Front. Biol. 2016, 11(3): 168–181
DOI 10.1007/s11515-016-1406-2

REVIEW

Adult neurogenesis and pattern separation in rodents: A critical evaluation of data, tasks and interpretation

Martha Hvoslef-Eide[1,2,3], Charlotte A. Oomen (✉)[4,5,6]

[1] *Department of Psychology, University of Cambridge, Cambridge CB2 3EB, United Kingdom*
[2] *Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge CB2 3 EB, United Kingdom*
[3] *Current addess Department of Biosciences, University of Oslo, 0370 Oslo, Norway*
[4] *Department of Cognitive Neuroscience, Radboud University Medical Center, Donders Institute, Geert Grooteplein 21, 6500 HB, Nijmegen, The Netherlands*
[5] *Current address Centre for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands*
[6] *Current address Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University Amsterdam Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

Abstract **The ability to discriminate and store similar inputs as distinct representations in memory is thought to rely on a process called pattern separation in the dentate gyrus of the hippocampus. Recent computational and empirical findings support a role for adult-born granule neurons in spatial pattern separation. We reviewed rodent studies that have manipulated both hippocampal adult neurogenesis and assessed pattern separation. The majority of studies report a supporting role of adult born neurons in pattern separation as measured at the behavioral level. However, closer evaluation of the published findings reveals variation in both pattern separation tasks and in the interpretation of behavioral performance that, taken together, suggests that the role of hippocampal adult neurogenesis in pattern separation may be less established than is currently assumed. Assessment of pattern separation at the network level through the use of immediate early gene expression, optogenetic, pharmacogenetic and/or *in vivo* electrophysiology studies could be instrumental in further confirming a role of adult born neurons in pattern separation further. Finally, hippocampal adult neurogenesis and pattern separation are not an exclusive pair, as evidence for hippocampal adult neurogenesis contributing to the temporal separation of events in memory, forgetting and cognitive flexibility has also been found. We conclude that whereas current empirical evidence for the involvement of hippocampal adult neurogenesis in pattern separation seems supportive, there is a need for careful interpretation of behavioral findings and an integration of the various proposed functions of adult born neurons.**

Keywords adult neurogenesis, memory, pattern separation, dentate gyrus, behavioral paradigms

Introduction

Since the discovery that the brain of adult mammals (including humans) is able to produce new neurons, *adult neurogenesis* has received considerable attention. Adult neurogenesis is restricted to the subventricular zone of the lateral ventricles (with neurons migrating into the olfactory bulb) and to the subgranular zone of the hippocampal dentate gyrus (Altman, 1962, 1969; Eriksson et al., 1998; Kempermann, 2005). Not surprisingly given the well-established role of the hippocampus in memory, hippocampal adult neurogenesis has been implicated in learning and memory processes (Shors et al., 2001; Shors et al., 2002) reviewed in (Abrous et al., 2005; Deng et al., 2010; Koehl and Abrous, 2011; Marín-Burgin and Schinder, 2012; Oomen et al., 2014). More recently, adult born neurons were proposed by computational studies to contribute specifically to pattern separation in the dentate gyrus (Becker, 2005). Pattern separation is the process by which a neural circuit reduces overlap between similar input patterns, resulting in more dissimilar output signals (Marr, 1971; McNaughton and Morris, 1987; Treves and

Received March 22, 2016; accepted May 20, 2016
Correspondence: Charlotte A. Oomen
E-mail: charlotte.oomen@gmail.com

Rolls, 1992). It is thought that pattern separation facilitates storage of *unique* representations, which decreases the probability of interference at a later stage, i.e. during memory recall (Neunuebel and Knierim, 2014). Since the first empirical study in 2009 (Clelland et al., 2009), the number of articles published on pattern separation and hippocampal adult neurogenesis has currently risen to 102. In this review, we evaluate current empirical evidence for an involvement of adult neurogenesis in the hippocampus (referred to as 'adult neurogenesis') in pattern separation in rodents, with a focus on behavior. We describe and discuss common pattern separation memory paradigms and emphasize critical test parameters in the behavioral assessment of pattern separation. Other measures by which pattern separation ability may be assessed are also discussed. Finally, we argue that adult neurogenesis and pattern separation may not be an exclusive pair, as pattern separation has also been suggested to occur outside of the dentate gyrus and thus may not rely on adult neurogenesis exclusively. In addition, there is evidence for other roles of adult neurogenesis in cognition such as temporal separation, forgetting and cognitive flexibility.

Background: adult neurogenesis in learning and memory

A multitude of studies have investigated how adult-born neurons contribute to learning and memory processes (for reviews see (Deng et al., 2010; Koehl and Abrous, 2011; Marín-Burgin and Schinder, 2012; Oomen et al., 2014)). Some studies have made use of factors that modulate neurogenesis (e.g., exercise or stress) and inferred a role of adult-born neurons from subsequent changes in cognitive performance, although in such studies the role of a third variable is difficult to exclude (Bekinschtein et al., 2011). A more direct strategy for studying the function of adult neurogenesis is to examine the consequence of adult neurogenesis ablation or upregulation using X-ray irradiation, chemical methods (methylazoxymethanol acetate or temozolomide, which act as anti-mitotic drugs) or genetic tools. It has been suggested that relative timing and age of the ablated neurons may be critical in this (Farioli-Vecchioli et al., 2008) making ablation methods less than straightforward to interpret. In addition, no study has yet incorporated an experimental group in which a similar number of *adult granule neurons* is ablated, but see (Nakashiba et al., 2012), which would better address the question whether adult neurogenesis subserves a truly unique role. Some studies have provided compelling evidence for a role of adult-born neurons in general memory function e.g., (Dupret et al., 2007; Shors et al., 2002; Zhang et al., 2008). Overall however, publications on the effect of adult neurogenesis ablation on behavioral outcomes have yielded mixed results (Deng et al., 2010; Oomen et al., 2014). Indeed, adult neurogenesis appears to have very little effect when using a broad range of behavioral tasks, as demonstrated in a recent meta-analysis of 98 data sets in which no significant effect of ablating neurogenesis was found on contextual and cued fear conditioning or spatial memory (Groves et al., 2013). In light of this, the field has aimed increasingly at more specific memory functions.

Behavioral evidence for a role of adult neurogenesis in pattern separation

Pattern separation in the dentate gyrus

Pattern separation may represent a more precise cognitive construct by which adult-born neurons contribute to memory function and thus offer a solution to the inconsistency with which manipulation of adult neurogenesis has been shown to affect behavioral outcomes. Pattern separation (the process by which a neural circuit reduces overlap between similar input signals, resulting in less similar output signals) is thought to be necessary for the formation of unique, non- (or less-) overlapping representations and through that, successful memory storage (Marr, 1971; McNaughton and Morris,1987; Treves and Rolls, 1992). Some classical learning and memory tasks may have inadvertently loaded on the construct of pattern separation (thus yielding sensitivity to changes in adult neurogenesis), while others did not (thus being insensitive to adult neurogenesis manipulation). Within the hippocampus the dentate gyrus is thought to perform pattern separation, which in turn facilitates pattern completion (retrieval of a complete memory from a partial cue) in the downstream CA3 attractor circuitry (Treves et al., 2008). Evidence for pattern separation in the dentate gyrus comes from *in vivo* electrophysiological studies (Leutgeb et al., 2007; Neunuebel and Knierim, 2014). These studies found that small contextual changes (either by morphing the shape of the surrounding open field, or by inducing a conflict between local and global cues in the environment) induces markedly different cellular responses in the dentate gyrus versus CA3, whereby the dentate gyrus was found to be more sensitive to small environmental changes. Direct evidence for a role of pattern separation and completion in the dentate gyrus-CA3 circuitry was found by increasing local-global cue conflicts and simultaneously measuring dentate gyrus and CA3 input/output signals. Increasing such conflict resulted in less coherent dentate gyrus outputs (pattern separation) whereas the downstream CA3 was able to retrieve more coherent contextual representations, pointing at a pattern completion-like mechanism (Neunuebel and Knierim, 2014). Further evidence for a pattern separation function of the dentate gyrus has been studied at the behavioral level. It was found that an intact dentate gyrus is necessary for successfully remembering similar, but not dissimilar spatial information in rodents (Bekinschtein et al., 2013; Gilbert et al., 2001). In humans, using high-resolution functional magnetic resonance

imaging in healthy volunteers, the dentate gyrus/CA3 area was found to be particularly active during trials that involve encoding of highly similar items (Bakker et al., 2008). While a discussion of the role of adult neurogenesis in pattern separation focusing on human studies is beyond the scope of this review, it is worth noting the clinical importance of understanding potential deficits in pattern separation ability in humans. This may be particularly interesting in the context of memory loss during aging and/or age-related pathology (Stark et al., 2010; Yassa et al., 2010; Stark et al., 2015). Additionally, changes in pattern separation/pattern completion have been hypothesized to underlie stress-related psychopathology, and in particular post-traumatic stress disorder, as this is hallmarked by overgeneralization of fear memories which may be indicative of a pattern separation deficit (Kheirbek et al., 2012a; Segal et al., 2012; Besnard and Sahay, 2016). Finally, pattern separation deficits have been found in schizophrenic patients, which may be caused by dentate gyrus pathology in this disorder (Das et al., 2014).

