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Invited Review

Neuronal signaling in central nervous system

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Abstract: A new method of axon recording through axon bleb has boosted the studies on the functional role of central nervous system (CNS) axons. Using this method, we have revealed the mechanisms underlying the initiation and propagation of the digital-mode signal, all-or-none action potentials (APs), in neocortical pyramidal neurons. Accumulation of the low-threshold Na^+ channel subtype $Na_v1.6$ at the distal end of the axon initial segment (AIS) determines the lowest threshold for AP initiation, whereas accumulation of the high-threshold subtype $Na_v1.2$ at the proximal region of the AIS promotes AP backpropagation to the soma and dendrites. Through dual recording from the soma and the axon, we have showed that subthreshold membrane potential (V_m) fluctuations in the soma propagate along the axon to a long distance and probably reach the axon terminals. Paired recording from cortical neurons has revealed that these V_m changes in the soma modulate AP-triggered synaptic transmission. This new V_m-dependent mode of synaptic transmission is called analog communication. Unique properties of axonal K^+ channels (K_v1 channels) may contribute to shaping the AP waveform, particularly its duration, and thus controlling synaptic strength at different levels of presynaptic V_m. The level of background Ca^{2+} may also participate in mediating the analog signaling. Together, these findings enrich our knowledge on the principles of neuronal signaling in the CNS and help understand how the brain works.

Key words: axon recording; action potential; digital signaling; analog signaling; Na^+ channel; pyramidal neuron

中枢神经系统内神经元信号处理

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摘　要：一种新颖的轴突断端(axon bleb)膜片钳记录方法大力促进了中枢神经系统轴突功能的研究。我们的工作应用这一方法揭示了大脑皮层锥体神经元的数码信号(具全或无特性的动作电位)的爆发和传播机制。在轴突始段(axon initial segment, AIS)远端高密度聚集的低阈值Na^+通道亚型$Na_v1.6$决定动作电位的爆发；而在AIS近端高密度聚集的高阈值Na^+通道亚型$Na_v1.2$促进动作电位向胞体和树突的反向传播。应用胞体和轴突的同时记录，我们发现胞体阈下膜电位的变化可以在轴突上传播较长的距离并可到达那些离胞体较近的突触前终末。进一步的研究证明了胞体膜电位的变化调控动作电位触发的突触传递，该膜电位依赖的突触传递是一种模拟式的信号传递。轴突上一类特殊K^+通道(K_v1)的活动调制动作电位的波形，特别是其波宽，从而调控各种突触前膜电位水平下突触强度的变化。突触前终末的背景Ca^{2+}浓度也可能参与模拟信号的传递。这些发现深化了我们对中枢神经系统内神经信号处理基本原理的认识，进而帮助我们理解脑如何工作。

关键词：轴突记录；动作电位；数码式信号传递；模拟式信号传递；Na^+通道；锥体神经元
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The capability of producing regenerative electrical impulses, the action potentials (APs), defines a cell as a neuron. APs are normally initiated at the axon initial segment (AIS) of a neuron[1-5], then propagate along the axon to the presynaptic terminals and mediate synaptic transmission, meanwhile they backpropagate to the somatodendritic compartments and mediate certain types of synaptic plasticity[4, 6-10]. AP is a digital signal due to its all-or-none (1 or 0) feature. Traditionally, this digital signal has been believed as the sole mode of communication between neurons in the central nervous system (CNS); however, recent studies[11,12] have revealed that presynaptic membrane potential (V_m) modulates AP-triggered synaptic transmission, an analog-mode signaling. Here, I will review recent findings on the mechanism underlying AP-mediated digital signaling and V_m-dependent analog signaling in the CNS.

