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Issues to ponder when correlating hippocampal neurogenesis to a hippocampal-dependent memory function

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Abstract

Simple correlations between the overall hippocampal neurogenesis and the hippocampal-dependent learning and memory functions are common in the neurogenesis field. There is considerable evidence in the literature to link hippocampal neurogenesis to the hippocampal-dependent memory function. However, simple correlations between neurogenesis and memory function, particularly in studies where neither the cause-effect relationship is established nor the other relevant variables are considered, can lead to erroneous conclusions. As reliable and selective neurogenesis ablation techniques are yet to be developed for rat and higher animal models, it is likely that correlative studies between the overall neurogenesis and the memory function will continue in different conditions in these animal models. Such correlations should be acceptable as long as the other variables are considered adequately. Furthermore, in correlative analyses of the learning and memory function with the newly born granule cells, one needs to consider the age of the newly born granule cells because the newly born granule cells will require at least a few weeks of time after their birth to participate in the learning and memory function in rodent models.

Keywords

adult neurogenesis; hippocampal neurogenesis; doublecortin; newly born neurons; learning and memory; neurogenesis and behavior

The commentary, “*Relating hippocampal neurogenesis to behavior: the dangers of ignoring confounding variables*” by Lazic (2010) raises interesting properties of the data pertaining to an association between the status of the hippocampal neurogenesis and a working memory function, which was illustrated in the original article, “*Abnormal differentiation of newborn granule cells in age-related working memory impairments*” (see Nyffeler et al., 2010).

Nyffeler and colleagues illustrate an association between the working memory index (depicted by average escape latencies from trials 2–4 of the working memory task in a water maze) and the numbers of doublecortin (DCX) immunopositive newly born neurons in the subgranular zone-granule cell layer (SGZ-GCL) of individual subjects belonging to three different age groups of rats (3-month old, n=4; 6-months old, n=4; and 24 months old, n=8). The 24 months

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old group comprised old-good (n=4) and old-bad (n=4) subgroups based on their relative ability for a working memory function. By comparing across the three age-groups of rats (young adult, adult, and aged) using a linear correlation analysis, the authors concluded that better working memory performance is associated with the greater numbers of DCX+ newly born neurons (see Fig. 6[F] in Nyffeler et al., 2010). The commentary by Lazic selects these data as an example of an inappropriate correlation analysis because it did not take into account “the age of the animal” as an important variable. Through additional analyses Lazic demonstrates that, within group associations in three out of the four groups imply a sharply contrasting inference that the working memory deficit is correlated with the greater numbers of DCX+ newly born neurons (Lazic, 2010). This is an interesting and important observation.

The most important assertion made by Lazic (2010) that one needs to take into account other significant variables (which is age in Nyffeler et al, 2010) in correlation analysis is valid. Indeed, if Nyffeler et al (2010) had performed correlation analysis within the aged group (comprising both old-good and old-bad subgroups), it would not have given the broader inference (as implied in the legend for Fig. 6F) that the working memory deficit is associated with the lower numbers of newly born neurons. Although Nyffeler et al (2010) showed that the numbers of DCX+ neurons did not statistically differ between the old-good and the old-bad subgroups ($p>0.05$), they have somewhat emphasized on the trend that the old-good subgroup comprised more DCX+ neurons than the old-bad subgroup. Such links are fraught with danger when comparisons involve smaller sample sizes such as the n=4 employed in the study by Nyffeler et al (2010).

Lazic (2010) also points out another interesting observation that simple correlations between neurogenesis and the hippocampal-dependent learning and memory function are common in the neurogenesis field, which is true if one takes the statistics of all articles published in this exciting and the fastest growing field of neuroscience. One of the likely reasons for linking neurogenesis with the memory in most studies (i.e. in the absence of any cause-effect experiments) is that the overall knowledge pertaining to the role of hippocampal neurogenesis in the learning and memory function has progressed significantly in the past 4–5 years (see the recent review by Deng et al., 2010 for details). For example, there is now credible evidence that the spatial learning in the water maze increases the survival of new granule cells that were born one-week prior to the commencement of the water maze training but promotes the apoptosis of new granule cells that were born 3 days before the onset of the water maze training and new granule cells that were born in the early segment of the 6-day water maze training (Dobrossy et al., 2003; Dupret et al., 2007; Tronel et al., 2010). Interestingly, preventing apoptosis of the new granule cells that were born 3 days prior to the water maze training or granule cells that were born in the later stage of the water maze learning thwarts not only the increased survival of new granule cells that were born 7 days prior to the onset of the training but also diminishes the overall learning performance in the water maze (Dupret et al., 2007; Tronel et al., 2010). These results imply that learning astutely inserts or eliminates newly born granule cells depending on their stage of the development and the functional importance, and that the populations of new granule cells rescued are likely those that are successfully integrated into the learning and memory circuitry (Tronel et al., 2010, Deng et al., 2010). Furthermore, a memory retrieval task after the water maze training preferentially activates the new granule cells that are ~4–6 weeks old at the time of learning the task and ~10 weeks old at the time of a memory retrieval test in mice (Kee et al., 2007). Moreover, computational models suggest a role for newly born granule cells in the encoding of temporal information as well as in the maintenance of old memories during the encoding of new information (see Aimone et al., 2006, 2009; Deng et al., 2010). Likewise, two recent studies utilizing a specific genetic ablation technique demonstrated spatial learning deficits with the loss of hippocampal neurogenesis (Dupret et al., 2008; Zhang et al., 2008). These studies have received wider attention because the genetic ablation of neurogenesis is considered superior to the previously employed