Adult neurogenesis and pattern separation

A role for adult neurogenesis in pattern separation was first proposed by computational studies (Becker, 2005; Aimone et al., 2006, 2009). Empirical evidence for this link was then shown in rodents using two techniques for neurogenesis ablation; focal X-ray irradiation and lentiviral expression of dominant-negative version of the Wnt protein, accomplished through intra-dentate gyrus injection (Clelland et al., 2009). In this initial study both ablation methods produced impairments in discriminating similar, but not dissimilar locations in two different memory tasks: a delayed non-matching to place task in a radial arm maze and a touchscreen-based automated location discrimination task. Since this first demonstration, the interest in neurogenesis and pattern separation has increased substantially and the search terms ‘pattern separation’ and ‘neurogenesis’ in PubMed currently yields 100 results (two additional studies were found via references). Of these 102 publications, 53, report empirical findings; of these, 38 describe a manipulation of adult neurogenesis in combination with an assessment of pattern separation ability (listed in Table 1). The majority (31) of these 38 studies conclude that adult-born neurons are involved in pattern separation. However, the nature of the experimental design varies across studies, both in terms of methods and in the interpretation of behavioral results.

Behavioral pattern separation tasks are usually designed to place a high demand on resolving the confusability of inputs requiring discrimination (and memory formation) of, for example, similar contexts, places, or episodes (Gilbert et al., 1998; Gilbert et al., 2001; Yassa and Stark, 2011; Bakker et al., 2008; Bekinschtein et al., 2013; Kesner, 2013). To ensure that changes in task performance are caused by altered pattern separation ability as opposed to general changes in learning ability (or confounding factors), the inclusion of dissimilar within-task control conditions is critical (Yassa and Stark, 2011; Kesner, 2013; Liu et al., 2016). A pattern separation deficit can be inferred from impaired memory of the more similar task condition specifically, and the graded nature of memory impairments and a significant linear improvement in performance as a function of decreased similarity, supports an involvement of pattern separation specifically (Kesner, 2013). Thus, in the event that an experimental manipulation does not affect performance on the similar condition (or when both the similar and dissimilar condition are affected) validation of task parameters may be required to ensure that the similar condition is sufficiently demanding and that the dissimilar condition is sufficiently less taxing.

We have reviewed the 38 studies by evaluating the effects of changes in adult neurogenesis on pattern separation performance, according to the task requirements and interpretation of findings described above. Of the 38 studies, 20 presented their work with the primary aim to directly manipulate levels of adult neurogenesis, or affect functionality of adult born neurons specifically, whereas the remaining studies were generally designed to study the impact of other factors on hippocampal function thereby also affecting adult neurogenesis (e.g., aging, exercise, hormonal changes). The 20 studies using direct adult neurogenesis manipulations will be discussed in more detail below, grouped according to the type of behavioral paradigm used (for task examples, see Fig. 1) and include the following studies (Clelland et al., 2009; Sahay et al., 2011; Burghardt et al., 2012; Cushman et al., 2012; Kheirbek et al., 2012b; Luu et al., 2012; Nakashiba et al., 2012; Niibori et al., 2012; Pan et al., 2012; Tronel et al., 2012; Groves et al., 2013; Bekinschtein et al., 2014; Farioli-Vecchioli et al., 2014; Garthe et al., 2014; Kesner et al., 2014; Swan et al., 2014; Wu and Hen, 2014;Zhang et al., 2014; Bonds et al., 2015; Wu et al., 2015).

Evidence from contextual fear memory

Many studies that directly manipulated adult neurogenesis used contextual fear memory to assess pattern separation (Sahay et al., 2011; Cushman et al., 2012; Kheirbek et al., 2012a; Nakashiba et al., 2012; Niibori et al., 2012; Tronel et al., 2012; Farioli-Vecchioli et al., 2014; Wu and Hen, 2014; Bonds et al., 2015; Wu et al., 2015). Of those, most have used an extended contextual fear discrimination paradigm (see Fig. 1A.), (Frankland et al., 1998). In this task animals (usually mice) are exposed to context A, which is paired with a foot shock on day 1. This is followed by extended discrimination training in daily exposure sessions to context A (paired with shock) alongside exposure to a similar context B (no shock). Animals usually show equal levels of freezing on the first day of exposure to both contexts indicating fear generalization. Over time they gradually learn to inhibit freezing in the non-aversive context (B). Acquired discrimination between contexts was shown to require intact NMDA receptors in dentate gyrus granule cells (McHugh et al., 2007). Using

Table 1 Empirical animal studies of *neurogenesis* and *pattern separation*

	Effect on task(s)	Dissimilar condition?	Support role of NG in PS?	Change in NG/experimental manipulation	Species/Sex/Age	Reference
1	Impaired novel object in place recognition memory	No	Yes	Decreased NG after early malnutrition	Rat/m/-	Perez-Garcia et al., 2016
2	No effect on location discrimination learning (touchscreen)	Yes	No	Increased NG after electroconvulsive seazures	Rat/m/8+ weeks	Svensson et al., 2016
3	Improved performance on the DNMTP radial arm maze	Yes	Yes	Innate higher levels of NG correlate positively with task performance in male spatial strategy users.	Rat/m and f/10+ weeks	Yagi et al., 2016
4	Improved performance on contextual fear discrimination (in 3 month old mice)	No	Yes	Increased NG after fingolimod treatment	Mice/m/ 3,7,12 months	Efstathopoulos et al., 2015
5	No effect on contextual fear discrimination	No	No	Decreased NG in a Dorfin homozygous knockout mouse	Mice/-/-	Park et al., 2015
6	No effect on trial-unique nonmatching to location (touchscreen)	Yes	No	Increased neurogenesis after electroconvulsive seazures	Rat/m/8+ weeks	Svensson et al., 2015
7	Improved contextual fear discrimination	No	Yes	Increased NG after postnatal sevoflurane treatment	Rat/m/5–8 weeks	Chen et al., 2015
8	Impaired learning in a high-interference condition of submerged T-maze paradigm	Yes, low interference	Yes	Decreased NG after chemotherapy drug treatment	Rat/f/5 months	Winocur et al., 2015
9	Improved contextual fear discrimination after running in aged animals	No	Yes	Decreased NG in aging, increased neurogenesis after voluntary exercise	Mice/m/2,17 months	Wu et al., 2015
10	Improved novel object recognition of similar, but not distinct objects	Yes	Yes	Increased NG after voluntary exercise	Mice/f/8+ weeks	Bolz et al., 2015
11	Impaired contextual fear discrimination	No	Yes	Decreased survival and changed dendritic complexity after PS1 knockdown of adult-born neurons	Mice/m/8 months	Bonds et al., 2015
12	Improved spontaneous location recognition memory	Yes	Yes	Increased NG after systemic ghrelin administration	Rats/m/8+ weeks	Kent et al., 2015
13	Impaired performance on a spatial metric task and temporal object association task	No	Yes	Decreased NG in DNMT-1 knockout mice	Mice/m/1–3 months	Kesner et al., 2014
14	Impaired performance on similar and dissimilar trials in the location discrimination task during reversal only.	Yes	No	Decreased NG in GFAP-TK mice	Mice/m/8+ weeks	Swan et al., 2014
15	Impaired object-in-place memory	No	Yes	Decreased NG due to inflammatory factors (and normalization through ibuprofen treatment)	Mice/f/8+ weeks	Llorens-Martin et al., 2014
16	Impaired spontaneous location recognition memory	Yes	Yes	Decreased NG through viral delivery of Wnt	Rats/m/8+ weeks	Bekinschtein et al.,2014

