Cognitive enhancing effects of voluntary exercise, caloric restriction and environmental enrichment: a role for adult hippocampal neurogenesis and pattern separation?

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Abstract

Several behavioural interventions, such as physical exercise, dietary restriction, and enriched environments are associated with both improved cognition and increased adult hippocampal neurogenesis. Whether the learning and memory improvements associated with these interventions are causally dependent on the upregulated neurogenesis has not yet been conclusively determined. However, with the accumulating evidence of a role for adult-born hippocampal neurons in spatial pattern separation, it is possible that the improvements in learning and memory result, at least in part, from an improvement in pattern separation. The following review focuses on three major behavioural manipulations associated with cognitive enhancement: voluntary exercise, caloric restriction, and environmental enrichment (including learning), and how increased neurogenesis may contribute to the enhancement by improving pattern separation.

Introduction

There is substantial evidence that two regions of the postnatal mammalian brain, the subgranular zone and subventricular zone, maintain a unique form of plasticity that enables the continual production of new neurons throughout adulthood (see Aimone et al., 2014 for a review; Altman, 1962). This process, referred to as adult neurogenesis, produces new neurons that during their differentiation into mature neurons, possess unique biological properties and eventually become functionally integrated into neural circuits (reviewed by Mongiat & Schinder, 2011).

Because of the well-established role of the hippocampus in learning and memory, the function of adult hippocampal neurogenesis, in particular, has received substantial interest from researchers. Progenitor cells in the subgranular zone of the dentate gyrus (DG) subregion of the hippocampus generate approximately 5000 new neurons each day in 9-10 week old adult rats (Gould et al., 1999). These neurons, produced along the border between the granule cell layer and hilus, are initially innervated by septal neurons and mature granule cells, and receive feedback from CA3 pyramidal neurons (Vivar & van Praag, 2013). By one month of age, these new neurons are innervated by cells in the perirhinal cortex and lateral entorhinal cortex (Vivar & van Praag, 2013), grow axons onto target cells in CA3 (Toni et al., 2008; Sun et al., 2013) and CA2 pyramidal neurons (Llorens-Martin et al., 2015), evoke stable action potentials (Gu et al., 2012), and show enhanced synaptic plasticity that is maintained until 7-8 weeks of age (Lemaire et al., 2012). In addition to their unique electrophysiological properties, adult born neurons retain a long-term capacity to alter the shape of their dendritic tree in response to experiences such as spatial learning (Lemaire et al., 2012).
There is accumulating evidence that immature hippocampal neurons make distinct contributions to learning and memory (reviewed by Koehl & Abrous, 2011 and Oomen et al., 2014). For example, in rodents, down-regulating DG neurogenesis can impair performance on hippocampus-dependent tasks such as the Morris Water Maze (e.g., Dupret et al., 2008), trace fear conditioning (e.g., Shors et al., 2002), nonmatching-to-sample (e.g., Winocur et al., 2006), radial-arm maze (e.g., Clelland et al., 2009), contextual fear conditioning (e.g., Pan et al., 2012), and olfactory discrimination (e.g., Luu et al., 2012). In contrast, upregulating DG neurogenesis has been shown to provide cognitive enhancing effects on similar hippocampus-dependent tasks (e.g., Nilsson et al., 1999; Sahay et al., 2011; Marlatt et al., 2012).

In particular, there is accumulating evidence that adult born neurons contribute to DG function by facilitating a specific component of memory processing, pattern separation (Oomen et al., 2014). Pattern separation is a theoretical computational mechanism involving the transformation of input representations into output representations that are less correlated with each other. By transforming similar experiences into discrete non-overlapping representations, pattern separation increases the likelihood of accurate encoding and subsequent retrieval. Some computational models suggest that adult born DG granule cells in particular play an important role in pattern separation (Aimone & Gage, 2011) and there is growing evidence in support of this claim from behavioural experiments (Clelland et al., 2009; Creer et al., 2010; Sahay et al., 2011; Nakashiba et al., 2012; Tronel et al., 2012; Bekinschtein et al., 2014; Kent et al., 2015).

However, whether neurogenesis contributes to cognition at all is a topic of debate, in part because of highly variable results in the literature (Groves et al., 2013). We have pointed out, however, that the source of variability in these studies could be due to differences in the load on pattern separation, which was not manipulated or considered in the majority of studies (Bekinschtein et al., 2011). The finding that several labs have reported neurogenesis reduction-induced impairments and sparing of function, dependant on pattern separation load within the same task (such that other factors are well-controlled) provides good evidence for this idea.

