Editor's Note: These short, critical reviews of recent papers in the Journal, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

The Calcium Kinetics and Inositol Trisphosphate Receptor Properties Shape the Asymmetric Timing Window of Coincidence Detection

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Review of Sarkisov and Wang (<http://www.jneurosci.org/cgi/content/full/28/1/133>)

The cerebellum is thought to be a specialized organ for supervised learning (also known as associative learning), through which each input signal is specifically associated with a desired output. Purkinje cells provide the sole output from the cerebellar cortex, and each Purkinje cell receives two types of excitatory inputs: one from hundreds of thousands of parallel fibers and the other from a single climbing fiber. The transmission efficacy of the parallel fiber–Purkinje cell synapse is depressed when the climbing fiber and parallel fiber are repetitively and synchronously activated [long-term depression (LTD)]. This spike-timing-dependent plasticity is widely accepted as the cellular correlate of cerebellar associative learning.

Parallel fibers and climbing fibers activate different signaling pathways in Purkinje cells. On the one hand, parallel fiber firing activates the mGluR1 metabotropic glutamate receptor pathway, resulting in activation of phospholipase C (PLC) and production of inositol 1,4,5-trisphosphate (IP_3). However, climbing

fiber firing depolarizes the Purkinje cell and induces Ca^{2+} influx through voltage-gated calcium channels. Therefore, IP_3 and Ca^{2+} represent parallel-fiber and climbing-fiber activities, respectively. By sensing sequential binding of IP_3 and Ca^{2+} , the IP_3 receptor acts as a coincidence detector that associates parallel fiber inputs with climbing fiber inputs. The output signal is the release of more Ca^{2+} from internal stores, which leads to LTD (Wang et al., 2000; Doi et al., 2005). IP_3 receptor-mediated calcium-induced calcium release is greatest when parallel fiber stimuli precede climbing fiber stimuli by ~ 100 ms (half-maximal width, ~ 200 ms).

The characteristics of the IP_3 receptor that shape the timing window of coincidence detection are not fully understood. To answer this question, Sarkisov and Wang (2008) explored the coincidence detector properties of the IP_3 receptor in Purkinje cells by photolyzing double-caged IP_3 , which is less antagonistic to the IP_3 receptor and offers a higher spatial resolution of focal uncaging than its single-caged counterpart (Sarkisov et al., 2007). Coincident presentation of IP_3 and climbing fiber activation triggered calcium signals in Purkinje cells that were substantially larger than the linear sum of the signals from climbing fiber and IP_3 alone [Sarkisov and Wang (2008), their Fig. 1

(<http://www.jneurosci.org/cgi/content/full/28/1/133/F1>)]. Climbing fiber activation up to 100 ms before or up to 500 ms after IP_3 uncaging resulted in synergistic calcium release [Sarkisov and Wang (2008), their Fig. 2 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)]. Calcium responses were sensitive to the order of IP_3 uncaging and climbing fiber activation, and the maximal signals were observed when the climbing fiber was activated 100–200 ms after IP_3 uncaging; they reached 0.6–1.8 μM in spines and 0.3–0.7 μM in spiny dendrites [Sarkisov and Wang (2008), their Figs. 1D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F1>), 2C,D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)]. This optimal delay of climbing fiber inputs is consistent with their putative role in associative learning as teaching signals. Coincidence-dependent supralinearity emerged in a shorter time than enzymatic reactions take [Sarkisov and Wang (2008), their Fig. 3 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F3>)], which, in agreement with previous simulation studies (Doi et al., 2005; Hernjak et al., 2005), suggests that PLC is not involved in the positive feedback loop of calcium-induced calcium release.

Next, by using a calcium-store depletor and an IP_3 receptor blocker, the authors demonstrated that supralinear cal-

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cium signals resulted from IP₃ receptor-mediated calcium-induced calcium release from internal stores [Sarkisov and Wang (2008), their Fig. 4D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F4>)], as reported previously (Wang et al., 2000). To rule out the possibility that IP₃-induced calcium release activates potassium conductance and enhances climbing fiber-evoked calcium entry to the hyperpolarized dendrite, they visualized calcium signals along the dendrite. As expected, the spatial distribution of supralinear calcium responses was much more restricted than that of electrical potentials [Sarkisov and Wang (2008), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F4>)], indicating that potassium conductances are unlikely to be responsible for the supralinearity.

