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Current Opinion in Neurobiology

The electrical significance of axon location diversity

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The axon initial segment (AIS) is a unique domain of the proximal axon serving critical electrical and structural roles including the initiation of action potentials and maintenance of cellular polarity. Recent experimental and theoretical advances demonstrate that the anatomical site for initiation is remarkably diverse. The AIS location varies not only axially, along the axon, but axons also emerge variably from either the soma or proximal dendrites. Here, we review the evidence that the diversity of AIS and axon location has a substantial impact on the electrical properties and speculate that the anatomical heterogeneity of axon locations expands synaptic integration within cell types and improves information processing in neural circuits.

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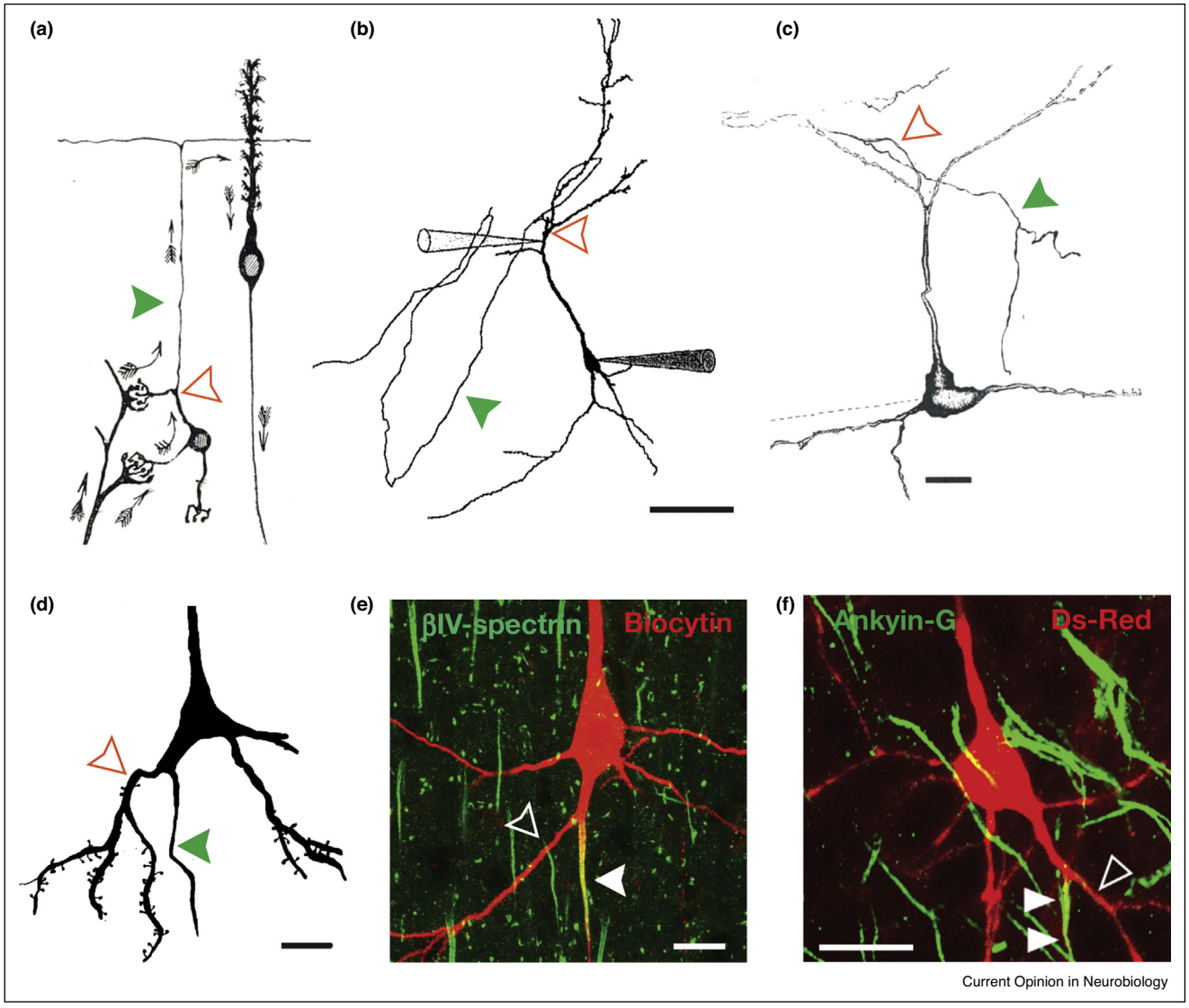
Introduction

