Neural Coding: Hybrid Analog and Digital Signalling in Axons

Mammalian axons are thought to act as digital signaling devices, conveying information only by the timing and rate of all-or-none action potentials. Two recent studies now show that synaptic potentials can also spread far down the axon and influence action potential-triggered transmitter release in a graded, ‘analog’ manner. Axons thus encode information both about subthreshold and suprathreshold synaptic activity.

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Axons connect neurons. They are signalling devices, transmitting action potentials which trigger neurotransmitter release from synaptic boutons along the axon. In the classical view, synaptic input to the soma and dendrites of a neuron is funnelled into the axon, and at the initiation site — in the axon initial segment or first node of Ranvier [1–3] — a decision is made to fire an action potential once a threshold level of depolarization is exceeded. As the action potential is an all-or-none signal, it can be viewed as a ‘digital’ signal, with its initiation thus representing an analog-to-digital conversion. The action potential is then transmitted down the axon to the somatic recording site, where it serves as the necessary trigger for synaptic release. Action potential propagation is thought to be quite reliable, making axons relatively uninteresting in signal processing terms, acting as simple point-to-point relays. Furthermore, in this view, the information transmitted by axons is solely encoded by the number and timing of action potentials in the axon.

Two recent studies [4,5] have challenged this view. Using sophisticated techniques to make whole-cell patch-clamp recordings directly from the axons of neurons in brain slices, Alle and Geiger [4] and Shu et al. [5] have shown, in two different neuronal types, that in addition to action potentials, axons can transmit subthreshold synaptic potentials surprisingly efficiently (Figure 1), and that this spread of subthreshold potentials can have a significant impact on synaptic transmission triggered by action potentials.

Alle and Geiger [4] recorded directly from mossy fiber boutons [6], several hundred microns from the soma of dentate gyrus granule cells. They found that activating synaptic input to the granule cells resulted in large subthreshold excitatory postsynaptic potentials (EPSPs) in the mossy fiber boutons, with EPSP amplitudes that were graded with stimulation intensity. They demonstrated by local application of blockers of synaptic transmission that these axonal EPSPs, which they called excitatory presynaptic potentials, or EPreSPs, are propagated from the soma and not locally generated in the axon. Thus, the axon must be capable of efficient transmission of subthreshold EPSPs down the axon. By measuring the decay of EPreSP amplitude as a function of distance from the soma, they estimated that the space constant — $\lambda$, the distance at which the voltage decays to 37% of its original value — for propagation of EPreSPs is 430 $\mu$m.

Shu et al. [5] used a novel approach to record intracellularly from axons. They noticed that the cut end of unmyelinated pyramidal cell axons in brain slices often forms a membrane ‘bleb’ which is large enough to permit whole-cell patch-clamp recording. By simultaneously recording from the soma and such axon blebs, they showed that subthreshold membrane potentials at the soma spread efficiently down the axon. In particular, spontaneous barrages of synaptic activity triggered by network ‘up’ states [7] could be observed hundreds of microns down the axon (Figure 1), with a space constant $\lambda$ of 417 $\mu$m. The space constant values obtained by the two studies are surprisingly large given the fine calibre of pyramidal cell and granule cell

Figure 1. Synaptic potentials spread efficiently down the axon.
A schematic representation of a mammalian central neuron showing a simultaneous recording at the soma and several hundred microns down the axon. Single EPSPs (left) and depolarizations due to synchronous network activity (right) propagate efficiently to the axonal recording site and thus can modulate action potential-driven neurotransmitter release.
terminals are comparable to space primary effect of subthreshold and Geiger two independent studies developed by both groups. Thus, compartmental models of axons requirements confirmed by low intracellular resistivity, resistivity (consistent with the slow axons must have high membrane passive in the axon appears to be relatively transmitter release not appear to be sufficient to trigger subthreshold potentials alone did synaptic transmission. Importantly, subthreshold potentials did not appear to affect propagation fidelity of action potentials per se (compare [13,15]). However, in the two preparations, different effects of subthreshold membrane potentials on axonal action potential shape were observed. While Shu et al. [5] showed that somatic depolarizations broaden axonal action potentials, which is in turn expected to enhance synaptic release [6,16], Alle and Geiger [4] observed little change in action potential waveform.

