Chapter 18
Excitatory and Inhibitory Synaptic Placement and Functional Implications

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Abstract Synaptic transmission between neurons is the basic unit of communication in neural circuits. The relative number and distribution of excitatory and inhibitory synaptic inputs across individual dendrites and neurons are the hardware of local dendritic and cellular computations. In this chapter, we discuss the structural and functional observations that have guided the understanding of excitatory and inhibitory synaptic organization across the neuronal arbor, the subcellular targeting properties of different neuronal subtypes, and the effects of synaptic placement on local integration within dendritic segments. We focus primarily on the adult mammalian cortex and hippocampus, where excitatory and inhibitory cell types, their connectivity, and its functional implications have been best characterized.

18.1 Introduction

The neurotransmitter synthesized and released at the synapse is the basis for the classification of neurons as excitatory or inhibitory. Glutamatergic neurons, releasing glutamate, excite postsynaptic cells, while GABAergic neurons, releasing GABA (γ-aminobutyric acid), inhibit them. The delicate balance between

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excitation and inhibition is essential for precise nervous system function and plasticity, as its perturbation has been associated with disorders ranging from epilepsy (Mohler et al. 2004), to autism (Rubenstein and Merzenich 2003), and mental retardation (Dani et al. 2005; Kleschevnikov et al. 2004), as well as neuropsychiatric disorders such as schizophrenia (Lewis et al. 2005). A key to this balance is the precise coordination of excitatory and inhibitory synaptic activity at specific cellular locales. Yet, our knowledge regarding global distribution, as well as the local placement, of excitatory and inhibitory synaptic inputs across different neuronal subtypes is still quite poor. Below we summarize the current state of the field and the evolution of our understanding to date.

18.2 Organization of Synapses Across the Dendritic Arbor

18.2.1 First Views Through Golgi Staining and Electron Microscopy

18.2.1.1 Pyramidal Neurons

In the late nineteenth century, development of the Golgi stain, a silver stain that sparsely but intensely labels neurons, enabled the first clear discrimination of individual neuronal morphology (Fig. 18.1A–C). In his meticulously detailed drawings, Ramon y Cajal characterized the elaborate dendritic arbors and axonal
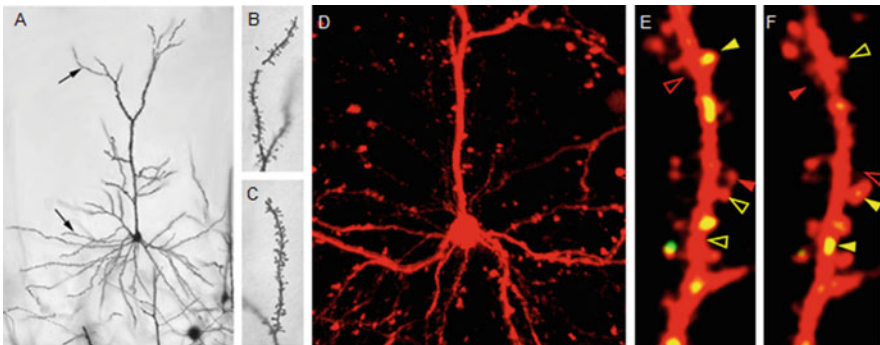

Fig. 18.1 Visualization of L2/3 pyramidal neurons. (**A**) Golgi-stained pyramidal neuron (Image kindly provided by Dr. Terry Robinson, University of Michigan). (**B**, **C**) High-magnification views of the two dendrites marked by *arrows* in panel **A**. Note dendritic spines. (**D**) 3D volume projection of a pseudo-colored pyramidal neuron imaged in vivo using two-photon microscopy. (**E**) An individual dendrite expressing YFP (*red*) and teal-gephyrin (*green*) to label inhibitory synapses. (**F**) The same dendrite imaged 8 days later. *Yellow arrows* indicate dynamic inhibitory synapses and *red arrows* indicate dynamic spines. *Filled arrows* show when structures are present and *open arrows* indicate their absence

processes of neurons with a wide range of morphologies. One obvious distinction was that some neurons have smooth dendrites, while others have many spiny protrusions. In general, most neurons with spiny dendrites were later revealed to be glutamatergic and excitatory, while neurons with smooth dendrites for the most part release GABA and are inhibitory (Gabbott and Somogyi 1986; Kubota 2014; Morishima et al. 2011; Thomson and Deuchars 1997). Elegant electron microscopy (EM) studies on Golgi-stained cells observed that each spine contains a synapse characterized by round presynaptic vesicles and a robust postsynaptic density (Hersch and White 1981; LeVay 1973; Parnavelas et al. 1977). These asymmetric synapses, classified as type 1 synapses, are innervated by axons of glutamatergic neurons (Baude et al. 1993). While there are a few reports of specific cell regions on particular types of neurons where type 1 synapses are localized directly on the dendritic shaft (Megias et al. 2001; Parnavelas et al. 1977), this is generally not the case. Given that the vast majority of spines, with the exception of some very thin spines (about 2–4 % of total cortical spines) (Arellano et al. 2007; Hersch and White 1981; White and Rock 1980), have a single type 1 excitatory synapse (Harris et al. 1992; LeVay 1973), it was reasonable to assume that each spine can serve as a readily identifiable structural surrogate for an excitatory synapse. Thus, for spiny pyramidal neurons, dendritic spine distributions as seen by Golgi stain or by filling cells with a fluorescent dye could inform as to the placement of excitatory synapses across the dendritic arbor (Elston and Rosa 1997; Larkman 1991; Trommald et al. 1995).