(*Continued*)

	Effect on task(s)	Dissimilar condition?	Support role of NG in PS?	Change in NG/experimental manipulation	Species/Sex/Age	Reference
17	Impaired contextual fear discrimination (use of randomized and non-randomized version)	No	Yes	Decreased NG (dorsal versus ventral) through X-ray irradiation	Mice/m/7+ weeks	Wu and Hen, 2014
18	Impaired contextual fear discrimination and subsequent normalization	No	Yes	Decreased NG (Btg1 mice) and normalization using voluntary exercise	Mice/-/8+ weeks	Farioli-Vecchioli et al., 2014
19	Impaired DNMTP in the radial arm maze at both small and large separations	Yes	Yes	Decreased NG in *Ezh2* knockout mice	Mice/m/-	Zhang et al., 2014
20	More perseverance in the Morris water maze, less precise search patterns.	No	Yes	Decreased NG in Cyclin-D2 knockout mice	Mice/f/8+ weeks	Garthe et al., 2014
21	Impaired contextual fear discrimination (reported in separate study: Jin et al., 2013)	No	Yes	Decreased NG in Ras/Grf1 knockout mice	Mice/-/-	Darcy et al., 2014 (Jin et al., 2013)
22	No effect on contextual fear discrimination	No	No	Increased NG in Pet knockout mice (serotonin depletion)	Mice/m/8–10 weeks	Diaz et al., 2013
23	No effect on DNMTP in the radial arm maze at small or large separations	Yes	No	Decreased NG in GFAP-TK rats	Rats/m/8 weeks	Groves et al., 2013
24	Impaired performance on small separation in touchscreen location discrimination	Yes	Yes	Decreased NG in TNiK knockout mice	Mice/m/8+ weeks	Coba et al., 2012
25	Impaired and improved novel object in place memory	Yes	Yes	Decreased NG in SREB2 transgenic mice/ Increased NG in SREB2 knockout mice	Mice/m/4–6 months	Chen et al., 2012
26	Impaired cognitive flexibility	No	Yes	Decreased NG in GFAP-TK mice and after X-ray irradiation	Mice/m/10+ weeks	Burghardt et al., 2012
27	Impaired contextual fear discrimination	Yes, in a separate experiment	Yes	Deletion of NR2B of the NMDAR in adult born neurons only;	Mice/m/14–16 weeks	Kheirbek et al., 2012
28	Impaired contextual fear discrimination after NG ablation (main finding: improved performance upon inhibited neurotransmission of adult cells)	Yes, in a separate experiment	Yes	Inhibited neurotransmission of adult granule cells in combination with decreased NG after X-ray irradiation.	Mice/m/14+ weeks	Nakashiba et al., 2012
29	Impaired performance on a high interference odor discrimination paradigm	No	Yes	Decreased NG after X-ray irradiation	Rats/m/3+ months	Luu et al., 2012
30	Impaired DNMTP in the radial arm maze at both small and large separations	Yes	Yes	Decreased NG in ERK5 inducable knockout (adult born neurons only)	Mice/m/12 weeks	Pan et al., 2012
31	Improved contextual fear discrimination in males, no effect in females	No	No	Ablation of postnatal NG in DNMT1 knockout mice	Mice/m and f/ 3–5 months	Cushman et al., 2012
32	Impaired contextual fear conditioning	Yes	Yes	Ablation of NG using HSV-tk under the nestin promotor and by means of systemic temozolomide treatment	Mice/m/10 weeks	Niibori et al., 2012

(*Continued*)

	Effect on task(s)	Dissimilar condition?	Support role of NG in PS?	Change in NG/experimental manipulation	Species/Sex/Age	Reference
33	Impaired performance on an adapted version of the Barnes' maze	No	Yes	NF-kB knockout results in a (pathological) increase in NG	Mice/m/-	Imielski et al., 2012
34	Improved performance in a submerged radial arm maze (and a lack thereof in knockout animals)	Yes	Yes	Increased NG in control animals after enrichment; and a lack thereof in mice with conditional knock-out of CREB binding protein	Mice/f/2–5 months(?)	Lopez-Atalaya et al., 2011
35	Improved (and impaired) contextual fear discrimination (normal absence of fear memory in novel context)	No	Yes	Increase (and decrease) in NG through genetic manipulation and X-ray irradiation.	Mice/m and f/14–18 weeks	Sahay et al., 2011
36	Impaired contextual fear discrimination	No	Yes	Decreased NG in Nestin rtTA/Tet mice	Mice/m/8 weeks	Tronel et al., 2010
37	Improved location discrimination learning (touchscreen)	Yes	Yes	Increased NG after running	Mice/m/3 and 22 months	Creer et al., 2010
38	Improved location discrimination learning and improved DNMTP in the radial arm maze (on similar conditions only)	Yes	Yes	Decreased NG after X-ray irradiation and viral delivery of Wnt knockdown	Mice/f/8+ weeks	Clelland et al., 2009

PS = pattern separation; NG = adult hippocampal neurogenesis; (-) indicates that this information is not clearly reported; DNMTP = delayed nonmatching to place.
Summary of studies in which effects of adult neurogenesis manipulations are studied at the level of behavioral pattern separation; discussed in the text or referred here (Creer et al., 2010; Lopez-Atalaya et al., 2011; Coba et al., 2012; Imielski et al., 2012; Diaz et al., 2013; Llorens-Martín et al., 2014; Bolz et al., 2015; Chen et al., 2015; Efstathopoulos et al., 2015; Kent et al., 2015; Park et al., 2015; Svensson et al., 2015; Winocur et al., 2015; Pérez-García et al., 2016; Yagi et al., 2016).

genetic ablation of adult neurogenesis, Tronel and colleagues (2012) were the first to show that adult-born neurons are also required for contextual discrimination of similar contexts, which was later replicated using focal X-ray irradiation (Sahay et al., 2011). Of interest, the latter study showed that genetically increasing adult neurogenesis is sufficient to improve contextual discrimination (Sahay et al., 2011). Furthermore, specific deletion of NR2B-containing NMDA receptors from adult-born granule cells impaired contextual fear discrimination of similar, but not dissimilar contexts (Kheirbek et al., 2012b). Deletion of PS-1 (again, in adult born neurons only) yielded similar impairments (Bonds et al., 2015). Finally, mice that lack the antiproliferative gene Btg1 had reduced levels of adult neurogenesis and show deficits in discriminating between contexts in this paradigm (Farioli-Vecchioli et al., 2014). Exposing mice to voluntary exercise normalized both adult neurogenesis and fear discrimination in this study. Cushman and colleagues (2012) ablated neurogenesis in juvenile animals by genetically blocking postnatal DNMT-1, resulting in a smaller granule cell layer. Of interest, this led to unimpaired discrimination of similar contexts in female animals and *enhanced* discrimination in male mice. Although this finding seems unexpected, it may be in line with another study that blocked neurotransmission in postnatally-born *adult* granule cells with a subsequent improvement in contextual discrimination (Nakashiba et al., 2012). This highlights a potential role for both immature and mature neurons in contextual discrimination and thus the importance of cell heterogeneity within the dentate gyrus.