Neurons, the cellular computational units of the central nervous system, are morphologically polarized cells and segregated into diverse functional compartments. A unique and critical site of the proximal axon of mammalian neurons is the axon initial segment (AIS), clustering high densities of voltage-gated sodium (Nav) channels [1]. The AIS defines the location from where all action potentials (APs) are initiated and propagate bidirectionally, towards the presynaptic terminals and back into the soma and dendrites (reviewed in [2,3]). Understanding the structural and molecular properties of the site of initiation is of fundamental importance as it affects the electrical function of neurons. The discovery that the AIS cytoskeleton and associated ion channel clustering change in response to network activity has led to the concept that the AIS may serve to homeostatically scale intrinsic excitability [4,5,6•]. In addition, evidence is accumulating that the morphological diversity of the AIS and its functional impact are greater than appreciated; not only is the AIS location variable along the axon branch, but axons themselves may also emerge either from the soma or from dendrites, and in some cell types even up to hundreds of micrometres from the soma. Here, we review recent electrophysiological and theoretical advances into this anatomical heterogeneity fuelling the idea that spike initiation location may not only impact excitability, but also the backpropagation of action potentials and synaptic integration.

Location, location, location

The position of the AIS along the axon varies substantially between neuronal cell types [7], within cell types and across early neuronal development [8,9]. In addition, already since the seminal studies of Ramón y Cajal, it is known that axons have variable sites from where they emerge, often from the soma but also from dendrites, such as the parallel fibres of cerebellar granule cells or the axons from the avian optical lobe neuron [10,11] (Figure 1a). Although neurons with dendritic axons were generally believed to be rare, detailed morphological reconstructions show that they exist across a wide variety of cell types including the hippocampal dentate gyrus basket cells and somatostatin interneurons [12,13], in approximately 75% of the population of dopaminergic cells of the substantia nigra [14–16] and in 40% of the cerebellar granule cells [11,17] (Figure 1a–d). Furthermore, staining for ankyrin-G or βIV-spectrin, markers for the AIS structure, combined with Cre recombinase reporter mice made it possible to obtain detailed information about the AIS location within the axon, as well as the axon location itself, across large populations of identified cell types. Also this recent body of work revealed that dendritic axons are present in approximately 60% of the hippocampal pyramidal neurons, 30% of the thick-tufted somatosensory layer 5 pyramidal neurons, 40% of cortical interneuron basket cells and 60% of Martinotti cells [7,18•,19••] (Figure 1e–f). Given the abundance of dendritically targeted axons the question arises: what could be the role of such asymmetry in axon position, and more broadly of the diversity of AIS location? As early as in 1898 Ramón y Cajal proposed that in cells with dendritic axons, " . . . *the soma, or cell body does not always take part in the conduction of the nerve impulses which are received. The afferent wave is sometimes propagated directly from the dendrites to the axon*" (reviewed in [20]). Recent patch-clamp recordings, combined with morphological reconstructions and theoretical advances,

