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Report

Sensory-Driven Enhancement of Calcium Signals in Individual Purkinje Cell Dendrites of Awake Mice

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SUMMARY

Climbing fibers (CFs) are thought to contribute to cerebellar plasticity and learning by triggering a large influx of dendritic calcium in the postsynaptic Purkinje cell (PC) to signal the occurrence of an unexpected sensory event. However, CFs fire about once per second whether or not an event occurs, raising the question of how sensory-driven signals might be distinguished from a background of ongoing spontaneous activity. Here, we report that in PC dendrites of awake mice, CF-triggered calcium signals are enhanced when the trigger is a sensory event. In addition, we show that a large fraction of the total enhancement in each PC dendrite can be accounted for by an additional boost of calcium provided by sensory activation of a non-CF input. We suggest that sensory stimulation may modulate dendritic voltage and calcium concentration in PCs to increase the strength of plasticity signals during cerebellar learning.

INTRODUCTION

Calcium is the trigger for cellular mechanisms of plasticity in many neurons throughout the brain and as such, it is thought to play a central role during learning and memory formation (Sjöström and Nelson, 2002; Zucker, 1999). For Purkinje cells (PCs) of the cerebellar cortex, the job of generating the calcium signals necessary for plasticity and learning is often attributed to the powerful climbing fiber (CF) input (De Zeeuw et al., 1998; Ito, 2013; Raymond et al., 1996; Simpson et al., 1996; Thach et al., 1992). This is because in many learning tasks, CFs seem to play the role of "teachers" by firing a burst of action potentials to signal that an unexpected sensory event has occurred (Gilbert and Thach, 1977; Kitazawa et al., 1998; Medina and Lisberger, 2008; Rasmussen et al., 2008; Raymond and Lisberger, 1998; Soetedjo et al., 2008). In turn, the CF burst produces a strong depolarization of the postsynaptic PC and causes a calcium-based dendritic spike that serves as the trigger for a variety of plasticity mechanisms (Kitamura and Kano, 2013; Schmolesky et al., 2002). In this report, we use calcium imaging of PC dendrites in awake mice to resolve a long-standing question about the way CF inputs encode information and generate plasticity signals in the cerebellum.

It was pointed out 40 years ago that from the perspective of an individual PC, CF signals are potentially ambiguous (Gilbert, 1975). In the adult cerebellum, each PC receives input from a single CF (Ito, 1984), and every CF fires bursts of action potentials spontaneously at a rate of about once per second in vivo (Ito, 1984). Furthermore, early investigators demonstrated that because CF bursts are "all or nothing" (Crill, 1970), they evoke the same electrical response in the soma of the postsynaptic PC (i.e., a complex spike [CS]) regardless of whether the CF input fired spontaneously or in response to a sensory event (Eccles et al., 1966). These classic electrophysiology studies led to the current view of cerebellar learning, according to which all individual CF bursts are equivalent, and therefore to obtain information about sensory-driven instructive signals, one must collect responses to CF inputs over many learning trials or many PCs (Gibson et al., 2004; Houk et al., 1996; Mauk and Donegan, 1997; Ozden et al., 2009; Schultz et al., 2009).

Our experiments challenge the classical view that spontaneous and sensory-driven CF inputs are equivalent. This view does not take into account the fact that the somatic and dendritic compartments of the PC are functionally separate and process CF signals independently during synaptic activation (Davie et al., 2008; Llinás and Sugimori, 1980). Therefore, we designed our experiments to test whether spontaneous and sensory-driven CF inputs are different from each other, not by recording the electrical CS response near the PC soma as in previous work (Eccles et al., 1966), but by measuring CF-triggered calcium spikes and visualizing dendritic plasticity signals directly.

RESULTS

We used two-photon microscopy to image spontaneous and sensory-driven calcium spikes triggered by activation of the CF input to individual PC dendrites. Awake mice were head-fixed on top of a cylindrical treadmill while we delivered periocular airpuffs via a needle pointed toward the eye (Figure 1A). We imaged 13 locations in five mice and found CF-triggered calcium spikes in response to the periocular airpuff in a total of 76 PCs distributed broadly within three separate zones of the cerebellar cortex

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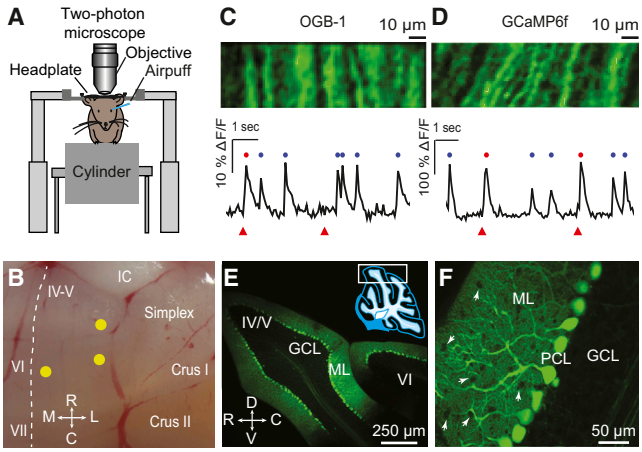

Figure 1. Imaging Calcium Spikes in PC Dendrites of Awake Mice

(A) Two-photon microscopy of cerebellar cortex in awake mice, head-fixed on a cylindrical treadmill. Airpuff stimuli were delivered to the ipsilateral eye.

(B) Imaged locations in cerebellar cortex (yellow circles). Dashed line indicates the midline.

