The Virtues of Youth and Maturity (in Dentate Granule Cells)

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How does adult neurogenesis contribute to memory? Nakashiba and colleagues generated mutant mice in which synaptic output from older hippocampal granule cells was specifically blocked. Experiments with these mice reveal an unpredicted age-dependent specialization of function, demonstrating that recently born cells support pattern separation, whereas older cells support pattern completion.

Episodic memory, which relies on the hippocampus, requires both pattern separation and pattern completion. Pattern separation is the ability to distinguish similar-but-different cues, such as your mother's face and her sister's face. Pattern completion is the process of retrieving more complete memories from partial cues. For instance, upon seeing your mother's face, you are able to recall where you last saw her. The dentate gyrus (DG) has long been thought to support pattern separation, consistent with the large number of infrequently firing granule cells (GCs), whereas area CA3, immediately downstream of the DG, has been associated with pattern completion, consistent with the high density of recurrent collaterals (Marr, 1971).

In mammals, the DG is one of only two brain regions where adult neurogenesis occurs. The birth and integration of neurons during adulthood underlie pattern separation. When neurogenesis is ablated by X-ray irradiation, the ability to discriminate highly similar locations or contexts is impaired (e.g., Clelland et al., 2009; Sahay et al., 2011), and provocatively, when neurogenesis is enhanced by promoting the survival of newly born cells, pattern separation improves (Sahay et al., 2011). However, the newborn cells are highly active for only a few weeks, then become increasingly silent. What are the relative contributions of young and older GCs? In this issue of Cell, Nakashiba et al. (2012) address this question by generating mutant (DG-TeTX) mice whose old (>4 weeks) dentate GCs cannot excite their CA3 cell targets (see Figures 1A and 1B).

They first used a sequence of three fear conditioning tests to assess spatial context discrimination. The mice learn to "freeze" upon re-exposure to a context in which they received a footshock (fear context). The mice should freeze much less in a context where they are never shocked (safe context).

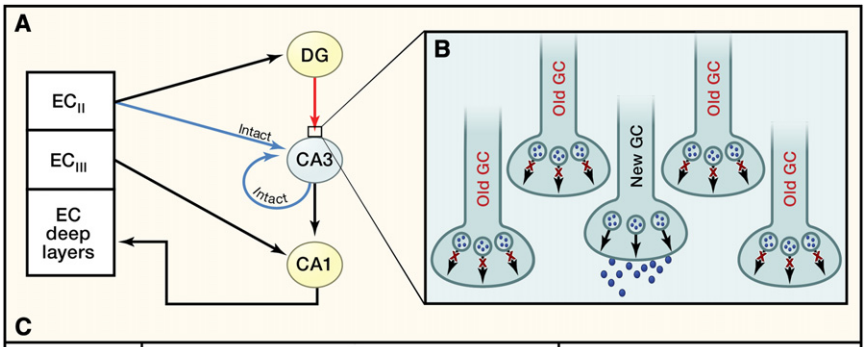
In the first test, the mice were shocked in the fear context for 3 days, then on days 4 and 5 were tested without shock in the fear context and an obviously different safe context. Both control and mutant mice exhibited equally good discrimination of fear and safe contexts, immediately freezing in the fear context and freezing much less in the safe context.

The second test proceeded as the first, except that the fear and safe contexts were physically very similar. Initially, neither group showed differential freezing across the two contexts. Accordingly, a further 12 days of training was given to promote this more difficult discrimination. Control mice did not reliably distinguish the two contexts until the last 4 days. Remarkably, the DG-TeTX mutants discriminated these very similar contexts over the entire 12 day period.

The third discrimination test used intermediately different contexts and was run on DG-TeTX mutants and controls with and without irradiation to ablate neurogenesis. Irradiation impaired context discrimination in both groups, confirming the importance of young GCs for context discrimination. One caveat is the mutant mice's somewhat *impaired* context discrimination in this test.

Together, these experiments suggest that recently born GCs (<4 weeks old, < 5% of GC population) play a privileged role in pattern separation, and that, remarkably, depriving CA3 of the output from old GCs (>95% of population) can actually improve the discrimination of very similar contexts (see Figure 1C). It would be interesting to see the neural representations in the DG and CA3 that support the mutants' enhanced context discrimination. Hippocampal place cells immediately "remap" to distinguish obviously different contexts (e.g., Wills et al., 2005), and the mutants show normal remapping in this situation. However, the situation is different for subtly different contexts, for which place cell representations slowly diverge over several days of experience (e.g., Lever et al., 2002), reminiscent of the controls' behavioral discrimination in the second test. Perhaps the mutant's rapid discrimination in this test reflects immediate remapping in CA3, driven by more distinct representations among the young DG GCs.