The following review describes some of this behavioural evidence in rodents and suggests that the cognitive enhancing effects of three behavioural interventions—exercise, caloric restriction, and environmental enrichment/learning—may result from improved pattern separation and increased adult hippocampal neurogenesis.

Cognitive enhancing effects of exercise

Accumulating evidence from both human and animal studies shows that exercise can be associated with profound cognitive benefits (reviewed by Hotting & Roder, 2013). In rodents, both voluntary and forced running paradigms are associated with improved learning and memory, increased synaptic plasticity, and increased neurogenesis (reviewed by van Praag, 2008). For example, Merkeley and colleagues (2014) demonstrate that following a running period of 30 days, young rats show pronounced and long-lasting changes in the rate of neuronal maturation and survival up to 9 months after the exercise period has ceased. This increased plasticity is thought to lead to improvements in hippocampal-dependent cognition (Speisman et al., 2013).

In particular, the ability to make fine spatial discriminations resulting from improved pattern separation may underlie the enhancement of spatial memory associated with physical activity. We previously showed that providing adult male mice with a running wheel improved performance on the Location Discrimination (LD) task (Creer et al., 2010). The LD task, conducted using an automated touchscreen apparatus, allows the experimenter to manipulate the similarity between locations on the screen, and thus the load on pattern separation (Oomen et al., 2013; Clelland et
In this study, the ability to discriminate between two locations was shown to be similarity- and neurogenesis-dependent. Adult mice provided with a running wheel had a higher density of newborn neurons in the DG and performed better than controls in the small separation condition. In contrast, there were no group differences in performance in the large separation condition. In the same study, aged mice showed impaired performance on the task and low levels of basal cell genesis. Access to a running wheel was not associated with increased density of newborn neurons in the DG in the aged group. These findings suggest that exercise alone, without enhanced neurogenesis, was not sufficient to improve performance on the task with a high load on pattern separation.

These findings support other claims of a positive correlation between physical activity, neurogenesis, and performance on spatial maze tasks (Speisman et al., 2013). However, studies in which newly born DG cells have been ablated have yielded conflicting results. Irradiation in mice selectively blocked exercise-induced enhancement of water maze learning, but not contextual fear conditioning (Clark et al., 2008), whereas others have reported the opposite effects (Wojtowicz, Askew, & Winocur, 2008). The discrepant findings may partially result from differing requirements for spatial pattern separation, a factor that was not explicitly manipulated in these studies. As the requirement for pattern separation increases, newly born neurons may become more important. It may therefore be crucial, in such studies, to ensure that similarity of to-be-remembered stimuli is manipulated, ideally parametrically (Oomen et al., 2014).

It should be noted that increased physical activity is also associated with several other functional changes, such as increased neurotrophin gene expression (Lee et al., 2012a), angiogenesis (Pereira et al., 2007), dendritic spine density (Stranahan, Khalil, & Gould, 2007), and changes in neurotransmitters (reviewed by Lin & Kuo, 2013). Thus, it is possible that a combination of increases in both neurotrophin signaling and adult hippocampal neurogenesis, are responsible for exercise-dependent enhancement in pattern separation. Evidence for this idea comes from experiments done using the Spontaneous Location Recognition task (SLR; Figure 1a), developed to evaluate spatial pattern separation (Bekinschtein et al., 2013). Performance on SLR is sensitive to both levels of BDNF and hippocampal neurogenesis, such that rats with inhibited BDNF or DG neurogenesis were impaired only in conditions with a high load on pattern separation (Bekinschtein et al., 2013; 2014). Furthermore, we found that BDNF exogenously injected into the DG improved performance, again only in conditions with a high load on pattern separation (Bekinschtein et al., 2013; 2014). This enhancement was found to be dependent upon the presence of immature adult-born neurons (Bekinschtein et al., 2013, 2014). Thus, physical exercise could be enhancing pattern separation through increases in BDNF, neurogenesis or both.

Interestingly, Akers and colleagues (2014) recently demonstrated that an increase in neurogenesis produced by exercise during the retention interval in contextual fear memory and water maze paradigms decreased memory performance (*Akers et al., 2014). However, exercise prior to conditioning did not affect memory. This study shows that under at least some specific circumstances, exercise can increase neurogenesis, yet impair memory.

