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# Dendritic calcium signaling in cerebellar Purkinje cell

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ABSTRACT

The Purkinje cells in the cerebellum are unique neurons that generate local and global $Ca^{2+}$ signals in response to two types of excitatory inputs, parallel fiber and climbing fiber, respectively. The spatiotemporal distribution and interaction of these synaptic inputs produce complex patterns of $Ca^{2+}$ dynamics in the Purkinje cell dendrites. The $Ca^{2+}$ signals originate from $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$ release from intracellular stores that are mediated by the metabotropic glutamate receptor signaling pathway. These $Ca^{2+}$ signals are essential for the induction of various forms of synaptic plasticity and for controlling the input–output relationship of Purkinje cells. In this article we review $Ca^{2+}$ signaling in Purkinje cell dendrites.



## 1. Introduction

$Ca^{2+}$ is a major intracellular signaling molecule in mammalian neurons, and it controls and modulates a wide range of neuronal functions including neuronal excitability, firing patterns, neurotransmitter release, synaptic plasticity and circuit formation. Purkinje cells, the solo output of the cerebellar cortex, have highly elaborate dendritic arbors and receive two types of excitatory inputs from more than 100,000 parallel fibers (PFs) and a single climbing fiber (CF). Purkinje cells exhibit a variety of dendritic $Ca^{2+}$ signals in response to these excitatory inputs (Fig. 1). Given the high expression level of voltage-gated $Ca^{2+}$ channels (VGCCs) in the dendrites (Westenbroek et al., 1995), Purkinje cells show prominent dendritic $Ca^{2+}$ spikes that are associated with substantial elevation of intracellular $Ca^{2+}$ concentration throughout their dendritic trees (Lev-Ram, Miyakawa, Lasser-Ross, & Ross, 1992; Miyakawa, Lev-Ram, Lasser-Ross, & Ross, 1992; Sugimori & Llinas, 1990; Tank, Sugimori, Connor, & Llinas, 1988). Another source of $Ca^{2+}$ signals in Purkinje cell dendrites is $Ca^{2+}$ release from intracellular stores. The type-1 metabotropic glutamate receptor (mGluR1) activated by PF inputs mediates inositol-1, 4, 5-triphosphate ($IP_3$) induced $Ca^{2+}$ release from internal stores (Finch & Augustine, 1998; Takechi, Eilers, & Konnerth, 1998).

Interplay between $Ca^{2+}$ influx through VGCCs and $IP_3$-induced $Ca^{2+}$ release promotes the complex spatiotemporal dynamics of dendritic $Ca^{2+}$ signals in Purkinje cells and results in nonlinear integration of synaptic inputs and long-lasting changes in synaptic efficacy at PF synapses (Inoue, Kato, Kohda, & Mikoshiba, 1998; Khodakhah & Armstrong, 1997; Miyata et al., 2000; Sarkisov & Wang, 2008; Wang, Denk, & Hausser, 2000). Furthermore, the highly elaborate structure and the nonlinear properties of Purkinje cell dendrites facilitate distinct levels of dendritic computation and other forms of synaptic plasticity, and $Ca^{2+}$ signals play a critical role in these processes. In this article we review the current understanding of $Ca^{2+}$ signaling in Purkinje cell dendrites and how it underlies various cellular functions in the cerebellum.

## 2. Dendritic calcium spikes

The advent of techniques that allow intracelluar recording from the dendrites in brain slice preparations has facilitated investigations into the electrophysiological properties of the dendrites (Hausser, Spruston, & Stuart, 2000; Llinas, Nicholson, Freeman, & Hillman, 1968; Llinas & Sugimori, 1980; Stuart, Dodt, & Sakmann, 1993; Wong, Prince, & Basbaum, 1979). In 1980, Llinas and Sugimori showed the electrophysiological properties of Purkinje cell dendrites in mammalian cerebellar slices (Llinas & Sugimori, 1980). Depolarizing current injection elicits a high frequency burst of fast sodium spikes (simple spikes; SS) and a bout of slow-rising $Ca^{2+}$ spikes interposed between SS. The amplitude of SS is highly attenuated in the dendrites, indicating passive propagation of sodium spikes into the dendrites (Llinas & Sugimori, 1980). This has been confirmed by simultaneous somatic and dendritic patch-clamp recordings (Stuart & Hausser, 1994) and modeling studies (Vetter, Roth, & Hausser, 2001) showing that weak and passive propagation of SS into the dendrites is primarily due to low sodium channel density in the dendrites and the extensive dendritic branching of Purkinje cells. In striking contrast,