Another possible mechanism is an increase in resting calcium in the presynaptic bouton triggered by the subthreshold depolarization [14]. This was tested by loading neurons with the calcium chelator EGTA to block presynaptic calcium accumulation, which reduced the enhancement of transmission in both cell types. This indicates that subthreshold potentials in the axon can trigger calcium entry via activation of low-threshold or ‘window’ calcium currents, which can raise presynaptic calcium levels sufficiently to enhance release (although this was not shown directly in these studies). However, some enhancement still remained even in the presence of high presynaptic EGTA concentrations (10 mM), particularly at the mossy fiber bouton synapse, suggesting that there may exist an additional, calcium-independent mechanism for subthreshold depolarizations to modulate release. One possibility is that there may be a direct voltage modulation of the synaptic release machinery. Thus, there could be multiple mechanisms, some of them cell-type specific, for modulation of synaptic transmission by subthreshold axonal EPSPs. Sorting out the relative importance of these different mechanisms is sure to stimulate future research.

Taken together, these studies [4,5] should prompt nothing less than a revolution of our view of signalling in axons of the mammalian central nervous system. Rather than just acting as simple digital signalling devices, axons can also act in an analog manner: they not only transmit information when the neuron has reached action potential threshold, but also signal subthreshold levels of activity in a graded manner. So even if an EPSP does not trigger an action potential, it can still influence transmission of information by spikes triggered by subsequent EPSPs.

Such a hybrid model of transmission (Figure 2), in which subthreshold potentials do not directly trigger release but rather modulate subsequent action-potential driven release, is not entirely unprecedented: there exist several invertebrate systems where changing membrane potential in the presynaptic neuron has been shown to alter synaptic transmission [17–19]. What is particularly important about the new studies [4,5] is that transient depolarizations provided by physiological stimuli (EPSPs) are capable of modulating

![Figure 2. Three modes of synaptic signalling in mammalian central axons.](image-url)
transmission. This suggests that the timing relationship of EPSPs and succeeding action potentials will be critical [4], favouring potentiation of transmission in response to facilitating synaptic inputs. It also provides a mechanism for short-term memory of recent activity, where synaptic release depends on the recent history of synaptic input to the neuron.

This new view has several additional interesting functional implications. EPSPs attenuate as they spread down the axon. Shu et al. [5] performed an anatomical analysis showing that more than one hundred synapses made by the axon of cortical pyramidal neurons are within a space constant of the soma and thus will be affected by subthreshold axonal modulation. However, the magnitude of any effect of subthreshold depolarization on synaptic transmission will depend on the distance of the individual synaptic contacts from the soma: proximal synaptic contacts will be strongly influenced, and distal ones less so. It is therefore likely that there exists mixed analog/digital signalling at proximal contacts, and purely digital signalling at distal contacts. This may reflect different functional roles of proximal and distal connections. Proximal connections should be influenced more by the local network context that generated the depolarization, for example oscillations or other forms of synchrony, such that the axonal depolarization represents a form of positive feedback for coordinating local networks. On the other hand, long-range connections exhibit entirely digital signalling, since local feedback is not necessary.

There are many questions left open by these two remarkably complementary papers [4,5]. Firstly, how general are these findings? Both studies recorded from the large, main trunk of unmyelinated axons exhibiting relatively small amounts of branching. In axons exhibiting more branching (such as many interneuron axons), or in thinner axons (particularly axon collaterals) attenuation of subthreshold potentials should be much steeper, and so will permit less hybrid signalling. It also remains to be determined how well subthreshold potentials can spread in myelinated axons.

Secondly, can inhibitory postsynaptic potentials (IPSPs) spread down the axon with similar efficacy to EPSPs, and if so, how do IPSPs influence synaptic release? Thirdly, how do axonal voltage-gated channels influence propagation? Alle and Geiger [4] showed that application of the sodium channel blocker tetrodotoxin had only a relatively small effect on the EPSP amplitude; however, the dependence on EPSP amplitude was not examined, and other channels may also contribute (particularly $I_h$, cation channels and also calcium channels, as expected from the fact that subthreshold calcium entry appears to influence action potential-dependent release).

Fourthly, as action potential propagation itself can be modulated by membrane potential [13,15], it is conceivable that synaptic potentials spreading in the axon can either promote or inhibit propagation in different regions of the axon. And lastly, if axo-axonic synapses are able to trigger presynaptic potentials, the present studies suggest that these potentials should be able to spread to other synapses to influence synaptic release, or even retrogradely to influence action potential propagation or initiation. Such two-way traffic of subthreshold potentials in the axon may have unexpected consequences for information processing.