Given the number of inter-study replications, the contextual fear discrimination is useful as a behavioral read-out in experiments that manipulate adult neurogenesis. However, the paradigm generally does not incorporate a dissimilar within-task control. Therefore, differences in discrimination performance may be caused by differences in general learning abilities such as the capacity to learn to inhibit freezing, and not pattern separation per se. Additionally, performance may be confounded by differences in anxiety or fear responsiveness, which is particularly relevant in the context of adult neurogenesis as animal models with less adult neurogenesis show increases in anxiety-related behaviors (Revest et al., 2009; Seo et al., 2015; Yun et al., 2016), whereas increasing adult neurogenesis was found to result in reduced stress and anxiety-related behavior (Kannangara et al., 2011; Hill et al., 2015). Ideally, contextual discrimination ability of animals should be tested between the shock-context (context A) and a similar context (context B) *and* between the shock context (context A) and a dissimilar context (C) within the same experiment using a counterbalanced design. Some studies in which adult neurogenesis was manipulated have addressed this issue, by providing additional experiments on fear conditioning performance (Sahay et al., 2011) or fear discrimination learning (Kheirbek et al., 2012b) using more dissimilar contexts.

Evidence from place learning with reinforcement

Paradigms using place learning have frequently been employed to assess the effect of adult neurogenesis. For example, delayed non-matching to place has been used in a radial arm maze (Fig. 1B), (Clelland et al., 2009; Pan et al., 2012; Groves et al., 2013; Zhang et al., 2014). Furthermore, location discrimination learning has been implemented using a touchscreen-equipped operant chamber (Clelland et al., 2009; Swan et al., 2014). From those studies, most report a functional role of adult neurogenesis in pattern separation with the exception of Groves *et al*. (2013).

Closer examination of the positive findings however, highlights issues at the level of data interpretation. First, Pan and colleagues (2012) studied inducible and conditional knockout of the gene coding for ERK5 mitogen activated protein kinase, exclusively in neural progenitor cells. In this study reduced neurogenesis was paralleled by impaired memory performance at both the similar and dissimilar spatial separations in the radial arm maze (90 and 180 degrees). The performance impairments therefore suggest a general memory deficit, as opposed to (or in addition to) a pattern separation deficit. Similarly, impaired pattern separation ability has been inferred in mice with a specific ablation of the gene for Enhancer of zeste homolog 2 (*Ezh2)* in dentate gyrus neural progenitor cells, which caused a robust reduction of adult-born neurons (Zhang et al., 2014). Again, memory deficits in the radial arm maze were found on both spatial separations, precluding a specific pattern separation deficit. Finally, Swan and colleagues (2014) used GFAP-TK mice to reduce adult neurogenesis and test animals on the touchscreen based location discrimination task. In this study, performance impairments were also found on both the small and large separation condition, but only during the reversal phase, which led the authors to conclude adult neurogenesis is mainly involved in cognitive flexibility. In Groves *et al*. (2013), delayed nonmatching to sample in the radial arm maze was not impaired in rats with genetic neurogenesis ablation (GFAP-TK rats). This may be due to the fact that performance level (even at the smallest separation) was still relatively high (70% correct in control animals). Although speculative, it is therefore possible that an increase in the load on pattern separation through the use of a smaller separation would have yielded a separation-dependent effect of genetic ablation.

A selection of studies that do not directly set out to assess pattern separation nevertheless report interesting results. Using the Morris water maze, memory impairments after adult neurogenesis ablation were reflected by less precise search patterns, which the authors suggest may involve pattern separation (Garthe et al., 2014). Furthermore, Burghardt and colleagues (2012) implemented an active place avoidance paradigm on a round platform that was rotated to create interference conditions (requiring animals to update the place-shock association) for which adult neurogenesis was critical. The study concluded that adult neurogenesis is necessary for cognitive flexibility, which may depend on pattern separation ability.

Overall, with the exception of *Clelland et al.* (2009), these examples of place learning tasks do not conclusively support a role of adult neurogenesis in pattern separation. This is based on the observation that in some studies pattern separation load is either not challenging enough (Groves et al., 2013) or too challenging (Pan et al., 2012; Swan et al., 2014; Zhang et al., 2014), which could be addressed by further optimisation of task parameters. For findings in the water maze paradigm and during active avoidance learning, pattern separation may be involved but is not directly tested (Burghardt et al., 2012; Garthe et al., 2014).

Evidence from spontaneous location recognition memory

Spontaneous location recognition memory tasks have generally generated data in support of a role for adult-born neurons in pattern separation (Bekinschtein et al., 2014; Kesner et al., 2014). In a so-called metric spatial processing task, spatial displacement of two objects was used. Adult neurogenesis ablation in DNMT-1 knockout mice resulted in impaired metric processing, suggestive of impaired pattern separation. Due to their one-trial learning structure, the spontaneous location recognition memory tasks allow for the study of the different stages of memory processing in pattern separation. Indeed, in a different spontaneous location recognition paradigm for pattern separation (Fig. 1C), brain-derived neurotrophic factor (BDNF) in the dentate gyrus was shown to be important specifically during the consolidation (but not retrieval) of memories for similar (but not dissimilar) spatial locations in an open field. BDNF was found to interact with adult-born neurons in the service of pattern separation (Bekinschtein et al., 2013; Bekinschtein et al., 2014).

Evidence from other tasks

One study involving direct ablation of adult neurogenesis inferred a pattern separation deficit using a task that does not involve space or context (Luu et al., 2012). In this study, an odor discrimination paradigm was implemented in which rats were required to discriminate between two odors. After acquisition of a number of odor pairs, a second set of odor pairs was learned in which previously remembered odors were repeated to introduce interference, prevention of which arguably involves pattern separation (Kent et al., 2016). Reducing adult neurogenesis, which was restricted to hippocampal- and not olfactory bulb neurogenesis, impaired performance on this task. Although this study is particularly interesting with regards to the involvement of adult neurogenesis in memory for non-spatial information, no