How do specifically the young GCs support pattern separation? Computational models suggest that GCs generate orthogonalized CA3 representations to represent similar-but-different contexts but then play a minor, if any, role in retrieval, which is driven by direct input to the CA3 from the entorhinal cortex



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Test for	Task		DG-TeTx performance relative to controls
Pattern separation	Discrimination of contexts (differential freezing)	Obviously different	=
		Highly similar	>>
		Intermediately different	< (small effect) Strong impairing effect of irradiation
	Spatial discrimination (radial arm maze)		=
	Spatial context discrimination (place cell remapping)		CA3 cells: = CA1 cells: =
Pattern completion	Go to platform location when most localizing cues are removed		Latency to platform location: < Hovering over correct location over 90 second period: =
	Context-recall-dependent freezing		10 seconds re-exposure: < 180 seconds re-exposure: =

Figure 1. Characterization of the DG-TeTx Mouse

(A) Basic entorhinal-hippocampal loop. Entorhinal cortex perforant path projects to the DG and hippocampal fields CA3 and CA1. GCs in the DG project via the mossy fiber pathway to CA3. CA3 cells project to themselves (recurrent collaterals, thought to support pattern completion) and to CA1. CA1 projection to the deep entorhinal cortex closes the loop. Nakashiba and colleagues generated a mutant mouse (DG-TeTx) in whom synaptic transmission from GCs to CA3 (red) is impaired, whereas synaptic transmission at perforant path and recurrent collateral synapses to CA3 (blue) is intact.

(B) In the DG-TeTx mouse, the synaptic output to CA3 cells from old GCs (>4 weeks old) is specifically inhibited when activated by tetanus toxin, such that vesicles containing transmitter cannot fuse with the presynaptic membrane at mossy fiber terminals. Output of newborn GCs is unaffected.

(C) Summary of behavioral performance of DG-TeTx mice. Overall, DG-TeTx mice are capable of good pattern separation, especially between similar spatial contexts, but show impaired pattern completion when time is restricted. Symbols for performance relative to controls: = equivalent; < worse than; > > much better than.

(e.g., Treves and Rolls, 1992). This could explain why the young GCs are useful for forthcoming pattern separation tasks and why old GCs are so inactive. In addition, it may be that the dynamic recruitment of different sets of young GCs over time, rather than their large absolute number, supports pattern separation. This conclusion raises a bigger question: what do the old GCs do?

The authors provide evidence that old GCs are required for rapid pattern completion. They used a standard reference-memory version of the water-maze task, where mice swim toward a platform hidden under opaque water. The platform location was fixed relative to four large distal cues hung on curtains around the maze. After training, the mice had to swim to the platform location with different numbers of distal cues present. DG-TeTX mutants and controls reached the platform location equally quickly with all four cues present and equally slowly with all four cues removed, nicely indicating that the mice did use these cues to navigate. Crucially, when only one distal cue was available to trigger retrieval of the complete water-maze representation, including the platform location, the mutants took longer than controls. Overall, this suggests that DG-TeTX mutants have a pattern-completion deficit. In another partial-cue triggering approach, the authors used contextual fear conditioning again and limited not cue number but cue exposure time. The DG-TeTX mutants were impaired in context-dependent freezing when the context re-exposure period was 10 s but not when it was 3 min, suggesting a deficit specifically in *rapid* pattern completion (see Figure 1C).

So how do old GCs contribute to rapid pattern completion? Pattern completion is supported by CA3 recurrent-collateral NMDA receptors (Nakazawa et al., 2002) and has been shown in the place cell representation of two distinct environments (Wills et al., 2005). CA3 is also the DG's output, so old GCs presumably must affect CA3 representations. One possibility is that old GCs simply provide tonic activation of CA3, which allows faster pattern completion. Another is that the GCs themselves contribute to pattern completion, perhaps via the recurrent connectivity from mossy cells (Lisman, 1999).

The finding that old GCs are unnecessary for pattern separation accords with current thinking that recruiting new pools of young, highly excitable GCs is useful for encoding new information, particularly when it overlaps with old information, by providing temporal context (Aimone et al., 2009). But the finding that old GCs are important for rapid pattern completion seems unexpected. As the authors note, however, separation and completion are two sides of the same coin. Both processes should operate along different dimensions to aid episodic memory, but if not, then one operation undoes the other. Thus the mutant mice might show improved discrimination of similar contexts precisely because pattern completion processes (producing generalization rather than discrimination) are impaired.

These exciting findings force new thinking on the question of how our brains store new events without forgetting old ones. Further, as adult neurogenesis helps to mediate antidepressants and may alleviate PTSD, these findings may also have wider significance.