There is a great variety in the density of spines between cells and between different branches on the same cell. To give a general sense of spine distributions, spine density is low within 40 μm of the cell body, reaching a maximum density in a region 40–130 μm from the soma, and then gradually decreasing toward a dendrite's distal tips (Megias et al. 2001). On the distal branches of pyramidal cells, spine density can range from 0 to 70 spines per 10 μm (Megias et al. 2001) with the highest spine density often found on the thickest dendrites, usually the primary apical dendrite. The total number of spines on spiny excitatory neurons typically ranges from 5,000 to 35,000 spines per cell, ultimately depending on the total dendritic length, with L5 pyramidal cells generally having longer total dendrite length and therefore more synapses than L2/3 pyramidal cells (Larkman 1991).

Inhibitory synapses innervated by the axons of GABAergic neurons, classified by EM as type 2, or symmetric synapses, are typified by a symmetric synaptic cleft, due to a minimal postsynaptic density, as well as flattened presynaptic vesicles (Davis and Sterling 1979). Unfortunately, since type 2 inhibitory synapses lack a morphological surrogate, their distribution patterns cannot be discriminated by Golgi staining alone. Laborious EM reconstructions of relatively small neuronal regions have shown excitatory to inhibitory synaptic ratios along pyramidal dendrites ranging from 6.5–12.5 to 1 (Davis and Sterling 1979). As previously mentioned, the cell body and proximal dendrites of excitatory neurons typically lack spines. However, they are often densely innervated by type 2 synapses. In the aspiny region of the proximal dendrites, inhibitory synapse density can be as high as 17 synapses per 10 μm (Megias et al. 2001). EM studies also show that on

excitatory dendrites, the majority of type 2 inhibitory synapses are located on the dendritic shaft at a density of about 3 per 10 μm (Hersch and White 1981). Inhibitory synapses can also be located on dendritic spines adjacent to excitatory synapses (Parnavelas et al. 1977), as well as on the axon initial segment (Hersch and White 1981; Westrum 1966). Inhibitory axons from different inhibitory neuron subtypes specifically target discrete regions of their postsynaptic pyramidal cell partners (we discuss this in greater detail further in the chapter).

18.2.1.2   Non-pyramidal Neurons

The dendrites of inhibitory neurons, in general, do not contain spines. However, a small subset of inhibitory neurons have dendritic spines (Azouz et al. 1997; Feldman and Peters 1978), with densities that range from 0.3 to 7 spines per 10 μm (Kawaguchi et al. 2006; Keck et al. 2011). Like spiny pyramidal cells, the cell body and most proximal dendrites of spiny interneurons within 30 μm of the soma lack spines (Kawaguchi et al. 2006). There are several distinct subtypes of spiny inhibitory neurons, but Martinotti cells are the ones with the highest spine density with about three to seven spines per 10 μm, approximately one fourth of the density on pyramidal cells (Gulyas et al. 1992; Kawaguchi et al. 2006). Martinotti cells also have longer spines than other spiny interneuron subtypes and more multiheaded spines (Kawaguchi et al. 2006). Immunohistochemistry experiments show that the majority of spines on these interneurons colocalize with Vglut1 but not VGAT, indicating that they mostly harbor excitatory synapses (Keck et al. 2011). Unlike dendritic spines on excitatory neurons, which typically contain only one excitatory synapse, the spines of some spiny inhibitory neurons in the hippocampus can contain up to six distinct excitatory synapses (Gulyas et al. 1992). The proportion of excitatory synapses located along the shaft of spiny inhibitory dendrites has not been established. We also know little about the distribution of inhibitory synapses on spiny interneurons (Gulyas et al. 1992).

Aspiny interneurons receive both excitatory and inhibitory inputs onto their soma and proximal dendrites (Davis and Sterling 1979), with a higher density along their distal dendrites (Parnavelas et al. 1977). Similarly to inhibitory synapses on pyramidal cells, both excitatory synapses and inhibitory synapses onto dendrites of aspiny inhibitory neurons cannot be visualized through a morphological surrogate and could initially be examined only by EM on relatively small arbor segments or by immunohistochemistry. EM reconstructions of isolated branches from different inhibitory neuron types provide anecdotal evidence that parvalbumin (PV)-positive cells have the highest density of synapses with a density of 30 excitatory and 2 inhibitory synapses per 10 μm of dendritic length for branches sampled (Gulyas et al. 1999). Inhibitory synapses are also relatively rare on calretinin (CR)-positive spiny interneurons (Gulyas et al. 1992) which have a lower density of seven excitatory synapses and two inhibitory synapses per 10 μm (Gulyas et al. 1999).

