the growth and length regulation of the flagellar filament of *Salmonella* Typhimurium.

The earlier work by Turner *et al.* [13] reporting that *E. coli* filaments grew at constant rates of ~13 nm/min was based on filament regrowth after shearing. In hindsight, this can be explained by the Renault *et al.* [16] injection-diffusion model, since Turner's measurements were extension rates at the ends of broken, long flagella and did not measure initial growth rates of nascent filaments, which the Renault study was able to achieve. The chain model proposed by Evans *et al.* [14] to explain filament elongation by a constant rate was based on interactions between amino- and carboxy-terminal regions in FlgE (hook) and FliC filament protein subunits through cysteine crosslinking experiments. However, it is known that the amino- and carboxy-termini of FlgE and FliC subunits are directly aligned in an anti-parallel orientation in the final folded conformation in the hook and filament, respectively [19,20]. Evans *et al.* [14] failed to demonstrate a parallel orientation of the amino- and carboxy-terminal helices as predicted by the chain model. They also argued that the 8 Å cysteine–cysteine spacing could not form between subunits in the assembled hook without testing this possibility. Given the dynamics of hook and filament subunit interactions in structures rotating at speeds of 300 to 1,000 revolutions per second, it would be difficult to assume that a static crystal structure model of hook and filament can accurately represent the dynamic subunit interactions *in vivo*.

REFERENCES

1. Chevance, F.F., and Hughes, K.T. (2008). Coordinating assembly of a bacterial macromolecular machine. Nat. Rev. Microbiol. *6*, 455–465.
2. Erhardt, M., Namba, K., and Hughes, K.T. (2010). Bacterial nanomachines: the flagellum and type III injectisome. Cold Spring Harb. Perspect. Biol. *2*, a000299.
3. Berg, H.C. (2003). The rotary motor of bacterial flagella. Annu. Rev. Biochem. *72*, 19–54.
4. Fujii, T., Kato, T., Hiraoka, K.D., Miyata, T., Minamino, T., Chevance, F.F., Hughes, K.T., and Namba, K. (2017). Identical folds used for distinct mechanical functions of the bacterial flagellar rod and hook. Nat. Commun. *8*, 14276.
5. Cohen, E.J., Ferreira, J.L., Ladinsky, M.S., Beeby, M., and Hughes, K.T. (2017). Nanoscale-length control of the flagellar driveshaft requires hitting the tethered outer membrane. Science *356*, 197–200.
6. Braun, V. (1975). Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta. *415*, 335–377.
7. Kovacs-Simon, A., Titball, R.W., and Michell, S.L. (2011). Lipoproteins of bacterial pathogens. Infect. Immun. *79*, 549–561.
8. Shu, W., Liu, J., Ji, H., and Lu, M. (2000). Core structure of the outer membrane lipoprotein from *Escherichia coli* at 1.9 Å resolution. J. Mol. Biol. *299*, 1101–1112.
9. Erhardt, M., Singer, H.M., Wee, D.H., Keener, J.P., and Hughes, K.T. (2011). An infrequent molecular ruler controls flagellar hook length in *Salmonella enterica*. EMBO J. *30*, 2948–2961.
10. Iino, T. (1974). Assembly of *Salmonella* flagellin *in vitro* and in vivo. J. Supramol. Struct. *2*, 372–384.
11. Iino, T. (1969). Polarity of flagellar growth in *Salmonella*. J. Gen. Microbiol. *56*, 227–239.
12. Keener, J.P. (2006). How *Salmonella* Typhimurium measures the length of flagellar filaments. Bull. Math. Biol. *68*, 1761–1778.
13. Turner, L., Stern, A.S., and Berg, H.C. (2012). Growth of flagellar filaments of *Escherichia coli* is independent of filament length. J. Bacteriol. *194*, 2437–2442.
14. Evans, L.D., Poulter, S., Terentjev, E.M., Hughes, C., and Fraser, G.M. (2013). A chain mechanism for flagellum growth. Nature *504*, 287–290.
15. Chen, M., Zhao, Z., Yang, J., Peng, K., Baker, M.A., Bai, F., and Lo, C.J. (2017). Length-dependent flagellar growth of *Vibrio alginolyticus* revealed by real time fluorescent imaging. eLife *6*, e22140.
16. Renault, T.T., Abraham, A.O., Bergmiller, T., Paradis, G., Rainville, S., Charpentier, E., Guet, C.C., Tu, Y., Namba, K., Keener, J.P., *et al*. (2017). Bacterial flagella grow through an injection-diffusion mechanism. eLife *6*, e23136.
17. Paul, K., Erhardt, M., Hirano, T., Blair, D.F., and Hughes, K.T. (2008). Energy source of flagellar type III secretion. Nature *451*, 489–492.
18. Minamino, T., and Namba, K. (2008). Distinct roles of the ATPase and proton motive force in bacterial flagellar protein export. Nature *451*, 485–488.
19. Fujii, T., Kato, T., and Namba, K. (2009). Specific arrangement of alpha-helical coiled coils in the core domain of the bacterial flagellar hook for the universal joint function. Structure *17*, 1485–1493.
20. Yonekura, K., Maki-Yonekura, S., and Namba, K. (2003). Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. Nature *424*, 643–650.

