COMMENTARY

Neurogenic Evangelism: Comment on Urbach et al. (2013)

Paul W. Frankland
The Hospital for Sick Children, Toronto, Canada, and University of Toronto, Canada

The continued production of new dentate granule cells throughout life has led to the idea that neurogenesis critically modulates hippocampal memory function. Consistent with this view, many studies have shown that experimental reduction of adult neurogenesis impairs hippocampal memory formation. However, impairments are not universally observed following suppression of adult neurogenesis. A new article by Urbach et al. falls into this latter category. A global deletion of the cyclin D2—a cell cycle regulation gene—induces a profound reduction in adult neurogenesis. Yet, despite this reduction, this knockout mouse was able to acquire a hippocampus-dependent spatial memory normally. These results suggest that ongoing adult neurogenesis is not necessary for many forms of hippocampus-dependent learning, and in this commentary, I discuss the implications of these types of results for the field.

Keywords: Barnes maze, adult neurogenesis, memory, hippocampus, cyclin D2

The discovery that new neurons are generated in the adult hippocampus has led to the popular idea that they are critical for memory function. Even within the field of adult neurogenesis there tends to be a mood of unwavering optimism: New neurons are good! Increasing their production must boost memory function. Decreasing their production must, conversely, impair memory. The danger of this *neurogenic evangelism* (a term a colleague of mine coined) is that it becomes tempting to ignore studies that do not fit snugly into this simple framework. A new article by Urbach and colleagues (2013) represents one such study, and in this commentary I will discuss what I think their findings mean for the field of neurogenesis and memory.

In exploring the relationship between adult hippocampal neurogenesis and memory, the vast majority of studies have conducted the following kind of experiment: first, in adult rodents, neurogenesis is up- or down-regulated, and then some time later (usually a few weeks) hippocampus-dependent memory formation is evaluated. Urbach and colleagues (2013) follow this now well-trodden path. In their case, they used mice lacking the gene for cyclin D2—a key cell cycle regulator. They found that this genetic manipulation powerfully suppressed neurogenesis in adult mice (Jaholkowski et al., 2009). They went on to test spatial memory in these mice and found, maybe surprisingly, it essentially unaltered. The spatial memory task they used was a Barnes maze, in which mice learn to locate an escape route in a circular arena. Detailed analysis of the behavior indicated that cyclin D2 knockout mice

acquired this form of spatial learning as quickly as controls, used equivalently "spatial" search strategies in finding the target hole and showed equivalent spatial bias in a probe test conducted at the end of training (Urbach et al., 2013). So, despite having no adult neurogenesis, cyclin D2 knockout mice were quite able to form a spatial memory.

How general are these effects? It might be easy to dismiss the findings of this article had the analyses been limited to single, albeit unquestionably hippocampus-dependent, paradigm. However, this is not the case. This work builds on previous articles from the same group where they tested the same mice in a collection of other hippocampus-dependent tasks (Jaholkowski et al., 2009; Jedynak et al., 2012). Similar to the results in the Barnes maze they observed normal learning in the water maze and contextual fear conditioning and even enhanced learning in a trace fear conditioning paradigm. The mice were additionally tested in assays of sensorimotor function and emotion and, again, passed with flying colors. These findings are not unprecedented either. There's a sizable (and growing) collection of articles that come to exactly the same conclusion as Urbach and colleagues: That suppressing ongoing adult neurogenesis does not always lead to impaired hippocampus-dependent memory formation (e.g., Arruda-Carvalho, Sakaguchi, Akers, Josselyn, & Frankland, 2011; Denny, Burghardt, Schachter, Hen, & Drew, 2012; Drew, Denny, & Hen, 2010; Martinez-Canabal, Akers, Josselyn, & Frankland, 2012; Meshi et al., 2006; Snyder, Hong, McDonald, & Wojtowicz, 2005).

These types of results challenge the conventional wisdom—that ongoing adult neurogenesis is required for normal hippocampus-dependent memory formation. So, where does this leave us? Are articles such as Urbach et al. the exception or the rule? How might we account for the absence of effects of suppressing adult neurogenesis on memory in this study?

Correspondence concerning this article should be addressed to Paul W. Frankland, Program in Neurosciences and Mental Health, The Hospital for Sick Children, Departments of Psychology and Physiology, University of Toronto, Toronto, Ontario, Canada M5G 1X8. E-mail: paul.frankland@sickkids.ca

Right Approach, Wrong Behavior?