18.2.2 Synaptic Visualization by New In Vivo Fluorescent Labeling Methods

Golgi staining gave us a first view of dendritic spine distributions, and EM studies were first to shed light on the fundamental layout of excitatory and inhibitory synaptic distributions on different neuronal types. Yet, both methods have inherent limitations. As a cell fill, Golgi stain can at best identify spines on cells with spiney dendrites, while all inhibitory synapses as well as excitatory synapses on aspiny dendrites remain invisible. EM is limited by the difficulty of reconstructing large dendritic segments. EM reconstruction of an entire cell would require a heroic effort, one rarely attempted (see White and Rock 1980 for a reconstruction of an entire spiny stellate neuron). Both Golgi staining and EM reconstructions necessitate tissue fixation and cannot be used for visualizing structural dynamics. Thus, our initial view of synaptic distributions was constrained by the limitations of the Golgi and EM methods.

Recently, imaging of fluorescently labeled cells in vivo has provided a new view not only of dendritic spine distributions but also of excitatory and inhibitory synapses as well as the dynamics of these structures (Fig. 18.1D–F). First studies on neurons fluorescently labeled with green fluorescent protein (GFP) showed that in adult animals dendrites of pyramidal neurons are very stable (Grutzendler et al. 2002; Mizrahi and Katz 2003; Trachtenberg et al. 2002), but dendritic spines are highly dynamic (Trachtenberg et al. 2002), implying a capacity for synaptic removal and addition. Spine dynamics of excitatory as well as spiny inhibitory neurons can be further increased upon sensory deprivation (Hofer et al. 2009; Holtmaat et al. 2006; Keck et al. 2008; Trachtenberg et al. 2002). Dynamics differ dependent on deprivation protocol and cortical lamina, consistent with the view that they reflect specific circuit alterations.

In contrast to the stability of excitatory dendritic branches, inhibitory dendrites are capable of growth and retraction in vivo (Chen et al. 2011a, b; Lee et al. 2006, 2008), and their dynamics are influenced by sensory manipulations (Chen et al. 2011b). The boutons of inhibitory neurons are also capable of remodeling, and their dynamics also increase in response to sensory deprivation (Chen et al. 2011b; Keck et al. 2011; Pieraut et al. 2014; Schuemann et al. 2013; Wierenga et al. 2008). Thus, synaptic distributions are not necessarily rigid. Rather, both excitatory and inhibitory synapses are dynamic structures, and their remodeling potentially underlies functional plasticity.

More recently, the expression of fluorescent proteins fused to postsynaptic scaffolding molecules has enabled direct synaptic visualization in vivo. Expression of fluorescent proteins fused either to PSD95, as a postsynaptic marker of excitatory synapses, or gephyrin, as a postsynaptic marker of inhibitory synapses, together with a separate cell fill to outline their location on the arbor has allowed for the first time a direct assessment of synaptic distribution patterns on cortical pyramidal cells, as well as synapse dynamics at different cellular locales (Cane et al. 2014; Chen et al. 2012; Isshiki et al. 2014; van Versendaal et al. 2012; Villa et al. 2016).

A critical aspect of this approach is the sparse labeling of only a subset of neurons so that accurate counting of synaptic distributions is feasible, in a way that is not possible with antibody staining. PSD95-GFP labeling in vivo shows that most stable spines have large PSD95 puncta, but a small population are devoid of a PSD95 label (Cane et al. 2014; Villa et al. 2016). While these spines may contain other PSD95 scaffolding molecules, such as PSD93 or SAP 102 typical of immature synapses (Aoki et al. 2001; Elias et al. 2008; Sans et al. 2000), this finding highlights the fact that spines are an imperfect surrogate for excitatory synapses.

When labeling postsynaptic gephyrin as a marker for inhibitory synapses on pyramidal neurons, a surprising finding was the prevalence of inhibitory synapses on dendritic spines (Chen et al. 2012; van Versendaal et al. 2012). Dually inner-vated spines harboring both an excitatory and an inhibitory synapse had previously been reported by EM (Fifkova et al. 1992; Kisvarday et al. 1985; Knott et al. 2002; Kubota et al. 2007; Megias et al. 2001; Parnavelas et al. 1977). However, the fluorescent labeling provided an orders of magnitude larger sample size as com-pared to EM, leading to the realization that on average, a third of inhibitory synapses on L2/3 pyramidal neurons reside on spines. Large volume imaging of entire L2/3 pyramidal neurons, labeled with fluorescent gephyrin in addition to a cell fill, allowed a first quantitative look at inhibitory synapse distributions across the dendritic arbors of this cell type: on average five spines and two inhibitory synapses per 10 μm (Chen et al. 2012). About 15 % of spines contain an inhibitory synapse, with an overall density of one inhibitory synapse on a spine per 10 μm (Chen et al. 2012). Both spines and inhibitory shaft synapses are distributed relatively uniformly across the spiny region of the dendritic arbor. In contrast, inhibitory spine synapses were found to be denser on the apical tuft than on basal and proximal dendrites (Chen et al. 2012).