Cerebellar Granule Cells: Dense, Rich and Evolving Representations

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For half a century it was assumed that granule cells use ultra-sparse encoding, but now *in vivo* calcium-imaging studies have shown that large ensembles of granule cells provide dense signals, which themselves evolve and adapt during training.

Cerebellar granule cells form the densest neuronal layer in the brain and they are critical for motor learning [1]. Their morphology is highly conserved throughout phylogeny [2]. Granule cells have a small cell body with only two to seven dendrites, each equipped with a claw receiving a single mossy fiber input. These mossy fibers are derived from many pre-cerebellar locations, which carry a large variety of signals including sensory, motor, and contextual information [2,3]. Partly based on this sparse synaptic connectivity, classical theories on cerebellar function have argued that granule cells relay sparse and fixed encodings of sensorimotor signals that could be used for generating specific associative connections at the granule cell to Purkinje cell synapse (Figure 1A)

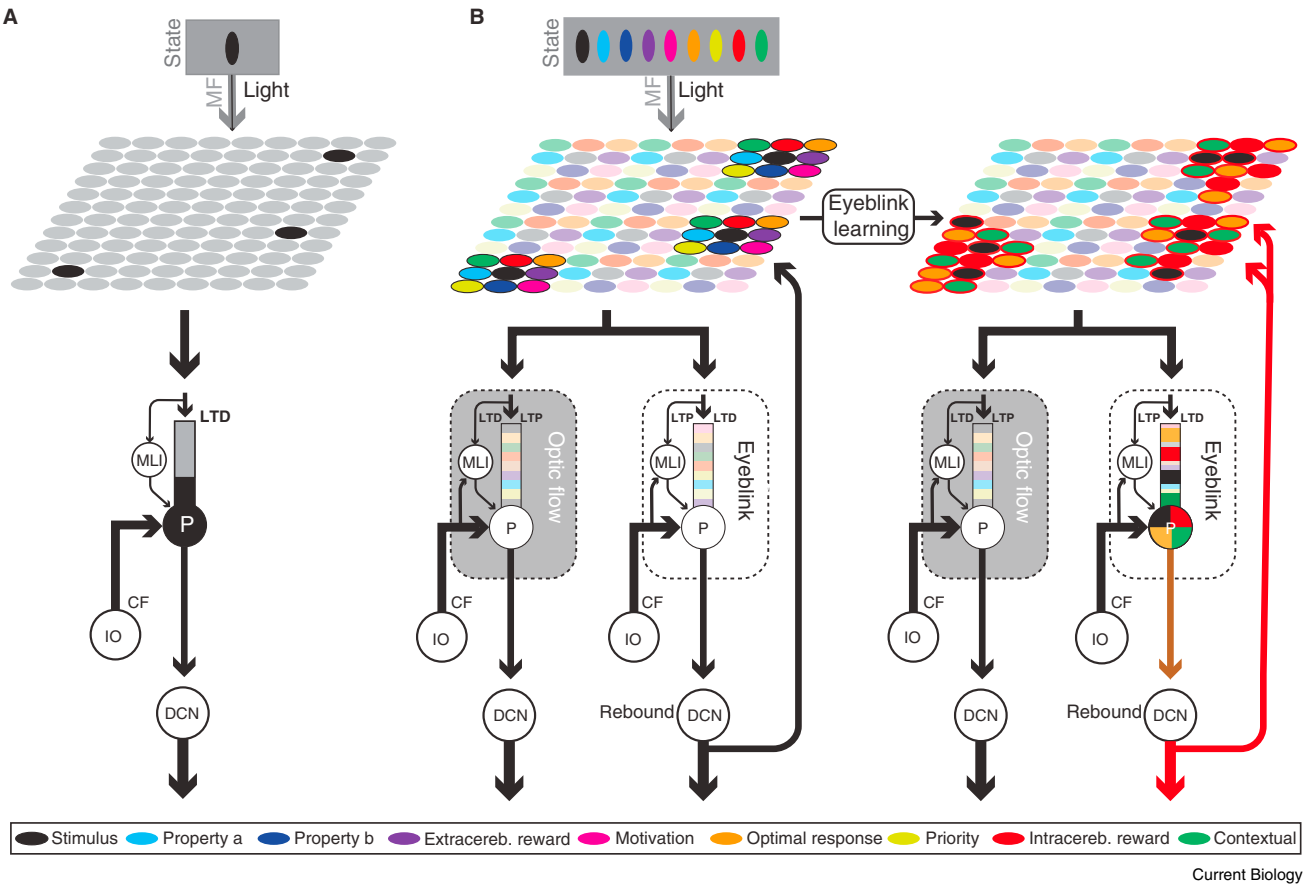

Figure 1. Signals in the granule cell layer are dense rather than sparse and evolve and adapt during training.
(A) Historically, granule cell signals (grey ovals) have been proposed to encode a sparse code of the stimuli. These signals arrive via the mossy fiber pathway (MF) and converge onto Purkinje cells (P) and the molecular layer interneurons (MLI). The inferior olive (IO) modulates the activity at the granule cell to P, granule cell to MLI, and MLI to P synapses through climbing fibers (CF). Temporal coincidence or separation of these two inputs changes the signals from the granule cells via long-term depression (LTD) or long-term potentiation (LTP), the plastic impact of which will be conveyed to the deep cerebellar nuclei (DCN). (B) New studies [6–8] have revealed that the coding in the granule cell layer is in fact dense and rich (colorful ovals), ranging from information about the physical nature of the external stimuli (black, light and dark blue ovals) and interpretation of the context (green ovals), to information about the internal state of the cerebellum (red ovals), or the reward expectation (purple ovals), priority (yellow ovals), motivation (pink ovals), and optimal response (orange ovals) of the organism. Moreover, these new studies indicate that the coding in the granule cell layer can be subject to learning, such as occurs during eyeblink conditioning. These evolving patterns of granule cell activity will affect the bidirectional plasticity at the Purkinje cell synapses, which is partially predetermined by expression patterns of intrinsic molecular pathways (reflected by the different background colors of the Purkinje cell modules involved; grey and white boxes).

