Re-engineering the Hippocampus

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As adult-generated neurons integrate into hippocampal circuits, they compete with mature neurons for inputs from the entorhinal cortex. By reducing spines on mature granule cells, McAvoy et al. (2016) find that new neurons integrate more efficiently, and this facilitates learning.

From ancient Greece to modern-day Hollywood, the ability to boost and optimize memory represents a recurring and alluring theme. By re-engineering the hippocampus—the brain region that is important for transforming our experiences into lasting memories— McAvoy et al. (2016) begin to turn fiction into fact with a new study (at least if you're a mouse).

To do this they ask a simple, yet lofty, question: is it possible to engineer a better hippocampus—one that encodes and stores information more efficiently? Their approach focused on adult neurogenesis—a unique and robust form of plasticity in the hippocampus. Throughout life, new neurons are generated in the subgranular zone of the dentate gyrus. These newly generated neurons then need to integrate into the existing hippocampal circuitry, and this is not a trivial task. As new adult-generated neurons mature, they must extend dendrites and axons through a maze of existing processes, establishing contact with the appropriate pre- and post-synaptic partners.

The hippocampus (much like the field that studies it) is a highly competitive environment. As new neurons integrate, they appear to compete with existing, mature granule cells for inputs from entorhinal cortex and outputs onto CA3 pyramidal cells. In a series of landmark papers, Nicolas Toni and colleagues used retroviral labeling techniques to visualize new neurons as they integrated into established adult hippocampal circuits. What they saw was striking. In the molecular layer of the dentate gyrus, spiny protrusions from newborn neurons preferentially contacted existing boutons. This suggested that new neurons compete with established neurons for inputs from the entorhinal cortex (Toni et al., 2007) and that receiving strong entorhinal input imparts a survival advantage for the new neurons. A similar pattern was observed on the output side. In CA3, large mossy fiber terminals from newborn granule cells formed synapses on thorny excrescences immediately adjacent to contacts from mature granule cells (Toni et al., 2008). Again, this suggested a competitive process in which new neurons form new output connections and, in doing so, potentially overwrite existing connections.

While these "snapshots" of the new neuron wiring strongly hinted that the integration of new neurons depended upon competitive interactions between new and mature populations of granule cells, the most direct support for this idea would be to perturb the system—advantage or disadvantage one or other population—and watch the competition play out. McAvoy et al. (2016) adopted this strategy in the current study. They selectively reduced dendritic spines in the mature population of granule cells in the dentate gyrus. They reasoned that by blunting the competitive edge of the mature cells, new neurons would be able to integrate more efficiently.

To do this, they took advantage of their previous work in which they had identified Krüppel-like factor 9 (*Klf9*) as a key negative transcriptional regulator of dendritic spines (Scobie et al., 2009). They generated mice in which *Klf9* could be inducibly and reversibly overexpressed in mature granule cells in the dentate gyrus. As predicted, overexpressing *Klf9* reduced dendritic spine density in mature dentate granule cells. Spines were reduced on the most distal dendrites in the outer molecular layer, the region in which entorhinal inputs contact the granule cells. Consistent with these cells receiving reduced excitatory input from the entorhinal cortex, the mature granule cells overexpressing *Klf9* were less active. Mature granule cells had reduced expression of the activity-regulated gene, *c-fos*, supporting the idea that spine loss blunted their activity.

This confirmed *Klf9*'s status as a negative regulator of spines, supporting earlier work (Scobie et al., 2009). But did disadvantaging mature granule cells in this fashion give an edge to their competition? Strikingly, reducing spines in mature granule cells led to a spike in neurogenesis. The number of new granule cells was increased immediately following 2 weeks of *Klf9* overexpression, but neurogenesis returned to baseline levels within an additional 2 weeks. Furthermore, neurons born 1 week prior to *Klf9* overexpression (i.e., those that were integrating at the time when Klf9 levels were elevated) had a higher rate of long-term survival. Some pathological conditions, including epilepsy, are also associated with increased neurogenesis (Jessberger et al., 2007). However, with these pathological conditions, new neurons are located ectopically and form aberrant connections. McAvoy et al. (2016) used a combined retrovirus and rabies virus labeling approach to examine the connectivity of the new neurons following *Klf9* overexpression. They found that the new neurons appear to connect up quite normally, suggesting that *Klf9* overexpression not only boosted neurogenesis, but these new neurons also integrated seamlessly in the hippocampus.