Figure 1



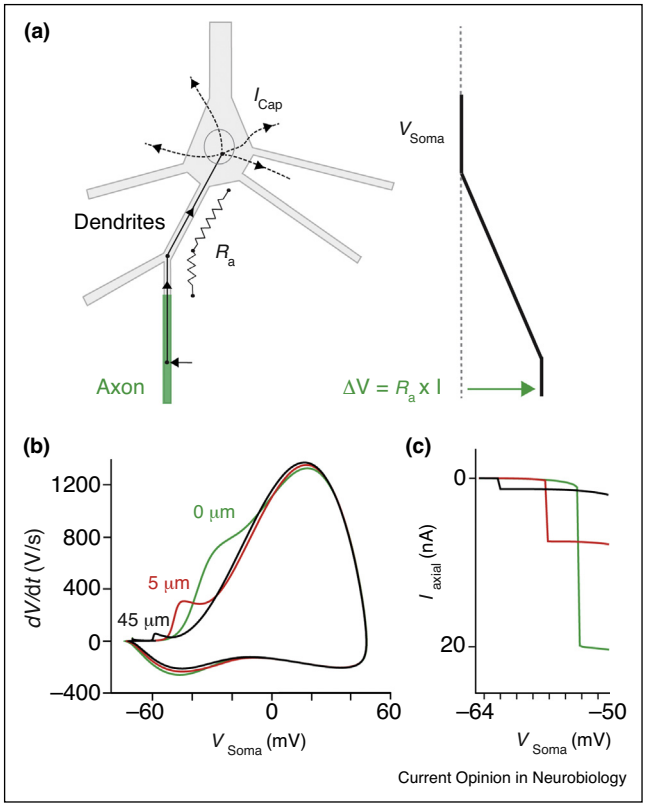
Dendritic axons are a common feature of various neuron types. **(a)** Example of a dendritic origin (red open arrow) of the parallel fibre (green closed arrow) from a cerebellar granule cell projecting to a Purkinje cell. Black arrows depict the axipetal current flow drawn by Ramón y Cajal. **(b)** A substantia nigra dopaminergic neuron with dendritic axon illustrated with patch pipette recording locations. The axon-soma distance of this cell is 215 μm. **(c)** Distal axon origin (~100 μm from the soma) in a hippocampal dentate gyrus basket cell. **(d)** Example of a rat parietal cortex layer 2/3 pyramidal neuron with axon emerging from thick basal dendrite. **(e)** Immunofluorescence of a biocytin-filled rat thick-tufted layer 5 pyramidal neuron (red) with dendritic origin of the AIS (βIV-spectrin identified, green). Filled arrows mark the axons. Open arrows indicate the axon-carrying dendrites. **(f)** Immunofluorescence image of a Thy1$^+$ mouse hippocampal CA1 pyramidal neuron (red) with Ankyrin-G (green). Scale bars; b, 100 μm; c–f, 20 μm.
Images are adapted from [11] (a); [14] (b); With permission from John Wiley and Sons [12] (c); [28] (d); [18•] (e) and [19••] (f).

are shedding more light on the excitability and the associated current flow.

AIS location diversity and intrinsic excitability; the theory

In order to understand the electrical impact of the spatial variation in axon location, it is imperative to consider the cellular morphology from a biophysical perspective. In a situation where the initiation site of the action potential is located to a thin axon, electrotonically close to a much larger somatodendritic compartment, resistive coupling theory applies [21•,22]. The sodium (Na^+) current generated in the AIS flows primarily towards the soma, which thus acts as a current sink, and subsequently exits as capacitive current through the large somatodendritic membrane (Figure 2a). Because the intracellular medium

Figure 2



Resistive coupling between the axon and soma. **(a)** *Left*, Circulation of current entering the axon emerging from a dendrite. The current mainly flows to the soma, a current sink, along a resistive path (R_a: axial resistance). *Right*, By Ohm's law, a voltage gradient forms between soma and injection site, proportional to R_a. **(b)** Phase-plane plot (dV/dt *versus* V) of somatic action potentials in a simple model with the AIS at 3 different locations (0, 5 and 45 μm), reveals a lower voltage threshold with increasing soma-axon distance. **(c)** Axial current entering the soma as a function of somatic potential: the threshold is lower and the current is smaller for larger soma-axon distance. b and c, Adapted from [22].

is resistive, the axial current follows Ohm's law: a voltage gradient develops between the soma and axonal site, equal to the product of the input current and axial coupling resistance (R_a) between the two sites, $\Delta V = R_a \times I$ (Figure 2a) [21•]. R_a is furthermore determined by the geometry of the axonal or dendritic branch in between the soma and AIS, proportional to the distance and inversely proportional to the cross-section area (diameter squared). This simplified model predicts a strong influence of the geometry of the AIS and neighbouring dendritic sites and makes several predictions about the functional impact that we will review in detail below.

