Figure 1. Pollinating bees.
(A) Native, wild bee (*Augochlora pura*) pollinating tomato. (B) European honey bee (*Apis mellifera*) pollinating watermelon. Photo credit: Lisa Mandle.

pollinator-dependent crops. There were no differences in price trends, however, between pollinator-dependent and pollinator-independent crops in the United States between 1966 and 2003 (J. Ghazoul and L.P. Koh, personal communication). A second indicator would be an increase in the price farmers pay to rent honey bees for pollination purposes. In fact, the prices paid by North American almond growers have increased from $35 per hive in the early 1990s to $150 per hive today [8].

A key question for those concerned with pollinator decline is whether changes in pollinator abundance translate into changes in crop production [18–20]. Determining this is not as easy as it would seem because a multitude of inputs can limit crop production, including soil fertility, pest control, irrigation, and weather, thus requiring large-scale experimental manipulations to determine how often pollination is a limiting factor at the field scale. Aizen *et al.*'s [11] findings, although non-experimental, suggest that such limitation has not yet occurred globally, though the lower yield of the most pollinator-dependent crops suggests that it may be beginning to occur. A second key question, and one that is more difficult to answer, has to do not with current yields but with risk. On this, Aizen *et al.*'s [11] results are unambiguous. Our increasing reliance on pollinator-dependent crops could act synergistically with our increasing reliance on single pollinator species to increase the risk of a future crisis in the global food supply. The time to act on diversifying our suite of pollinators and solving honey bee health problems is now — before we see significant changes in crop production.

References
1. Linder, H.P. (1998). Morphology and the evolution of wind pollination. In Reproductive Biology, S.J. Owens and P.J. Rudall, eds. (Richmond, UK: Royal Botanic Gardens, Kew), pp. 123–135.
2. Ashman, T., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R., Jongston, M.O., Mazer, S.J., Mitchell, R.J., *et al*. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85, 2408–2421.
3. Burd, M. (1994). Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Rev. 60, 83–139.
4. Knight, T.M., Steets, J.A., and Ashman, T.L. (2006). A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. Am. J. Botany 93, 271–277.
5. Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tschartke, T. (2007). Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond. B 274, 303–313.
6. Free, J.B. (1993). Insect Pollination of Crops, 2nd Edition (London: Academic Press).
7. Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B.E. (2008). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecolog. Econ., in press.
8. Johnson, R. (2007). Recent Honey Bee Declines (Washington, DC: Congressional Research Service), 14 pages.
9. National Research Council (2007). Status of Pollinators in North America (Washington, DC: The National Academies Press).
10. Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Oholemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., *et al*. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313, 351–354.
11. Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., and Klein, A.M. (2008). Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18, 1572–1575.
12. Kremen, C., Williams, N.M., and Thorp, R.W. (2002). Crop pollination from native bees at risk from agricultural intensification. Proc. Natl. Acad. Sci. USA 99, 16812–16816.
13. Winfree, R., Williams, N.M., Dushoff, J., and Kremen, C. (2007). Native bees provide insurance against ongoing honey bee losses. Ecol. Lett. 10, 1105–1113.
14. Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S.S., Klein, A.M., Mayfield, M.M., *et al*. (2008). Landscape effects on crop pollination services: Are there general patterns? Ecol. Lett. 11, 499–515.
15. Williams, N. (2008). Bee fears heighten. Curr. Biol. 18, R682–R683.
16. Stokstad, E. (2007). The case of the empty hives. Science 316, 970–972.
17. Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D.M., *et al*. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318, 283–286.
18. Ghazoul, J. (2005). Buzziness as usual? Questioning the global pollination crisis. Trends Ecol. Evol. 20, 367–373.
19. Ghazoul, J. (2007). Challenges to the uptake of the ecosystem service rationale for conservation. Cons. Biol. 21, 1651–1652.
20. Kremen, C., Daily, G.C., Klein, A.-M., and Scofield, D. (2008). Inadequate assessment of the ecosystem service rationale for conservation: A reply to Ghazoul. Cons. Biol. 22, 795–798.

Department of Entomology, Rutgers University, New Brunswick, New Jersey 08901, USA.
E-mail: rwinfree@rci.rutgers.edu

Purkinje Neurons: What Is the Signal for Complex Spikes?

Cerebellar Purkinje neurons generate characteristic complex spikes; but are these bursts of activity generated by somatic or dendritic excitability? A recent study may have settled this debate by giving the soma the dominant role, but it does not fully resolve the question of what information is transmitted downstream of the Purkinje cells.