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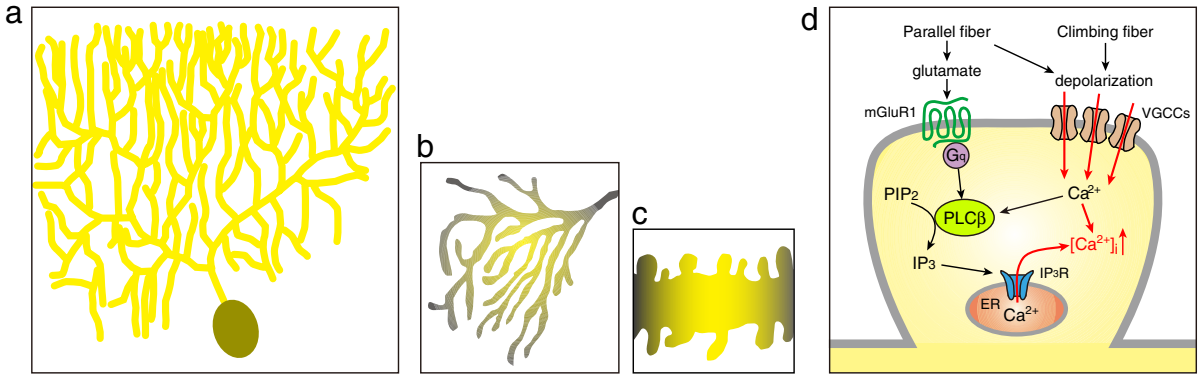


**Fig. 1.** A schematic illustrating dendritic $Ca^{2+}$ signaling in Purkinje cells. (a) Climbing fiber input and strong depolarization to the soma induce $Ca^{2+}$ spikes that are associated with global $Ca^{2+}$ signals (yellow) in the whole dendritic tree. (b), (c) Parallel fiber input induces localized $Ca^{2+}$ signals in the spiny branchlets (b) and in individual spines (c). (d) The intracellular signaling pathway for synaptically-induced $Ca^{2+}$ signals in parallel fiber synapses.

the amplitudes of $Ca^{2+}$ spikes are larger in more superficial dendrites than in the soma, indicating that sodium channels and $Ca^{2+}$ channels predominate at the soma and in the dendrites, respectively (Llinas & Sugimori, 1980).

The spatial profile and precise localization of $Ca^{2+}$ signals triggered by dendritic $Ca^{2+}$ spikes have been analyzed by $Ca^{2+}$ imaging studies (Lev-Ram et al., 1992; Miyakawa et al., 1992; Ross & Werman, 1987; Tank et al., 1988). $Ca^{2+}$ signals generated due to depolarization-induced $Ca^{2+}$ spikes spread throughout the whole dendritic tree. As expected, these $Ca^{2+}$ signals are largest in the distal dendrites and barely detectable at the soma (Lev-Ram et al., 1992). This is mainly due to surface-to-volume effects, and may also be influenced by non-uniform distribution of VGCCs (Denk, Sugimori, & Llinas, 1995; Isope & Murphy, 2005; Kulik et al., 2004) and passive and active properties of the dendrites that result in a non-uniform dendritic voltage profile. Although hot spots of $Ca^{2+}$ elevation during depolarization of the cell could be observed in the primary dendrites (Tank et al., 1988), the initiation site of dendritic $Ca^{2+}$ spikes induced by somatic depolarization is still unclear. Further improvement of recording techniques, i.e., increased detection sensitivity and spatiotemporal resolution (Otsu et al., 2008) might help to resolve this issue.

In addition to the somatic depolarization, strong PF stimulation can elicit dendritic $Ca^{2+}$ spikes (Llinas et al., 1968; Rancz & Hausser, 2006). These synaptically-evoked $Ca^{2+}$ spikes are confined to the stimulated region, and they trigger synaptic plasticity at the PF–Purkinje cell synapse (Hartell, 1996; Schreurs, Oh, & Alkon, 1996; Wang, Denk et al., 2000) and dendritic glutamate (Duguid, Pankratov, Moss, & Smart, 2007; Shin, Kim, & Linden, 2008) and endocannabinoid (Rancz & Hausser, 2006) release. It has also been shown that PF-evoked dendritic $Ca^{2+}$ spikes can regulate action potential output by activating large conductance $Ca^{2+}$-activated potassium (BK) channels (Rancz & Hausser, 2010). These lines of evidence suggest that PF-driven dendritic $Ca^{2+}$ spikes play an important role in regulating the input and output of Purkinje cells. The other excitatory input to Purkinje cells, CF, causes much stronger depolarization than PF input and triggers $Ca^{2+}$ spikes in the dendrites (Davie, Clark, & Hausser, 2008; Llinas & Sugimori, 1980; Miyakawa et al., 1992), as detailed below.