The amount of Na^+ current needed for a given axonal depolarization is inversely proportional to axial resistance ($I = \Delta V/R_a$). Therefore, a neuron with a more distal AIS or thinner axon should either be more excitable, reflected in a hyperpolarization of the voltage threshold and reduced rheobase (current to threshold) for AP generation or require a lower Na^+ channel density for a given level of excitability (Figure 2b and c). This theoretical prediction has also been confirmed in detailed multi-compartmental computational models in which membrane properties can be maintained constant while axonal compartments are varied in their location [18•,22,23] and is consistent with the notion that spike initiation occurs at the distal end of the AIS due to its larger electrotonic isolation [24,25].

Notably, some simulation studies showed that a distal location of the AIS can reduce neuronal excitability [5,23,26]. Theoretically, this can happen if the proximal axon and the somatodendritic compartment are comparable in size, so that the soma is no longer a current sink for the AIS [22]. However, in cortical pyramidal neurons, the axon diameter scales with soma size, and is always much smaller compared to the soma [27,28]. Another situation where excitability could be reduced with increasing soma-AIS distance is when a hyperpolarizing current is locally strongly activated at the AIS, for example by Kv1 or Kv7 channels [21•,26]. Direct recording from the AIS and estimates of conductance density show that below the spike threshold the slowly-activated M/Kv7 current and fast-activating Kv1 current are, however, relatively small compared to the inward Na^+ current [29–31]. Similar differences between Kv7 and Nav conductance densities were found in the avian nucleus magnocellularis [32]. Thus for these cells theory predicts that if all membrane properties remain constant, a distal shift of the AIS makes the cell more excitable. Finally, for *in vivo*-like fast excitatory postsynaptic potentials (EPSPs), a significant frequency-dependent voltage attenuation could occur along the axon. In this case, large distal shifts of the AIS could reduce excitability, resulting in a U-shaped relationship between AIS position and excitability [33••]. What is the experimental evidence for the theoretical predictions?

AIS location diversity and intrinsic excitability; the experimental observations

In agreement with the role of the AIS in intrinsic excitability, genetically disrupting the development of an AIS or pharmacologically inactivating the AIS Na^+ channels substantially depolarizes the AP threshold (>10 mV), increases the rheobase and reduces the firing rate [30,34,35]. In accord, a number of experimental studies showed that increasing or decreasing the AIS length raises or reduces the intrinsic excitability, respectively, by affecting voltage threshold and firing rate (reviewed in [36,37]). On the other hand, experimentally isolating and interpreting the specific role of the AIS or axon location has proven to be substantially more challenging and the interpretation of the results is contentious. The studies observing a distal relocation of the AIS found a decreased

intrinsic excitability which in some cases correlated with the extent of the distal relocation [5,26,38,39]. A reduction in intrinsic excitability is opposite to the above described theoretically predicted lower AP voltage threshold. One possible explanation for the discrepancy could be that the experimental conditions used to induce AIS/axon location changes, such as chronic elevation of neuronal activity by pharmacological approaches or optogenetic stimulation, were in all cases accompanied by a reduction in the neuronal input resistance (R_N), which directly implies an increase in rheobase [5,26,38,39]. Other changes that have an impact on excitability may also occur, such as phosphorylation of Na^+ channels [6•]. As mentioned above, another possibility is that a large Kv7 conductance is present in the AIS, which could lead to decreased excitability with more distal AIS locations [21•,26].