Sungho Hong[1]
and Erik De Schutter[1,2]

The cerebellar cortex receives two distinct types of afferent: mossy and climbing fibers. While mossy fibers carry inputs from many different regions of the central nervous system, climbing fibers originate only from the inferior olive and contact only one cell
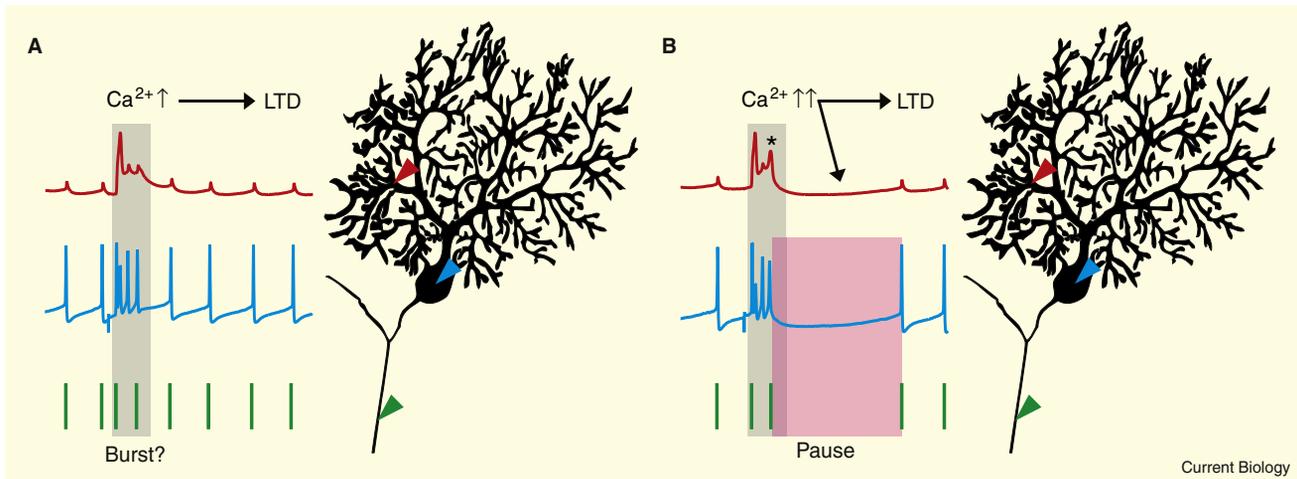
Figure 1. Effects of a complex spike at different stages.

(A) During a complex spike (shaded), the dendritic excitation (red) causes a large calcium inflow, which triggers LTD of activated parallel fiber synapses. However, the complex spike burst, seen at the soma (blue), is generated almost independently of the dendritic mechanisms, and then surprisingly most of its characteristics are lost in the axon (green). (B) An additional dendritic spike can trigger a hyperpolarization both at the dendrite and the soma, which leads to a longer pause in the spike train (magenta). This can become a reliable signal, transmitted by the axon to the deep cerebellar nuclei neurons. (Adapted with permission from [11].)

type, the Purkinje neuron. Even though each Purkinje cell receives only one climbing fiber input, this synaptic connection is unusually strong and can reliably evoke a strong depolarization to generate a characteristic burst: the complex spike. *In vivo*, a Purkinje cell emits simple spikes with a rate of ~50 Hz [1], while the inferior olive, the source of the climbing fiber inputs, has a much lower firing rate of ~1 Hz; as a result, complex spikes, which are usually followed by a 10–30 msec long pause [2], intermittently interrupt the simple spike trains.

The Purkinje neuron is equipped with the morphological and ionic mechanisms to support these two types of signaling. It has an extremely elaborate dendritic structure which, together with a low density of sodium channels [3], effectively prevents back-propagating spikes from invading and affecting the dendritic tree [4]. Furthermore, as already suggested in an early study by Llinás and Sugimori [5], there are abundant calcium-mediated mechanisms, such as voltage-activated calcium and calcium-dependent potassium channels, that can generate dendritic spikes. Historically, the voltage-gated dendritic calcium spike has been considered an important contribution to the climbing-fiber-evoked complex spike [6]. As observed in other neurons, this dendritic spike could possibly propagate toward the soma and axon,

and thereby cause the second and later spikes in a burst, or at least a plateau depolarization. But it has been unclear how much contribution from the dendritic tree is actually required [7], as later experiments raised doubts about the importance of the dendritic calcium spike for generating the somatic burst — even though the somatic complex spike waveform barely changes, dendritic local calcium influx can be greatly reduced by either localized inhibition [8] or climbing fiber plasticity [9]. Also, dissociated Purkinje neurons, stripped off their entire dendritic tree, can still generate a bursting response to a transient input [10].