18.3 Segregation of Distinct Inputs onto Specific Cellular Locales

18.3.1 Interneuron Targeting of Pyramidal Neurons

While synaptic distributions and the valance of the input at each location are important aspects of an individual neuron's wiring diagram, identifying the precise source of input at each synaptic locale is also critical for understanding a neuron's computational properties. GABAergic cells are particularly heterogeneous with respect to their axonal and dendrite morphology, firing patterns, molecular markers, and postsynaptic targeting (Klausberger and Somogyi 2008) and can be classified into subtypes based on these features (DeFelipe et al. 2013; Petilla Interneuron Nomenclature et al. 2008) or by staining for molecular markers, such as parvalbumin (PV), somatostatin (SOM), the serotonin 5HT3a receptor (5HT3aR),

vasoactive intestinal peptide (VIP), calbindin D (CB), and CR (Kawaguchi and Kubota 1997; Kubota et al. 1994, 2011). The PV, SOM, and 5HT3aR markers define nonoverlapping classes of neurons and together represent nearly 100 % of cortical interneurons (Lee et al. 2010; Rudy et al. 2011; Uematsu et al. 2008).

The majority of inhibitory neurons expressing the calcium-binding protein PV, classified as fast-spiking basket neurons, predominantly form synapses onto the soma and proximal dendrites of excitatory neurons (Miles et al. 1996) (Fig. 18.2a). In the G42 transgenic line, which labels a subset (~50 %) of PV-positive cells (Chattopadhyaya et al. 2004), 20 % of synapses onto pyramidal cells target the soma and 40 % the proximal dendrites (within 40 μm of the soma) (Di Cristo et al. 2004). The remaining 40 % target the more distal pyramidal neuron dendrites (Di Cristo et al. 2004). Thus, although PV cells are more likely to innervate the soma and proximal dendrites of pyramidal neurons, they are not strictly limited to these subcellular domains. PV cells strongly inhibit neighboring pyramidal neurons (60 % probability of connectivity) (Avermann et al. 2012; Lee et al. 2013). Because of the large number of PV cell inputs targeting the soma and proximal dendrites of pyramidal neurons, PV neurons are capable of regulating the timing and magnitude of pyramidal output and can limit the temporal window of action potential generation (Higley 2014; Pouille and Scanziani 2001).
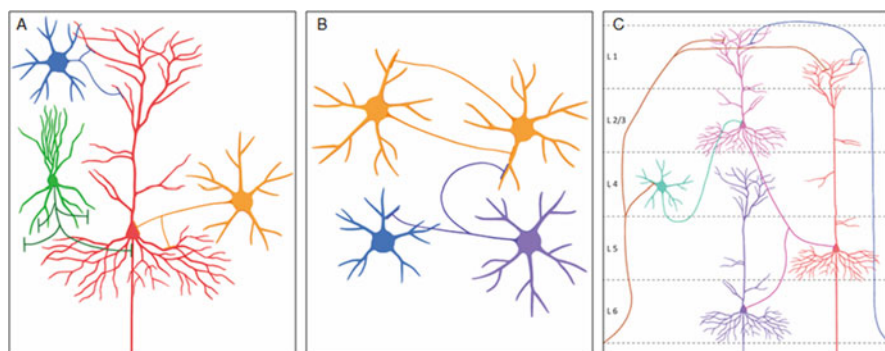

Fig. 18.2 Schematic circuit diagrams of cortical neurons illustrating general principles of connectivity. (**A**) Schematic of inhibitory innervation of a cortical pyramidal neuron (*red*) by cell types that target distinct cellular locations. Chandelier cells (*green*) target the axon initial segment. Parvalbumin (PV)-expressing interneurons (*orange*) mostly target the soma and proximal dendritic segments. Somatostatin (SOM)-expressing interneurons (*blue*) mostly target distal dendrites. (**B**) Schematic of how interneurons target other interneurons. In addition to pyramidal neurons, PV cells (*orange*) mostly innervate other PV cells. VIP-positive interneurons (*purple*) primarily target other interneurons and rarely innervate pyramidal neurons. In L2/3, SOM interneurons (*blue*) mostly target pyramidal neurons and are less likely to innervate other interneurons. (**C**) Schematic of excitatory cortical circuitry. Thalamic axons (*orange*) drive activity of L4 spiny stellate neurons (*light blue*) and innervate L1 apical tufts. Stellate neurons innervate the proximal dendrites of L2/3 neurons (*pink*) which in turn project distantly to other cortical regions and to the proximal dendrites of nearby L5 and L6 pyramidal neurons (*red* and *purple*), which project to subcortical and thalamic regions, respectively. Top-down cortical inputs (*blue*) primarily innervate L1 apical tufts