neurogenesis ablation techniques using the administration of methylazoxymethanol acetate (MAM) or the brain irradiation (Shors et al., 2001, 2002; Madsen et al., 2003). The concerns with the MAM administration or the brain irradiation approaches include the possibility of a collateral hippocampal damage or interference with the hippocampal function via mechanisms that are unrelated to the neurogenesis when treated at higher doses or the possibility of only a partial ablation of the neurogenesis when treated at lower doses. Consistent with the above genetic ablation studies, there are several other studies that implied impairments in the long-term spatial memory function with a significantly reduced hippocampal neurogenesis (Snyder et al., 2005; Imayoshi et al., 2008; Zhang et al., 2008; Jessberger et al., 2009). A recent study suggests that hippocampal neurogenesis is also important for the spatial pattern separation function (Clelland et al., 2009).

Thus, there is considerable evidence in the literature to link the hippocampal neurogenesis with the hippocampal-dependent memory function (see Leuner et al., 2006; Deng et al., 2010 for details on this issue). However, it is debatable whether this evidence is adequate enough to make a simple correlation between the neurogenesis and the memory function, particularly in studies where neither the cause-effect relationship is established nor the other relevant variables are considered. In neurogenesis studies performed in rat or primate models, a lack of appropriate techniques for selectively ablating or diminishing the overall neurogenesis without compromising the rest of the hippocampal cytoarchitecture has also contributed to the higher incidence of correlative studies between the neurogenesis and the memory function. The more reliable genetic ablation approaches are currently confined to only the mouse models, as transgenic rat models that are amenable for neurogenesis ablation are yet to be developed. On the other hand, it can be argued that studying neurogenesis in rats and non-human primates is more appropriate for understanding the role of neurogenesis in humans, as these species are considered much closer than mice to humans. For example, a recent study has shown that the young neurons in adult rats display a mature neuronal marker profile and an activity-induced immediate early gene expression 1–2 weeks earlier than those in mice, and newly born neurons in rats are 10 times more likely to be recruited into the learning circuits and the overall contribution of the young neurons to a fear memory is much greater in rats than in mice (Snyder et al., 2009). Thus, until selective neurogenesis ablation techniques are available, it is likely that correlative analysis between the overall neurogenesis and the memory function or the extent of neurogenesis and mood/anxiety function (Sahay and Hen, 2007) will continue in the hippocampal neurogenesis studies performed in rats and higher animal models. This should be acceptable as long as the other variables are considered adequately.

Another issue is whether it is appropriate to correlate the total numbers of DCX+ newly born neurons with the memory function, as illustrated in Nyffeler et al (2010). While quantifying the DCX+ immature neurons in the SGZ-GCL is indeed useful for gauging the status of the ongoing neurogenesis in different conditions (Rao and Shetty, 2004), it is unclear whether newly born neurons in the DCX expression phase have any role in encoding of the memory. This is because DCX+ neurons represent immature neurons, and a vast majority of which are likely to be younger than two weeks old in rats (Rao and Shetty, 2004; Rao et al., 2005, 2006). In mice, the events in the first week after birth for newly generated granule cells mostly comprise their short-distance migration into the GCL from the neurogenic SGZ and their tonic activation by the ambient GABA (Esposito et al., 2005; Ge et al., 2006; Snyder et al., 2009; Deng et al., 2010). The growth of dendrites into the molecular layer and axons into the dentate hilus and the CA3 region (Zhao et al., 2006), and establishment of afferent synaptic connectivity from the hippocampal GABA-ergic interneurons occur in the second week after birth (Ge et al., 2006; Overstreet Wadiche et al., 2005; Markwardt et al., 2009). However, they exhibit some distinct physiological properties during the second week after birth, which include higher membrane resistance and firing properties, and a GABA-mediated depolarization (Esposito et al., 2005; Ge et al., 2006). At this stage of the development, they also display

hyperexcitability during the water maze learning and hence are likely amenable to be influenced by the learning task or to be recruited to memory circuits (Deng et al., 2010). A recent study demonstrates that a spatial learning task accelerates the maturation of dendritic trees of the newly born neurons even when they are in the immature DCX expression phase, which eventually leads to a faster integration of the newly born neurons into the hippocampal network (Tronel et al., 2010). Thus, while the survival and dendritic growth of the newly born neurons in the DCX expression stage can be influenced by a learning task, there is no conclusive evidence hitherto to support a role for DCX-expressing newly born neurons in encoding of the memory.