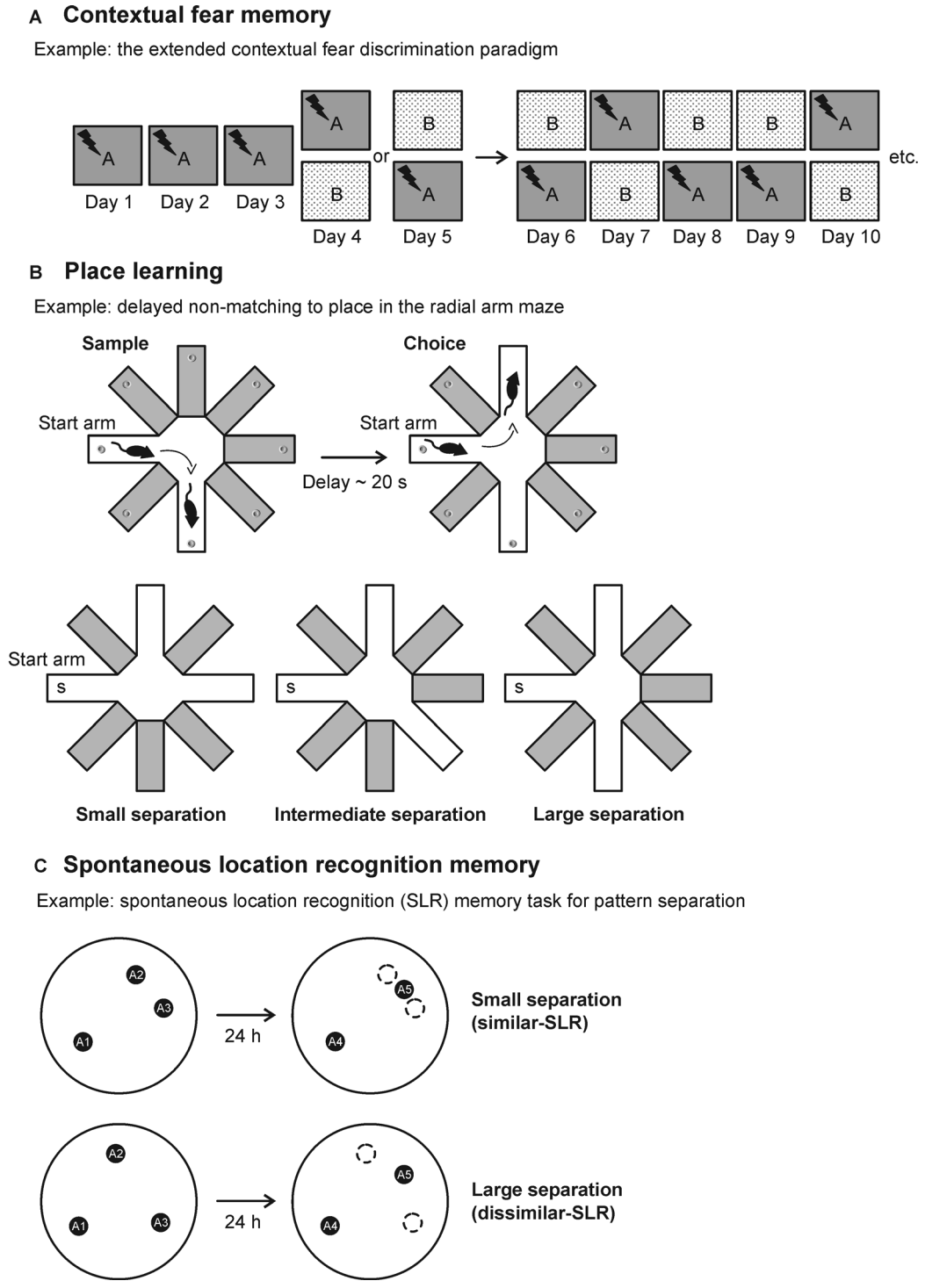

Figure 1 Pattern separation task examples. (A) Contextual fear discrimination in which animals are trained to discriminate context A (paired with a foot shock) from a safe context (B) Delayed non-matching to location in the radial arm maze. Trial example (top): during the sample phase animals collect a reward from one arm (sample arm) and after a delay, are required to choose between the sample arm (incorrect, unbaited) and a novel arm (correct, baited) during the choice phase. Below, three different choice phase configurations are shown, taxing similarity between the sample arm and novel arm by presenting them at different separations (2, 3 or 4). Note; arms and locations should vary between trials. S = start arm. (C) The spontaneous location recognition paradigm for pattern separation. Animals explore three identical copies (A1, A2, A3) of objects during a sample phase (left) and after a delay, memory is assessed using preference for the object placed in a novel location (A5) compared to the familiar location (A4) in the choice phase (right). Two trial types are used in randomized order, implementing a small or large distance between objects A2 and A3 thereby varying pattern separation load. (Figures drawn based on task descriptions in Tronel et al., 2012; Clelland et al., 2009 and Bekinschtein et al., 2013.)

explicit dissimilar control condition was implemented, which may hamper the interpretation of these findings in terms of pattern separation specifically.

Other evidence for a role of adult neurogenesis in pattern separation

Changes in pattern separation after neurogenesis manipulation can also be measured using read-outs other than behavior. An elegant contextual fear conditioning study by Niibori *et al*. (2012) involved mapping activity-dependent genetic markers. They showed that reducing adult neurogenesis led to an increased overlap in the CA3 neurons active in similar contexts (showing no involvement for adult born neurons in dissimilar contexts). In other words, the population code in the CA3 was less able to ensure the separation of similar patterns in the absence of adult neurogenesis. This study presents a valuable approach to identifying pattern separation changes beyond those at the behavioral level. Furthermore, *in vivo* electrophysiological measures of the degree of overlap in neural signaling (by measuring the degree of remapping) in downstream CA areas would greatly contribute to the involvement of adult neurogenesis in pattern separation. This was previously implemented in, for example, a mouse model of NR1 deficiency in the dentate gyrus (McHugh et al., 2007) and in a mouse model in which neurotransmission in adult dentate granule cells was blocked (Nakashiba et al., 2012). However, to our knowledge, this *in vivo* electrophysiological approach has not been implemented in studies aimed directly at the role of adult neurogenesis in behavioral pattern separation.

Pattern separation and adult neurogenesis may not be an exclusive coupling

In addition to the methodological issues associated with the field of neurogenesis and pattern separation, it is worth considering that pattern separation is not the only behavioral function that has been attributed to adult-born neurons. Vice versa, adult-born neurons may not be the only neural population supporting pattern separation, as this process has also been attributed to areas of the brain lacking adult neurogenesis (reviewed in Kent et al., 2016). Some of the evidence challenging an exclusive relationship between pattern separation and adult neurogenesis is presented below.

Temporal separation of events

Electrophysiological studies have shown that adult born neurons exhibit intrinsic and synaptic hyperexcitability (Schmidt-Hieber et al., 2004; Ge et al., 2007) and thus may act as 'pattern integrators' (Aimone et al., 2010; Marín-Burgin et al., 2012). From these observations it may be assumed that events occurring at the same time will activate cells from the same immature granule cell population, whereas events occurring days or (more likely) weeks apart will activate a different set of immature granule cells. The activation of these different populations would therefore increase the ability to "pattern separate" events across a temporal scale (Aimone et al., 2006, 2009), but see (Alme et al., 2010). Indeed, behavioral studies have found that adult born neurons are important for the temporal context of events (Sisti et al., 2007; Kesner et al., 2014).

Recently more direct evidence for this was found using *in vivo* electrophysiology (Rangel et al., 2014). In rats, temporally separated experiences (i.e. exposure to an environment) were coded by selective sets of neurons, whereby temporal separation resulted in an increase in the percentage of cells that fired more specifically to one context only (instead of firing non-specific in multiple contexts). Of particular interest, a reduction in adult neurogenesis after temozolomide treatment (which acts as an anti-mitotic drug) resulted in a reduced percentage of cells firing specifically to one (or two) contexts and an increase in the percentage of cells firing non-selectively, indicating that adult-born neurons are involved in coding of specific events when segregated across time.

Memory clearance

Adult neurogenesis results in the integration of new neurons into established connections in the dentate gyrus and may therefore serve as an on-going, nonspecific decay function helping to clear encoded memories (Deisseroth et al., 2004; Frankland et al., 2013). This is supported by the observation that reduced adult neurogenesis results in slowing of the decay of long-term potentiation (LTP) in dentate gyrus perforant path synapses (Kitamura et al., 2009).

As the level of non-developmental neurogenesis is higher in infancy than in adulthood, infancy amnesia (whereby few episodic memories are retrievable in adulthood) may be explained by memory clearance through neurogenesis. Indeed, infant mice not only show high levels of neurogenesis, but also are able to retain contextual fear memory for only 1 day after exposure, while adult mice show a comparable reaction up to 28 days after shock (Akers et al., 2014). Reducing neurogenesis in infancy was found to improve contextual fear memory. Contextual fear conditioning was also disrupted in adult mice if increases in neurogenesis occurred between encoding and retrieval (induced by running, among others), (Akers et al., 2014), but see (Van der Borght et al., 2007). This impaired freezing was not observed if the running-induced increase in neurogenesis was limited genetically (Akers et al., 2014). The experiments in Akers et al. (2014) used a post-acquisition neurogenesis manipulation, focusing on assessing the effect of neurogenesis on established memories. Similarly, post-acquisition exercise (which increased neurogenesis) disrupted Morris water maze retention in adult mice, but resulted in

initial improved performance when platform locations were reversed, presumably due to a weakened memory of the original platform location (Epp et al., 2016). Furthermore, neurogenesis-induced facilitation of learning a second pair of odor-place associations only occurs when the second pair are a reversal of previous reward contingencies (but not when using a novel pair; Epp et al., 2016, also see Van der Borght et al., 2007). This indicates that a degree of conflict, or interference with previously established associations, is necessary for changes in neurogenesis to be critical. Importantly, whilst impaired retention performance following exercise has been evident on traditionally hippocampus-sensitive tasks (contextual fear conditioning, incidental context learning, Morris water maze, odor-place paired associates learning), it has not been observed in a conditioned taste aversion paradigm (Akers et al., 2014).

While the evidence for a role of adult neurogenesis in forgetting may seem at odds with the literature on adult neurogenesis and pattern separation, the two may well work in parallel. As the focus of studies using pattern separation as a readout have mainly studied the effects of adult neurogenesis on anterograde memories (that is; how does manipulating adult neurogenesis affect the learning of new information), studies focused on forgetting have instead studied the effects of adult neurogenesis on retrograde memories (that is; how does manipulating adult neurogenesis affect the retention of previously learnt information; Frankland et al., 2013). Importantly, the role of neurogenesis in forgetting has not been assessed using a pattern separation task, raising the possibility that these experiments are highlighting an alternative role for neurogenesis that does not include pattern separation. It is worth noting however that Frankland and colleagues would predict the same effect of an post-acquisition neurogenesis manipulation irrespective of specific content within the range of hippocampal tasks (Frankland et al., 2013).