An alternative strategy to empirically investigate the electrical role of the AIS-soma separation is a comparative analysis between subpopulations of neurons with identified anatomical location of the AIS [14,16,18•,19••,33••]. In layer 5 of the somatosensory cortex, pyramidal neurons with axon-carrying dendrites have a ~3 mV more hyperpolarized action potential voltage threshold compared to neurons with somatic axons [18•]. However, detailed geometrical quantification and correlation analysis showed that the AIS-soma distance was not a strong predictor of the voltage threshold and neither for the firing rate. Furthermore, comparative electrophysiological recordings from CA1 pyramidal neurons also failed to show differences in action potential voltage threshold as a function of distance between the AIS and the soma, across a range of ~40 μm [19••]. Interestingly, recent *in vivo* recordings from dopaminergic cells in the substantia nigra followed by reconstruction and computational modelling found that a low firing rate in neurons with a distal AIS was best explained by the negative correlation between the soma-AIS distance and the AIS length [40]. Together, the experimental studies illustrate that disentangling and understanding the role of AIS location in intrinsic neuronal excitability requires extensive and joint quantitative assessments of multiple factors. However, the general problem is that correlation does not imply causation. Conclusively demonstrating the specific impact of AIS/axon location on intrinsic excitability through experimental means awaits further investigation and methodologies to manipulate each factor separately and selectively. Perhaps optogenetic tools to control organelle positions [41] may in the future be leveraged to selectively relocate the initial segment.

AIS location diversity and backpropagation

A critical function of the AIS is the generation of an AP backpropagating into the soma and the dendritic tree. Somatically recorded APs acquired with sufficiently high temporal resolution show, in most mammalian neurons, an early component reflecting the spike generated in the AIS and a late component reflecting spike generation in the somatodendritic compartment [30,42]. According to resistive coupling theory, the current transmitted to the soma by the AIS spike is inversely proportional to axial resistance [18•] and in particular the soma should therefore receive less current from a dendritic axon compared to a somatic axon (Figure 2). Surprisingly, heterogeneity in AIS and axon position is not always reflected in changes in the initial rising phase of the AP. In hippocampal CA1 pyramidal neurons and thick-tufted layer 5 pyramidal neurons the somatically recorded AP is indistinguishable between dendritic and somatic axons [18•,19••]. In thick-tufted layer 5 cells detailed geometric analysis showed that the smaller antidromic current, with a more distal AIS position, matches the smaller somatodendritic capacitance of neurons with axon-carrying dendrites, which have a thinner apical dendrite [18•]. As a result of the cable properties between the axon and dendrites, the antidromic current flow maintains a constant rise-time of the somatodendritic spike. Whether a tuning between AIS location and the dendritic tree is a general rule in building neurons remains to be further investigated but evidence for covaration is indeed seen in other cell types. For example, in the avian nucleus laminaris (NL) of the chick, a region involved in auditory processing, neurons tuned to a high characteristic frequency have large soma-AIS distances but also shorter dendrites when compared to those with low characteristic frequency, which have a short soma-AIS distances and larger dendrites [33••,43]. In addition, ion channel properties also correlate with frequency tuning, including expression of dendritic T-type Ca^{2+} channels [44]. However, unlike cortical pyramidal neurons, NL neurons do not regenerate the AP at the soma with Na^+ current and the AP amplitudes between the NL subpopulations differ as a function of AIS position and frequency tuning [33••].

Further examples of covariation between AIS location and dendrites are found in midbrain dopaminergic neurons with dendritic axons (Figure 1b). In these cells the axon-carrying dendrite is ~2-fold larger in diameter compared to non-axon carrying dendrites, which will facilitate backpropagation towards the soma [15,16]. Despite such morphological adaptations, the distal location of the AIS seems to be electrically significant: in these cells the rising phase of the somatic AP is slow, unreliable and can even fail during the integration of fluctuating *in vivo*-like synaptic inputs [15,45]. Collectively, the findings from various studies show that the distance between soma and AIS impacts AP backpropagation but the functional consequences are highly variable between cell-types.