Contrary to the dendritic paradigm, Davie *et al.* [11] have now reported that a complex spike can be evoked exclusively by a large conductance input at the soma, delivered via a dynamic clamp, without leading to any significant excitation of the distal dendrite. Furthermore, the influence of a dendritic spike on the somatic burst waveform turned out to be quite small: an extra dendritic spike rarely leads to an extra somatic spike during the burst. The reason for this is that the Purkinje cell dendritic spike has a small amplitude and a short duration, plus it arrives at the soma with high attenuation (~40%) and usually during the somatic refractory period. Note that the results of Davie *et al.* [11] apply only to the intracellular

waveform — presumably dendritic excitation is required to generate the classic extracellular signal [12].

There is an ongoing debate about what is signaled by climbing fibers and how it affects the output of Purkinje cells [13]. In the Marr-Albus-Ito theory of cerebellar learning, the climbing fiber carries an error feedback teaching signal which induces long-term depression (LTD) of activated parallel fiber synapses on the Purkinje dendrite [12]. In this view, the climbing fiber delivers a Purkinje-neuron-specific signal which, by its large calcium influx, evokes LTD (Figure 1). An alternative view is that the synchronized firing of olivary neurons, transmitted via the climbing fiber input, convey precise timing information that is important for motor control [14]. In this case, regardless of synaptic plasticity, climbing fiber input evokes distinctive timing signals in Purkinje cells, which propagate to the deep cerebellar nuclei. Davie *et al.* [11], together with an old finding of Callaway *et al.* [8], suggest that the complex spike somatic signal is almost indifferent to what is happening in the dendrites, which sounds compatible with the latter viewpoint.

It seems unlikely, however, that the complex spike would be propagated in a distinguishable manner: even though a Purkinje cell axon can reliably transmit a spike train of up to about 200 Hz, depending on conditions

such as baseline potential and upward speed, propagation failure rapidly increases above this firing rate and, at 500 Hz, more than 50% of spikes are lost during transmission [15,16]. Because complex spike bursts can instantaneously exceed 500 Hz, only two or three spikes of a single burst are transmitted and this will hardly change the output rate (Figure 1A). This makes the observation by Davie et al. [11] that the somatic burst shape is evoked independent of dendritic signaling puzzling and difficult to interpret.

Surprisingly, Davie et al. [11] also found that dendritic calcium spikes influence another aspect of the Purkinje cell response to climbing fiber input: the length of the pause after a complex spike increases by ∼50% with an additional dendritic spike. This elongation is caused by a significant increase of the afterhyperpolarization following the dendritic spike, possibly due to calcium-activated potassium currents, which play an important role in regulating the Purkinje neuron spike patterns [17].

It has previously been suggested that pause coding might be important in Purkinje cell signaling. Analysis of spontaneous and evoked simple spike trains has shown that they can be divided into two components: regular patterns with relatively short interspike intervals and longer pauses [1]. While it has been shown that complex spikes, and presumably also their pauses, can be synchronized over long distances [18], the simple spike pauses are only synchronized among neighboring Purkinje cells [2]. A closely related recent result is that parallel fiber patterns generate a simple spike pause which encodes the dissimilarity between the input and patterns previously learned by LTD [19]. The proposed mechanism is similar to that of Davie et al. [11]: LTD modulates the voltage-gated calcium entry in dendrites, which in turn determines the activation of calcium-dependent potassium channels and the level of hyperpolarization. Taken together, this suggests that the influence of the climbing fiber evoked dendritic spike on the duration of the pause following the complex spike might be the more important coding principle (Figure 1B).

The final question is then how this output is decoded in the deep cerebellar nuclei. The simple spike pause has been proposed to be an effective signal [1,2] because deep cerebellar nuclei neurons show a strong rebound depolarization and subsequent bursts when released from a deep continuous hyperpolarization. Interestingly, rebound spiking has originally been proposed as a mechanism that allows a deep cerebellar nuclei neuron to identify climbing fiber activation [20], but now this can be extended to any pause. But to fully understand how pause coding works in this system we need a better understanding of the synchronization of pauses across the Purkinje cell population and of the quantitative anatomy of the Purkinje cell to deep cerebellar nuclei projection [1].