1 Recording from CNS axons

In general, the cell body and dendrites of a neuron receive synaptic inputs from other neurons, the interaction between these inputs and intrinsic membrane properties of the postsynaptic neuron results in the generation of APs at the axon, the main output signal that a neuron used to communicate with other neurons. Although many sites of a neuron could initiate APs[3,4,13-17], the AIS turns out to be the location where APs are normally initiated[1-5]. In order to reveal the mechanism underlying neuronal signaling, researchers need to perform direct electrophysiological recordings from all the structures of a neuron including the dendrites, the soma and the axon. Since the size of dendrites (~2 μm in diameter) and the soma (10–20 μm) are large enough for whole-cell recording and sharp electrode recording, extensive studies have been carried out to investigate their role in the integration of synaptic inputs. Due to the small size of CNS axons (<1 μm), it has been impossible to perform direct recording from them. Therefore, the biophysical properties and functional roles of axons remain largely unknown. As we know that the fundamental stone of the modern electrophysiology is the intracellular studies carried out in giant squid axons by Hodgkin and Huxley half century ago[18], the studies on axons in mammalian CNS were hampered by the difficulties of direct recording. Some investigators did obtain successful whole-cell recordings from axons, but only in some special structures of the axon such as the axon hillock of cortical pyramidal neurons[3,4,15], the giant mossy fiber button of hippocampal granule cells[19,20] and the calyx of Held in the brain stem[21-23]. To fully probe the functional role of CNS axons in signal processing, we need direct recordings from every segments of the axon to study the properties of ion channels and neurotransmitter receptors distributed along the axon.

A new method (Fig. 1 and Fig. 2) of direct patch-clamp recording from CNS axons has been developed recently[2,12,24,25]. In cortical brain slices, axons of projection neurons such as the pyramidal cells are cut during slicing procedures. At the cut end, the axon reseals and forms a bleb-like structure with a diameter of several microns (3–6 μm, Fig. 1*A*). This axon bleb is large enough for whole-cell recording as well as membrane patch recording. For dual recording from the soma and the axon, somatic recording is achieved first with pipette solution containing fluorescent dye; the morphology of the recorded neuron can be visualized under fluorescent microscope in couple of minutes. It is then easy to trace the axon from the axon hillock down to the cut-end bleb; whole-cell recording can be obtained from the axon bleb. To study the biophysical properties of the axonal ion channels, one could perform cell-attached, outside-out patch and inside-out patch recordings from the axon bleb (Fig. 1*A*). Equipped with these techniques, we have identified some unique properties of axonal ion channels and revealed important roles of the axon in signal processing[2,12,24,25].

2 Digital signaling – AP initiation and propagation

Since AP is the main output signal of a given neuron and AP generation determines the excitability of the neuron, it is critical to identify the AP initiation site (the final integration site of synaptic inputs) and reveal the underlying mechanism for AP initiation and propagation. Previous studies in spinal motorneurons[26-28] and cortical pyramidal neurons[1-5] have revealed that the neuronal compartment with the lowest threshold for AP initiation is the AIS, which has a threshold 15 mV lower than the soma[5, 26,27]. It has been long believed that a high concentration of Na^+ channels distributed at the AIS determines the lowest threshold[16, 29-31]. Interestingly, although the distal and the proximal region of the AIS have a similar density of Na^+ channels[5, 15, 32], APs preferred to initiate at the distal end of the AIS, rather than at the proximal AIS, in layer 5 pyramidal neurons. Differences in biophysical properties of Na^+ channels

may be attributable to this site preference. Initial cell-attached and outside-out patch recordings revealed a ~7 mV hyperpolarizing shift in the activation curve of Na^+ channels in the axon, in comparison with that at the soma[15], suggesting that the voltage-dependent property of the AIS Na^+ channels play an important role in determining the AP initiation site. Recent immunostaining results have demonstrated that, in different neuronal types, the Na^+ channel subtypes may be targeted to the AIS differentially[32-36]. Since Na^+ channel subtypes have different activation thresholds[37], their subcellular distribution and density at the AIS may contribute to the AP initiation and regulation[33,38-40]. Therefore, a full resolution of the role of density *vs* voltage-dependent property of Na^+ channels at the AIS requires mapping of the distribution of Na^+ channel subtypes and direct recording from the axonal membrane at both the proximal and the distal regions of the AIS.