Cognitive flexibility

Another proposed role for adult neurogenesis in cognition is that of cognitive flexibility. Burghardt *et al.* (2012) showed that mice without adult neurogenesis were impaired on an active place avoidance, but only under conditions of conflict. In this study, animals were placed on a round platform surrounded by spatial cues. A shock was delivered when the mouse entered a certain zone of the platform. Mice without adult neurogenesis showed normal acquisition of the place-shock association and were unaffected during the extinction of this association. Subsequently, a conflicting condition was created by rotating the platform 180 degrees, which required animals to update information on the precise location of shock zone in the same environment. Neurogenesis ablation was found to hamper re-learning during this conflict condition, which indicates that adult neurogenesis may be involved in cognitive flexibility. In line with these findings, animals without adult neurogenesis show more perseverance in a Morris water maze reversal learning paradigm (Pan et al., 2012; Garthe et al., 2014). In addition, using touchscreen location discrimination and reversal, impaired place memory was found to occur only during reversal (Swan et al., 2014), while a study using touchscreen location discrimination in a rat model of electroconvulsive shocks (which increased adult neurogenesis), found that this improved performance on the reversal learning phase only, without this correlating with the number of adult-born neurons (Svensson et al., 2015).

Again, it is possible that the experimental evidence for a role of adult neurogenesis in cognitive flexibility could be compatible with the studies of adult neurogenesis in pattern separation. Notably, manipulating adult neurogenesis appears to specifically affect performance when there is a requirement to learn new information that directly conflicts with old information (Burghardt et al., 2012; Svensson et al., 2015; Epp et al., 2016). Although speculative, this sensitivity to interference is not unlike the sensitivity in pattern separation tasks to manipulations of similarity of simultaneously presented stimuli. It is possible that adult born neurons serve a role in resolving interference both when two similar stimuli are presented simultaneously, as well as when new information overlaps significantly with a previous memory.

Pattern separation without adult neurogenesis

Finally, there is evidence that pattern separation is not unique to the dentate gyrus. For example, tasks designed to test the use of "pattern-separated" or interference-reducing conjunctive object representations are reliably impaired by perirhinal cortex lesions, but are completely unaffected, or even facilitated, by hippocampal lesions e.g., (Saksida et al., 2006, 2007). It has been argued that unique conjunctive representations that reduce interference exist throughout ventral visual stream, continuing into the temporal lobe (Barense et al., 2005; Cowell et al., 2010; Nadel and Peterson, 2013; Kent et al., 2016). Pattern separation during processing of reward values has also been suggested, whereby the amygdala is involved in identifying relative small (but not large) differences in reward value (Gilbert and Kesner, 2002). The ability to produce outputs that are less correlated than their inputs is likely a ubiquitous property fundamental to neural networks in general, and although plasticity mechanisms involved in non-hippocampal pattern separation are not fully understood it seems that pattern separation (as a behavioral readout) does not solely rely on the presence of adult born hippocampal neurons.

Conclusion and discussion

A specific role for adult-born neurons in pattern separation presents a promising and exciting avenue of research and several studies have generated convincing evidence for this idea. In addition, pattern separation (and the potential

involvement of adult born neurons therein) may provide an interesting novel angle in human memory research in the context of aging and psychopathology. However, whereas the majority of current empirical findings seem to suggest an established role for adult neurogenesis in pattern separation, there are a number of methodological considerations and issues at the level of interpretation of results. As discussed, in order to measure changes in pattern separation specifically, 1) tasks should ideally include a dissimilar condition; 2) similarity should be sufficiently taxed; and 3) only if an experimental manipulation changes performance in similar but not (or to a lesser extent) in dissimilar conditions may an effect on pattern separation be concluded. When applying these criteria, it becomes challenging for several of the studies reviewed here to claim a definite role for adult born neurons in pattern separation. Furthermore, pattern separation does not solely rely on adult neurogenesis as other plasticity mechanisms (such as BDNF, Bekinschtein et al., 2013) have been found to be involved in this process, and because pattern separation likely occurs outside of the hippocampal dentate gyrus as well. Overall, this emphasizes the need for a degree of caution in concluding an established role for adult neurogenesis in pattern separation.

Finally, there are a number of ideas on the role of adult neurogenesis in memory as discussed here (such as pattern separation, temporal separations of events, forgetting and cognitive flexibility). Whether these views represent complementary processes or whether they can be integrated, remains to be shown. So far, no studies we are aware of have tested experimental hypotheses of different theories against each other, which would be a valuable contribution to the field.

Acknowledgements

CAO is supported by Amsterdam Brain & Cognition (grant #ABC2014-11 - 02) and the Amsterdam Brain and Mind Project.

Compliance with ethics guidelines

Martha Hvoslef-Eide and Charlotte A. Oomen declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

Abrous D N, Koehl M, Le Moal M (2005). Adult neurogenesis: from precursors to network and physiology. Physiol Rev, 85(2): 523–569

Aimone J B, Deng W, Gage F H (2010). Adult neurogenesis: integrating theories and separating functions. Trends Cogn Sci, 14 (7): 325–337

Aimone J B, Wiles J, Gage F H (2006). Potential role for adult neurogenesis in the encoding of time in new memories. Nat Neurosci, 9(6): 723–727

Aimone J B, Wiles J, Gage F H (2009). Computational influence of adult neurogenesis on memory encoding. Neuron, 61(2): 187–202

Akers K G, Martinez-Canabal A, Restivo L, Yiu A P, De Cristofaro A, Hsiang H L, Wheeler A L, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards B A, Miyakawa T, Josselyn S A, Frankland P W (2014). Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science, 344(6184): 598–602

Alme C B, Buzzetti R A, Marrone D F, Leutgeb J K, Chawla M K, Schaner M J, Bohanick J D, Khoboko T, Leutgeb S, Moser E I, Moser M B, McNaughton B L, Barnes C A (2010). Hippocampal granule cells opt for early retirement. Hippocampus, 20(10): 1109–1123

Altman J (1962). Are new neurons formed in the brains of adult mammals? Science, 135(3509): 1127–1128

Altman J (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol, 137(4): 433–457

Bakker A, Kirwan C B, Miller M, Stark C E (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. Science, 319 (5870): 1640–1642

Barense M D, Bussey T J, Lee A C, Rogers T T, Davies R R, Saksida L M, Murray E A, Graham K S (2005). Functional specialization in the human medial temporal lobe. J Neurosci, 25(44): 10239–10246

Becker S (2005). A computational principle for hippocampal learning and neurogenesis. Hippocampus, 15(6): 722–738

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, 24(8): 905–911

Bekinschtein P, Kent B A, Oomen C A, Clemenson G D, Gage, F H, Jr., Saksida L M, Bussey T J(2013). BDNF in the Dentate Gyrus Is Required for Consolidation of "Pattern-Separated" Memories. Cell Reports, 5: 1–10

Bekinschtein P, Oomen C A, Saksida L M, Bussey T J (2011). Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: BDNF as a critical variable? Semin Cell Dev Biol, 22(5): 536–542

Besnard A, Sahay A (2016). Adult Hippocampal Neurogenesis, Fear Generalization, and Stress. Neuropsychopharmacology, 41(1): 24–44

Bolz L, Heigele S, Bischofberger J (2015). Running improves pattern separation during novel object recognition. Br Plast, 1(1): 129–141

Bonds J A, Kuttner-Hirshler Y, Bartolotti N, Tobin M K, Pizzi M, Marr R, Lazarov O (2015). Presenilin-1 Dependent Neurogenesis Regulates Hippocampal Learning and Memory. PLoS ONE, 10(6): e0131266

Burghardt N S, Park E H, Hen R, Fenton A A (2012). Adult-born hippocampal neurons promote cognitive flexibility in mice. Hippocampus, 22(9): 1795–1808