Calcium is stimulatory to the IP₃ receptor at low concentrations, whereas it is inhibitory at high concentrations (Iino, 1990). Therefore, to test whether climbing fiber-evoked calcium influx had positive or negative effects on calcium release from internal stores, the authors measured calcium signals after stimulating the climbing fiber variable times and photolyzing IP₃ at the end of the stimuli [Sarkisov and Wang (2008), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F5>)]. The more times the climbing fiber was stimulated, the greater amount of calcium was released from internal stores, indicating that the climbing fiber-evoked calcium responses in their experimental settings were within a range that stimulates the IP₃ receptor.

The IP₃-before-climbing-fiber timing window of several hundred milliseconds [Sarkisov and Wang (2008), their Fig. 2C,D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)] is similar to the binding dissociation time of IP₃ from its receptor, suggesting that the timing window is defined by this dissociation. IP₃ degradation also might shape the timing window, although some theoretical studies have indicated that it is unlikely (Doi et al., 2005; Hernjak et al., 2005). To experimentally rule out IP₃ degradation as a timing window constraint, the authors took advantage of a poorly hydrolyzable agonist of the IP₃ receptor, gPIP₂. gPIP₂ uncaging at two time points revealed that the size of paired-pulse enhancement was a monotonically decreasing function of the interval, with a half-maximal width of 400 ms, which corresponded to the bind-

ing dissociation timescale [Sarkisov and Wang (2008), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F6>)]. This finding supports the previous hypothesis that the timing window was set by dissociation of IP₃ from its receptor, not by IP₃ degradation.

Collectively, these remarkable experiments indicate that the asymmetric timing window of coincidence detection in the Purkinje cell is shaped by the calcium kinetics and IP₃ receptor properties; but some intriguing questions still need to be answered. First, could LTD be induced in a coincidence-dependent manner by the coincident activation protocol the authors used? If and only if so, coincidence signals explored in this study could be discussed in the context of spike-timing-dependent plasticity mechanisms. The above question arises because IP₃ uncaging and climbing fiber activation resulted in supralinear calcium signals [Sarkisov and Wang (2008), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F1>)] that were considerably smaller (<2 μ M) than those resulting from conjunctive parallel-fiber–climbing-fiber activation (>10 μ M) (Wang et al., 2000). Although the authors cite another study indicating that 2 μ M of calcium was sufficient for LTD induction (Tanaka et al., 2007), in that study, LTD required a sustained calcium increase lasting for more than several seconds. Thus, the calcium transients that decayed within hundreds of milliseconds in Sarkisov and Wang (2008) may not have been sufficient to produce LTD.

The second remaining question concerns the order dependence of the peak supralinearity. The authors observed maximal supralinearity at $\Delta t = \sim 100$ ms [Sarkisov and Wang (2008), their Fig. 2C,D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)]. But if the timing window were shaped solely by the calcium kinetics and IP₃ receptor–IP₃ dissociation profile, the supralinearity should have been largest when IP₃ uncaging and climbing fiber activation were exactly coincident, leading to a simultaneous rise of IP₃ and Ca²⁺ concentrations and the most efficient activation of the IP₃ receptor. In other words, the difference in rate between calcium decay and binding dissociation of IP₃ from its receptor [Sarkisov and Wang (2008), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/28/1/133/F7>)] only accounts for the difference

in slopes on the left and right sides of each asymmetric supralinearity plot [Sarkisov and Wang (2008), their Fig. 2C,D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)], leaving the deviation of its peak from the exact point of coincidence unexplained. UV-induced IP₃ photolysis and climbing fiber-induced calcium influx are fast processes that take place and affect the intracellular Ca²⁺ concentrations within 10–20 ms [Sarkisov and Wang (2008), their Figs. 1C (<http://www.jneurosci.org/cgi/content/full/28/1/133/F1>), 6A (<http://www.jneurosci.org/cgi/content/full/28/1/133/F6>)], and their timing does not account for the shift of the peak, either. In addition to the lack of explanatory factors for the order-dependence of the maximal supralinearity, the order-dependence itself is unclear. Fitting a Gaussian distribution to an asymmetric plot [as was done in this study Sarkisov and Wang (2008), their Fig. 2C,D (<http://www.jneurosci.org/cgi/content/full/28/1/133/F2>)] can lead to erroneous statistical conclusions as well as a misinterpretation of the peak location. Considering that, it might be better not to reject for now the null hypothesis that the peak of the supralinearity lies at $\Delta t = 0$.

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