[4,5]. One of the hypothesized advantages of sparse encoding was an efficient separation of input patterns, which as a consequence would increase the speed of formation of novel associations at the level of Purkinje cells. This memory formation at the Purkinje cell dendrites would, in turn, be guided by a powerful error signal mediated by the olivary climbing fibers, selecting the proper sets of separated, sparsely encoded inputs generated in the granular layer. Surprisingly, three new studies, including Knogler *et al.* [6], Giovannucci *et al.* [7], and Wagner *et al.* [8], in recent issues of *Current Biology*, *Nature Neuroscience*, and *Nature*, respectively, provide compelling evidence that the encoding of granule cells is dense in that large ensembles of up to about half of the cells in a particular lobule can be activated during sensorimotor integration.

This groundbreaking discovery was made possible by utilizing technological advances in monitoring neuronal activity in awake, behaving animals. Until recently, granule cell signals were measured using electrophysiological approaches, often in anesthetized or decerebrated animals, procedures which significantly limit the activity in the granule cell layer. The few studies performed in awake mice [9,10] showed rich firing patterns in single granule cells, but failed to report population coding because of technical limitations. Granule cells are simply too small and too dense for it to be possible to readily discriminate and identify abundant numbers of single-units by electrophysiological means. The three new studies [6–8] highlighted here took advantage of large field, high-speed two-photon calcium imaging, making it possible to simultaneously observe the activity of hundreds of granule cells in a single field of view. Combined with the latest advances in genetically encodable calcium indicators [11], which increase the temporal resolution of observed signals, and computational methods, which allow separation of signals from overlapping cells, it is now possible to monitor large populations of granule cells in awake behaving animals at a single cell resolution.