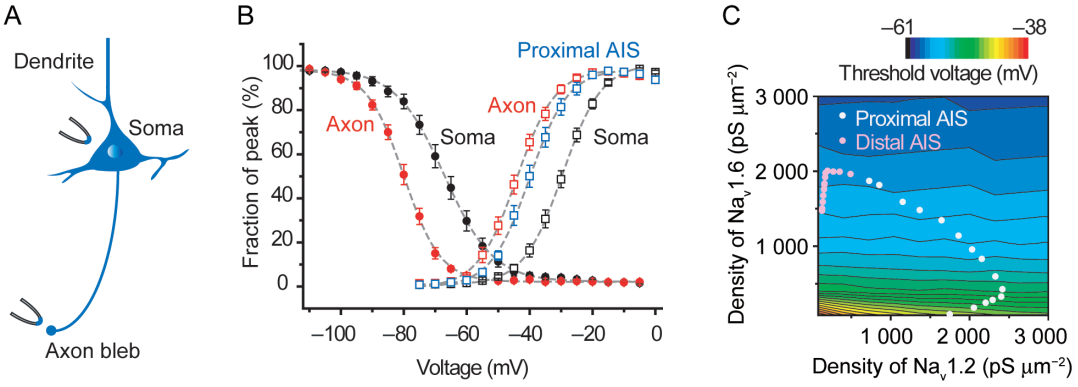

Fig. 1. The lowest threshold of AP initiation is determined by Na^+ channel subtype $Na_v1.6$. *A*: Schematic diagram showing direct patch-clamp recording from the soma and the axon of a cortical pyramidal neuron. The axon bleb formed at the cut end of the axon during slicing is large enough for patch recording and whole-cell recording (see Fig. 2). *B*: Activation and availability curves for somatic and axonal (>50 µm from the soma) Na^+ current. The activation curve for proximal-AIS is inserted for comparison. *C*: Contour map of the AP threshold with various density combinations of $Na_v1.2$ and $Na_v1.6$. Dots indicate the local density for individual channel subtypes at the AIS (2-µm interval). Adapted from Hu *et al*., 2009 [25].

Axon bleb recording method mentioned above perfectly meet the technical needs. Through regular outside-out patch recording as well as whole-bleb recording (isolated axon bleb)[25], we found that the AIS has the highest Na^+ channel density in comparison with the soma and other compartments of the axon, consistent with previous immunostaining results[1,33,35,41-45] and theoretical predictions[16,31]. Importantly, we found that the activation threshold of Na^+ channels progressively decreases (up to 14 mV) as the distance from the soma increases, and reaches the lowest threshold at the distal end of the AIS and the following axonal regions (Fig. 1*B*). Interestingly, immunostaining results revealed that Na^+ channel subtype $Na_v1.2$ and $Na_v1.6$ selectively target to the proximal and the distal AIS, respectively, with some overlap in between[25]. These findings are consistent with previous reports showing that $Na_v1.6$ channels have a half-activation voltage ~15 mV lower than $Na_v1.2$ channels in expression system. Simulation studies reveal that the low-threshold $Na_v1.6$ channels that locate at the distal region of the AIS determine the AP initiation site (Fig. 1*C*). Further dual recording from the soma and the axon (Fig. 2*A*) showed that axonal AP may not be able to propagate back to the somatodendritic compartments if the somatic V_m is hyperpolarized[25], suggesting that proximal AIS $Na_v1.2$ channels may promote AP backpropagation in physiological conditions (Fig. 2*B*). As we know that AP backpropagation is critical for certain types of synaptic plasticity (such as the spike-timing dependent plasticity) [3, 6-10], $Na_v1.2$ channels may participate in these events through gating AP propagation to the dendrites. Together, these findings reveal the mechanism underlying AP initiation and backpropagation in cortical pyramidal neurons.