Chandelier cells, or axo-axonic cells, are a morphologically distinct GABAergic cell type recognizable by their axonal arborizations. These cells innervate the axon initial segment of neighboring pyramidal cells (Fig. 18.2a), giving their own axonal arbor a stereotypical chandelier-like appearance (Somogyi 1977; Somogyi et al. 1982; Woodruff et al. 2010, 2011). A widely held notion is that chandelier cells are a subset of PV-positive interneurons, but recent work shows that only a subset of chandelier cells stain positive for PV (Kawaguchi and Kubota 1998; Taniguchi et al. 2013). Through their innervation of the axon initial segment, chandelier cells can be seen as the last line of defense for preventing the post-synaptic cell from firing an action potential, although they can also have depolarizing effects, depending on the membrane potential of the postsynaptic neuron (Khirug et al. 2008; Szabadics et al. 2006; Woodruff et al. 2009).

SOM interneurons expressing the peptide hormone somatostatin typically target the dendrites of pyramidal neurons (Kawaguchi and Kubota 1998) (Fig. 18.2a). One well-known SOM cell subtype, the Martinotti cell, has a clearly identifiable morphology, with an axon that projects apically to layer 1 and there inhibits the apical tufts of deep-layer pyramidal cells. Although SOM-positive neurons are more inclined to innervate the most distal dendrites (Jiang et al. 2013), it is inaccurate to categorize their synapses as exclusive to that cellular locale. In the GIN transgenic line which labels a subset (~20 %) of SOM-positive cells (Oliva et al. 2000), pyramidal cell targeting by these neurons is such that 4 % of their synapses are directly onto the soma, 20 % are onto the proximal dendrites (within 40 μm of the soma), and the remaining 76 % are onto the more distal dendrites (Di Cristo et al. 2004). SOM interneurons are highly likely to innervate neighboring pyramidal cells (71 % probability of innervating cells within 200 μm) and also target other interneuron subtypes, but specifically avoid innervation of other SOM-positive neurons (Fig. 18.2b) (Fino and Yuste 2011; Pfeffer et al. 2013). By innervating the dendrites of pyramidal cells, SOM interneurons are likely to play an important role in controlling the generation of dendritic Ca^{2+} spikes (discussed later), and they can deliver more localized control over particular excitatory inputs. They are positioned to modulate the excitability of individual dendritic spines, segments, or branches.

18.3.2 Interneuron Targeting of Other Interneurons

The different subsets of interneurons also have specific targeting patterns onto other interneurons (Fig. 18.2b). PV cells have also been shown to strongly inhibit neighboring PV cells (with 55 % probability of connectivity), but are less likely to innervate other interneuron subtypes (24 % probability of connectivity with neighboring 5HT3aR neurons) (Avermann et al. 2012; Pfeffer et al. 2013). 5HT3aR-positive neurons have only been recently described and can be divided into two distinct subgroups. The first is VIP negative and typically reelin positive, which has a late-spiking firing pattern and a neurogliaform axonal arbor

morphology restricted to L1 (Lee et al. 2010; Rudy et al. 2011). The second is VIP positive and represents about 15 % of all GABAergic cells. These neurons mostly target SOM and some PV interneurons, and thus their firing results in disinhibition of pyramidal cells (Hioki et al. 2013; Pfeffer et al. 2013; Pi et al. 2013). VIP-positive interneurons are highly unlikely to innervate neighboring pyramidal cells. Paired recordings show that only 12 % of VIP neurons innervate a pyramidal neighbor (Pfeffer et al. 2013). In a recently described mouse circuit, pyramidal neurons within vibrissal motor cortex (vM1) that project to somatosensory barrel cortex (S1) strongly innervate VIP neurons, which in turn innervate SOM neurons that target S1 pyramidal neurons (Lee et al. 2013). This circuit activates S1 VIP neurons during whisking, resulting in the inhibition of S1 SOM neurons and thus disinhibition of S1 pyramidal neurons, potentially influencing the coincident detection of sensory information and altering whisker movement (Lee et al. 2013). More generally, through their inhibitory control over other inhibitory circuits and their location in superficial cortical layers, VIP cells likely play important roles in regulating inter-areal cross-talk between different cortical regions.