By imaging the entire cerebellum of awake, head-embedded larval zebrafish, Knogler *et al.* [6] were able to show that activity in the granule cell layer is dense across different modalities. The researchers made use of the fact that larval zebrafish are optically transparent and relatively small, allowing modern two-photon microscopes to image the entire volume of the cerebellum in one experiment and at high sampling rate. In a separate set of experiments, the authors used genetic tools available in the zebrafish model to sparsely label granule cells in the larval cerebellum to carefully describe the granule cell anatomy at this stage of the fish development. This additional dataset revealed that, even in immature fish, the cerebellum is already well-established and separated into three distinctive layers, and that the majority of granule cells 6–7 days post-fertilization show several distinctive dendrites (claws) capable of integrating multiple mossy fiber inputs. Interestingly, most of the zebrafish granule cells at this stage showed between two and three claws, consistent with the morphology of young mammalian granule cells [12], which are on their way to develop about four dendrites when they reach adulthood [2].

While imaging the whole cerebellar volume of larval zebrafish, Knogler *et al.* [6] observed that granule cells were densely active in response to sensory inputs, including whole-field light flashes, whole-field moving bars and mild shocks, as well as to stimulus-evoked motor behaviors captured by monitoring the movement of the tail. In general, the cells responded robustly to either one sensory and/or one motor-related variable. Thus, multimodal signaling did occur, but not for different sensory stimuli (Figure 1B, left). In addition, they showed using both calcium-imaging and electrophysiological recordings that cells with the same or similar responses were spatially clustered and temporally homogeneous rather than continuously varying. In this respect, the responses also deviated from the theoretical considerations of Marr [4] and Albus [5], who predicted that the granule cells would encode signals in higher temporal dimensions than those conveyed by their mossy fiber inputs. Such signals enriched in both the spatial and temporal domain would generate more diversity in coding, allowing the climbing fibers in the molecular layer to select the proper signals from a wider range of inputs. However, temporal patterning did not turn out to be an obvious feature, at least not in developing larval zebrafish subjected to sensory stimulation and/or executing motor tasks that did not entail training. It remains possible that temporal diversification in granule cell signaling becomes more apparent in adult animals during motor learning, which was studied by Giovannucci *et al.* [7].

Giovannucci *et al.* [7] also showed the general property of dense, rather than sparse, coding in the cerebellar granule cells, but they took their experimental approach to adult mammals subjected to a motor learning task. Consistent with the findings in the zebrafish, they found that, during classical eyeblink conditioning — a cerebellar form of motor learning in which adult mice are taught to associate a flash of light (conditioned stimulus) with a puff of air to the eye (unconditioned stimulus) — many granule cells responded to either the conditioned or unconditioned stimulus (light or puff), the triggered behavior (closing the eye), spontaneous behavior (whisking or walking) or a combination of these (Figure 1B, left). Most strikingly, as the animals started to learn to respond to the light with a pre-emptive blink, some of the granule cells showed activity that encoded this prediction. As learning further progressed, more granule cells acquired this predictive signal that ultimately highly correlated with the absence or presence of the learned response (Figure 1B, right), together enhancing the level of decorrelation and temporal dispersion among different sets of granule cells.

Whereas the decorrelation of the encodings of granule cells was predicted by Marr [4] and Albus [5], they did not envision the widespread adaptive features of these cells at the input stage. In their models, the learning was predominantly attributed to the output stage of the cerebellum, with its robust integrative properties provided by the large dendritic trees of the Purkinje cells. However, while Giovannucci *et al.* [7] uncovered the evidence for learning at the input stage analyzing sensorimotor signaling, they did not combine their imaging and behavioral studies with concomitant electrophysiological recordings to establish the level of the fine-temporal dimensions of granule cell activity during eyeblink conditioning. Moreover, it was also not investigated to what extent granule cells may also encode higher order cognitive signals.