Cognitive enhancing effects of caloric restriction

Another behavioural intervention that is associated with both cognitive enhancing effects and increased neurogenesis is caloric restriction. The cognitive benefits following a reduction in daily food intake was one of the earliest clues for the important relationship between metabolic state and cognitive functioning. Consistently, caloric restriction has been shown to enhance cognitive performance, increase the production and survival of adult-born neurons, and exert
neuroprotective effects in animal models of aging and neurodegenerative disease (reviewed by Gillette-Guyonnet & Vellas, 2008).

One mechanism by which such cognitive enhancing effects may occur is via hormones associated with caloric restriction, such as the peptide hormone ghrelin. Ghrelin is predominately known for its growth hormone releasing and orexigenic properties, acting in the pituitary and hypothalamus to regulate energy homeostasis, appetite, body weight, and adiposity (reviewed by Chen et al., 2009). Circulating ghrelin levels increase during periods of fasting and caloric restriction (Lutter et al., 2008).

Recently, the peptide’s extra-hypothalamic actions and additional biological functions have been identified, such as pro-cognitive, antidepressant, and neuroprotective effects (reviewed by Andrews, 2011). Consistently, intracranial infusions and systemic ghrelin treatments have been shown to have beneficial mnemonic effects in rodents, affect measures of hippocampal synaptic plasticity, and increase hippocampal cell proliferation and neurogenesis (Chen et al., 2012; Zhao et al., 2014).

To help elucidate the cognitive enhancing effects of ghrelin, we administered a treatment of acyl-ghrelin, peripherally, to adult rats (*Kent et al., 2015). We tested spatial memory using the SLR task and compared performance of the ghrelin-treated rats to a saline-treated control group. In our study, the ghrelin-treated rats had a 58% increase in the number of new adult-born neurons in the DG, compared to the saline-treated rats. The ghrelin-treated group demonstrated successful spatial memory encoding and retrieval in the most challenging condition of SLR, whereas the saline-treated group performed only at chance level (Figure 1B). Behavioural testing took place 22 days after the first acyl-ghrelin injection and at least 8 days after the final injection, in order to evaluate the long-term effects of elevating acyl-ghrelin levels, instead of acute effects. Because circulating levels of ghrelin were no longer elevated during the time of testing, it suggests that the improved performance on SLR was a result of lasting changes in the brain, such as the increased level of newborn brain cells in the DG.

Another possible mediator of the cognitive enhancing effects of caloric restriction is BDNF (Lee et al., 2002b). Caloric restriction has been shown to increase BDNF expression and the number of newly generated cells (Lee et al., 2000). Thus caloric restriction could lead to upregulation of BDNF, which could in turn lead to upregulation of neurogenesis. This upregulation of neurogenesis could then improve cognition possibly through improving pattern separation, for the reasons given above. Note that this mechanism of BDNF is different from the acute effects of BDNF on pattern separation in the Bekinschtein et al. (2013) study described previously.

Cognitive enhancing effects of environmental enrichment including learning

Finally, we focus here on the enhancing effects of environmental enrichment on cognition (see Gage et al., this issue). It has been shown that exposure to a cognitively challenging and stimulating environment enhances subsequent learning and memory performance (e.g., Wainwright et al., 1993; Kempermann, Kuhn, & Gage, 1998; Nilsson et al., 1999), and that training in explicit learning tasks can also act as a cognitive enhancer as measured by performance on subsequent tests (e.g., Light et al., 2010; Nokia et al., 2012). Evidence suggests that upregulated hippocampal neurogenesis may underlie these cognitive enhancing effects of environmental enrichment and learning (Gould et al, 1999; Nokia et al., 2012; *Clemenson et al. 2014).
For the effects of enrichment, rodent studies show that environmental enrichment can enhance cognitive performance on subsequent learning and memory tests, and increase adult neurogenesis (Nilsson et al., 1999). Some have argued that the benefits of environmental enrichment may be due to increased physical activity (e.g., Mustroph et al., 2012); however, there is some evidence that environmental enrichment and exercise have different effects on adult-born neurons, such that environmental enrichment increases survival, whereas exercise leads to an increase in proliferation (reviewed by Olson et al., 2006). Recently, *Clemenson and colleagues (2014)* reported that mice with increased neurogenesis due to environmental enrichment had an improved ability to discriminate between similar contexts, whereas mice with increased neurogenesis after being provided a running wheel, without further enrichment, did not show this enhancement. This suggests that there may be a fundamental functional difference between neurogenesis induced by different methods (i.e., exercise or environmental enrichment), and its role in pattern separation.