Some SOM neurons in L4 of the somatosensory cortex have also been shown to target other GABAergic interneurons, so that their activation results in a disinhibition of pyramidal cells (Jiang et al. 2013; Xu et al. 2013). The SOM neurons in layer 4 differ in morphology, intrinsic electrophysiological properties, and output connectivity from L2/3 SOM interneurons. Specifically, L2/3 SOM interneurons make many strong contacts with pyramidal neurons and suppress pyramidal activity, while L4 SOM neurons make frequent strong contacts with PV-positive fast-spiking interneurons, which themselves inhibit L4 pyramidal neurons (Xu et al. 2013). Thus, increasing the activity of L4 SOM neurons results in increased activity of L4 pyramidal neurons (Xu et al. 2013). These L4 SOM neurons are not strongly activated by thalamic inputs, but are likely activated by acetylcholine (Fanselow et al. 2008; Kawaguchi 1997), which suggests that during arousal and attention, L4 SOM neurons can become activated, resulting in greater activation of L4 pyramidal neurons.

18.3.3   Pyramidal Cell Targeting of Other Pyramidal Neurons

Is there a subcellular bias to excitatory axon innervation of cortical excitatory targets? Historically, unlike inhibitory neurons, excitatory pyramidal cells have been considered a generally uniform population, with circuit specificity resulting from their laminar location. Recent molecular studies have identified genes that are expressed in specific cortical layers (Belgard et al. 2011; Molyneaux et al. 2007), as well as genetic markers resolving intra-layer subsets of excitatory neurons distinguished by their projections to specific target structures (Sorensen et al. 2015). When excitatory neurons are defined according to their axonal projection and target

innervation, pyramidal neuron diversity may exceed that of inhibitory neurons (Huang 2014). Potentially, resolving excitatory neuron subclasses may also reveal distinct subcellular target specificity analogous to that of interneuron subclasses. In the hippocampus, considered a simplified cortical structure containing only one pyramidal cell layer (Thomson and Lamy 2007), genetically distinguishable pyramidal neuron subsets have not yet been identified. Cre recombinase driver lines created by the GENSAT project should prove useful for further resolution of pyramidal neuron subtypes (Gerfen et al. 2013; Gong et al. 2007).

Within the cortex, pyramidal cell dendrites can receive excitatory inputs from several sources: from local axons within the same cortical column, from adjacent cortical column axons, from other cortical regions in the same hemisphere, from commissural axons originating in the opposite hemisphere, as well as from direct feed-forward inputs that originate from the thalamus (Fig. 18.2c). The main target of feed-forward thalamic axons is L4, and indeed L4 cells are strongly activated by the thalamus despite the fact that cortical synapses onto L4 outnumber thalamic synapses by a factor of 10 (Benshalom and White 1986; Peters and Payne 1993). Thalamic synapses onto L4 are localized slightly more proximal to the soma than corticocortical synapses, but their synaptic strengths are approximately equal, suggesting that coincident activation of thalamic synapses is the main contributing factor to the strength of thalamocortical synapses (Schoonover et al. 2014). The classic thalamic information flow through the cortex is considered to pass from L4 to L2/3, then down to L5 and L6, and out to other cortical areas and back down to the thalamus and other subcortical regions. However, L2/3, L5, and L6 receive sparse thalamic inputs as well (for excellent reviews of cortical innervation patterns in the barrel cortex, see (Feldmeyer 2012; Lefort et al. 2009)), and recent in vivo studies show that thalamic inputs can drive L5/L6 independently of L4 (Constantinople and Bruno 2013).

Given the laminar nature of information flow within the cortex, the relative placement of a dendritic or other cellular locale within the cortical lamina is the strongest determinant of excitatory input specificity onto that locale. Apical dendrites of L2/3, L5, and L6 pyramidal cells arborizing in L1 receive feedback excitatory inputs from other cortical areas as well as from the posterior medial thalamic nucleus (POm) axons that travel in this lamina (Felleman and Van Essen 1991; Larkum 2013). Local, interlaminar cortical axons carrying feed-forward information are more likely to innervate the basal and proximal dendrites of their cortical targets (Feldmeyer 2012; Spruston 2008). For example, L4 axons mainly innervate the basal dendrites and proximal apical dendrites of L2/3 pyramidal cells within 70 μm of the soma (Feldmeyer et al. 2002; Silver et al. 2003), and L2/3 pyramids predominantly innervate the basal dendrites of L5 pyramidal neurons (Schubert et al. 2001). There is also a bias for thalamic axons to innervate the basal dendrites of L5 pyramidal neurons (Feldmeyer 2012). On the other hand, corticocallosally projecting L5 pyramidal neurons synapse mainly onto the distal region of the basal dendritic tree of their L5 partners at an average distance of about 130 μm (Larsen and Callaway 2006), suggesting that commissural axons are more likely to innervate the distal dendrites of their targets. There are also examples of

subcellular innervation bias in the hippocampus, where the CA1 pyramidal cells are innervated by axons of CA3 and entorhinal cortex (EC); the EC axons preferentially target the distal dendrites of CA1 neurons, and the CA3 axons innervate the basal and proximal dendrites of the same CA1 neurons (Megias et al. 2001). Additionally, distant CA3 neurons are more likely to project to the apical CA1 dendrites, while CA3 neurons with cell bodies located close to CA1 are more likely to project to the basal dendrites of CA1 neurons (Li et al. 1994). Thus, a general theme seems to be that long-range feedback excitatory inputs are more likely to innervate distal dendrites, while feed-forward thalamic and interlaminar projections are more likely to target basal and proximal dendrites of their cortical targets. Although we have yet to delineate the full extent of cellular and subcellular targeting specificity for each cortical cell type, the precision we see thus far suggests that excitatory and inhibitory synaptic placement onto pyramidal cell dendrites is a critical aspect of their function within the circuit.