This ability of granule cells to carry a cognitive signal was revealed by Wagner *et al.* [8], who subjected awake behaving mice to an operant, voluntary forelimb motor task, during which they were trained to push a lever in order to receive a sucrose-water reward with varied delay times. They showed that some granule cells responded preferentially to reward or reward omission, whereas others selectively encoded expectation of reward. Moreover, here too granule cell encoding was dense, showing high levels of decorrelation and temporal dispersion while being subject to changes over time as learning progressed. Whereas the intracerebellar feedback signals to the granule cells have been shown to originate directly in the cerebellar nuclei [13] (Figure 1B, right), the source of the extracerebellar reward signals remain unclear. The neuro-anatomical tracing studies by Wagner *et al.* [8] raise the possibility that these reward signals might be derived from areas like the pons, which receive extensive input from higher brain regions like the cerebral cortex. The ability of the cerebellum to integrate cognitive signals of reward expectation in the context of sensorimotor processing may endow the system to even further refine coordination of its output and speed up the learning process.

The new experimental data provided by Knogler *et al.* [6], Giovannucci *et al.* [7] and Wagner *et al.* [8] are well in line with recent theoretical work by Cayco-Gajic *et al.* [14]. Their work suggests that, while granule cells are well suited to sparsen mossy fiber input patterns, expansion of coding space and decorrelation of activity patterns are the main determinants of pattern separation and learning speed for biologically realistic, spatially correlated input patterns [14]. In fact, it seems that it is the sparsity in synaptic connectivity rather than ultra-sparsening of activity patterns that appears critical. The small number of granule cell dendrites each receiving a single mossy fiber enables efficient transmission of information, introduces few correlations, and also provides a thresholding operation that

acts to decorrelate signals [15]. Moreover, the new experimental data also align well with the recent concepts of D'Angelo and De Zeeuw [16] and Gao *et al.* [13,17], who predicted that learning would also take place at the cerebellar input stage — the granular layer, which is also studded with ample forms of synaptic, intrinsic and hardwired plasticity.

Inarguably, the three new papers [6–8] have significantly advanced our knowledge of cerebellar function in intact, behaving animals. Yet, there are still a few unanswered questions left that need to be addressed by future studies. Specifically, it will be crucial to unravel whether the learning-related granule cell signals truly emerge in granule cells themselves or rather reflect changes in the upstream information carried by the mossy fibers. In addition, we need to determine how stable the granule cell representations are over time after the learning is completed, and finally, we need to identify the exact source of the extracerebellar reward signals (Figure 1B). Answering these questions will require measurements of activity in mossy fibers and granule cells over large neuronal populations with the ability to simultaneously manipulate and disrupt the inputs from a wide variety of sources. Recent advancements in designing multicolor indicators [18], high-speed volumetric imaging [19] and all optical control of neuronal firing [20] will make it better possible to tackle these last outstanding questions.

REFERENCES

1. Galliano, E., Gao, Z., Schonewille, M., Todorov, B., Simons, E., Pop, A.S., D'Angelo, E., van den Maagdenberg, A.M., Hoebeek, F.E., and De Zeeuw, C.I. (2013). Silencing the majority of cerebellar granule cells uncovers their essential role in motor learning and consolidation. Cell Rep. *3*, 1239–1251.

2. Wittenberg, G.M., and Wang, S.S.-H. (2007). Evolution and scaling of dendrites. In Dendrites, G. Stuart, N. Spruston, and M. Hausser, eds. (New York: Oxford University Press), pp. 43–67.

3. Huang, C.C., Sugino, K., Shima, Y., Guo, C., Bai, S., Mensh, B.D., Nelson, S.B., and Hantman, A.W. (2013). Convergence of pontine and proprioceptive streams onto multimodal cerebellar granule cells. eLife *2*, e00400.

4. Marr, D. (1969). A theory of cerebellar cortex. J. Physiol. *202*, 437–470.

5. Albus, J.S. (1971). A theory of cerebellar function. Math. Biosci. *10*, 25–61.

6. Knogler, L.D., Markov, D.A., Dragomir, E.I., Štih, V., and Portugues, R. (2017). Sensorimotor representations in cerebellar granule cells in larval zebrafish are dense, spatially organized and nontemporally patterned. Curr. Biol. *27*, 1288–1302.