With respect to the effects of explicit learning tasks on neurogenesis and cognition, enhanced neurogenesis has been demonstrated in some (e.g., Gould et al, 1999) but not all (e.g., Van der Borght et al, 2005) studies. For example, Gould and colleagues (1999) were the first to report that learning in the Morris Water Maze resulted in a three-fold increase in the survival of neurons born one week before training. Since this initial study, others have replicated the finding, demonstrating an increase in DG neurogenesis after maze training (e.g., Sisti, Glass, & Shors, 2007).

The effect on cognition of previous learning was tested by Light and colleagues (2010), who demonstrated that training mice on two distinct eight-arm radial mazes was associated with better performance on a subsequent battery of five independent learning tasks. Similarly, Nokia and colleagues (2012) trained rats on one of two hippocampal-dependent associative learning tasks, and found that this training facilitated performance on the subsequent task when tested one week later. They also demonstrated that there was a positive impact on neuronal survival for cells generated after learning. However, several others have found no effect of training on hippocampal-dependent tasks or on levels of new DG neurons. In some cases even the opposite association has been demonstrated, such that Morris Water Maze training caused a reduction in neurogenesis (e.g., Mohapel et al., 2006).

One of the reasons for the contradictory results may be that Morris Water Maze involves both physical activity and stress, factors that are known to modulate adult hippocampal neurogenesis as well (Ehninger & Kempermann, 2006). For example, adult-born neurons have been shown to be involved in hypothalamic-pituitary-adrenal (HPA) axis regulation (Snyder et al., 2011), and through this, may potentially control stress hormone (e.g., corticosterone) levels. As corticosterone is known to be a potent modulator of memory, this may be a way by which neurogenesis changes memory performance.

Another possibility is the inconsistency of setups, protocols, and parameters, and age of new cells during training (Epp et al., 2010), which can also affect DG neurogenesis, independent of learning. Furthermore, while the Morris Water Maze has proven to be an effective testing paradigm for hippocampus-dependent spatial memory, it may not be the ideal behavioural test for examining the role of new adult born neurons (*Garthe et al., 2014*), in part also because the varying protocols may change the load on pattern separation (Bekinschtein et al., 2011).

Conclusions
Adult hippocampal neurogenesis is positively correlated with exercise, caloric restriction, and environmental enrichment, all of which are also associated with cognitive enhancement. Therefore, at least in animal models, the cognitive enhancing effects may be driven by increases in adult hippocampal neurogenesis and -- given the evidence for a role for adult-born neurons in pattern-separation -- at least some of these effects may be due specifically to improvements in this function, and some of the discrepancies in the literature due to differences in the load on pattern separation in the behavioural paradigms used.

These speculations could be the basis of future experiments. If the cognitive enhancement associated with these interventions results from heightened pattern separation, then we would expect improved performance on tasks requiring the discrimination between similar stored representations. For example, we would expect improved performance on tasks requiring a subject to discriminate between similar spatial locations in a radial arm maze, open field, or on a touchscreen, such as those described above (Clelland et al., 2009; Bekinschtein et al., 2014; Creer et al., 2010), and on tasks requiring the subject to discriminate between similar contexts such as in modified contextual fear conditioning paradigms that use similar shock and no-shock contexts (Nakashiba et al., 2012). We would not expect to see improvements on tasks requiring the discrimination between dissimilar representations, such as the standard contextual fear conditioning paradigm that uses two distinct chambers. If the improved pattern separation associated with these interventions occurs in human subjects as well, then we would also expect performance to improve on tasks requiring subjects to discriminate between similar representations, such as discriminating between repeated stimuli, novel stimuli, and perceptually similar lure stimuli, which is a common paradigm used for studying pattern separation in humans (Kirwan & Stark, 2007).

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Figure 1. Modified with permission from Kent et al., (2015). A. Schematic of Spontaneous location recognition (SLR) task showing the small (top) and x-small (bottom) conditions. Each trial contains two phases, separated by 24 h: Sample Phase and Choice Phase. B. Discrimination ratios during the test phase for ‘novel’ conditions in the SLR task. **p<0.0001, one-way repeated measures ANOVA followed by Bonferroni post-hoc comparisons, n=12 per group.