References
1. Shin, S.-L., Hoebeek, F.E., Schonewille, M., De Zeeuw, C.I., Aertsen, A., and De Schutter, E. (2007). Regular patterns in cerebellar Purkinje cell simple spike trains. PLoS ONE 2, e485.
2. Shin, S.-L., and De Schutter, E. (2006). Dynamic synchronization of Purkinje cell simple spikes. J. Neurophysiol. 96, 3485–3491.
3. Stuart, G., and Häusser, M. (1994). Initiation and spread of sodium action potentials in cerebellar Purkinje cells. Neuron 13, 703–712.
4. Vetter, P., Roth, A., and Häusser, M. (2001). Propagation of action potentials in dendrites depends on dendritic morphology. J. Neurophysiol. 85, 926–937.
5. Llinás, R., and Sugimori, M. (1980). Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. J. Physiol. 305, 197–213.
6. Knöpfel, T., Vranesic, I., Staub, C., and Gähwiler, B.H. (1991). Climbing fibre responses in olivo-cerebellar slice cultures. II. Dynamics of cytosolic calcium in Purkinje cells. Eur. J. Neurosci. 3, 343–348.
7. Schmolesky, M.T., Weber, J.T., De Zeeuw, C.I., and Hansel, C. (2002). The making of a complex spike: ionic composition and plasticity. Ann. NY Acad. Sci. 978, 359–390.
8. Callaway, J.C., Lasser-Ross, N., and Ross, W.N. (1995). IPSPs strongly inhibit climbing fiber-activated [Ca2+]i increases in the dendrites of cerebellar Purkinje neurons. J. Neurosci. 15, 2777–2787.
9. Weber, J.T., De Zeeuw, C.I., Linden, D.J., and Hansel, C. (2003). Long-term depression of climbing fiber-evoked calcium transients in Purkinje cell dendrites. Proc. Natl. Acad. Sci. USA 100, 2878–2883.
10. Swensen, A.M., and Bean, B.P. (2003). Ionic mechanisms of burst firing in dissociated Purkinje neurons. J. Neurosci. 23, 9650–9663.
11. Davie, J.T., Clark, B.A., and Häusser, M. (2008). The origin of the complex spike in cerebellar Purkinje cells. J. Neurosci. 28, 7599–7609.
12. Ito, M. (2001). Cerebellar long-term depression: characterization, signal transduction, and functional roles. Physiol. Rev. 81, 1143–1195.
13. Simpson, J.I., Wylie, D.R., and De Zeeuw, C.I. (1996). On climbing fiber signals and their consequence(s). Behav. Brain Sci. 19, 363–383.
14. Kitazawa, S., and Wolpert, D.M. (2005). Rhythmicity, randomness and synchrony in climbing fiber signals. Trends Neurosci. 28, 611–619.
15. Khaliq, Z.M., and Raman, I.M. (2005). Axonal propagation of simple and complex spikes in cerebellar Purkinje neurons. J. Neurosci. 25, 454–463.
16. Monsivais, P., Clark, B.A., Roth, A., and Häusser, M. (2005). Determinants of action potential propagation in cerebellar Purkinje cell axons. J. Neurosci. 25, 464–472.
17. Womack, M.D., and Khodakhah, K. (2003). Somatic and dendritic small-conductance calcium-activated potassium channels regulate the output of cerebellar purkinje neurons. J. Neurosci. 23, 2600–2607.
18. Welsh, J.P., Lang, E.J., Sugihara, I., and Llinás, R. (1995). Dynamic organization of motor control within the olivocerebellar system. Nature 374, 453–457.
19. Steuber, V., Mittmann, W., Hoebeek, F.E., Silver, R.A., De Zeeuw, C.I., Häusser, M., and De Schutter, E. (2007). Cerebellar LTD and pattern recognition by Purkinje cells. Neuron 54, 121–136.
20. Aizenman, C.D., and Linden, D.J. (1999). Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. J. Neurophysiol. 82, 1697–1709.

[1]Computational Neuroscience Unit, Okinawa Institute of Science and Technology, 7542 Onna, Onna-son, Okinawa 904-0411, Japan.
[2]Theoretical Neurobiology, University of Antwerp, 2610 Antwerp, Belgium.
E-mail: sungho.hong@gmail.com