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REFERENCES

Aimone, J.B., Wiles, J., and Gage, F.H. (2009). Neuron *61*, 187–202.

Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Jr., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R.A., Gage, F.H., and Bussey, T.J. (2009). Science *325*, 210–213.

Lever, C., Wills, T., Cacucci, F., Burgess, N., and O'Keefe, J. (2002). Nature *416*, 90–94.

Lisman, J.E. (1999). Neuron *22*, 233–242.

Marr, D. (1971). Philos. Trans. R. Soc. Lond. B Biol. Sci. *262*, 23–81.

Nakashiba, T., Cushman, J.D., Pelkey, K.A., Renaudineau, S., Buhl, D.L., McHugh, T.J., Rodriguez Barrera, V., Chittajallu, R., Iwamomot, K.S., McBain, C.J., et al. (2012). Cell *148*, this issue, 188–201.

Nakazawa, K., Quirk, M.C., Chitwood, R.A., Watanabe, M., Yeckel, M.F., Sun, L.D., Kato, A., Carr, C.A., Johnston, D., Wilson, M.A., and Tonegawa, S. (2002). Science *297*, 211–218.

Sahay, A., Scobie, K.N., Hill, A.S., O'Carroll, C.M., Kheirbek, M.A., Burghardt, N.S., Fenton, A.A., Dranovsky, A., and Hen, R. (2011). Nature *472*, 466–470.

Treves, A., and Rolls, E.T. (1992). Hippocampus *2*, 189–199.

Wills, T.J., Lever, C., Cacucci, F., Burgess, N., and O'Keefe, J. (2005). Science *308*, 873–876.

COPII Vesicles Get Supersized by Ubiquitin

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Some proteins are too big to fit into conventional COPII-coated vesicles, which raises the question of how large cargo, such as procollagen fibrils, are exported from the endoplasmic reticulum. Jin et al. (2012) in *Nature* now report that the creation of oversized vesicles is facilitated by the ubiquitination of the COPII component Sec31p.

In all eukaryotes, COPII vesicles form at endoplasmic reticulum (ER) exit sites for cargo export to the Golgi apparatus. Formation of these vesicles can be achieved with six polypeptides in vitro: Sec12 (an integral ER membrane protein and a guanine nucleotide exchange factor for the cytoplasmic protein Sar1p); Sar1p (a GTPase that, in its GTP-bound form, is recruited to the ER); Sec23/24, which are recruited to membranes by Sar1p-GTP and then recruit Sec13/31, the final subunits. The assembly of these proteins at ER exit sites generates a COPII-coated vesicle with an average diameter of 60–90 nm (Jensen and Schekman, 2011). It has remained unclear, however, how the COPII machinery generates larger carriers to accomplish the transport of bulky cargoes, such as procollagens. In a new paper, Rape and colleagues provide an important clue to this puzzle (Jin et al., 2012). They report that the ubiquitin ligase Cul3 and its specific BTB-containing adaptor protein KLHL12 monoubiquitinate the COPII component Sec31 to drive assembly of large coats to accommodate the secretion of collagen I and IV.

Vertebrates secrete many different kinds of collagens, which have critical roles in chondrocyte development and mineralization of the bone and in the assembly of extracellular matrix that facilitates cell-cell interactions, signaling, and organization. For a number of reasons, it is unlikely that procollagen export is independent of COPII-mediated secretion. Mutations in Sec23A that affect interaction with Sec31 affect procollagen export from the ER (Kim et al., 2012); depletion of Sec13/31 inhibits collagen secretion in fibroblasts and causes skeletal deformation in zebrafish (Townley et al., 2008); structural studies of the individual COPII components and their assemblies in vitro has revealed that binding of Sec13/31 to Sec23/24 has the essential properties to generate a structure big enough to encapsulate procollagens (Stagg et al., 2008). Yet, it has remained unclear how cells use the same COPII components to generate megacarriers for procollagen export as for the secretion of much smaller cargo.

Jin et al. show that the creation of megacarriers involves the monoubiquitination of Sec31 by KLHL12-Cul3. They demonstrate that depletion of Cul3 by small interfering RNA or short hairpin RNA in mouse embryonic stem cells inhibits collagen IV secretion. Conversely, overexpression of KLHL12 in the transformed human fibroblast line IMR90 results in elevated secretion of procollagen type I. In cells overexpressing KLHL12, Sec31 and KLHL12 are observed attached to large spherical membranes of 200–500 nm internal diameter. These findings suggest that monoubiquitination of Sec31 somehow results in the generation of larger Sec31- and KLHL12-coated membranes that permit secretion of collagens.