It would be of interest to reveal the mechanism for AP initiation and propagation in intrinsic neurons (in-

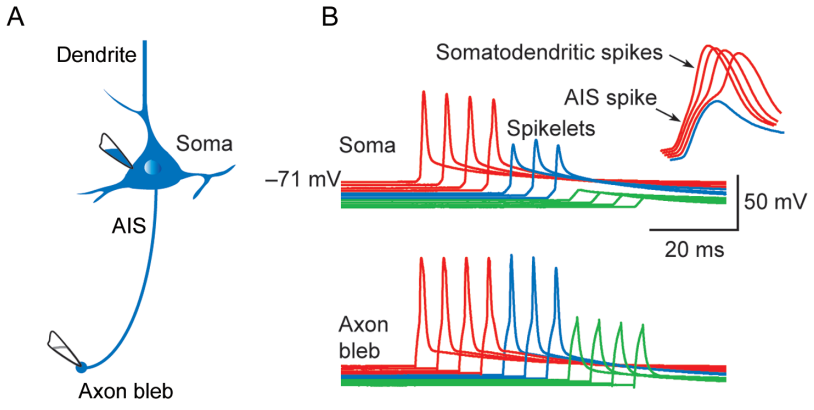

Fig. 2. Perisomatic high-threshold Na^+ channels regulate AP backpropagation. *A*: Schematic diagram (for panel *B*) of the direct whole-cell recording from the soma and the axon. *B*: Somatic hyperpolarization causes failures of AP backpropagation to the soma, as reflected by the occurrence of spikelets at hyperpolarizing membrane potentials. Red traces, axonal APs evoked by brief current injections at the axon bleb are able to successfully backpropagate to the soma. Blue traces, hyperpolarization causes AP backpropagation failures. Green traces, further hyperpolarization prevents AP generation at both the axon and the soma. Note the propagation of subthreshold membrane potentials. Inset, overlay of somatic APs and a spikelet showing the two components of somatic APs, the somatodendritic spike and the AIS spike. Adapted from Hu *et al*., 2009 [25].

terneurons within a brain region). In the cerebral cortex, the inhibitory interneurons are important elements in neural networks. They could not only control the precise timing of AP initiation in pyramidal neurons but also maintain the overall balance of the excitation and inhibition in the cortex[46]. Inhibitory interneurons normally formed synapses onto the somatodendritic compartments of pyramidal neurons; however one type interneurons called chandelier cells send their axons to innervate the AIS of pyramidal neurons[41, 47] and thereby may control their spike timing. Therefore, the excitability of these inhibitory interneurons actually plays an important role in cortical information processing. Whether the interneurons use similar mechanism for AP initiation as pyramidal neurons and whether they have different Na^+ channel subtypes in their axons need further examination.

3 Propagation of subthreshold V_m changes along axon

The axon is a cable-like structure, and its main function is to generate and propagate APs, the digital form signal. Since the discovery of the ionic mechanisms underlying APs in the giant squid axon, extensive studies have focused on the mechanisms for AP generation and propagation; however, the propagation of subthreshold V_m changes, another important function of the cable-like axon, is largely ignored because researchers assume that subthreshold V_m fluctuations at the soma and the AIS only help generate APs as soon as the firing threshold is reached. In cortical layer 5 pyramidal neurons, simultaneous recording (Fig. 3*A*) from the soma and the axon[12] revealed that V_m fluctuations (summation of postsynaptic potentials during network activities, Fig. 3*B*) in the soma could propagate along the axon with a length constant of ~400 μm (far distant from the AIS), indicating that somatic voltage changes attenuate to 37% when propagate to this axonal location. The waveform of V_m changes in the axon is a close copy of the somatic voltage waveform with larger attenuation at high frequencies, presumably resulting from the low-pass filter property of the axonal cable (Fig. 3*B*). Similar results were obtained when compared the V_m waveforms at the soma and the axon in response to step current injection at the soma (length constant: 455 μm)[12]. The spreading of subthreshold potentials not only occurs in the axons of cortical pyramidal neurons, but also takes place in mossy fibers of hippocampal granule cells. Recording from the mossy fiber boutons detects passive propagation of transient subthreshold V_m changes along the axon with a length constant of ~400 μm[11].