This article uses two key methodologies: first, a genetic approach to experimentally reduce adult neurogenesis, and, second, behavioral analyses to evaluate the impact on memory function. We'll start with the approach taken to reduce neurogenesis. A wide range of approaches have been used to experimentally manipulate adult neurogenesis ranging from chemical (e.g., antimitotic agents such methylazoxymethanol acetate [MAM]) to irradiation to genetic. In the current study, they used mice where the cell cycle regulator gene, cyclin D2, is deleted in all tissues and throughout development. They concede that there are possible "off-target" effects of this manipulation. Furthermore, more targeted genetic ablation strategies are widely used that allow the specific elimination of neural precursor cells and their progeny (Imayoshi, Sakamoto, & Kageyama, 2011). However, because the mice learned normally in a number of different situations, it is hard to imagine how a potential lack of manipulation specificity could confound the interpretation of the results. Is the cyclin D2 associated reduction in adult neurogenesis sufficient in magnitude? If neurogenesis was only modestly reduced, then it might be trivial to find hippocampus-dependent memory function unaffected. However, this doesn't appear to be the case—previous analyses of these mice revealed an approximate 95% reduction in neurogenesis (measured by numbers of cells expressing the immature neuronal marker, DCX; Jaholkowski et al., 2009). This is a sizable reduction, matching or exceeding what is observed using other manipulations.

So, if the approach to reducing adult neurogenesis is adequate, then what about the behavior? The initial observations of adult neurogenesis in the hippocampus were made in the 1960s (Altman, 1962, 1963; Altman & Das, 1965). However, it was not until 30 years later, and the advent of more efficient techniques for labeling and phenotyping adult-generated neurons (Cameron, Woolley, McEwen, & Gould, 1993), that interest in adult neurogenesis was rekindled. Soon after, attention quickly turned to the question of how new neurons in the hippocampus might impact behavior, and in particular, memory function. These studies, not unreasonably, asked whether reducing adult neurogenesis would produce similar deficits to those observed after hippocampal lesions. The first study of this kind used MAM to reduce adult neurogenesis in rats and showed that the acquisition of trace eyeblink conditioning was blocked (Shors et al., 2001). Subsequent studies used other popular hippocampus-dependent paradigms, including various forms of spatial learning, fear learning, and recognition tasks (Deng, Aimone, & Gage, 2010).

While these were the tasks of choice initially, more recently there's been a shift toward tasks that emphasize spatial discrimination. This is mainly motivated by recognition that dentate gyrus may be important for pattern separation (Treves & Rolls, 1994)—the process of transforming overlapping input representations into less overlapping or distinct output representations—and, by extension, that dentate neurogenesis may modulate the efficiency of such a process (Sahay, Wilson, & Hen, 2011). Indeed, genetic or chemical suppression of adult neurogenesis impairs population coding of similar (but not dissimilar) contexts in the CA3 region of the hippocampus, consistent with the idea that perturbing hippocampal neurogenesis perturbs a pattern separation-like process in the hippocampus (Niibori et al., 2012). To study behavioral correlates of pattern separation a variety of tasks have been used. These include contextual fear discrimination (where animals learn to discriminate between similar vs. dissimilar contexts), radial-arm maze (where animals learn to discriminate adjacent vs. nonadjacent arms), and touchscreen-based operant tasks (where animals learn to discriminate close vs. distant locations on a touchscreen). In these tasks, suppression of adult neurogenesis usually impedes discrimination (Clelland et al., 2009; Kheirbek, Tannenholz, & Hen, 2012; Sahay et al., 2011; Tronel et al., 2012), whereas promoting adult neurogenesis may facilitate discrimination (Nakashiba et al., 2012; Sahay, Scobie, et al., 2011). Therefore, it is plausible that testing cyclin D2 knockout mice in these tasks would reveal deficits relevant to reduced adult neurogenesis. Whether or not any potential deficits in these paradigms would reflect deficient pattern separation is less clear, however. For example, it is questionable as to whether discriminating between two similar contexts or between two adjacent arms in the radial arm maze engages a pattern separation process any more than discriminating between an array of potential escape holes in the Barnes maze. Clearly, these tasks differ in several dimensions (e.g., difficulty), and therefore it is possible that dimensions other than dependency on pattern separation makes these paradigms sensitive to fluctuations in levels of adult neurogenesis.

Wrong Approach, Right Behavior?

Let's instead consider the alternate possibility that there is nothing wrong with the behavior. The behavioral analyses of these mice—spanning both the current article as well as a previous article—are certainly comprehensive, encompassing many of the paradigms that have been used (successfully) in these types of studies. Moreover, they have been conducted thoughtfully, with careful attention to detail. Maybe it's something about the approach used to suppress neurogenesis?