(C) Top: field of view showing PC dendrites in an example OGB-1/AM experiment. Bottom: an example fluorescence trace representing spontaneous (blue dots) and sensory-evoked (red dots) calcium spikes in response to periocular airpuff stimuli (triangles).

(D) Same as (C), for an example GCaMP6f experiment.

(E and F) Expression of GCaMP6f (green) in PCs (PCL, Purkinje cell layer) located in the area marked by a rectangle in the inset of (E). Notice the absence of staining in the GC layer (GCL) and MLIs (white arrows in F). C, caudal; D, dorsal; IC, inferior colliculus; L, lateral; M, medial; R, rostral; V, ventral. IV-V, VI, VII, simplex, and crusI/II are different lobules.

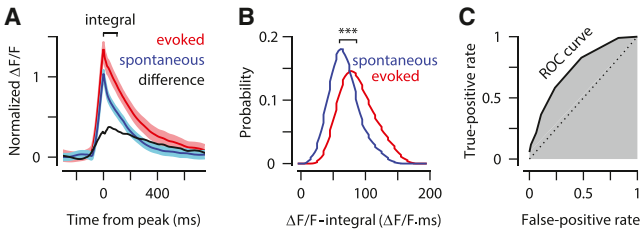

Figure 2. Sensory-Driven Enhancement of Calcium Spikes in a Representative Dendrite

(A) Average of spontaneous (blue) and sensory-evoked (red) calcium spikes (± SEM). The difference trace (black) is the difference between evoked and spontaneous traces. All traces are normalized to the peak of the spontaneous trace.

(B) Histograms of the size of spontaneous (blue) and evoked (red) calcium spikes computed by taking the ΔF/F integral in the window shown in (A) ("integral"). Bin width = 20 ΔF/F × ms.

(C) ROC curve (black solid line) and the corresponding area under the curve (shaded gray) computed from the histograms shown in (B). The dotted diagonal is the ROC curve if the two histograms were completely overlapping.

(Figure 1B): the vermis of lobule VI and the paravermis of lobules IV/V and VI.

As in earlier imaging studies (Ozden et al., 2008; Sullivan et al., 2005), PC dendrites appeared as elongated structures separated from each other by 5–10 µm (Figures 1C and 1D, top). We used two different fluorescent calcium indicators with complementary advantages: OGB-1/AM (Figure 1C), a fast-responding synthetic indicator that can be bulk loaded and is taken up by a variety of cell types in the cerebellar cortex (Sullivan et al., 2005), and GCaMP6f (Figure 1D), a genetically encoded calcium indicator with slower kinetics that allows cell-type-specific expression (Figures 1E and 1F; see Experimental Procedures). With GCaMP6f, we observed labeled PCs at an average density of 13.6 ± 1.4 cells per 10^4 µm^2 cortical surface area, an estimated 76% of all PCs (percentages were calculated in comparison with cell densities reported in Sturrock, 1989). In comparison, other cell types were sparser and dimmer: 7% of molecular layer interneurons (MLIs) were visible at an average of 0.4 times the brightness of PC dendrites, and 1% of granule cells (GCs) were visible at 0.06 times the brightness of PC dendrites. Thus, under our expression conditions, nearly all of the GCaMP6f signal in the ML neuropil arose from PC dendrites, even before regions of interest (ROIs) were identified.

Calcium spikes in individual PC dendrites were apparent as a rapidly rising transient increase in the fluorescence signal (Figures 1C and 1D, bottom). We have shown previously that these dendritic calcium spikes are generated when the CF input fires and generates a CS in the PC (Ozden et al., 2008). Our imaging tools and template-and-threshold algorithm can detect approximately 95% of all CSs with a <8% false-positive rate (Ozden et al., 2008). In PC dendrites, calcium spikes occurred at the expected spontaneous rate of once per second (Figures 1C and 1D, blue dots; rate = 0.90 ± 0.25 Hz, mean ± SD), and also in response to periocular airpuff stimulation (Figures 1C and 1D, red dots; response probability = 0.58 ± 0.24, mean ± SD). We termed these events spontaneous and evoked calcium spikes, respectively.

Sensory-Driven Enhancement of Calcium Spikes in a Representative Dendrite

Figure 2 shows data for an example PC dendrite in a GCaMP6f experiment. The average evoked calcium spike (Figure 2A, red; 27 spikes) was larger in amplitude than the average spontaneous calcium spike (Figure 2A, blue; 65 spikes; difference between evoked and spontaneous spikes shown in black). Note that all fluorescence traces have been normalized so that the peak of the average signal for spontaneous calcium spikes in the PC is "1" (see Experimental Procedures). As an index of the size of the calcium spike, we computed the integral of each normalized fluorescence signal in the 100 ms window following its peak (Figure 2A; "integral"). We will refer to this quantity as the ΔF/F-integral. In the example shown, the ΔF/F-integral of the calcium spike was on average 50% larger for evoked versus spontaneous events (Figure 2B; red versus blue, Kolmogorov-Smirnov test, $p < 0.001$).

Next, we used receiver operating characteristic (ROC) analysis to quantify the degree to which the ΔF/F-integral of the calcium spike was sufficient to distinguish sensory-evoked signals from spontaneous ones (see Experimental Procedures). We constructed an ROC curve (Figure 2C) by sliding the value of a discriminability criterion along the horizontal axis of Figure 2B. For each value of the criterion, we plotted a point in the ROC curve with coordinates FPR and TPR, where FPR is the false-positive rate (i.e., the fraction of calcium spikes in the spontaneous