It is of interest to know the functional role of these propagating V_m changes along the axon. We speculate that the subthreshold V_m changes arriving at the presyn-

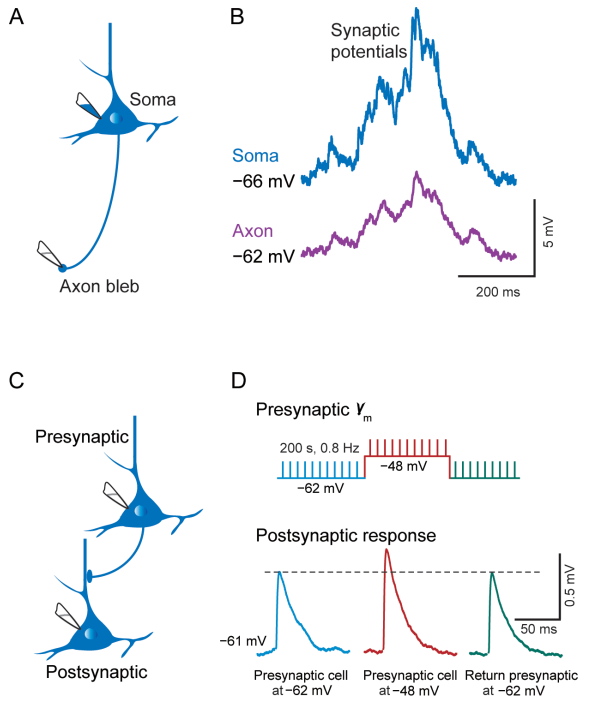

Fig. 3. Analog signaling between cortical pyramidal neurons. *A*: Schematic diagram (for panel *B*) showing dual recording from the soma and the axon. *B*: V_m fluctuations recorded at the soma and the axon during network activity (Up state). Note the size difference and the similarity of the voltage waveforms in the soma and axon. The axonal recording site is 266 μm away from the soma. *C*: Schematic diagram (for panel *D*) showing paired recordings from pyramidal neurons. *D*: Presynaptic depolarization (from −62 to −48 mV) increases the average amplitude of excitatory postsynaptic potentials evoked by single APs (with brief current injection at 0.8 Hz). Adapted from Shu *et al*., 2006[12].

aptic terminal may regulate synaptic transmission. In addition to the AP-triggered synaptic transmission, there is another type of synaptic transmission named graded transmission, which is dependent of presynaptic V_m changes rather than AP generation[48-51]. This V_m-dependent graded transmission occurs in some specific synapses of invertebrate nervous systems where the release rate of presynaptic vesicles depends on the V_m level of the presynaptic terminal[21,52-56]. This type of transmission also exists in vertebrate nervous system, but it was only found in photoreceptors and some interneurons in the retinal, in hair cells of the cochlea, and in electroreceptors of the lateral line organ of fish and amphibians[48-50,57]. Both the AP-triggered and the V_m-dependent graded transmission occur in some invertebrate synaptic contacts, where presynaptic depolarization alone may cause neurotransmitter release in addition to AP-triggered release[58-61]. Some invertebrate synapses may use a hybrid-form synaptic transmission, meaning that the amplitude of AP-triggered postsynaptic potential is subjected to change in response to a V_m change in the presynaptic neuron[52-56]. Therefore, one would speculate that, in mammalian CNS, V_m changes in the soma may propagate down the axon to presynaptic terminals and influence synaptic transmission through a way akin the graded transmission.

4 Analog signaling – V_m-dependent modulation of synaptic transmission

The graded and the hybrid synaptic transmission described above require electronically close distance between the soma and the axonal terminal. Indeed, in layer 5 pyramidal neurons, over 150 putative synaptic boutons (presynaptic terminals) distribute at axon collaterals within 500 μm and ~270 boutons within 1 mm from the soma [12]. Using paired recording from pyramidal neurons (Fig. 3*C*)[12], we have found that modest depolarization (~15 mV) in the presynaptic soma causes a significant increase in the average amplitude of postsynaptic responses evoked by single APs (Fig. 3*D*). Further examination has revealed that the amplitude of postsynaptic response is a linear function of presynaptic V_m levels, depolarization in the presynaptic soma results in facilitation of postsynaptic responses at a rate of ~30% per 10 mV. These results indicate that the synaptic transmission between cortical pyramidal neurons not only via AP-mediated digital communication but also through V_m-dependent graded (or analog) communication[12]. This hybrid form of synaptic transmission also occurs at synaptic connections between mossy fiber boutons and the dendrites of CA3 pyramidal neurons[11], and between molecular layer interneurons of the cerebellum[62].