18.4 Functional Implications of Synaptic Placement Across the Dendritic Arbor

18.4.1 Spatial Clustering of Excitatory Inputs

Experiments suggest that neurons are capable of three different types of dendritic integration. Inputs can be summed sublinearly, when there is a decrease in driving force as the membrane potential nears the reversal potential; linearly, when inputs occur on different dendritic branches or are separated by sufficient distance to act independently; and supralinearly, when local conditions facilitate depolarization (Palmer 2014). These different modes of processing are possible within the same neuron, for example, if the dendrites operate linearly when activated by a non-preferred stimulus and nonlinearly when activated by a preferred stimulus (Grienberger et al. 2015). The type of integration performed is strongly influenced by the relative placement of excitatory and inhibitory synapses on the dendrite as well as the dendrite's location within the arbor.

Historically, synapses distant from the soma were thought to have less influence over action potential initiation in the axon, due to loss of charge as current flows from the dendrites to the soma and axon hillock in a model of passive dendrites (Golding et al. 2005). However, the discovery of dendritic spikes explained how distal excitatory synapses are able to propagate their signals effectively to the soma. Dendritic spikes are essentially action potentials that are generated in the dendrite, which acts as an active rather than passive signaling compartment. As dendritic spikes propagate toward the soma, they are capable of triggering action potentials (Golding and Spruston 1998), also recently shown in vivo (Grienberger et al. 2014). Dendritic Ca^{2+} spikes can be initiated by strong synchronous activation of several synapses (Gasparini and Magee 2006), and the probability of dendritic spike

initiation in distal branches can be further increased by nearby initiation of NMDA spikes (Larkum et al. 2009). Theoretical models and in vitro experiments both support the view that spatially clustered, temporally coactivated excitatory inputs are more likely to initiate dendritic spikes than inputs that are distributed across multiple branches (Gasparini and Magee 2006; Larkum et al. 2009; Mel 1993; Winnubst and Lohmann 2012). Dendritic spikes can thus improve the computational properties of individual neurons (Larkum 2013; Major et al. 2013), by enabling the discrimination of patterns delivered to a single dendrite from those delivered randomly across the dendritic tree (Branco et al. 2010).

Another consequence of the fact that dendritic spikes elicited by nearby coactive synapses are capable of triggering action potential firing is that neighboring synapses would thus be more likely to undergo long-term potentiation (LTP) than synapses that are spatially distant. There are also examples of dendritic spike-induced LTP independent of action potential firing in vitro (Golding et al. 2002; Gordon et al. 2006) and recently in vivo (Gambino et al. 2014). Since dendritic spikes have a smaller spread than action potentials, these would preferentially produce LTP on locally clustered coactive synapses, especially in regions of the distal dendritic tree that are beyond the reach of backpropagating action potentials (Golding et al. 2001). The pervasiveness of functionally clustered inputs in vivo is under debate. Studies in the visual (Jia et al. 2010), auditory (Chen et al. 2011c), and barrel cortex (Varga et al. 2011) have suggested that functionally distinct excitatory inputs are randomly distributed along pyramidal dendrites. Other experiments provide evidence for clustering of functionally related excitatory inputs in the hippocampus (Druckmann et al. 2014; Kleindienst et al. 2011; Takahashi et al. 2012) and cortex (Chen et al. 2013; Makino and Malinow 2011; McBride et al. 2008; Takahashi et al. 2012) (reviewed in DeBello et al. 2014). These findings are not necessarily in conflict. Excitatory synapses that are functionally similar may have a higher probability of spatial clustering along the dendrite, but at the same time, dendrites are interspersed with a wide range of functional inputs. Spatial clustering of functionally related inputs may also be more prominent in certain subsets of neurons.

18.4.2 Effects of Inhibitory Synaptic Placement on Pyramidal Cell Dendritic Integration

Individual pyramidal cells receive thousands of excitatory synaptic inputs that can be functionally diverse. Yet, the action potential output of most pyramidal neurons is sparse and narrowly tuned. This restriction of action potential firing to a defined sensory range within a limited temporal window is brought about by the integration of excitatory synaptic events combined with the local influence of inhibition (reviewed in Chadderton et al. 2014). Inhibition has a major influence on the effective output of neurons at multiple levels. Individual inhibitory synapses on

spines can veto individual spine conductances (Chiu et al. 2013). Inhibitory shaft synapses can influence excitatory integration within local dendritic segments (Kim et al. 1995; Perez-Garci et al. 2006; Willadt et al. 2013). On a more cellular scale, PV cell innervation of the soma (Pouille and Scanziani 2001) or chandelier cell innervation of the axon initial segment (Woodruff et al. 2011) can cancel axonal output. In the latter cases, the high density of inhibitory synapses on the cell body and axon initial segment (Di Cristo et al. 2004; Miles et al. 1996; Woodruff et al. 2010) suggests that synchronous firing of many synapses would be needed for effective inhibition in these locales, and a single inhibitory synapse would have a negligible effect on outcome. This is in contrast to dendritically targeted inhibition, where individual synapse changes could have a dramatic effect on the computation of dendritic integration for specific branches.

