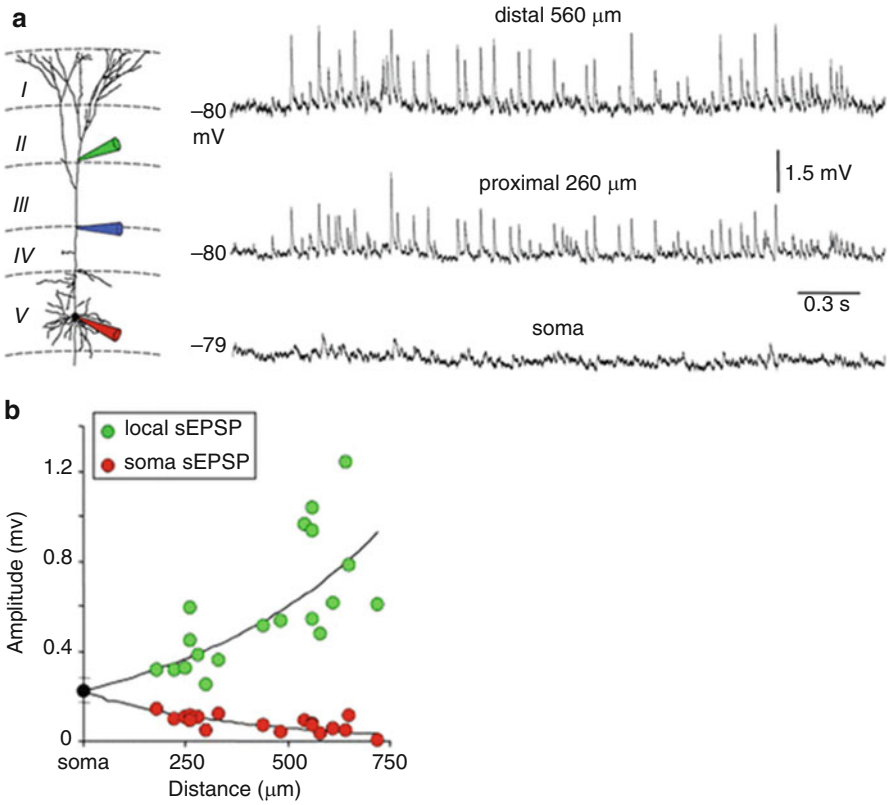

Fig. 18 Site dependence of spontaneous EPSP amplitude. (**a**) Spontaneous EPSPs (sEPSPs) were simultaneously recorded at the soma and two apical dendritic sites from layer V neocortical pyramidal neurons. (**b**) Note how the local amplitude of the sEPSPs increased exponentially with distance from the soma (*green circles*) and how the somatic amplitude of sEPSPs decreased exponentially as their generation site became more distal (Modified from Williams and Stuart (2002))

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