Chen C, Shen F Y, Zhao X, Zhou T, Xu D J, Wang Z R, Wang Y W (2015). Low-dose sevoflurane promotes hippocampal neurogenesis and facilitates the development of dentate gyrus-dependent learning in neonatal rats. ASN Neuro, 7(2): 7

Chen Q, Kogan J H, Gross A K, Zhou Y, Walton N M, Shin R, Heusner C L, Miyake S, Tajinda K, Tamura K, Matsumoto M (2012). SREB2/GPR85, a schizophrenia risk factor, negatively regulates hippocampal adult neurogenesis and neurogenesis-dependent learning and memory. Eur J Neurosci, 36(5): 2597–2608

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, Bussey T J (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science, 325(5937): 210–213

Coba M P, Komiyama N H, Nithianantharajah J, Kopanitsa M V, Indersmitten T, Skene N G, Tuck E J, Fricker D G, Elsegood K A, Stanford L E, Afinowi N O, Saksida L M, Bussey T J, O'Dell T J, Grant S G (2012). TNiK is required for postsynaptic and nuclear signaling pathways and cognitive function. J Neurosci, 32(40): 13987–13999

Cowell R A, Bussey T J, Saksida L M (2010). Components of recognition memory: dissociable cognitive processes or just differences in representational complexity? Hippocampus, 20(11): 1245–1262

Creer D J, Romberg C, Saksida L M, van Praag H, Bussey T J (2010). Running enhances spatial pattern separation in mice. Proc Natl Acad Sci USA, 107(5): 2367–2372

Cushman J D, Maldonado J, Kwon E E, Garcia A D, Fan G, Imura T, Sofroniew M V, Fanselow M S (2012). Juvenile neurogenesis makes essential contributions to adult brain structure and plays a sex-dependent role in fear memories. Front Behav Neurosci, 6: 3

Das T, Ivleva E I, Wagner A D, Stark C E, Tamminga C A (2014). Loss of pattern separation performance in schizophrenia suggests dentate gyrus dysfunction. Schizophr Res, 159(1): 193–197

Deisseroth K, Singla S, Toda H, Monje M, Palmer T D, Malenka R C (2004). Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron, 42(4): 535–552

Deng W, Aimone J B, Gage F H (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci, 11(5): 339–350

Diaz S L, Narboux-Nême N, Trowbridge S, Scotto-Lomassese S, Kleine Borgmann F B, Jessberger S, Giros B, Maroteaux L, Deneris E, Gaspar P (2013). Paradoxical increase in survival of newborn neurons in the dentate gyrus of mice with constitutive depletion of serotonin. Eur J Neurosci, 38(5): 2650–2658

Dupret D, Fabre A, Döbrössy M D, Panatier A, Rodríguez J J, Lamarque S, Lemaire V, Oliet S H, Piazza P V, Abrous D N (2007). Spatial learning depends on both the addition and removal of new hippocampal neurons. PLoS Biol, 5(8): e214

Efstathopoulos P, Kourgiantaki A, Karali K, Sidiropoulou K, Margioris A N, Gravanis A, Charalampopoulos I (2015). Fingolimod induces neurogenesis in adult mouse hippocampus and improves contextual fear memory. Transl Psychiatry, 5(11): e685

Epp J R, Silva Mera R, Köhler S, Josselyn S A, Frankland P W (2016). Neurogenesis-mediated forgetting minimizes proactive interference. Nat Commun, 7: 10838

Eriksson P S, Perfilieva E, Björk-Eriksson T, Alborn A M, Nordborg C, Peterson D A, Gage F H (1998). Neurogenesis in the adult human hippocampus. Nat Med, 4(11): 1313–1317

Farioli-Vecchioli S, Mattera A, Micheli L, Ceccarelli M, Leonardi L, Saraulli D, Costanzi M, Cestari V, Rouault J P, Tirone F (2014). Running rescues defective adult neurogenesis by shortening the length of the cell cycle of neural stem and progenitor cells. Stem Cells, 32(7): 1968–1982

Farioli-Vecchioli S, Saraulli D, Costanzi M, Pacioni S, Cinà I, Aceti M, Micheli L, Bacci A, Cestari V, Tirone F (2008). The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. PLoS Biol, 6(10): e246

Frankland P W, Cestari V, Filipkowski R K, McDonald R J, Silva A J (1998). The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav Neurosci, 112(4): 863–874

Frankland P W, Köhler S, Josselyn S A (2013). Hippocampal neurogenesis and forgetting. Trends Neurosci, 36(9): 497–503

Garthe A, Huang Z, Kaczmarek L, Filipkowski R K, Kempermann G (2014). Not all water mazes are created equal: cyclin D2 knockout mice with constitutively suppressed adult hippocampal neurogenesis do show specific spatial learning deficits. Genes Brain Behav, 13(4): 357–364

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(4): 559–566

Gilbert P E, Kesner R P (2002). The amygdala but not the hippocampus is involved in pattern separation based on reward value. Neurobiol Learn Mem, 77(3): 338–353

Gilbert P E, Kesner R P, DeCoteau W E (1998). Memory for spatial location: role of the hippocampus in mediating spatial pattern separation. J Neurosci, 18(2): 804–810

Gilbert P E, Kesner R P, Lee I (2001). Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. Hippocampus, 11(6): 626–636

Groves J O, Leslie I, Huang G J, McHugh S B, Taylor A, Mott R, Munafò M, Bannerman D M, Flint J (2013). Ablating adult neurogenesis in the rat has no effect on spatial processing: evidence from a novel pharmacogenetic model. PLoS Genet, 9(9): e1003718

Hill A S, Sahay A, Hen R (2015). Increasing Adult Hippocampal Neurogenesis is Sufficient to Reduce Anxiety and Depression-Like Behaviors. Neuropsychopharmacology, 40(10): 2368–2378

Imielski Y, Schwamborn J C, Lüningschrör P, Heimann P, Holzberg M, Werner H, Leske O, Püschel A W, Memet S, Heumann R, Israel A, Kaltschmidt C, Kaltschmidt B (2012). Regrowing the adult brain: NF-κB controls functional circuit formation and tissue homeostasis in the dentate gyrus. PLoS ONE, 7(2): e30838

Kannangara T S, Lucero M J, Gil-Mohapel J, Drapala R J, Simpson J M, Christie B R, van Praag H (2011). Running reduces stress and enhances cell genesis in aged mice. Neurobiol Aging, 32(12): 2279–2286

Kempermann G (2005). Adult neurogenesis, Stem Cells and Neuronal Development in the Adult Brain. Oxford University Press, New York

Kent B A, Beynon A L, Hornsby A K, Bekinschtein P, Bussey T J, Davies J S, Saksida L M (2015). The orexigenic hormone acyl-ghrelin increases adult hippocampal neurogenesis and enhances pattern separation. Psychoneuroendocrinology, 51: 431–439

Kent B A, Hvoslef-Eide M, Saksida L M, Bussey T J (2016). The representational-hierarchical view of pattern separation: Not just hippocampus, not just space, not just memory? Neurobiol Learn Mem, 129: 99–106

Kesner R P (2013). An analysis of the dentate gyrus function. Behav Brain Res, 254: 1–7

Kesner R P, Hui X, Sommer T, Wright C, Barrera V R, Fanselow M S (2014). The role of postnatal neurogenesis in supporting remote memory and spatial metric processing. Hippocampus, 24(12): 1663–1671

Kheirbek M A, Klemenhagen K C, Sahay A, Hen R (2012a). Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. Nat Neurosci, 15(12): 1613–1620

Kheirbek M A, Tannenholz L, Hen R (2012b). NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. J Neurosci, 32(25): 8696–8702

Kitamura T, Saitoh Y, Takashima N, Murayama A, Niibori Y, Ageta H, Sekiguchi M, Sugiyama H, Inokuchi K (2009). Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory. Cell, 139(4): 814–827

Koehl M, Abrous D N (2011). A new chapter in the field of memory: adult hippocampal neurogenesis. Eur J Neurosci, 33(6): 1101–1114

Leutgeb J K, Leutgeb S, Moser M B, Moser E I (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science, 315(5814): 961–966