Many factors regulate the stability of spines. In principle, therefore, there should be alternate ways of perturbing the competition between mature and new granule

cells, establishing that the current observations are not peculiar to the *Klf9* intervention. To do this, McAvoy et al. (2016) targeted the Rho family GTP-ase, *Rac1*. In contrast to *Klf9*, *Rac1* is a positive regulator of dendritic spines. Accordingly, conditional deletion of *Rac1* from the mature granule cell population led to a decrease in spine density (similar to *Klf9* overexpression). Moreover, spine loss in the mature population led to an increase in the number of immature neurons in the dentate gyrus. These findings confirm the interaction between the morphology/activity of mature granule cells and the integration of immature neurons and, therefore, provide direct support for the ideas originally proposed by Toni et al. (2007, 2008). Improved methods for imaging new neurons will now make it possible to watch this competition play out in real time (Gonçalves et al., 2016).

How does this transient boost in neurogenesis affect hippocampal function? With the development of more effective methods for labeling newborn neurons in the 1990s, the study of hippocampal neurogenesis underwent something of a renaissance, and this question about function has been at the forefront. In the typical study, experimenters introduce some intervention to increase neurogenesis (e.g., from drugs to exercise to genetic interventions) and then wait several weeks before training animals in a task that engages the hippocampus. The typical finding is that boosting hippocampal neurogenesis improves hippocampal learning (e.g., Sahay et al., 2011a).

However, these interventions all produce global changes in hippocampal neurogenesis. At the time of testing, the hippocampus would contain new neurons in a broad range of maturational states (from days to several weeks old). The biophysical and functional features of newborn granule cells change dramatically as they mature, with neurons that are 4–8 weeks old being especially plastic and excitable (Toni and Schinder, 2016). It is at this point that they appear to be most important for hippocampal memory function—they are recruited into hippocampal memories in great numbers and subsequent inhibition of these cells impairs memory (Gu et al., 2012). It would therefore be advantageous to furnish the hippocampus with a cohort of new neurons at just this right age. The clever, reversible strategy developed by McAvoy et al. (2016) allows them to do exactly that and flood the hippocampus with a cohort of new neurons of exactly the right vintage.

To examine whether memory improved, they took their re-engineered mice with boosted cohorts of 5- to 8-week-old new neurons and tested them in two tasks, the water maze and contextual fear discrimination. They found improvements in both tests. In the water maze, the mice with boosted neurogenesis could use spatial information more flexibly. In the context discrimination task, the mice with boosted neurogenesis discriminated more persistently between a dangerous context (in which they received a shock) and a safe context (in which nothing unpleasant happened). These effects were more pronounced in middle-aged and old-aged mice, suggesting that the beneficial effects of boosting neurogenesis may be most obvious against a backdrop of declining cognitive function.

The ability to discriminate dangerous places from safe places is thought to depend on pattern separation. Pattern separation refers to the process in which similar input patterns of activity are transformed into dissimilar output patterns of activity (Sahay et al., 2011b). In the dentate gyrus, neurogenesis is thought to facilitate this computation, in part, by helping to sparsify the neural code. With a sparser code, it should be easier to represent different places (or experiences) by non-overlapping populations of dentate granule cells.

Therefore, the authors used a molecular imaging approach (catFISH) to test the idea that improved pattern separation might underlie improvements in contextual fear discrimination in the re-engineered mice. They visualized neural ensembles in the dentate gyrus activated by two distinct experiences (placement in the "dangerous" context in which they had been shocked versus placement in a similar "safe" context in which nothing untoward happened). Remarkably, the degree of overlap between the populations engaged by these two experiences was reduced in the re-engineered mice, consistent with their ability to more efficiently discriminate these contexts. Chemically preventing the increase in neurogenesis eliminated this difference, and also the improved context discrimination. These findings provide strong support for the notion that the integration of adult generated neurons plays an important role in context discrimination by minimizing the co-activation of neurons in dangerous versus safe contexts.

So far, so good. But does improved memory come at a cost? The McAvoy et al. (2016) study doesn't address this potential downside. However, the cost of adding plasticity to any system (e.g., the brain or computer) is instability, referred to as the plasticity-stability dilemma (Grossberg, 1987). Given this, we predict that similarly boosting neurogenesis after training (rather than before) would highlight the cost of more efficient encoding: the loss of established memories (Frankland et al., 2013). So we are left pondering what precisely an optimized hippocampus would look like.

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