Dendritic axons enrich synaptic integration

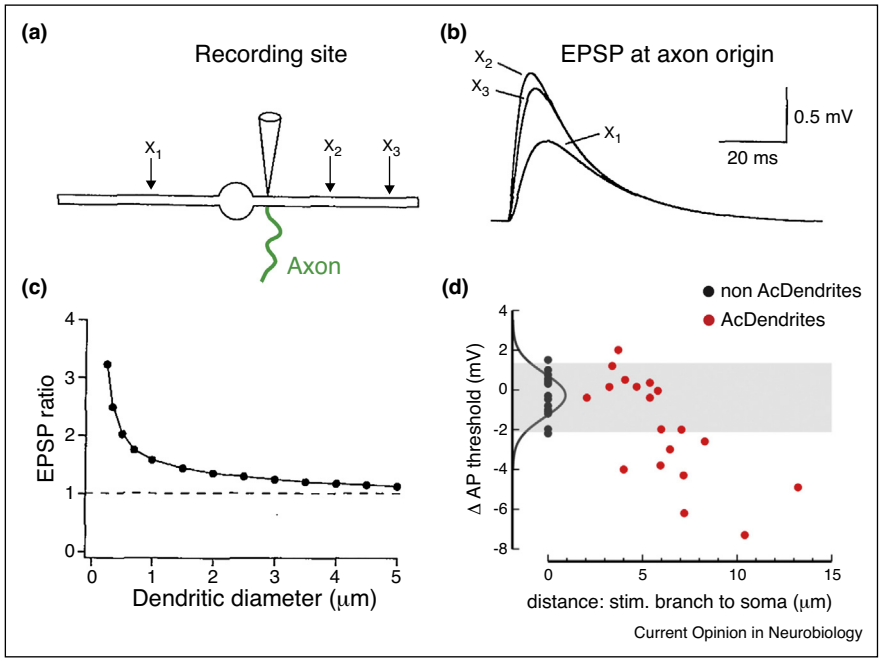
According to resistive coupling theory, when synaptic current enters the axon-carrying dendrite, it flows towards the soma and thereby produces a voltage gradient

between the soma and the point where the axon emerges from the dendrite, which adds up to the somatic potential, producing a larger synaptic potential in the axon. In accord with the theory, compartmental simulations based on substantia nigra dopaminergic neurons show EPSP amplification in axon-carrying dendrites (Figure 3a and b). The same current input will produce a smaller synaptic depolarization at the axon when entering the cell at the non-axon carrying dendrites or when the axon emerges from the soma. The difference is theoretically proportional to the axial resistance between the soma and the branching point of the axon, in particular inversely related to dendrite diameter (Figure 3c). Experiments addressing synaptic integration in these cells have been performed with simultaneous dual or triple patch-clamp recording from the axon-carrying and non axon-carrying dendrites [14–16]. In support of the theoretical predictions that EPSPs are amplified due to resistive coupling, barrages of simulated EPSPs mimicking *in vivo* activity were observed to trigger APs more reliably when generated in the axon-carrying dendrite compared to the non-axon carrying dendrite [15]. Recently, increased coupling of EPSP input to AP output has also been demonstrated with two-photon uncaging in CA1 pyramidal neurons comparing excitatory synapses on the axon-carrying dendrites with other dendrites [19••]. The difference in integration is reflected by a somatically recorded lower AP voltage threshold when uncaging glutamate at the axon-bearing dendrite, the difference being again due to the voltage gradient between soma and axon branching point during stimulation (Figure 3d).

From single axons to neural circuits

The studies reviewed so far show that biophysical differences between the axon-carrying and non-axon carrying dendrites mostly leads to differential computational processing of synaptic inputs. This raises the tantalizing possibility that variation of axon location in single cell classes enriches the repertoire of synaptic integration at the circuit level. Even further, within an individual cell with dendritic axons, the distinct branch types may be receiving specific afferent inputs. Evidence for branch-specific synaptic clustering comes from single-cell analysis of dopaminergic neurons [46]. Anatomical identification of inputs revealed a higher density of glutamatergic synapses on the dendrites located near the axon in the substantia nigra pars compacta (SNc) compared to dendrites in the substantia nigra pars reticulata (SNr), making the SNc inputs more likely to drive the activity of dopaminergic neurons [46]. Notably, in the hippocampus, pyramidal neurons with dendritic axons are preferentially observed in the superficial layers of the stratum