## 3. Calcium signaling evoked by climbing fiber input

In adult rodents, each Purkinje cell is innervated by a single CF, and each CF makes hundreds of synaptic contacts onto the proximal dendrites of the Purkinje cell (Palay & Chan-Palay, 1974), providing powerful excitatory drive. Thus, CF input produces massive depolarization of the dendrites and evokes the stereotypic complex spike (CS), which is comprised of a prominent spike followed by a burst of several small spikelets (Eccles, Llinas, & Sasaki, 1966; Fujita, 1968; Llinas & Sugimori, 1980). A CS evoked by a CF is associated with dendritic $Ca^{2+}$ spikes that are mediated by the high-threshold P/Q-type VGCCs richly expressed in Purkinje cell dendrites (Westenbroek et al., 1995). The CF-evoked dendritic $Ca^{2+}$ spikes can propagate down to the soma (Eccles et al., 1966; Llinas & Nicholson, 1971; Llinas et al., 1968; Llinas & Sugimori, 1980) and often show a burst like pattern (Fujita, 1968; Llinas & Sugimori, 1980), and the secondary spikelets in a CS have therefore been assumed to reflect dendritic $Ca^{2+}$ spikes. However, by using simultaneous somatic and dendritic (or axonal) patch-clamp recordings together with dynamic clamp techniques, it has recently been shown that CF-evoked dendritic spikes are not directly linked to spikelets in CS, and that all spikelets in somatic CS are generated in the axon (Davie et al., 2008). Thus, the generation of CS and CF-evoked dendritic $Ca^{2+}$ spikes are derived from distinct mechanisms. Although generation of CS and CF-evoked dendritic spikes is primarily mediated by sodium channels and P/Q-type VGCCs, respectively, other channels also participate in shaping the CS waveform. Low-threshold VGCCs can be activated by CF inputs, and blocking these channels results in a change in the CS waveform (Cavelier et al., 2008). Voltage-gated potassium channel subunit Kv3.3 at the soma is required for the generation of secondary spikelets in CS (Hurlock, McMahon, & Joho, 2008).

CF-evoked dendritic $Ca^{2+}$ spikes can propagate throughout the dendritic tree and are associated with large $Ca^{2+}$ influx even in the terminal spiny branchlets (Konnerth, Dreessen, & Augustine, 1992; Miyakawa et al., 1992). This indicates a role for CF input as an associative signal for triggering plasticity at PF synapses (Brenowitz & Regehr, 2003; Coesmans, Weber, De Zeeuw, & Hansel, 2004; Konnerth et al., 1992; Miyata et al., 2000; Sakurai, 1990; Wang, Denk et al., 2000). Dendritic $Ca^{2+}$ signals evoked by CF are highly reliable but can be modulated by various factors. As is the case with dendritic $Ca^{2+}$ spikes evoked by depolarizing current injections, CF-evoked dendritic $Ca^{2+}$ signals are dependent on their location in the dendrites (Miyakawa et al., 1992), the membrane potential of the cell (Midtgaard, Lasser-Ross, & Ross, 1993; Miyakawa et al., 1992) and inhibitory inputs from molecular layer interneurons (Callaway, Lasser-Ross, & Ross, 1995).

CF-evoked dendritic $Ca^{2+}$ signals have also been shown to be modulatable *in vivo* (Kitamura & Hausser, 2011). The membrane potential of Purkinje cells *in vivo* is bistable between a hyperpolarizing down state and a depolarizing up state, and the state transition is triggered by CS (Loewenstein et al., 2005; Rokni, Tal, Byk, & Yarom, 2009; Yartsev, Givon-Mayo, Maller, & Donchin, 2009). Spontaneous and sensory-evoked CF inputs are associated with dendritic $Ca^{2+}$ spikes, and the amplitude of $Ca^{2+}$ transients depends on the membrane potential of the cell; $Ca^{2+}$ signals are