

K⁺ Channel Regulation of Multicompartmental Signal Integration

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Determining how neurons integrate different streams of information is critical to understanding circuit computational functions. In this issue of *Neuron*, Harnett et al. (2013) show that voltage-gated K⁺ channels control multiple layers of dendritic integration in layer 5 pyramidal neurons.

A basic but enduring problem facing neuroscientists is to understand the computations performed by the brain at the cellular level. How do neurons integrate tens of thousands of synaptic inputs, which are widely dispersed across varied and complex dendritic architectures to produce meaningful output? The spatial dispersion of inputs, together with fundamental physical properties of dendrites that act to severely filter synaptic conductances, means that synaptic inputs do not simply sum linearly. Rather, a given synapse's location and relative timing greatly impacts its ability to influence the neuron's action potential (AP) output.

This problem acutely affects cortical layer 5 pyramidal neurons (L5), which have dendrites spanning all six layers of the cortex (Figure 1). These cells are the major source of cortical output and so are decisive integrators in the cortical column. Previous reports have shown that active dendritic conductances can be recruited to produce regenerative events (spikes) to boost the propagation of synaptic signals to the axosomatic area where classical action potentials are initiated (Figure 1) (Larkum et al., 1999, 2009; Schiller et al., 2000; Williams and Stuart, 2002). Dendritic spikes carried by voltage-gated Na⁺ and Ca²⁺ currents, along with regenerative N-methyl-D-aspartic acid (NMDA) receptor currents, have led to a multilayered compartmental model for dendritic integration (Figure 1). Such a model is intriguing given the general cortical design in which feedforward sensory information is delivered to middle layers (layer 4), while top-down feedback internal representations of context, feature, attention, etc. arrive at layer 1

(Gilbert and Sigman, 2007). How then do these different streams of information interact? The different compartments of integration must somehow convene to provide contextualized output. Larkum et al. (2009) addressed this issue, showing that while individual branches of dendrites in the apical dendritic tuft produce NMDA receptor-mediated spikes in isolation, when multiple branches are activated together they can elicit a Ca²⁺ spike in the dendritic trunk, which can then propagate to the axosomatic initiation zone to affect AP output (Figure 1).

In this issue of *Neuron*, Harnett et al. (2013) have extended these findings, using a remarkable array of challenging electrophysiological and imaging techniques to describe a multilayer integration scheme in which regenerative signals are compartmentalized by voltage-gated K⁺ channels. Blocking these channels decreased the threshold for initiating spikes in multiple compartments to enhance their coupling. Moreover, they show that these principles apply in vivo during a sensory-motor object localization task.

In the first set of experiments, recording at the soma and the base of the apical dendritic tuft (termed the nexus, Figure 1), Harnett et al. (2013) confirmed previous findings by injecting suprathreshold current into the nexus, which resulted in large-amplitude spikes initiated in the distal dendritic trunk, which then forward propagated to the axosomatic integration zone to set off a classical action potential (Larkum and Zhu, 2002; Williams and Stuart, 2002). As previously proposed, this suggests that, in addition to the axosomatic integration zone, the distal apical

trunk nonlinearly integrates synaptic signals from the tuft (Larkum et al., 2009; Williams and Stuart, 2002).

Next, with electrodes placed at the nexus and tuft, simulated subthreshold synaptic input into the tuft was dramatically attenuated by the time it arrived at the nexus due to dendritic filtering. And unlike the trunk spikes, tuft spikes did not propagate well. When current was injected close to the nexus, tuft spikes were able to then detonate dendritic trunk spikes. However, in more distal tuft regions, the tuft spike only decrementally spread to the nexus, failing to induce trunk spikes. The local tuft spikes were prevented by tetrodotoxin, suggesting that they were initiated by voltage-gated Na⁺ channels. Harnett et al. (2013) provided support for this finding with glutamate uncaging/Ca²⁺ imaging experiments showing that activation of multiple dendritic spines resulted in large-amplitude Ca²⁺ influx into the stimulated branches. These NMDA receptor-dependent signals too, however, failed to actively propagate to the trunk.