Figure 3



Differential synaptic integration in dendritic axons. **(a)** Simulated morphology with a dendritic axon emerging 50 μm from the soma, and synaptic inputs at different locations; on a dendrite (X_1) and on the axon-carrying dendrite (locations X_2 and X_3). **(b)** Simulated EPSPs measured at the axon origin for the three locations shown in a, showing larger and more rapid EPSPs when synapses on the axon-carrying dendrite are activated. **(c)** The EPSP amplification varies inversely with the diameter of the axon-carrying dendrite (decreasing R_a). **(d)** Somatic recordings of action potentials in CA1 pyramidal cells reveal a more hyperpolarized voltage threshold when the axon-carrying dendrite (AcDendrite, red) is stimulated compared to non-axon carrying dendrites (black), with lower threshold potentials when the AIS is further from the soma (equivalent with increasing R_a). Adapted from [14] (a–c) and [19••] (d).

pyramidale [19••]. A subdivision of dendritic and somatic-axon pyramidal neurons along the superficial-to-deeper layer axis is in accord with experimentally observed gradients at the level of genetics, type of inhibitory inputs, sharp wave ripples, and activity recruitment during behavioural tasks [47,48]. In addition, recent data showed that the layer 5 neurons with dendritic axons have a distinct dendritic morphology, putatively reflecting a morphological subtype [18•]. Whether the subdivision has a genetic basis is not known but classification based on single-cell transcriptome analysis revealed three distinct types in the deeper layers of layer 5 [49]. Recent retrograde tracer analysis showed that thick-tufted layer 5 neurons can be subdivided based on their projection area, dendritic morphology and *in vivo* firing properties, with cells projecting to the posterior medial division of the thalamus (POm) showing reduced dendritic branching in the layer 5 lamina and higher spontaneous firing rate [50]. Whether the POm projecting layer 5 cells are characterized by dendritic axons, however, is not known and remains to be examined.

Interestingly, reconstructions and modelling of the cerebellar granule cells (CGCs) showed that CGCs with axon-carrying dendrites are characterized by more complex claw-shaped endings receiving a greater excitatory input from mossy-fibres [17]. The significance for processing in the CGC layer remains to be further examined and may be different from the examples described above since CGCs are electrotonically extremely compact, showing uniform EPSPs along their dendrite and soma [51]. Nevertheless, it is tempting to speculate that resistive coupling of axons within the thin CGC dendrites impacts the temporal processing of the high-frequency information from mossy fibre inputs and expands the integrative capabilities of the CGC layer to relay information to the Purkinje cells. Both theoretical and experimental studies thus converge to the idea that diverse dendrite-axon architectures in neurons expand synaptic integration and the encoding capabilities of cellular circuits.

Conclusion

In summary, recent studies are slowly shedding more light on both the abundance and significance of dendritic axons but many questions remain to be resolved. First, while in some cell types the AIS/axon location co-varies with the structure of the dendritic tree, it is not well understood whether this is a generic phenomenon. Addressing this would require an integrated imaging approach for both the axonal and dendritic tree in conjunction with electrical recordings. Secondly, what are the molecular mechanisms involved in establishing the axon origin and are neurons with dendritic axons representing genetic subtypes? Recently developed patch-clamp RNA-sequencing approaches [52•,53•] could in principle be employed to harvest the cytoplasmic content of identified neurons with or without dendritic axons and link the morphological types to the single-cell transcriptome to resolve whether they represent genetic subtypes. Furthermore, the emerging theoretical and experimental evidence that is reviewed indicates that although intrinsic excitability is only marginally different in neurons with dendritic axons, the neuronal architecture provides a unique integrative pathway for synaptic potentials. Addressing the question of significance of axon placement will therefore require linking the cellular properties to connectivity and resolving its role at the level of information processing in the circuit. Ultimately, combining computational, anatomical and functional recording approaches will answer the long-standing question of the functional significance of AIS/axon location variability.

Conflicts of interest statement

Nothing declared.

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See annotation to Ref. [52•].