Multiple mechanisms may contribute to this presynaptic V_m-dependent analog signaling between CNS neurons. Background Ca^{2+} concentration in the presynaptic terminal is an important factor influencing the release probability of synaptic vesicles. Subthreshold depolarization that arrived at the axon terminal may increase the background Ca^{2+} concentration via the activation of voltage-gated Ca^{2+} channels distributed at the presynaptic membrane. In the Calyx of Held, depolarization of the presynaptic terminal causes the activation of P/Q-type Ca^{2+} channels, which subsequently increase the amplitude of postsynaptic current[21]. In

cortical pyramidal neurons, the activation of P/Q and N-type Ca^{2+} channels may be attributable to the increase in baseline Ca^{2+} level and AP-evoked Ca^{2+} transients at both the AIS and axon terminals (that close to the soma) in response to somatic depolarization[63]. Blocking voltage-gated Ca^{2+} channels diminished presynaptic depolarization-induced increase in asynchronous release in molecular layer interneurons of the cerebellum[62], consistent with the notion that V_m changes alter the background Ca^{2+} concentration and thus regulate neurotransmitter release. In the presence of a high concentration of EGTA (a slow Ca^{2+} buffer) in the recording pipette, the success rate of finding somatic depolarization-induced facilitation of postsynaptic responses in pyramidal neuron pairs was substantially reduced[12]. Together, these findings support a role of background Ca^{2+} in analog signaling. In hippocampal mossy fiber boutons, however, depolarization sufficient to increase neurotransmitter release causes no changes in AP-evoked Ca^{2+} transients[11]. Imaging of Ca^{2+}-dependent fluorescence in these giant boutons reveals no detectable changes in the basal fluorescence or AP-induced fluorescence transients[64], suggesting that presynaptic Ca^{2+} may be not involved in the analog signaling in these special synapses.

The waveforms of presynaptic APs play an important role in determining the amount of neurotransmitter release, broader APs may trigger more Ca^{2+} influx at the presynaptic terminal[20]. Therefore, we speculate that the waveform of axonal APs may be regulated by somatic V_m changes. Interestingly, simultaneous recording from the soma and the axon of layer 5 pyramidal neurons shows that somatic subthreshold depolarization selectively broadens the axonal APs but not the somatic APs[12]. Further examination of the K^+ current at the soma and the axon revealed that a rapidly activating but slowly inactivating K^+ current, known as the D-current[65], was only observed at the axon[24]. The time course of inactivation lasts for several seconds[65], consistent with the time course of depolarization-induced AP broadening and facilitation of postsynaptic responses[12]. Pharmacological experiments show that this axonal K^+ current is mediated by K_v1 channels, blocking these channels abolishes depolarization-induced facilitation of postsynaptic responses[24, 66]. In sharp contrast, depolarization has no effect on the waveform of APs at the hippocampal mossy fiber boutons[11], suggesting that the mechanism for analog signaling may differ in different brain regions.

These studies opened a new window from which we can understand how CNS neurons signal each other. Additional studies are required to investigate whether the analog signaling is a universal mode of synaptic transmission in the CNS, whether there are brain region-specific mechanisms, whether this mode of signaling influences the operation of local circuitry and whether it has a functional role in the intact brain. Equipped with the new method of axon recording, we are ready to address these important questions.

In conclusion, the studies reviewed above provide deep insight into the mechanism for the initiation and propagation of the digital signals – all-or-none APs. Selective accumulation of Na^+ subtype $Na_v1.6$ at the distal end of AIS determines the lowest threshold for AP initiation, whereas accumulation of $Na_v1.2$ at the proximal AIS may promote, if not guarantee, AP backpropagation into the somatodendritic compartments. We have discovered another mode of synaptic transmission, the analog-mode signaling between cortical neurons – presynaptic V_m-dependent modulation of synaptic responses. This mode of transmission depends on the passive propagation of subthreshold depolarization along the axon, which inactivates axonal K_v1 channels and broadens axonal APs, and thereby facilitates postsynaptic responses. All these studies have benefited from the new method of axon bleb recording, which will further promote studies on the function of CNS axons.

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