Therefore, the tuft can be considered yet another integration zone, capable of amplifying local excitatory input through regenerative spiking. However, these spikes cannot overcome electrical compartmentalization to propagate to the dendritic trunk and axosomatic integrative zones. How then do distal tuft inputs influence neuronal output? Recently, the same group obtained in vivo two-photon imaging results showing that large, synchronous, tuft-wide Ca²⁺ transients are induced during sensory-motor behavior in mice (Xu et al., 2012). These could be induced experimentally by

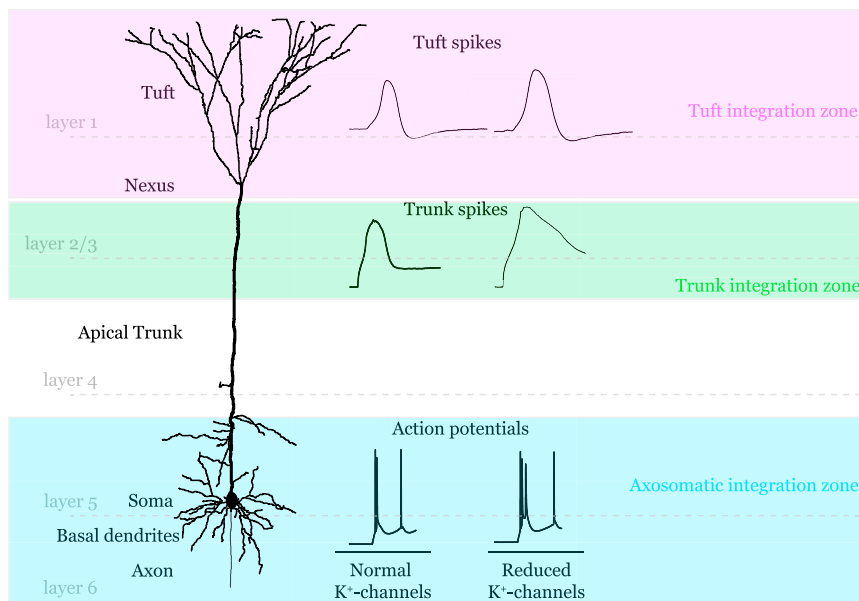


Figure 1. Schematic Diagram of the Integration Zones in a Reconstructed Layer 5 Pyramidal Neuron

Representative traces from signals in each zone are shown before and after K^+ channel blockade.

pairing trunk spikes with tuft depolarization, leading to increased frequency and duration of dendritic trunk Ca^{2+} spikes, which influenced AP output. Guided by previous findings in hippocampal CA1 pyramidal neuron dendrites showing that dendritic signaling is controlled by voltage-gated K^+ channels (Cai et al., 2004; Hoffman et al., 1997; Losonczy et al., 2008), Harnett et al. (2013) reasoned that these may also compartmentalize signals between L5 integration zones.

In outside-out patches from the trunk and tuft, Harnett et al. (2013) mapped the expression pattern and measured the properties of both transient (rapidly inactivating) and sustained (slowly/non-inactivating) voltage-gated K^+ channels. The data revealed a similar distribution pattern for both currents throughout the apical dendritic trunk and tuft. Harnett et al. (2013) then investigated the pharmacological profile of the currents, finding two drugs (quinidine and barium), which, at the concentrations used, appeared to selectively reduce both types of K^+ currents. These K^+ channel blockers were then used to determine in which ways K^+ channels affected excitability for each compartment.

With recording electrodes in the soma and nexus, K^+ channel blockers boosted

trunk spikes initiated with nexus current injection, which induced repetitive AP firing. Blockers did not, however, affect AP firing induced by somatic current injection, demonstrating specific K^+ channel control spiking in the dendritic trunk. This finding was supported by an additional set of experiments in which sub-threshold current injections into the soma, to simulate barrages of synaptic input, were paired with simulated synaptic input to the trunk. The enhanced trunk electrogenesis upon K^+ channel block was found to increase AP output.

Recording simultaneously in the trunk and the tuft, K^+ channel block decreased the threshold current required for trunk spike initiation and enhanced their propagation into the tuft, allowing full invasion of tuft branches. Signals traveling from the tuft to the trunk were also enhanced, with blockers again reducing the threshold current required to induce tuft spikes, which were increased in both amplitude and duration. Simulated sub-threshold synaptic input delivered simultaneously into the tuft and trunk generated plateau potentials in the tuft, which then spread to the trunk. This same group had recently shown that such signals are induced during whisking behavior during an object localization task in mouse L5

neurons (Xu et al., 2012). Here, while performing the same task in the presence of K^+ channel blockers, Harnett et al. (2013) found increased occurrence, amplitude, and duration of tuft Ca^{2+} signals evoked by whisker-object contact.