7. Giovannucci, A., Badura, A., Deverett, B., Najafi, F., Pereira, T.D., Gao, Z., Ozden, I., Kloth, A.D., Pnevmatikakis, E., Paninski, L., *et al*. (2017). Cerebellar granule cells acquire a widespread predictive feedback signal during motor learning. Nat. Neurosci. *20*, 727–734.

8. Wagner, M.J., Kim, T.H., Savall, J., Schnitzer, M.J., and Luo, L. (2017). Cerebellar granule cells encode the expectation of reward. Nature *544*, 96–100.

9. van Beugen, B.J., Gao, Z., Boele, H.J., Hoebeek, F., and De Zeeuw, C.I. (2013). High frequency burst firing of granule cells ensures transmission at the parallel fiber to purkinje cell synapse at the cost of temporal coding. Front. Neural Circuits *7*, 95.

10. Powell, K., Mathy, A., Duguid, I., and Hausser, M. (2015). Synaptic representation of locomotion in single cerebellar granule cells. eLife *4*, e07290.

11. Badura, A., Sun, X.R., Giovannucci, A., Lynch, L.A., and Wang, S.S. (2014). Fast calcium sensor proteins for monitoring neural activity. Neurophotonics *1*, 025008.

12. Hsieh, C.P., Chang, W.T., Lee, Y.C., and Huang, A.M. (2015). Deficits in cerebellar granule cell development and social interactions in CD47 knockout mice. Dev. Neurobiol. *75*, 463–484.

13. Gao, Z., Proietti-Onori, M., Lin, Z., Ten Brinke, M.M., Boele, H.J., Potters, J.W., Ruigrok, T.J., Hoebeek, F.E., and De Zeeuw, C.I. (2016). Excitatory cerebellar nucleocortical circuit provides internal amplification during associative conditioning. Neuron *89*, 645–657.

14. Cayco-Gajic, A., Clopath, C., and Silver, R.A. (2017). Sparse synaptic connectivity is required for decorrelation and pattern separation in feedforward networks. bioRxiv., http://dx.doi.org/10.1101/108431.

15. Billings, G., Piasini, E., Lorincz, A., Nusser, Z., and Silver, R.A. (2014). Network structure within the cerebellar input layer enables lossless sparse encoding. Neuron *83*, 960–974.

16. D'Angelo, E., Koekkoek, S.K., Lombardo, P., Solinas, S., Ros, E., Garrido, J., Schonewille, M., and De Zeeuw, C.I. (2009). Timing in the cerebellum: oscillations and resonance in the granular layer. Neurosci. *162*, 805–815.

17. Gao, Z., van Beugen, B.J., and De Zeeuw, C.I. (2012). Distributed synergistic plasticity and cerebellar learning. Nat. Rev. Neurosci. *13*, 619–635.

18. Dana, H., Mohar, B., Sun, Y., Narayan, S., Gordus, A., Hasseman, J.P., Tsegaye, G., Holt, G.T., Hu, A., Walpita, D., *et al*. (2016). Sensitive red protein calcium indicators for imaging neural activity. eLife *5*, e12727.

19. Song, A., Charles, A.S., Koay, S.A., Gauthier, J.L., Thiberge, S.Y., Pillow, J.W., and Tank, D.W. (2017). Volumetric two-photon imaging of neurons using stereoscopy (vTwINS). Nat. Methods *14*, 420–426.

20. Packer, A.M., Russell, L.E., Dalgleish, H.W., and Hausser, M. (2015). Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. Nat. Methods *12*, 140–146.

Prefrontal Cortex: A Mystery of Belated Memories

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A recent study suggests that the prefrontal cortex gradually becomes critical as a storage site for remotely acquired memories. How do we interpret this observation in light of the well-known functional role of the prefrontal cortex in cognition and memory?

The prefrontal cortex has long been viewed as the site of working memory, our ability to hold newly acquired information in mind for brief periods [1]. A new study by Kitamura *et al.* [2], however, suggests that prefrontal cortex plays a key role in memory for a different time period, specifically for maintaining remotely