From the above perspectives, it emerges that in correlative analysis of the learning and memory function with the newly born granule cells, one need to consider the age of the newly born granule cells. Based on studies that examined an association between the expression of immediate early genes such as c-fos and arc and the learning and memory function, it appears that the newly born granule cells need at least over 2 weeks of time after their birth to participate in the learning and memory function in rats and mice (Kee et al., 2007; Snyder et al., 2009). As most newly born granule cells lose their DCX expression after two weeks of birth (Rao and Shetty, 2004) and a learning task itself can alter the survival of different fractions of DCX+ granule cells in distinct stages of the development (Tronel et al., 2010), correlating the total numbers of DCX+ newly born neurons with the memory function does not seem appropriate for ascertaining the role of neurogenesis in the memory function in different experimental conditions.

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References

- Aimone JB, Wiles J, Gage FH. Potential role for adult neurogenesis in the encoding of time in new memories. *Nat Neurosci* 2006;9:723–727. [PubMed: 16732202]
- Aimone JB, Wiles J, Gage FH. Computational influence of adult neurogenesis on memory encoding. *Neuron* 2009;61:187–202. [PubMed: 19186162]
- Deng W, Aimone JB, Gage FH. New neuron and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nature Review Neurosci* 2010;11:339–350.
- Döbrössy MD, Drapeau E, Aourasseau C, Le Moal M, Piazza PV, Abrous DN. Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Mol Psychiatry* 2003;8:974–982. [PubMed: 14647395]
- Dupret D, Fabre A, Döbrössy MD, Panatier A, Rodríguez JJ, Lamarque S, Lemaire V, Oliet SH, Piazza PV, Abrous DN. Spatial learning depends on both the addition and removal of new hippocampal neurons. *PLoS Biol* 2007;5(8):e214. [PubMed: 17683201]
- Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN, Piazza PV. Spatial relational memory requires hippocampal adult neurogenesis. *PLoS ONE* 2008;3(4):e1959. [PubMed: 18509506]
- Espósito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J Neurosci* 2005;25:10074–10086. [PubMed: 16267214]
- Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 2006;439:589–593. [PubMed: 16341203]
- Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itohara S, Kageyama R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci* 2008;11:1153–1161. [PubMed: 18758458]

- Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, Squire LR, Gage FH. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem* 2009;16:147–154. [PubMed: 19181621]
- Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat Neurosci* 2007;10:355–362. [PubMed: 1727773]
- Lazic SE. Relating hippocampal neurogenesis to behavior: the dangers of ignoring confounding variables. *Neurobiol Aging*. 2010 In press.
- Leuner B, Gould E, Shors TJ. Is there a link between adult neurogenesis and learning? *Hippocampus* 2006;16:216–224. [PubMed: 16421862]
- Madsen TM, Kristjansen PE, Bolwig TG, Wortwein G. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* 2003;119:635–642. [PubMed: 12809684]
- Markwardt SJ, Wadiche JI, Overstreet-Wadiche LS. Input-specific GABA-ergic signaling to newborn neurons in adult dentate gyrus. *J Neurosci* 2009;29:15063–15072. [PubMed: 19955357]
- Nyffeler M, Yee BK, Feldon J, Knuesel I. Abnormal differentiation of newborn granule cells in age-related working memory impairments. *Neurobiol Aging*. 2010 [Epub ahead of print, Dec 17, 2008].
- Overstreet Wadiche L, Bromberg DA, Bensen AL, Westbrook GL. GABA-ergic signaling to newborn neurons in dentate gyrus. *J Neurophysiol* 2005;94:4528–4532. [PubMed: 16033936]
- Rao MS, Shetty AK. Efficacy of doublecortin as a marker to analyze the absolute number and dendritic growth of newly generated neurons in the adult DG. *Eur J Neurosci* 2004;19:234–246. [PubMed: 14725617]
- Rao MS, Hattiangady B, Abdel-Rahman A, Stanley DP, Shetty AK. Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. *Eur J Neurosci* 2005;21:464–476. [PubMed: 15673445]
- Rao MS, Hattiangady B, Shetty AK. The window and mechanisms of major age-related decline in the production of new neurons within the dentate gyrus of the hippocampus. *Aging Cell* 2006;5:545–558. [PubMed: 17129216]
- Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci* 2007;10:1110–1115. [PubMed: 17726477]
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001;410:372–376. [PubMed: 11268214]
- Shors TJ, Townsend DA, Zhao M, Kozorovitsky Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 2002;12:578–584. [PubMed: 12440573]
- Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM. A role for adult neurogenesis in spatial long-term memory. *Neuroscience* 2005;13:843–852. [PubMed: 15652983]
- Snyder JS, Choe JS, Clifford MA, Jeurling SI, Hurley P, Brown A, Kamhi JF, Cameron HA. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. *J Neurosci* 2009;29:14484–14495. [PubMed: 19923282]
- Tronel S, Fabre A, Charrier V, Olier SH, Gage FH, Abrous DN. Spatial learning sculpts the dendritic arbor of adult-born hippocampal neurons. *Proc Natl Acad Sci USA* 2010;107:7963–7968. [PubMed: 20375283]
- Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behavior. *Nature* 2008;451:1004–1007. [PubMed: 18235445]
- Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 2006;26:3–11. [PubMed: 16399667]