10^* References


The authors present a series of experiments demonstrating that increasing neurogenesis through either voluntary running or memantine treatments in adult mice, after the formation of memories, was sufficient to induce forgetting in contextual fear conditioning and water maze paradigms. In infant mice, decreasing neurogenesis after memory formation mitigated forgetting. They also examined infantile forgetting and compared neurogenesis levels in infant guinea pigs and degus, which had lower levels of postnatal hippocampal neurogenesis and normal memory retention; however, after increasing neurogenesis the authors were able to induce forgetting in these species.

Bekinschtein, P., Kent, B. A., Oomen, C. A., Clemenson, G. D., Gage, F. H., Saksida, L. M., & Bussey, T. J. (2014). Brain ‐ derived neurotrophic factor interacts with adult ‐ born immature cells in the dentate gyrus during consolidation of overlapping memories. Hippocampus. The authors demonstrate that the enhancement associated with brain ‐ derived neurotrophic factor (BDNF) for consolidation of “pattern ‐ separated” memories, necessary for successful performance on the spontaneous location recognition task (SLR), requires adult ‐ born hippocampal neurons. Rats with down ‐ regulated neurogenesis, after being treated with a lentivirus ‐ expressing dnWnt, did not show improved performance on SLR after a BDNF infusion, providing evidence that BDNF is one of the upstream signals that affects the plasticity of adult ‐ born young neurons in the process of pattern separation.

Clemenson, G. D., Lee, S. W., Deng, W., Barrera, V. R., Iwamoto, K. S., Fanselow, M. S., & Gage, F. H. (2014). Enrichment rescues contextual discrimination deficit associated with immediate shock. Hippocampus. The authors developed a novel behavioural test using a modified and more sensitive version of the immediate shock deficit (ISD) procedure, with an added discrimination trial in order to
examine whether the method by which newborn neurons are induced has an effect on the cells’ function. They compared the functional differences between exercise-induced neurogenesis and environmental enrichment-induced neurogenesis and found that only mice exposed to environmental enrichment, and not the mice with exercise-induced neurogenesis were able to discriminate between similar contexts.

The authors provide evidence that voluntary running increased the number of newly born neurons in the DG of adult mice, and improved spatial pattern separation, when assayed using a two-choice spatial discrimination task run on a mouse touchscreen. In aged mice, running did not increase the number of new neurons and they did not show an enhancement in their ability to make fine spatial discriminations.

The authors evaluate differences in setup and protocol of the Morris water maze in attempts to explain some of the contradictory results surrounding the functional relevance of adult hippocampal neurogenesis for performance. Cyclin D2 knockout mice showed reduced levels of neurogenesis and were impaired only when the performance required precise and flexible encoding. This demonstrated that the specific behavioural phenotype depends on test parameters, which are not uniform or standardized between experiments, and highlights the importance of identifying factors within task setup that may affect the functional role of new neurons.

The authors used a combination of retroviral and optogenetics approaches to characterize the development of DG-CA3 output circuit function. Their findings revealed a precise restricted time window when adult-born hippocampal neurons exhibit enhanced plasticity at CA3 synapses and are essential in hippocampal memory retrieval, demonstrated by reversibly silencing a population of 4-week-old cells. Memory retrieval was unaffected when reversibly silencing 2- or 8-week-old cells.

The authors were the first to show that administration of physiological doses of acyl-ghrelin has long lasting effects on adult hippocampal neurogenesis and pattern separation-dependent memory in rodents. After two weeks of daily administration of acyl-ghrelin, rats were able to perform difficult spatial discriminations on the Spontaneous Location Recognition task, whereas the saline-treated rats were not.

The authors provide evidence that a 30-day period of voluntary running in juvenile rats results in a temporary increase of proliferation and differentiation of neuronal precursors, and long-term (up to 11 months of age) increases in the rate of neuronal maturation and survival.

The authors provide the direct and quantitative neurophysiological evidence confirming predictions of a longstanding model of hippocampal memory processing that CA3 performs pattern completion and DG performs pattern separation. By simultaneously recording single-unit activity from CA3 and DG of behaving rats, the authors were able to show that after parametric changes to the environment, CA3 produced output patterns closer to the originally stored representation than the degraded input patterns from the DG.


The authors provide an excellent review of the developmental time-course of adult born hippocampal neurons. They describe findings from their experiments that used a novel combination of viral vectors for synapse specific trans-neuronal tracing to identify the unique afferents of adult-born neurons. They combine this review with a discussion of how their findings identifying specific (sub) cortical inputs to new granule cells in the dentate gyrus support claims of a functional contribution to pattern separation.

References


Gage et al., this issue


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.