K^+ channels therefore contribute to the electrical compartmentalization of both the dendritic trunk and tuft. Because K^+ channels inactivate with depolarization, Harnett et al. (2013) suggested that activation of multiple compartments might lead to their interaction. Harnett et al. (2013) tested this in triple whole-cell recordings at the soma, trunk, and tuft. While the rate of axonal firing induced with somatic current injection was mostly unaffected by subthreshold trunk or tuft excitatory input, pairing tuft and trunk inputs generated large plateau potentials that altered the pattern of neuronal output, inducing high-frequency burst firing.

In summary, the paper by Harnett et al. (2013) presents a convincing case for voltage-gated K^+ channel regulation of the interaction between dendritic integration compartments in cortical pyramidal neurons. These findings provide a mechanism for nonlinear dendritic integration of incoming sensory information with intrinsic feedback information streams in an individual neuron, demonstrating the importance of active dendritic properties in shaping cortical output. Tuft inputs can produce regenerative signals, but these do not actively forward propagate, limiting their ability to influence on trunk spike initiation and thus axonal output. K^+ channel inactivation during multicompartment excitation can allow for such forward propagation. While Harnett et al. (2013)'s in vivo results introduce some object localization data, it will be interesting to see if and how these mechanisms are engaged with different behaviors. Such active dendritic integration schemes may play a general role in integrating sensory information with top-down influences encoding attention, expectation, perception, and action command in other cortical areas (Gilbert and Sigman, 2007).

The widespread applicability of a commonly organized, cell-based integration design is exciting but more work remains in describing the basic principles involved. The precise nature and timing of the various input streams and their subcellular localization are yet to be

resolved. The extreme electrical compartmentalization in the tuft suggests that presynaptic inputs must temporally and spatially coordinate to initiate spikes. Are the related inputs required to initiate spikes clustered early in development or by experience to bind behaviorally relevant information onto dendritic branches (Makino and Malinow, 2011)? The nature of the tuft spikes is still in question, given differences between the present study (mixed Na⁺ and NMDA receptor dependent) and previous work (mediated predominantly by NMDA receptors) (Larkum et al., 2009), and the role of synaptic inhibition still needs to be incorporated into the compartmentalized integration framework.

The next step in characterizing the K⁺ channel contribution to dendritic integration will be to uncover the molecular identity of channels involved. The kinetics, pharmacology, and expression level of K⁺ channels clearly differed between the soma and apical dendrite/dendritic tuft recordings, probably indicating a different complement of pore-forming and/or auxiliary subunits. However, while the density of both the transient and sustained components appeared relatively constant throughout the apical trunk and tufts, a more thorough investigation into the location-dependent properties of activation and inactivation seem warranted, given the important role of their inactivation proposed for the coupling of tuft inputs and integration zones. This data could reveal subtle compartmental or dis-

tance-dependent differences in auxiliary subunit composition as found for CA1 dendrites (Sun et al., 2011). After identifying the primary and auxiliary subunits, their genetic knockdown may help to define their role in behaviorally relevant dendritic integration.

An important K⁺ channel feature is their high degree of modulation (Shah et al., 2010). Expression levels and location, along with their voltage dependence and timing, can be rapidly modified in dendrites in response to activity and neuromodulation through posttranslational modifications (Hoffman and Johnston, 1999). This active modulation of K⁺ channel function could dynamically regulate compartmentalization and thus the integration of information pathways.

Finally, combining the techniques used by Harnett et al. (2013) with mouse models of CNS disorders, it is possible to examine the disease implications of aberrant dendritic excitability and synaptic integration. Investigations into the molecular mechanisms behind CNS disorders have uncovered synaptic dysfunction in diverse diseases such as autism, schizophrenia, depression, and Alzheimer's disease. However dendritic integration of synaptic signals, linking synaptic molecular pathways and higher-ordered circuit functions, are also probably affected, either by propagating synaptic errors to integration and cortical circuit and network abnormalities or through direct disease mechanisms acting on voltage- or ligand-gated channel proteins and their

regulation, providing potential treatment options.

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