Liu K Y, Gould R L, Coulson M C, Ward E V, Howard R J (2016). Tests of pattern separation and pattern completion in humans-A systematic review. Hippocampus, 26(6): 705–717

Llorens-Martín M, Jurado-Arjona J, Fuster-Matanzo A, Hernández F, Rábano A, Ávila J (2014). Peripherally triggered and GSK-3β-driven brain inflammation differentially skew adult hippocampal neurogenesis, behavioral pattern separation and microglial activation in response to ibuprofen. Transl Psychiatry, 4(10): e463

Lopez-Atalaya J P, Ciccarelli A, Viosca J, Valor L M, Jimenez-Minchan M, Canals S, Giustetto M, Barco A (2011). CBP is required for environmental enrichment-induced neurogenesis and cognitive enhancement. EMBO J, 30(20): 4287–4298

Luu P, Sill O C, Gao L, Becker S, Wojtowicz J M, Smith D M (2012). The role of adult hippocampal neurogenesis in reducing interference. Behav Neurosci, 126(3): 381–391

Marín-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(6073): 1238–1242

Marín-Burgin A, Schinder A F (2012). Requirement of adult-born neurons for hippocampus-dependent learning. Behav Brain Res, 227 (2): 391–399

Marr D (1971). Simple memory: a theory for archicortex. Philos Trans R Soc Lond B Biol Sci, 262(841): 23–81

McHugh T J, Jones M W, Quinn J J, Balthasar N, Coppari R, Elmquist J K, Lowell B B, Fanselow M S, Wilson M A, Tonegawa S (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science, 317(5834): 94–99

McNaughton B L, Morris R G M (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends Neurosci, 10(10): 408–415

Nadel L, Peterson M A (2013). The hippocampus: part of an interactive posterior representational system spanning perceptual and memorial systems. J Exp Psychol Gen, 142(4): 1242–1254

Nakashiba T, Cushman J D, Pelkey K A, Renaudineau S, Buhl D L, McHugh T J, Rodriguez Barrera V, Chittajallu R, Iwamoto K S, McBain C J, Fanselow M S, Tonegawa S (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell, 149(1): 188–201

Neunuebel J P, Knierim J J (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. Neuron, 81(2): 416–427

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

Oomen C A, Bekinschtein P, Kent B A, Saksida L M, Bussey T J (2014). Adult hippocampal neurogenesis and its role in cognition. Wiley Interdiscip Rev Cogn Sci, 5(5): 573–587

Pan Y W, Chan G C, Kuo C T, Storm D R, Xia Z (2012). Inhibition of adult neurogenesis by inducible and targeted deletion of ERK5 mitogen-activated protein kinase specifically in adult neurogenic regions impairs contextual fear extinction and remote fear memory. J Neurosci, 32(19): 6444–6455

Park H, Yang J, Kim R, Li Y, Lee Y, Lee C, Park J, Lee D, Kim H, Kim E (2015). Mice lacking the PSD-95-interacting E3 ligase, Dorfin/Rnf19a, display reduced adult neurogenesis, enhanced long-term potentiation, and impaired contextual fear conditioning. Sci Rep, 5: 16410

Pérez-García G, Guzmán-Quevedo O, Da Silva Aragão R, Bolaños-Jiménez F (2016). Early malnutrition results in long-lasting impairments in pattern-separation for overlapping novel object and novel location memories and reduced hippocampal neurogenesis. Sci Rep, 6: 21275

Rangel L M, Alexander A S, Aimone J B, Wiles J, Gage F H, Chiba A A, Quinn L K (2014). Temporally selective contextual encoding in the dentate gyrus of the hippocampus. Nat Commun, 5: 3181

Revest J M, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza P V, Abrous D N (2009). Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry, 14(10): 959–967

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

Saksida L M, Bussey T J, Buckmaster C A, Murray E A (2006). No effect of hippocampal lesions on perirhinal cortex-dependent feature-ambiguous visual discriminations. Hippocampus, 16(4): 421–430

Saksida L M, Bussey T J, Buckmaster C A, Murray E A (2007). Impairment and facilitation of transverse patterning after lesions of the perirhinal cortex and hippocampus, respectively. Cereb Cortex, 17(1): 108–115

Schmidt-Hieber C, Jonas P, Bischofberger J (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature, 429(6988): 184–187

Segal S K, Stark S M, Kattan D, Stark C E, Yassa M A (2012). Norepinephrine-mediated emotional arousal facilitates subsequent pattern separation. Neurobiol Learn Mem, 97(4): 465–469

Seo D O, Carillo M A, Chih-Hsiung Lim S, Tanaka K F, Drew M R (2015). Adult Hippocampal Neurogenesis Modulates Fear Learning through Associative and Nonassociative Mechanisms. J Neurosci, 35 (32): 11330–11345

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

Shors T J, Townsend D A, Zhao M, Kozorovitskiy Y, Gould E (2002).

Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus, 12(5): 578–584

Sisti H M, Glass A L, Shors T J (2007). Neurogenesis and the spacing effect: learning over time enhances memory and the survival of new neurons. Learn Mem, 14(5): 368–375

Stark S M, Stevenson R, Wu C, Rutledge S, Stark C E (2015). Stability of age-related deficits in the mnemonic similarity task across task variations. Behav Neurosci, 129(3): 257–268

Stark S M, Yassa M A, Stark C E (2010). Individual differences in spatial pattern separation performance associated with healthy aging in humans. Learn Mem, 17(6): 284–288

Svensson M, Grahm M, Ekstrand J, Movahed-Rad P, Johansson M, Tingström A (2015). Effect of electroconvulsive seizures on pattern separation. Hippocampus, 25(11): 1351–1360

Swan A A, Clutton J E, Chary P K, Cook S G, Liu G G, Drew M R (2014). Characterization of the role of adult neurogenesis in touch-screen discrimination learning. Hippocampus, 24(12): 1581–1591

Treves A, Rolls E T (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. Hippocampus, 2(2): 189–199

Treves A, Tashiro A, Witter M P, Moser E I (2008). What is the mammalian dentate gyrus good for? Neuroscience, 154(4): 1155–1172

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

Van der Borght K, Havekes R, Bos T, Eggen B J, Van der Zee E A (2007). Exercise improves memory acquisition and retrieval in the Y-maze task: relationship with hippocampal neurogenesis. Behav Neurosci, 121(2): 324–334

Winocur G, Wojtowicz J M, Tannock I F (2015). Memory loss in chemotherapy-treated rats is exacerbated in high-interference conditions and related to suppression of hippocampal neurogenesis. Behav Brain Res, 281: 239–244

Wu M V, Hen R (2014). Functional dissociation of adult-born neurons along the dorsoventral axis of the dentate gyrus. Hippocampus, 24 (7): 751–761

Wu M V, Luna V M, Hen R (2015). Running rescues a fear-based contextual discrimination deficit in aged mice. Front Syst Neurosci, 9: 114

Yagi S, Chow C, Lieblich S E, Galea L A (2016). Sex and strategy use matters for pattern separation, adult neurogenesis, and immediate early gene expression in the hippocampus. Hippocampus, 26(1): 87–101

Yassa M A, Muftuler L T, Stark C E (2010). Ultrahigh-resolution microstructural diffusion tensor imaging reveals perforant path degradation in aged humans *in vivo*. Proc Natl Acad Sci USA, 107(28): 12687–12691

Yassa M A, Stark C E (2011). Pattern separation in the hippocampus. Trends Neurosci, 34(10): 515–525

Yun S, Donovan M H, Ross M N, Richardson D R, Reister R, Farnbauch L A, Fischer S J, Riethmacher D, Gershenfeld H K, Lagace D C, Eisch A J (2016). Stress-induced anxiety- and depressive-like phenotype associated with transient reduction in neurogenesis in adult nestin-CreERT2/Diphtheria toxin fragment A transgenic mice. PLoS ONE, 11(1): e0147256

Zhang C L, Zou Y, He W, Gage F H, Evans R M (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. Nature, 451(7181): 1004–1007

Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, Yuan Z, Jiao J (2014). Ezh2 regulates adult hippocampal neurogenesis and memory. J Neurosci, 34(15): 5184–5199