I've already covered issues of specificity and magnitude of the decrease in neurogenesis in the cyclin D2 knockout mice, and neither of these can account for the absence of memory phenotype. What other features of the approach might account for normal memory? The strategy that Urbach et al. (2013) adopted is the strategy that has been used in most (if not all) previous studies: That is, they have examined the impact of reducing neurogenesis on subsequent learning. The limitation of this type of pretraining manipulation is that it leaves open the possibility of compensation: Adult-generated neurons represent only a small fraction of all dentate granule cells, and when they're removed before learning, there's huge potential for compensation by the large numbers of remaining developmentally generated, granule cells. This potential for compensation is further compounded by the fact that adult-generated and developmentally generated neurons converge on similar cellular phenotypes: While adult-generated cells are transiently more excitable and more plastic, they eventually assume similar physiological and morphological properties to those generated earlier on during development (Ge, Yang, Hsu, Ming, & Song, 2007; Gu et al., 2012; Laplagne et al., 2006; Marin-Burgin, Mongiat, Pardi, & Schinder, 2012; Mongiat, Esposito, Lombardi, & Schinder, 2009; Stone et al., 2011). Therefore, developmentally generated cells can be thought of as perfect substitutes for (at least mature) adult-generated cells.

One way to circumvent this potential for compensation is to target adult-generated granule cells only *after* they have had the opportunity to be integrated into a memory trace (i.e., to become memory-committed). Two recent studies have done exactly this. First, Arruda-Carvalho et al. (2011) developed a genetic approach to conditionally ablate populations of adult-generated neurons, and contrasted the effects of removing these cells before or after memory formation. Only removal of cells after training led to memory deficits, suggesting that if new neurons are present at the time of learning (and sufficiently mature), they become integrated into memory traces and come to represent a critical component of that trace. However, if they are absent at the time of learning, then existing granule cells (for the most part generated during development) can do the job. A very similar dissociation between pre- and posttraining manipulations of adult-generated granule cells was found using an optogenetic approach (Gu et al., 2012). Using retroviral vectors to express light-activatable channels only in adult-generated neurons, they found that silencing at the time of retrieval impaired memory expression. Silencing during training failed to impair acquisition, again, presumably, because residual granule cells were able to compensate once adult-generated granule cells had been taken "offline."

Right Conclusion?

Urbach and colleagues conclude their article by saying that adult neurogenesis is not necessary for learning in the Barnes maze. Based on their previous work (Jaholkowski et al., 2009; Jedynak et al., 2012), I am sure they would extend this conclusion to many other forms of hippocampus-dependent paradigms. While this might offend the die-hard evangelist, this is almost certainly the right conclusion when coupled with the two caveats highlighted in this commentary. First, it may be possible to detect memory deficits in cyclin D2 knockout mice using other, more sensitive behavioral paradigms. Nonetheless, it is noteworthy that these mice with greatly reduced adult neurogenesis are not profoundly impacted in hippocampus-dependent memory paradigms. Second, the absence of any profound memory phenotype in these mice certainly does not exclude the likelihood that new neurons *normally* play a role in hippocampal memory formation. However, because of the potential for compensation, manipulating these cells once they are committed to memory storage is perhaps a more powerful approach to reveal their role in hippocampal memory function.

References

Altman, J. (1962). Are new neurons formed in the brains of adult mammals? *Science, 135,* 1127–1128. doi:10.1126/science.135.3509.1127

Altman, J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anatomical Record, 145,* 573–591. doi:10.1002/ar.1091450409

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology, 124,* 319–335. doi:10.1002/cne.901240303

Arruda-Carvalho, M., Sakaguchi, M., Akers, K. G., Josselyn, S. A., & Frankland, P. W. (2011). Posttraining ablation of adult-generated neurons degrades previously acquired memories. *The Journal of Neuroscience, 31,* 15113–15127. doi:10.1523/JNEUROSCI.3432-11.2011

Cameron, H. A., Woolley, C. S., McEwen, B. S., & Gould, E. (1993). Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience, 56,* 337–344. doi:10.1016/0306-4522(93)90335-D

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Jr., Fragniere, A., Tyers, P., . . . Bussey, T. J. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science, 325,* 210–213. doi:10.1126/science.1173215

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nature Reviews Neuroscience, 11,* 339–350. doi:10.1038/nrn2822

Denny, C. A., Burghardt, N. S., Schachter, D. M., Hen, R., & Drew, M. R. (2012). 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. *Hippocampus, 22,* 1188–1201. doi:10.1002/hipo.20964

Drew, M. R., Denny, C. A., & Hen, R. (2010). Arrest of adult hippocampal neurogenesis in mice impairs single- but not multiple-trial contextual fear conditioning. *Behavioral Neuroscience, 124,* 446–454. doi:10.1037/a0020081

Ge, S., Yang, C. H., Hsu, K. S., Ming, G. L., & Song, H. (2007). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron, 54,* 559–566. doi:10.1016/j.neuron.2007.05.002

Gu, Y., Arruda-Carvalho, M., Wang, J., Janoschka, S. R., Josselyn, S. A., Frankland, P. W., & Ge, S. (2012). Optical controlling reveals time-dependent roles for adult-born dentate granule cells. *Nature Neuroscience, 15,* 1700–1706. doi:10.1038/nn.3260