The new studies [4,5] should provide a major stimulus to the growing recent change in perspective on axons as signalling devices [20]. Rather than acting as simple, reliable transmission lines for action potentials, axons have now been shown to exhibit a rich repertoire of behaviour, in which both action potentials and synaptic potentials can be transmitted to synapses, and can interact to modulate synaptic release. This suggests that the action potential initiation site is no longer the final site of synaptic integration, but that action potentials and synaptic potentials continue to interact along the length of the axon. The next step will be to understand the computational significance of these interactions and how they can be exploited to enhance the normal functioning of the mammalian central nervous system. Now that the axons of mammalian neurons are finally becoming accessible to direct investigation using both imaging and electrophysiological techniques, we can expect many more breakthroughs from these tiny structures.

References
IKKε Signaling: Not Just NF-κB

IkB kinases (IKKs) are key components of NF-κB signaling pathways in innate immunity and inflammation. Surprisingly, three recent reports implicate IKKs in Drosophila in seemingly unrelated functions, including non-apoptotic caspase activation and cytoskeleton organization.

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NF-κB transcription factors are kept inactive by cytoplasmic sequestration through complex formation with inhibitory IkB proteins [1]. NF-κB stimulation—for example initiated by Toll-like receptors in innate immunity or in response to proinflammatory cytokines such as TNFα—requires dissociation of the NF-κB/IkB complex. IkB kinases (IKKs) were initially identified as a high-molecular weight complex capable of site-specific phosphorylation of IkB-α [2]. This phosphorylation triggers ubiquitin-mediated degradation of IkB-α, and the release of NF-κB transcription factors, which translocate into the nucleus [2]. Subsequent analysis identified two catalytic subunits (IKKα and IKKβ) and a structural component of this complex (IKKγ/NEMO). While the IKKαβγ complex is required for NF-κB activation in response to most NF-κB inducers, the role of two related kinases known as IKKe/IKKi and TBK1/NAK/T2K is less clear.

In Drosophila, two independent immune signaling pathways control the activity of distinct NF-κB-like proteins [3]. While the Toll/anti-fungal pathway requires Dorsal and Dif, the IMD/anti-bacterial pathway leads to activation of Relish [3]. Dorsal and Dif are rendered cytoplasmic in complex with the only IkB-like protein, termed Cactus. Phosphorylation of Cactus is required for its degradation [4–6], but the responsible kinase has not been identified. The Drosophila genome encodes two IKK genes. DmIKKβ (or DLAK) is most similar to human IKKβ, and is involved in Relish activation [7]. That leaves the second Drosophila IKK, DmIKKε (also known as Ik2), as a candidate for the Cactus kinase. However, recent reports [8,9], including one in this issue of Current Biology [10], rule out a function of DmIKKε as Cactus kinase. Instead, DmIKKε modulates caspases for a non-apoptotic function and controls both actin and microtubule cytoskeletons.

DmIKKε as a Negative Regulator of Diap1 Protein Stability

As in vertebrates, apoptosis in Drosophila is triggered by activation of caspases, a highly specialized class of cell death proteases. In surviving cells, caspases are kept inactive through complex formation with inhibitor of apoptosis proteins (IAPs), most notably Drosophila IAP1 (Diap1) [11]. In response to cell death-inducing signals, pro-apoptotic proteins such as Reaper stimulate the ubiquitylation and degradation of Diap1, releasing caspases from IAP inhibition and triggering apoptosis [11]. Interestingly, the recent paper by Kuranaga et al. [9] identifies mutations in DmIKKe as dominant suppressors of Reaper-induced cell death [9]. Subsequent analysis showed that loss of DmIKKe increases the stability of the Diap1 protein, providing an explanation for the observed suppression of Reaper-induced apoptosis [9]. These observations suggest that wild-type DmIKKe destabilizes Diap1, leading to Caspase activation, a conclusion which was confirmed in cell culture experiments and in transgenic flies.

Destabilization of Diap1 appears to be the result of phosphorylation by DmIKKe. Interestingly, human TBK1/NAK/T2K was able to promote phosphorylation and degradation of human XIAP [9], suggesting conservation of IKKe-mediated control of IAP stability. DmIKKe-mediated Diap1 degradation is independent of Reaper. Overexpression of DmIKKe in cell death deficient (i.e. reaper mutant) background still induced Diap1 instability and apoptosis [9]. This is a striking finding, as it suggests that control of Diap1 stability and thus caspase activation in Drosophila occurs through distinct pathways, including the classical apoptotic pathway and as well as by IKKe signaling.

DmIKKe Controls Diap1 in a Non-Apoptotic Setting

Despite the fact that overexpression of DmIKKe induces a strong apoptotic phenotype, developmental cell death appears