How are excitatory and inhibitory inputs integrated functionally at the level of individual dendritic branches and how does that influence overall neuronal firing? Clearly, local integration of excitatory and inhibitory synaptic activity is influenced by individual synaptic strength, timing, and location on the dendrite. Theoretical modeling predicts that activation of multiple distributed inhibitory synapses across the dendritic arbor can result in a global inhibition (Gidon and Segev 2012). However, experiments suggest that the influence of individual inhibitory synapses is locally restricted to the branch they target (Stokes et al. 2014), perhaps even to individual spines (Chiu et al. 2013). Thus, functional pairing or clustering of relevant excitatory synapses on particular dendritic segments with their inhibitory neighbors is highly relevant to dendritic integration. Moreover, the local constellation of such pairing or clustering is likely to have a different influence depending on their more global location along the arbor.

Dendritically targeted inhibition can limit initiation of dendritic Ca^{2+} spikes in both hippocampal and cortical pyramidal neurons (Buhl et al. 1994; Karube et al. 2004; Kim et al. 1995; Larkum et al. 1999; Perez-Garci et al. 2006; Willadt et al. 2013), so that they are initiated only in response to inputs encoding particular functional features. For example, in retinal ganglion cells, local integration of excitation and inhibition leads to initiation of dendritic spikes only in the preferred direction of motion, with inhibition preventing dendritic spike initiation in the non-preferred direction (Sivyer and Williams 2013). Mathematical models focusing on spike activity within dendrites have shown that "off path" inhibitory synapses, where the inhibitory synapse is located distally to the excitatory synapse, are more effective at preventing the initiation of dendritic spikes (Gidon and Segev 2012). Thus, the influence of inhibition on spike initiation can actually be more powerful when inhibitory synapses are located on distal dendrites rather than proximal dendrites and in a constellation where the inhibitory synapse is distal to its functionally paired excitatory synapse (Gidon and Segev 2012; Li et al. 2014). Conversely, in cases where a dendritic spike is successfully initiated, an "on path" inhibitory synapse, located between the excitatory synapse and the soma, is the most effective way to dampen the signal (Gidon and Segev 2012; Hao et al. 2009; Zhang et al. 2013).

Recent in vivo work has demonstrated that dynamic inhibitory synapses are likely to be located within 10 μm of dynamic spines, and this clustering of dynamic events increases upon sensory deprivation (Chen et al. 2012). It has been suggested that a loss of inhibition creates a permissive environment for excitatory synaptic changes (Chen et al. 2011b; Harauzov et al. 2010; Maya Vetencourt et al. 2008) because it broadens the window of spike-timing-dependent plasticity (Bi and Poo 2001; Higley and Contreras 2006; Song et al. 2000; Spruston 2008). The loss of an inhibitory synapse could create a permissive environment for neighboring excitatory synaptic gain or loss, while the addition of an inhibitory synapse could suppress plasticity in neighboring dendritic regions. Inhibitory inputs could also limit the ability to promote excitatory synaptic clustering through plasticity mechanisms. A recent study using calcium imaging in slice culture showed that GABA uncaging diminishes the Ca^{2+} transient resulting from a backpropagating action potential 20 μm in either direction of the uncaging site (Hayama et al. 2013). Thus, the specific placement of inhibitory synapses can regulate which excitatory synapses are able to undergo plasticity. Further, a recent modeling study suggests that the placement of inhibitory synapses can result in LTP, LTD, or a lack of plasticity in specific neighboring dendritic segments (Bar-Ilan et al. 2012), highlighting the importance on the placement of inhibitory synapses on the valance of plasticity at neighboring excitatory synapses.

18.5 Conclusion

How the excitatory and inhibitory inputs are integrated within a single neuron and how this integration supports computation in functioning networks are still critical questions in neuroscience today. Despite advances in our knowledge, we still have limited experimental evidence of the fine-scale synaptic architecture across the neuronal arbor of individual cells of different types, and we know even less of the functional interactions between synaptic excitation and inhibition (Chadderton et al. 2014). As we look to the future, combining structural two-photon imaging of synaptic locations with calcium functional imaging or electrophysiology provides a potential avenue to address these questions, in cell culture, in acute brain slices, and ideally in an intact in vivo system.

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