Imayoshi, I., Sakamoto, M., & Kageyama, R. (2011). Genetic methods to identify and manipulate newly born neurons in the adult brain. *Frontiers in Neuroscience, 5,* 64. doi:10.3389/fnins.2011.00064

Jaholkowski, P., Kiryk, A., Jedynak, P., Ben Abdallah, N. M., Knapska, E., Kowalczyk, A, . . . Filipkowski, R. K. (2009). New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. *Learning Memory, 16,* 439–451. doi:10.1101/lm.1459709

Jedynak, P., Jaholkowski, P., Wozniak, G., Sandi, C., Kaczmarek, L., & Filipkowski, R. K. (2012). Lack of cyclin D2 impairing adult brain neurogenesis alters hippocampal-dependent behavioral tasks without reducing learning ability. *Behavioural Brain Research, 227,* 159–166. doi:10.1016/j.bbr.2011.11.007

Kheirbek, M. A., Tannenholz, L., & Hen, R. (2012). NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. *Journal of Neuroscience, 32,* 8696–8702. doi:10.1523/JNEUROSCI.1692-12.2012

Laplagne, D. A., Esposito, M. S., Piatti, V. C., Morgenstern, N. A., Zhao, C., van Praag, H., . . . Schinder, A. F. (2006). Functional convergence of neurons generated in the developing and adult hippocampus. *PLoS Biol, 4,* e409. doi:10.1371/journal.pbio.0040409

Marin-Burgin, A., Mongiat, L. A., Pardi, M. B., & Schinder, A. F. (2012). Unique processing during a period of high excitation/inhibition balance in adult-born neurons. *Science, 335,* 1238–1242. doi:10.1126/science.1214956

Martinez-Canabal, A., Akers, K. G., Josselyn, S. A., & Frankland, P. W. (2013). Age-dependent effects of hippocampal neurogenesis suppression on spatial learning. *Hippocampus, 23,* 66–74. doi:10.1002/hipo.22054

Meshi, D., Drew, M. R., Saxe, M., Ansorge, M. S., David, D., Santarelli, L., . . . Hen, R. (2006). Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat Neurosci, 9,* 729–731. doi:10.1038/nn1696

Mongiat, L. A., Esposito, M. S., Lombardi, G., & Schinder, A. F. (2009). Reliable activation of immature neurons in the adult hippocampus. *PLoS One, 4,* e5320. doi:10.1371/journal.pone.0005320

Nakashiba, T., Cushman, J. D., Pelkey, K. A., Renaudineau, S., Buhl, D. L., McHugh, T. J., . . . Tonegawa, S. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate

pattern completion. *Cell, 149*(1), 188–201. doi:10.1016/j.cell.2012.01 .046

Niibori, Y., Yu, T. S., Epp, J. R., Akers, K. G., Josselyn, S. A., & Frankland, P. W. (2012). Suppression of adult neurogenesis impairs population coding of similar contexts in hippocampal CA3 region. *Nat Commun, 3,* 1253. doi:10.1038/ncomms2261

Sahay, A., Scobie, K. N., Hill, A. S., O'Carroll, C. M., Kheirbek, M. A., Burghardt, N. S., . . . Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature, 472*(7344), 466–470. doi:10.1038/nature09817

Sahay, A., Wilson, D. A., & Hen, R. (2011). Pattern separation: A common function for new neurons in hippocampus and olfactory bulb. *Neuron, 70*(4), 582–588. doi:10.1016/j.neuron.2011.05.012

Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., & Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature, 410*(6826), 372–376. doi:10.1038/35066584

Snyder, J. S., Hong, N. S., McDonald, R. J., & Wojtowicz, J. M. (2005). A role for adult neurogenesis in spatial long-term memory. *Neuroscience, 130*(4), 843–852. doi:10.1016/j.neuroscience.2004.10.009

Stone, S. S., Teixeira, C. M., Zaslavsky, K., Wheeler, A. L., Martinez-Canabal, A., Wang, A. H., . . . Frankland, P. W. (2011). Functional convergence of developmentally and adult-generated granule cells in dentate gyrus circuits supporting hippocampus-dependent memory. *Hippocampus, 21*(12), 1348–1362. doi:10.1002/hipo.20845

Treves, A., & Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus, 4,* 374–391. doi:10.1002/hipo .450040319

Tronel, S., Belnoue, L., Grosjean, N., Revest, J. M., Piazza, P. V., Koehl, M., & Abrous, D. N. (2012). Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus, 22*(2), 292–298. doi: 10.1002/hipo.20895

Urbach, A., Robakiewicz, I., Baum, E., Kaczmarek, L., Witte, O. W., & Filipkowski, R. K. (2013). Cyclin D2 knockout mice with depleted adult neurogenesis learn Barnes maze task. *Behavioral Neuroscience, 127,* 1–8.